

## ANNEX D

### Effects of ionizing radiation on the immune system

#### Contents

	<i>Page</i>
INTRODUCTION .....	85
I. GENERAL FEATURES OF THE IMMUNE SYSTEM.....	87
A. Introduction .....	87
B. Organs and tissues of the immune system .....	89
1. Central lymphoid organs .....	89
2. Peripheral lymphoid tissues .....	89
3. Remarks concerning organs and tissues of the immune system .....	91
C. Cells and molecules of the immune system.....	91
1. Innate immunity .....	91
2. Acquired immunity .....	95
3. Human major histocompatibility complex .....	99
4. Antigen-presenting cells .....	102
5. Self tolerance and self-HLA-associated recognition .....	103
6. Cytokines .....	104
7. Remarks concerning cells and molecules of the immune system .....	105
D. Physiological immunosenescence .....	106
1. Concept of immunosenescence .....	106
2. Main features of immunosenescence .....	106
3. Remarks concerning immunosenescence .....	107
E. Summary .....	107
II. RADIATION-INDUCED ALTERATIONS OF THE IMMUNE SYSTEM.....	109
A. Introduction .....	109
B. Data concerning low-dose irradiation .....	109
1. Animal data .....	109
2. Human data .....	113
3. Remarks concerning low-dose/low-dose-rate irradiation .....	115
C. Data concerning high-dose irradiation .....	116
1. High-dose-induced immunosuppression .....	116
2. Immune reconstitution .....	117
3. Remarks concerning high-dose irradiation .....	118
D. Influence of dose rate and radiation quality on immune response .....	118
1. Low-LET exposures .....	118
2. High-LET exposures .....	119
3. Remarks concerning the influence of dose rate and radiation quality .....	121
E. Radiosensitivity of lymphocyte subsets .....	122
1. General considerations .....	122
2. Review of published data .....	122

	<i>Page</i>
3. Remarks concerning the radiosensitivity of lymphocyte subsets . . . . .	123
F. Alterations of the immune response after prenatal irradiation . . . . .	124
1. Animal data . . . . .	124
2. Human data . . . . .	125
3. Remarks concerning the effects of prenatal irradiation. . . . .	126
G. Immune dysfunction and hypersensitivity to ionizing radiation . . . . .	126
1. Human genetic disorders . . . . .	126
2. Autoimmune diseases and radiation response . . . . .	130
3. Ionizing radiation and acquired immune deficiency syndrome . . . . .	130
4. Remarks concerning human pathologies with immune dysfunction and hypersensitivity to ionizing radiation . . . . .	131
H. Summary. . . . .	131
III. POSSIBLE MECHANISMS INVOLVED AND IMPLICATIONS . . . . .	133
A. Lymphocyte apoptosis . . . . .	133
1. Review of published data . . . . .	133
2. Remarks concerning lymphocyte apoptosis . . . . .	134
B. TCR mutations . . . . .	135
1. Review of published data . . . . .	135
2. Remarks concerning TCR mutations . . . . .	136
C. Modification of Th1/Th2 balance . . . . .	136
1. Review of published data . . . . .	136
2. Remarks concerning modification of Th1/Th2 balance . . . . .	137
D. Bystander effects and genomic instability . . . . .	138
1. Review of published data . . . . .	138
2. Remarks concerning bystander effects and genomic instability . . . . .	139
E. Shift towards an inflammatory profile . . . . .	139
1. Review of published data . . . . .	139
2. Remarks concerning the shift towards an inflammatory profile . . . . .	140
F. Acceleration of immunological ageing . . . . .	140
1. Review of published data . . . . .	140
2. Remarks concerning the acceleration of immunological ageing . . . . .	140
G. Modification of antigen presentation . . . . .	143
1. Review of published data . . . . .	143
2. Remarks concerning modification of antigen presentation . . . . .	143
H. Autoimmune reactions . . . . .	143
1. Review of published data . . . . .	143
2. Remarks concerning autoimmune reactions. . . . .	144
I. Perturbation of immunological homeostasis . . . . .	144
1. Review of published data . . . . .	144
2. Remarks concerning perturbation of immunological homeostasis . . . . .	144
J. Other possible mechanisms involved . . . . .	145
1. Review of published data . . . . .	145
2. Remarks concerning other possible mechanisms involved . . . . .	145
K. Immune mechanisms and cancer . . . . .	146
1. General considerations. . . . .	146
2. Immune surveillance theory . . . . .	146
3. Immune response against tumours . . . . .	147
4. Immunologic promotion of tumours . . . . .	148

	<i>Page</i>
5. Remarks concerning immune mechanisms and cancer .....	149
L. Summary .....	149
IV. EPIDEMIOLOGICAL STUDIES .....	151
A. Atomic bombing survivors .....	151
1. General considerations .....	151
2. Short-term effects .....	151
3. Long-term effects .....	151
4. Remarks concerning data on survivors of the atomic bombings .....	155
B. Chernobyl workers and residents .....	155
1. General considerations .....	155
2. Emergency and clean-up workers .....	156
3. Residents of contaminated areas .....	157
4. Radioiodine contamination, immune status and thyroid diseases .....	159
5. Remarks concerning data on Chernobyl workers and residents .....	160
C. Techa River study .....	160
1. General considerations .....	160
2. Epidemiological data .....	160
3. Remarks on the Techa River study .....	162
D. Hanford nuclear site .....	162
1. General considerations .....	162
2. Hanford Thyroid Disease Study .....	163
3. Remarks concerning the Hanford nuclear site .....	163
E. Patients undergoing radiotherapy .....	163
1. General considerations .....	163
2. Review of published data .....	163
3. Remarks concerning data on patients undergoing radiotherapy .....	164
F. Summary .....	164
V. FINAL SUMMARY .....	167
VI. CONCLUDING REMARKS .....	171
ABBREVIATIONS .....	173
REFERENCES .....	177



## INTRODUCTION

1. DNA lesions and defective DNA repair are usually considered to be the key mechanisms that explain the biological effects of ionizing radiation. Recently, radiation-induced non-targeted effects, e.g. genomic instability or the bystander effect, have been described; they may represent other critical mechanisms in the initiation and development of late radiation-induced effects at the cellular or tissue level. Acute effects of whole-body irradiation may manifest as multiple organ failures (involving essentially the haematopoietic, digestive and central nervous systems as well as the skin). In this context, alterations of the immune system by ionizing radiation represent a field that has not recently been thoroughly assessed.

2. The effects of ionizing radiation upon immune system function were first reviewed in detail by the Committee in 1972 [U10]. Aspects of the subject were discussed in further UNSCEAR reports, as summarized in table 1 [U2, U4, U6,

U7, U8, U9]. A large number of new papers and technological developments since then have fostered progress in understanding the basic concepts concerning mechanisms underlying the effects of ionizing radiation on the immune system. In addition, there exists today a considerable amount of new data related to impaired immunological functions and the risk of diseases or mortality from causes other than cancer.

3. The scope of this annex includes reviews of:

- Radiation-induced alterations of the immune response, including immunosuppression (depression) or immunostimulation (activation);
- Possible mechanisms by which the immune system is altered following exposure to ionizing radiation;
- Epidemiological assessments of immune system alterations in various diseases, with emphasis on the effects of ionizing radiation.

**Table 1 Aspects covered by UNSCEAR documents concerning the effects of ionizing radiation upon immune system function**

<i>Content</i>	<i>Reference</i>
Volume II, annex F ("Effects of radiation on the immune system"): the general components of the immune response, effects of radiation on susceptibility to infection, effects of radiation on antibody formation, effects of radiation on cellular immune reactions, radiation and immunological tolerance, immunological aspects of radiation-induced carcinogenesis, effects of variation of condition of irradiation on immunological responses	[U10]
Radiocarcinogenesis and the immune system. Effects of prenatal irradiation on the haematopoietic and immune systems	[U9]
Effects of ionizing radiation on the haematopoietic and immune systems	[U8]
Radiation-induced cancer: impairment of immunological surveillance	[U7]
Haematological and immunological effects of ionizing radiation	[U6]
Annex B, part III: Effects of ionizing radiation on the immune system in the context of adaptive response	[U4]
Radiosensitivity and defective recombination in the immune system; immunological effects of exposure to radiation from the Chernobyl accident	[U2]



## I. GENERAL FEATURES OF THE IMMUNE SYSTEM

### A. Introduction

4. The immune system consists of cells and tissues spread widely throughout the body that protect against infections and cancer. Following the recognition of foreign or novel antigens, which are present in an immense variety of organisms or neoplastic cells, this system executes a complex response in order to give protection. Organs or tissues transplanted from other individuals are also recognized by the immune system as foreign agents. Immune recognition of these antigens generally leads to their elimination through the destruction and removal of the foreign cells.

5. The immune response is mediated by a number of different cells and molecules. The majority of these cells are white blood cells (leucocytes) produced in the bone marrow as precursors. Some develop into mature cells within the bone marrow, and others are transported by the blood to other tissues where they develop and mature further. Many molecules of the immune system are soluble or cell surface proteins. The immunocompetent cells are strategically

located in areas that come into close contact with foreign substances. In these locations, they are perfectly positioned to recognize those substances as “non-self” or foreign. Upon such recognition, immune cells are activated and function to neutralize or destroy the invading foreign substance.

6. Immunophenotyping with combinations of antibodies to various cell surface and cytoplasmic proteins allows the identification of specific cell types, determination of the degree of cell differentiation and recognition of abnormal cells. Most of these antibodies are against surface glycoproteins, which are often not only associated with particular cell lineages but also vary in expression with maturation, and are thus referred to as differentiation antigens. These antigens have been grouped together in clusters of differentiation (CD), numbered in the order in which they were identified. Therefore, by incubating cell suspensions with monoclonal antibodies that bind selectively to these cell membrane components, it is possible to identify the phenotype of different subsets of immune cells and determine their relative and absolute abundance (see table 2).

**Table 2 List of CD antigens cited in the text**

Adapted from reference [J1]

<i>CD</i>	<i>Cell population<sup>a</sup></i>	<i>Function<sup>b</sup></i>	<i>Molecular family</i>
CD1 a, b, c, d, e	Dendritic cells, Langerhans cells, B-cells, thymocytes	Antigen presentation (glycolipids)	Immunoglobulin
CD2	Thymocytes, NK cells, T-cells	Adhesion molecules; binding to CD58 and Lck activating T-cells	Immunoglobulin
CD3	T-cells, thymocytes	TCR-associated	Immunoglobulin
CD4	Subpopulation of thymocytes, Th1 cells, Th2 cells, monocytes, macrophages	Co-receptor of TCR for class II MHC; receptor of HIV-1/2 gp120; fixation of Lck (src-family tyrosine kinase) to the inner surface of the cell membrane	Immunoglobulin
CD5	Thymocytes, mature T-cells and B-cells	Positive or negative modulation of TCR signalling	Receptor
CD8	Subpopulation of thymocytes, cytotoxic cells	Co-receptor of TCR for class I MHC; binding to Lck on the inner surface of the cell membrane	Immunoglobulin
CD11b	NK cells and myeloid cells	Binding with CD54, iC3b components and $\alpha$ M subunit of integrins	$\alpha$ -integrins
CD14	Monocytes, macrophages, weakly expressed in neutrophils and myeloid dendritic cells	Receptor for the complex of LPS and LPB	Receptor
CD16	Neutrophils, NK cells, macrophages	Low-affinity FcR component (FcR $\gamma$ III); mediator of phagocytosis and cytotoxicity	$\alpha$ -integrins
CD19	B-cells	Formation of complexes with CD21 and CD81; B-cell co-receptor	$\alpha$ -integrins

<i>CD</i>	<i>Cell population<sup>a</sup></i>	<i>Function<sup>b</sup></i>	<i>Molecular family</i>
CD20	B-cells	Ca <sup>2+</sup> channel formation; possible role in B-cell activation	Possesses four transmembrane segments
CD23	Mature B-cells, activated macrophages, eosinophils, megakaryocytes, dendritic cells	Receptor with low affinity for IgE	Lectin-C-like
CD25	Activated T-cells, B-cells, monocytes	Subunit $\alpha$ of the human interleukin-2 receptor	$\alpha$ -chain type I glycoprotein containing two complement control protein domains
CD27	Medullar thymocytes, T-cells, NK cells and certain B-cells	Co-stimulator of T- and B-cells	TNF receptor
CD28	T-cell subpopulations, activated B-cells	Naive T-cell activation; co-stimulatory pathway involving CD80 and CD86	Immunoglobulin and CD86
CD38	Activated T- and B-cells	Multifunctional enzyme (NAD glycohydrolase, ADP-ribosyl cyclase, cyclic ADP ribose hydrolase); regulation of T- and B-cell activation; also functions in cell adhesion, signal transduction and calcium signalling	Type II glycoprotein
CD40	B-cells, macrophages, dendritic cells, basal cells, epithelial cells	Co-stimulator of B-cells; cytokine production by macrophages and dendritic cells	TNF receptor
CD43	Leucocytes (except resting B-cells)	Anti-adhesive	Mucine
CD44	Leucocytes, erythrocytes	Link with hyaluronic acid; adhesion receptor	Adhesion molecule
CD45	All haematopoietic cells	Tyrosine phosphatase	Fibronectin type III
CD45RO	T- and B-cell subpopulations, monocytes, macrophages	Isoforms of CD45 without A, B and C exons	Fibronectin type II
CD45RA	Naive T-cells, monocytes	Isoforms of CD45 with the A exon	Fibronectin type II
CD48	All leucocytes except neutrophils	Involved in lymphocyte activation through the interaction with its receptor (CD2)	Adhesion molecule
CD56	NK cells	Isoform of NCAM molecules	Immunoglobulin
CD69	Activated B- and T-cells, activated macrophages and NK cells	Antigen of early activation	Lectin C
CD80 (B7.1)	B-cell subpopulation	Co-stimulator; ligand for CD28 and CTLA-4	Immunoglobulin
CD86 (B7.2)	Monocytes, activated B-cells, dendritic cells	Ligand for CD28 and CTLA-4	Immunoglobulin
CD95	Broadly expressed in different cell lines	Binding with FasL (TNF-like), apoptosis induction	TNF receptor
CD117 (c-Kit)	Haematopoietic progenitor cells	Stem cell factor receptor	Immunoglobulin, tyrosine kinase
CD119	B-cells, monocytes, macrophages, endothelium	Interferon- $\gamma$ receptor	Fibronectin type III
CD120	Broadly expressed	TNF receptor	TNF receptor
CD121	T-cells, thymocytes, B-cells, macrophages, monocytes	IL-1 $\alpha$ and IL-1 $\beta$ receptor	Immunoglobulin
CD124	T-cells, B-cells, haematopoietic precursors	IL-4 receptor	Cytokine receptor, fibronectin type III
CD125	Activated B-cells, eosinophils, basophils	IL-5 receptor	Cytokine receptor, fibronectin type III
CD127	Monocytes, pro-B-cells, T-cells, lymphoid precursors	IL-7 receptor	Fibronectin type III
CD130	Broadly expressed	Common subunit shared with IL-6, IL-11 and leukaemia inhibitor factor	Immunoglobulin, cytokine receptor, fibronectin type III
CD132	B-cells, T-cells, NK cells, mastocytes, neutrophils	$\gamma$ -chain of the IL-2 receptor; common subunit shared with IL-4, IL-7, IL-9 and IL-15	Cytokine receptor

<sup>a</sup> NK = natural killer.

<sup>b</sup> TCR = T-cell receptor; MHC = major histocompatibility complex; HIV = human immunodeficiency virus; LPS = lipopolysaccharide; LPB = LPS-binding protein; TNF = tumour necrosis factor.



## B. Organs and tissues of the immune system

7. Like red blood cells (erythrocytes) and platelets (thrombocytes), the cells of the immune system arise in the bone marrow from pluripotent stem cells by a process called haematopoiesis. Myeloid precursors develop into a group of white blood cells known as phagocytes. Phagocytes include monocytes, macrophages and neutrophils. Other myeloid descendants become granule-containing inflammatory cells such as eosinophils, basophils and mast cells. Lymphoid precursors develop into the small white blood cells called lymphocytes. The two major classes of lymphocytes are B-cells and T-cells. Cells and molecules of the immune system are discussed in detail in section I.C.

8. The bone marrow and thymus are termed the primary (or central) lymphoid tissues, because mature lymphocytes are produced within these organs. Mature lymphocytes then enter the blood and travel to the secondary (or peripheral) lymphoid tissues such as lymph nodes, spleen and mucosa-associated lymphoid tissue. A notable feature of lymphocytes is that they can cross from the blood into the lymphatic circulation and then return, a phenomenon referred to as lymphocyte traffic or recirculation. The direct migration of lymphocytes to specific tissues is called homing.

### 1. Central lymphoid organs

9. All cells of the immune system originate from just one cell type, called a haematopoietic stem cell, in the bone marrow of adults (for prenatal life, see section II.F below). Pluripotent stem cells multiply to produce more pluripotent stem cells (self-renewal), thus ensuring the steady and lasting supply of stem cells. Homeostatic regulation stabilizes the number of pluripotent stem cells. Some of the pluripotent stem cells differentiate to become committed to one of two blood cell lineages: lymphoid or myeloid. Then lymphoid and myeloid stem cells become progenitor cells for each type of mature blood cell. These cells have lost the capacity for self-renewal and are committed to a given cell lineage: T- and B-cell progenitors, and progenitor cells for granulocytes, monocytes, eosinophils, basophils, mast cells, platelets and erythrocytes. Dendritic cells belong to the myeloid cell lineage, and it is likely that monocytes and dendritic cells arose from a common myeloid precursor. Progenitor commitment depends upon the acquisition of responsiveness to different growth factors such as colony-stimulating factors (CSFs), erythropoietin (EPO) and interleukins (ILs). The particular microenvironment within which the progenitor cell resides controls differentiation. The haematopoietic cells grow and mature on a meshwork of stromal cells, which are non-haematopoietic cells that support the growth and differentiation of the haematopoietic cells (figure I). In the absence of infection, bone marrow stromal cells are the major source of haematopoietic cytokines. In the presence of infection, cytokines produced by certain activated immunocompetent

cells induce additional haematopoietic activity, resulting in the rapid expansion of the white blood cells that participate in fighting infection. After a period of maturation and expansion within the bone marrow, more differentiated cells enter the bloodstream and are distributed throughout the body in different tissues. B-lymphocytes develop and mature in the bone marrow before entering the bloodstream, in contrast to T-lymphocytes, which leave the bone marrow as T-cell precursors and complete further maturation in the thymus [A25].

10. The thymus is an organ located anterior in the upper mediastinum. Immature T-lymphocytes migrate from the bone marrow into the thymus, where they become mature immunocompetent T-cells. A minority of lymphocytes become mature T-lymphocytes by extrathymic maturation processes. After thymic involution (see below), this pathway of extrathymic lymphocyte maturation becomes more relevant [B32]. Thymus cells are called thymocytes and are predominantly immature T-cells at various stages of development. There are also scattered epithelial cells, macrophages and dendritic cells [A25]. The thymus is very large in the first years of life, reaches maximum size at puberty and then becomes smaller in a process called involution. During this degenerative process, connective tissue, fibres and fat cells replace the previously functional tissue. Although only a few pieces of functional tissue remain, they suffice to continue to supply the organism with enough mature lymphocytes. Cervical lymphoid organs with thymic structure and function have recently been found in BALB/c and C57BL/6 mouse strains. Although cervical thymus tissue has been observed in other species, the presence of cervical thymus tissue in humans is considered rare after birth [T18].

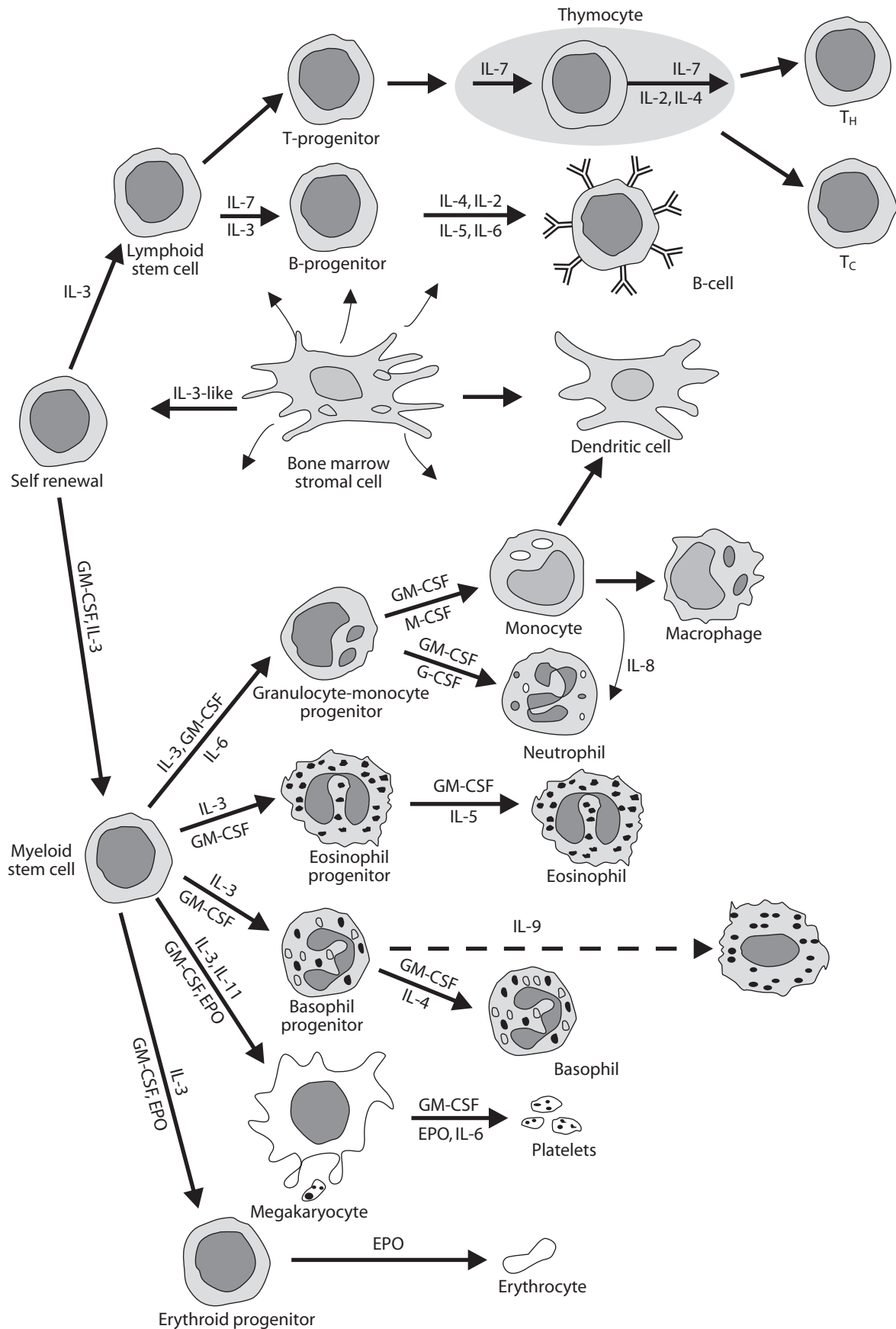
11. The main functions of the thymus include:

- Production of immunocompetent T-lymphocytes;
- Production of mature T-cells for peripheral tissues and circulation;
- Regulation of T-cell maturation, proliferation and function;
- Immunological self tolerance via positive and negative selection.

### 2. Peripheral lymphoid tissues

12. After maturing in the thymus, T-cells move through the circulation to other organs. Lymph nodes are small, bean-shaped structures that are spread throughout the body along the lymphatic routes. They contain specialized compartments where immune cells congregate and where they can encounter antigens. Lymph nodes constitute groups in areas where lymphatic vessels come together to form larger vessels, such as in the groin, neck and axilla. All lymphatic vessels draining back to the venous circulation from the tissues pass through lymph nodes, which filter and purify the drain fluid of the lymphatic vessels (lymph) before it flows

**Figure I. Regulation of haematopoiesis in the bone marrow by cytokines that stimulate the proliferation and/or differentiation of various haematopoietic cells (adapted from reference [G14]).**  
 GM-CSF: Granulocyte-macrophage colony-stimulating factor.



into the venous system [J1]. Several immune functions take place in the lymph nodes:

- Phagocytosis of microorganisms by fixed macrophages;
- T-lymphocyte activation;
- B-lymphocyte activation and proliferation (plasma cell formation and antibody production);
- Interaction between antigen-presenting cells and circulating lymphocytes.

13. The spleen is a soft organ located in the left hypochondrium that removes abnormal blood cells through phagocytosis, collects and disposes of senescent red blood cells, provides storage of iron from recycled red blood cells and plays a role in foetal and sometimes adult haematopoiesis. Spleen cells are called splenocytes. It is in the spleen that the initiation of immune responses by B-cells and T-cells takes place in response to antigens circulating in the blood. The spleen has a “red pulp” (its colour due to the presence of large numbers of erythrocytes in the blood vessels) that is characterized by a parenchyma that consists of macrophages and blood cells surrounded by numerous venous sinuses. The “white pulp” of the spleen is characterized by the lack of these sinuses and consequently the presence of fewer erythrocytes, and consists of B- and T-lymphocytes located in two different areas of the spleen. B-cells are located in the lymphoid follicles scattered throughout the organ. T-cells are located around the central arteries and form a kind of sheath (periarteriolar lymphoid sheath) [G27].

14. Mucosa-associated lymphoid tissue (MALT) consists of connective tissue containing lymphocytes and is located beneath mucous membranes of the respiratory, gastrointestinal, urinary and reproductive tracts. MALT has no distinct capsule like that of the lymph nodes but does often have a germinal centre containing actively dividing lymphocytes. Waldeyer’s throat ring (comprising the adenoids and the palatine, lingual and pharyngeal tonsils), Peyer’s patches of the small intestine, and the appendix are all examples of mucosa-associated lymphoid tissue [J1].

15. The skin is the largest organ in the human body. It protects against injury, infection, heat and cold, and it stores water, fat and vitamins [C30]. Although classically described as an anatomic barrier of the innate immune system (see section I.C.1 below), the skin may also be considered as an organ of the immune system. The ability of the cutaneous barrier to help defend the body against pathogens depends on both acquired and innate immune responses [B17, B27].

16. The “skin immune system” (SIS) has been proposed as the term for the complex of immune-response-associated cells present in normal human skin. Approximately half of human skin cells are directly or indirectly involved in the immune response (e.g. keratinocytes, endothelial cells, dendritic cells, T-lymphocytes, monocytes/macrophages, granulocytes and mast cells). Some of these cells are skin

residents, others can be recruited or are recirculating cells. This concept of the SIS also includes human skin humoral constituents such as immunoglobulins, cytokines, chemokines, complement, cathelicidins, defensins, dermcidins, prostaglandins and free radicals [B28, R13].

### 3. Remarks concerning organs and tissues of the immune system

17. On the basis of the preceding discussion, some concepts may be highlighted. The bone marrow and the thymus are the central lymphoid tissues. All cells of the immune system originate in the bone marrow from haematopoietic stem cells. After maturation and expansion, more differentiated immune cells enter the bloodstream to be distributed in the different tissues. Some immature lymphocytes move from the bone marrow to the thymus, where they become immunocompetent T-cells. Spread throughout the body, lymph nodes are peripheral lymphoid tissues where immune cells congregate and where these cells can encounter antigens. It is in the spleen, another peripheral lymphoid organ, that the initiation of the B- and T-cell immune responses to circulating antigens takes place. MALT consists of connective tissue containing lymphocytes. The skin and mucous membranes are the first line of defence against pathogens. Classically they have been described as anatomic barriers of the innate immune system alone; however, around half of human skin cells are involved in both innate and acquired immunity.

### C. Cells and molecules of the immune system

18. The cells and molecules of the immune system execute two different but related forms of immunity: innate and acquired. While innate immunity is fully functional before any foreign agent enters the body, acquired immunity is activated only after a pathogen has entered the organism. Acquired immunity mediated by B- and T-lymphocytes recognizes pathogens by rearranged high-affinity receptors. However, as acquired immunity involves activation and gene expression as well as cell proliferation, it is often not rapid enough to eradicate microorganisms. Innate immunity provides more rapid defence mechanisms.

#### 1. Innate immunity

19. The innate immune system comprises several defensive barriers:

- Anatomic barrier: skin and mucous membranes;
- Physiological barrier: temperature, pH, lysozymes and circulating factors such as interferon and complement;
- Inflammatory barrier: cellular and chemical mediators of the inflammatory response (histamine, acute phase proteins, fibrin, kinin);
- Phagocytic barrier (see below).

20. Phagocytes include granulocytes, peripheral monocytes, tissue macrophages and dendritic cells. Phagocytes are cells capable of surrounding, engulfing and digesting complete microorganisms by phagocytosis. Some of them play an important role in producing molecules involved in the inflammatory response associated with infections. They migrate towards the site of infection by margination, diapedesis and chemotaxis.

21. Granulocytes include three types of cell: neutrophils, eosinophils and basophils. Neutrophils play an essential role in the body's innate immune defence and are one of the primary mediators of the inflammatory response. They are highly specialized for their primary function, which is the phagocytosis and destruction of microorganisms. To defend the host, neutrophils employ a wide range of microbicidal products, such as oxidants, microbicidal peptides and lytic enzymes. The generation of microbicidal oxidants by neutrophils results in a respiratory burst with generation of highly reactive oxygen and nitrogen species (ROS/RNS) [G13, Q1]. Eosinophils attack parasites and phagocytose antigen-antibody complexes. Basophils secrete anticoagulant and vasodilatory substances, such as histamines and serotonin. Even though they possess phagocytic capability, their main function is the secretion of substances that mediate hypersensitivity reactions.

22. Monocytes in peripheral blood are young cells that already possess phagocytic capabilities. After migration into tissues, monocytes undergo further differentiation to become multifunctional tissue macrophages. Macrophages and dendritic cells present antigens to be recognized by lymphocytes, and are thus called antigen-presenting cells (APCs). (Although less efficient, B-lymphocytes are also APCs, as will be discussed later.) Macrophages play a central

role in the immune response. Among other functions, they seek out, ingest and destroy bacteria, viruses, tumour cells and other foreign material. They present foreign material to the cells of the immune system and in this way regulate the immune response. The understanding of the role of macrophages has changed since the identification of a particular family of receptors called pattern recognition receptors (PRRs). These PRRs recognize highly conserved antigenic structures, termed pathogen-associated molecular patterns (PAMPs), shared by large groups of pathogens. PRRs are secreted (complement, lectins) or expressed at the surface of cells (Toll-like receptors (TLRs)) [B23].

23. The TLR family constitutes an important component of the innate immune system. Although most commonly considered to be expressed on immune cells, e.g. phagocytes and T-regulatory cells, TLRs are also known to be functionally expressed on a variety of other cell types, such as airway and gut epithelial cells [A3, A16, C3, G31, J2, K40]. Ten members of the TLR family have been identified in humans; each recognizes a small range of conserved pathogen molecules (table 3). The binding of PAMPs to TLRs induces the production of pro-inflammatory cytokines and the up-regulation of surface co-stimulatory molecules. Recent studies have identified intracellular signalling pathways specific for individual TLRs that lead to the release of cytokine profiles specific for particular PAMPs. The ability of individual TLRs to discriminate among different PAMPs is an important determinant of the unique gene expression profiles activated in the host by different invading pathogens or environmental "danger signals" [V6]. Thus TLRs confer a certain degree of specificity to the innate immune response. Moreover, TLR-mediated recognition represents a cross-talk between the innate and the acquired immune system [A2, N8, R4].

**Table 3 Ligands recognized by Toll-like receptors**

<i>TLR</i>	<i>PAMP</i>	<i>Ligand</i>
TLR2	Gram (+) bacteria Gram (+) bacteria Bacteria Spirochaetes Leptospiras and porphyromonas Mycobacteria Yeast Trypanosoma Schistosoma Neisseria Klebsiella Host	Peptidoglycan (PGN) Lipoteichoic acid (LTA) Lipoproteins Glycolipids Lipopolysaccharide (LPS) Lipoarabinomannan Zymosan Glycosylphosphatidylinositol (GPI) Phosphatidylserine (PS) Porin Membrane protein A Heat shock proteins (HSPs)
TLR2/TLR1	Chemicals Bacteria Neisseria meningitidis	JBT-3002 Lipoproteins Soluble factor
TLR2/TLR6	Staphylococcus Streptococcus (Group B) Mycoplasma	Modulin Soluble factor Lipoprotein
TLR3	Chemicals Virus	Poly (I:C) Double-stranded RNA

<i>TLR</i>	<i>PAMP</i>	<i>Ligand</i>
TLR4	Gram (-) bacteria Chlamydia Murine retroviruses RS virus Plant Host	LPS, LTA HSP60 Envelope proteins F protein Taxol HSPs
TLR5	Bacterial flagellum	Flagellin
TLR7	Chemicals	Imidazoquinolines
TLR9	Bacteria, virus, insects Host	Unmethylated DNA rich in CpG Chromatin-IgG complexes
TLR10	Unknown	Specific ligands and functions are currently unknown

24. Natural killer (NK) cells are large granular lymphocytes able to kill a number of different target cells and that, in contrast to T- or B-lymphocytes, do not express clonally distributed receptors for antigens [M10]. They belong to the innate arm of the immune response because their cytotoxic activity is spontaneous, although activation may be mediated by cytokines. NK cells do not show secondary or memory responses. NK cells may recognize, bind and kill virus-infected host cells and tumour cells [H18, T12].

25. The heterogeneity of NK cells presents a major problem in their identification. They do not express CD3, but exhibit subsets expressing CD16 and CD56. Most human NK cells are mainly involved in cytotoxicity, have low-density expression of CD56 and have high levels of CD16 (CD3<sup>-</sup> CD56<sup>low</sup> CD16<sup>high</sup>), but around 10% of NK cells play a role in cytokine-mediated immunoregulation and are CD3<sup>-</sup> CD56<sup>high</sup> CD16<sup>-/low</sup> [C14, S19]. It was recently reported that peripheral CD56<sup>high</sup> NK cells are terminally differentiated cells indistinguishable from mature CD56<sup>low</sup> NK cells activated by IL-12, and do not constitute a functionally distinct NK cell subset [L23].

26. The different NK cell subsets have receptors on their surface that are not antigen specific. Depending on

their function, NK cell receptors can be divided into activation and inhibition receptors; NK cells are regulated by the integration of these opposing signals [L3, M10]. Normally NK cells are prevented from lysing “self” cells by the interactions of inhibitory receptors on the NK cell surface with the major histocompatibility complex (MHC). The MHC in humans is called the human leucocyte antigen (HLA), which will be discussed in more detail in section I.C.3 below. Upon self class I HLA binding, inhibitor NK receptors trigger a signalling pathway resulting in inhibition of cytotoxicity, thus providing protection for normal cells. Cells that have lost class I HLA molecules, or express insufficient amounts of them, are frequently found in viral infections or tumour transformation, are not able to trigger these inhibitory signals and thus are lysed by NK cells. Although a combination of several activating receptors may also boost lysis by NK cells, the higher affinity of the inhibitory receptors by self class I HLA prevents autoimmunity [B7, C5, L2, M11]. NK receptors may be classed within several families: killer cell immunoglobulin-like receptors (KIRs), lectin-like receptors (NKG2 and CD95), leucocyte Ig-like transcripts (ILTs), natural cytotoxicity receptors (NCRs) and Fcγ receptor III (CD16). These are described in table 4.

**Table 4 Natural killer cell receptors**

<i>Family<sup>a</sup></i>	<i>Receptor</i>	<i>Function<sup>b</sup></i>	<i>Ligand</i>	<i>Comments</i>
KIR	KIR2DL (KIR2DL4)	(-)	HLA-C (HLA-G)	2D: two extracellular Ig-like domains; L: long cytoplasmic tail
	KIR3DL	(-)	HLA-B HLA-A	3D: three extracellular Ig-like domains; L: long cytoplasmic tail
	KIR2DS	(+)	HLA-C	2D: two extracellular Ig-like domains; S: short cytoplasmic tail
	KIR3DS	(+)	HLA-B	3D: three extracellular Ig-like domains; S: short cytoplasmic tail



Family <sup>a</sup>	Receptor	Function <sup>b</sup>	Ligand	Comments
Lectin C	CD94/NKG2A (heterodimer)	(-)	HLA-E (indirect recognition of HLA-G as peptide presented by HLA-E)	CD94 functions as a chaperone (transports NKG2 to cell surface)
	CD94/NKG2C (heterodimer)	(+)	HLA-E (indirect recognition of HLA-G as peptide presented by HLA-E)	
	CD94/NKG2E (heterodimer)	(+)	HLA-E (indirect recognition of HLA-G as peptide presented by HLA-E)	
	NKG2D/NKG2D (homodimer)	(+)	MICA MICB ULBP	MICA/MICB: up-regulated on stressed cells and overexpressed in many tumours; ULBP: UL16-binding proteins from human cytomegalovirus
ILT	ILT2	(-)	HLA-A HLA-B HLA-F HLA-G	Also termed leucocyte Ig-like receptors or CD85j
NCR	NKp46	(+)	Viral and tumour proteins	Expressed in resting and activated NK cells
	NKp30	(+)	Viral and tumour proteins	
	NKp44	(+)	Viral and tumour proteins	Expressed only in activated NK cells
Fc $\gamma$ receptor III	CD16	(+)	Low-affinity receptor for IgG	Facilitates antibody-dependent cellular cytotoxicity

<sup>a</sup> KIR = killer cell Ig-like receptor (Ig superfamily); ILT = Ig-like transcript; NCR = natural cytotoxicity receptor.

<sup>b</sup> Functions: (-) = inhibitory; (+) = activating.

27. A particular type of lymphocyte called an NKT cell exhibits certain characteristics of both T-cells and NK cells. NKT cells, by definition, are T-lymphocytes, as they express a T-cell receptor (TCR) on the surface of their membranes. This distinguishes them from NK cells, although NKT cells do share some markers characteristic of NK cells. In contrast to conventional T-lymphocytes, the NKT cell TCR does not interact with peptide antigen presented by classical class I or II HLA molecules but instead recognizes glycolipids presented by CD1d, a non-classical antigen-presenting molecule. NKT cells also express a far more limited range of TCR variable (V) region genes. Unlike NK cells, NKT cells develop in the thymus and are either CD4+ or CD4-. When activated, NKT cells secrete large amounts of cytokines, such as interferon- $\gamma$  (IFN- $\gamma$ ) and interleukin-4 (IL-4). The activation of NKT cells can lead, paradoxically, either to suppression or to stimulation of immune responses, depending on the type of signal received. The cells play a critical and probably unique role in the immune system by linking innate and acquired immunity. NKT cells have been implicated in the regulation of immune responses associated with a broad range of diseases, including autoimmunity, infection and cancer [S9].

28. Mast cells (or mastocytes) are considered part of the immune system, where they play a role in innate immunity. Although best known for their role in allergy and anaphylaxis, mast cells are also involved in wound healing and in defence against pathogens. Mast cells circulate in an immature form,

only maturing once in a tissue site. They are present in most tissues in the vicinity of blood vessels, and are especially prominent near the boundaries between the outside world and the internal milieu (skin and mucosa). When activated, a mast cell rapidly releases its characteristic granules and various molecules into the intercellular environment: histamine, heparin, serine proteases, prostaglandin, leukotriene and cytokines.

29. The complement system refers to a series of serum proteins produced by different tissues and cells, including hepatocytes, macrophages and gut epithelial cells. These proteins circulate in an inactive form, but in response to the recognition of molecular components of a microorganism, they become sequentially activated in a cascade where the binding of one protein promotes the binding of the next one. Three pathways may activate the complement system: the classical complement pathway, the lectin pathway and the alternative complement pathway. These pathways differ in the manner in which they are activated. Antibody-antigen complexes activate the classical pathway. The lectin pathway is mediated by the mannan-binding protein (MBP), a protein that binds to the mannose groups found in many microbial carbohydrates but not usually found in human carbohydrates. The alternative pathway provides a means of non-specific resistance against infection without the participation of antibodies and hence provides a first line of defence against a number of infectious agents. Activation of the complement system results in the production of several

biologically active molecules (e.g. kinins, chemotactic factors and opsonins), which may lead to lysis, opsonization, inflammation or clearance of immune complexes.

## 2. Acquired immunity

30. The main features of acquired immunity are the following:

- Memory: recovery from one infection frequently protects against subsequent infection by the same organism (the individual is said to have become “immune”);
- Specificity: recovery from infection by one pathogen does not usually give protection against another;
- Diversity: the immune system can respond to an immense variety of foreign antigens;
- Self and non-self discrimination: an individual does not normally make an immune response against the antigens usually present in the body and distinguishes such antigens from those that do not belong to that individual (immune tolerance).

31. Lymphocytes are the predominant cells involved in acquired immunity, which includes humoral and cell-mediated responses. Soluble antibodies present in the serum mediate humoral responses, while cell-mediated responses result from the interaction between different types of cell. This distinction correlates, respectively, with the existence of two types of lymphocyte: B-cells and T-cells.

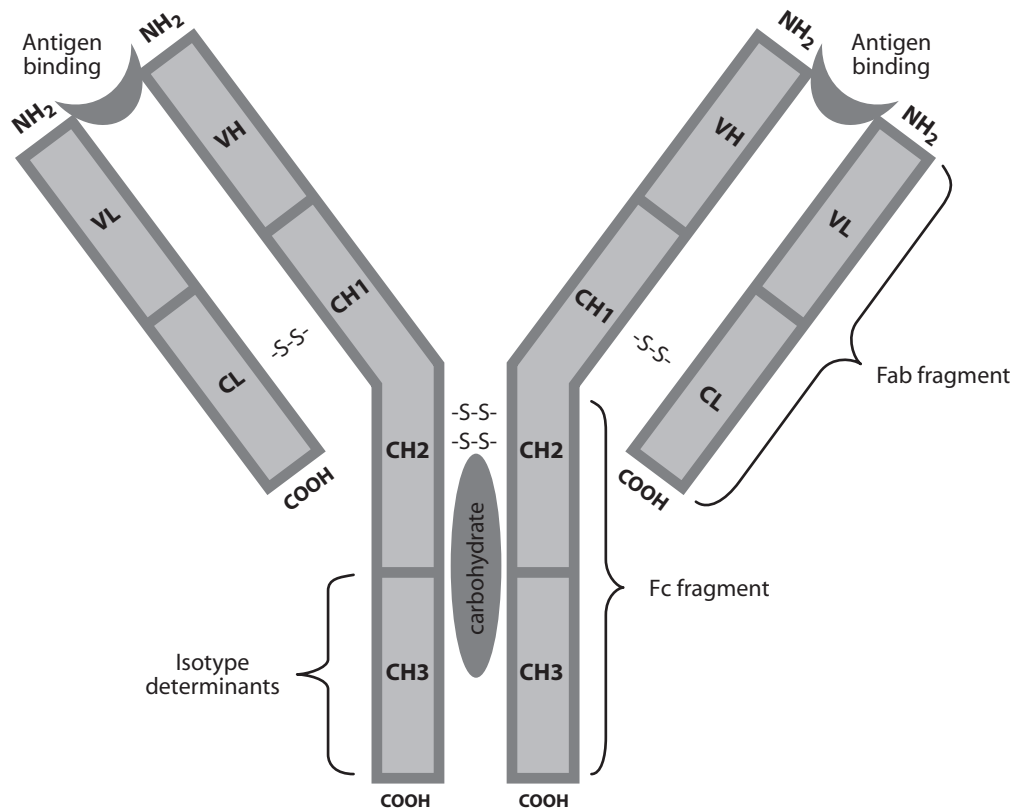
32. Cells involved in immune recognition have surface molecules called receptors that allow them to interact with other cell-attached or soluble molecules in their environment. Each receptor has a conformation complementary to the specific molecule (ligand) to be recognized. The ligand becomes bound to the receptor when recognition occurs. The term “antigen” includes any molecule that can be recognized by an antibody or by a lymphocyte receptor. However, lymphocyte receptors recognize only a small part of a whole molecule or ligand. The different parts of an antigen that can be recognized by lymphocyte receptors are called antigenic determinants or epitopes. The diversity and specificity of immune response imply that an enormous number of different specific receptors should exist. The diversity of the receptor repertoire is acquired during the maturation process of lymphocytes, which involves a large number of gene rearrangements and results, among the clones existing in the final mature population, in more than  $10^8$  types of receptor for different specific epitopes [J1].

33. B-lymphocytes are the effector cells of the humoral response. They originate and mature in the bone marrow, then they move through the circulation to various sites throughout the body. Upon interaction with a foreign

antigen, B-lymphocytes become mature antibody-secreting cells called plasma cells. Plasma cells are rarely found in the circulation but reside mostly in connective tissue, beneath epithelia, in the medullary cords of lymph nodes and in the white pulp of the spleen. Most plasma cells in the spleen and lymph nodes migrate into bone marrow.

34. B-cell receptors (BCRs) are able to recognize “free” soluble antigens in unmodified form, referred to as native antigens. B-cells can secrete antibodies in a soluble form when they are activated and develop into plasma cells. The BCRs found on mature B-cells consist of a membrane immunoglobulin (Ig) acting as an antigen-binding subunit, associated with a signalling subunit, which is a disulphide-linked Ig- $\alpha$ /Ig- $\beta$  heterodimer [M31]. Immunoglobulins are usually Y-shaped and consist of two light chains ( $\lambda$  or  $\kappa$ ) and two heavy chains ( $\alpha$ ,  $\delta$ ,  $\gamma$ ,  $\epsilon$  or  $\mu$ ). The type of heavy chain determines the immunoglobulin isotype (IgA, IgD, IgG, IgE and IgM, respectively). Each light chain has a molecular weight of ~25 000 daltons and is composed of two domains, one variable domain (VL) and one constant domain (CL). The heavy chain has a molecular weight of ~50 000 daltons and contains one variable domain (VH) and either three or four constant domains (CH1, CH2, CH3 and CH4), depending on the antibody class or isotype. Variable regions are contained within the amino ( $\text{NH}_2$ ) terminal end of the polypeptide chain and serve as the antigen-binding sites. Constant regions are, in comparison, rather uniform from one antibody to another within the same isotype. The four polypeptide chains are held together by covalent disulphide (-S-S-) bonds (figure II). While IgG is the predominant serum form, IgA is the main class of immunoglobulin found in secretions such as saliva, breast milk and tears, and in the digestive tract [G27].

35. Although in certain conditions B-cells may be susceptible to T-cell-independent activation [F15], the majority of B-cells require interaction with helper CD4+ T-cells to become activated and proliferate. The B-cell first expresses Ig on the cell surface as the B-cell receptor. If the B-cell receptor binds specific antigen, then the cell internalizes the antigen and presents it to T-cells, where it is recognized by the T-cell receptor (TCR). T-cell interaction with the B-cell involves additional regulatory signals involving stimulation, e.g. interactions CD40/CD40L and CD28/CD80. Fas ligand binding to Fas between B- and T-cells may negatively modulate B-cell activation, inducing apoptosis that limits B-cell proliferation and activation. Cytokines such as IL-2, IL-4 and IL-10 also play an important role in B-cell activation. B-cells express class II HLA molecules. They can find T-cells in secondary lymphoid organs shortly after antigen entrance, BCR-mediated endocytosis allows them to concentrate small amounts of specific antigen, and BCR signalling and class II HLA expression direct their antigen-processing machinery to favour presentation of antigens internalized through the BCR. These characteristics allow B-cells to be considered as antigen-presenting cells (APCs).

**Figure II. Molecular structure of immunoglobulins (adapted from reference [J1]).**

36. T-lymphocytes move from the bone marrow into the thymus as immature cells, take up residence and become thymus-dependent or mature T-lymphocytes. Prothymocytes in the superficial cortex of the thymus (CD2+) give rise to cortical thymocytes (CD1a+, CD2+, CD3+) and mature T-cells (CD4+ or CD8+). Medullary thymocytes are fewer and larger, and express CD4 or CD8 [A25]. Mature T-cells pass through the circulation to find homes in lymph nodes, mucosa-associated lymphoid tissue or the spleen. Most of the T-cells belong to one of two subpopulations, distinguished by the presence on their surface of one of two glycoproteins, designated CD8 and CD4. The type of these glycoproteins present determines the cell type to which T-lymphocytes can bind (see section I.C.3 below). The majority of CD8+ T-lymphocytes are cytotoxic T-cells, and the majority of CD4+ T-lymphocytes are helper T-cells. Cytotoxic and helper T-lymphocytes perform very different

functions in the immune system. Cytotoxic T-lymphocytes are effector cells that, once activated, can remove foreign organisms. Helper T-lymphocytes induce other immune cells to become better effectors. There are at least two subsets of helper T-cells (Th1 and Th2); they secrete very different cytokines upon activation [S36]. While the Th1 subset produces large amounts of cytokines that promote cell-mediated immune response, the Th2 subset produces an environment favouring humoral immunity by providing B-cell help for antibody production. The Th1 response (now sometimes called “type 1 immunity”) is characterized by production of IFN- $\gamma$ , IL-2, TNF- $\beta$  and TNF- $\alpha$ . Characteristics of the Th2 response (“type 2 immunity”) include production of IL-4, IL-10, IL-13 and IL-5, and stimulation of the production of IgE and IgG1 antibodies [K11]. Immune regulation involves homeostasis between Th1 and Th2 activity directing different immune response pathways (table 5).

**Table 5 Characteristic molecules produced by CD8+ and CD4+ T-cells**

Adapted from reference [J1]

<i>T-cell type</i>	<i>Molecule</i>	<i>Action</i>
CD8+ Production of molecules with cytotoxic activity	Perforins Granzymes Fas ligand IFN- $\gamma$ TNF- $\beta$ TNF- $\alpha$	Pore-forming proteins Proapoptotic intracellular proteases Transmembrane death activator Inhibition of viral replication Cell death Cell death



<i>T-cell type</i>	<i>Molecule</i>	<i>Action</i>
CD4+/Th1 Production of molecules capable of activating macrophages	IFN- $\gamma$ TNF- $\alpha$ Fas ligand GM-CSF CD-40-L IL-3 TNF- $\beta$ IL-2	Control of viral replication, Th2 inhibition Cell death Transmembrane death activator Granulocyte–macrophage colony stimulation Activation of target cells (via CD40 receptor) Growth and differentiation of target cells Cell death Lymphocyte activation and proliferation
CD4+/Th2 Production of molecules capable of activating B-cells	IL-4 IL-5 IL-6 CD-40-L IL-3 GM-CSF IL-10 TGF- $\beta$ Eotaxin	B-cell activation Eosinophil growth and differentiation Plasma cell and stem cell differentiation, antibody secretion, acute phase response Activation of target cells (via CD40 receptor) Growth and differentiation of target cells Granulocyte–macrophage colony stimulation Macrophage inhibition Chemotaxis and IL-1 synthesis Attracting eosinophils

37. Although most CD4+ T-cells belong to either the Th1 or Th2 subsets, 5–10% of CD4+ T-cells do not belong to these two subsets; these cells express CD25, the receptor for IL-2, and the  $\alpha\beta$  TCR. Their repertoire of antigen specificities is as broad as that of naive T-cells. These “T-regulatory” (Treg) cells are activated by binding to the class II HLA molecule, and they also receive co-stimulatory signals that serve to initiate, maintain and regulate the activation cascade. Most CD25+ CD4+ Treg cells are produced by the normal thymus as a functionally distinct and mature subpopulation of T-cells [S5]. The major function of Treg cells is to inhibit other T-cells from mounting an immune response against self components, thus protecting against autoimmunity. Upon TCR stimulation, Treg cells secrete large amounts of powerful immunosuppressor cytokines, which inhibit both Th1 help for cell-mediated immunity and Th2 help for antibody production.

38. Treg cells also express several members of the TLR family. Stimulation of Treg cells through TLRs can expand their numbers and strengthen their suppressive activity [S4]. This effect is apparently in opposition to the TLR-dependent stimulation of APCs, which enhances production of pro-inflammatory cytokines, thereby augmenting T-cell-mediated acquired immunity. However, the PAMP concentrations required for stimulation of Treg cells through TLRs are several orders of magnitude higher than those required for activation of APCs. If a large amount of PAMP, e.g. bacterial lipopolysaccharide (LPS), is produced, Treg cell activation may prevent severe systemic reactions, such as septic shock due to the production of large amounts of pro-inflammatory cytokines [S4]. Therefore CD4+ CD25+ Treg cells are an evolutionarily unique T-cell subpopulation bearing two kinds of receptor: TCRs and TLRs. This dual signaling source, together with other signals, may enable CD4+ CD25+ Treg cells to finely tune their activity to modulate acquired response against self and non-self antigens.

39. Normally lymphocytes are in a resting state, and before any contact with an antigen they are referred to as

“naive”. They become activated to carry out their specific functions when an immune response is triggered. Activated lymphocytes begin to proliferate in a process termed “clonal expansion”, giving rise to a clone of descendant cells. This activation also induces the cells to differentiate into primary effector T-cells, which can secrete cytokines and kill infected cells, and leads to rapid clearance of the pathogen. Once the response has ceased, lymphocytes revert to their previous inactive state, the primary effector cell population begins to contract and the majority of the cells die by apoptosis over the following weeks. A minority of cells, however, escape this period of cell death; the cells that survive constitute an expanded population of “memory” cells able to trigger a vigorous and effective secondary response should the same antigen be encountered in the future. The maturation status of CD4+ and CD8+ lymphocytes (naive/memory) can be determined on the basis of expression of CD45RA molecules. The phenotypes are CD45RA+ for naive T-cells and CD45RA– for memory T-cells. Although T-cells express CD45RO, the expression of CD45RO by T-cells is not a marker per se for memory cells, because other types of immune cell also express it. Phenotypically naive T-cells also express high levels of CD27 and CD28 molecules (CD45RA+ CD27+ CD28+). T-cells belonging to the memory pool have lost CD45RA expression (CD45RA– CD27+ CD28+). After repeated stimulation by antigen, memory T-cells down-regulate CD28 and subsequently CD27, and give rise to memory/effector cells (CD45RA– CD27– CD28–) and terminally differentiated effector T-cells (CD45RA+ CD27– CD28–) [V2]. The number of memory cells remains remarkably constant over time; this is due to their ability for self-renewal by undergoing slow, periodic turnover, referred to as “homeostatic turnover”, and these cells can protect against secondary infections [A10, C1, J3].

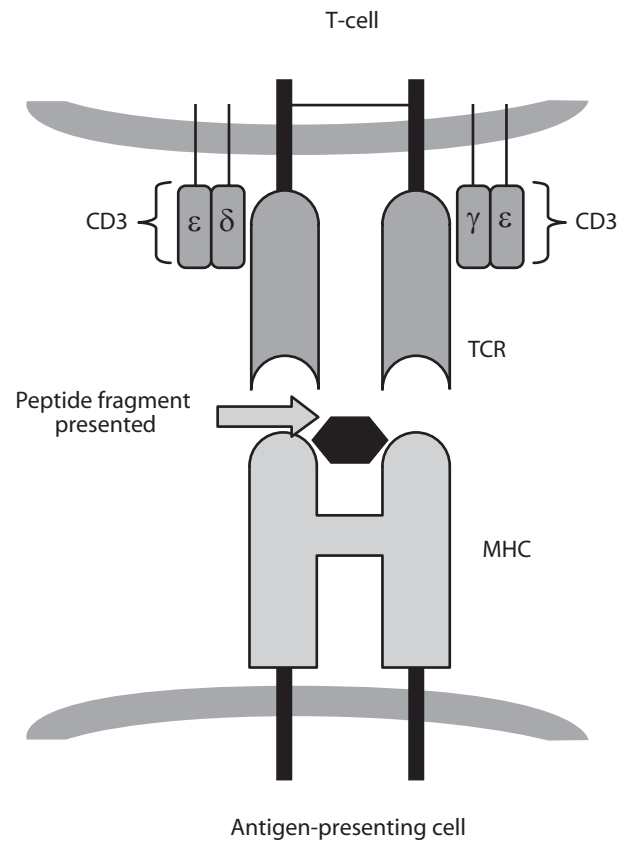
40. T-cells can be divided into two subsets on the basis of the structure of their TCRs. Most T-cells express a TCR heterodimer consisting of  $\alpha$ - and  $\beta$ -chains ( $\alpha\beta$  T-cells), whereas a minor subpopulation expresses an alternative TCR isoform made of  $\gamma$ - and  $\delta$ -chains ( $\gamma\delta$  T-cells). Each chain consists

of a variable domain, a constant domain, a transmembrane domain and a cytoplasmic domain. The TCR is encoded by multiple constant (*C*), joining (*J*), diversity (*D*) and variable (*V*) gene segments that are selectively recombined to generate TCR diversity. This diversity is necessary for the recognition of the many antigenic peptides possible. First, *V(D)J* recombination assembles unique *BCR* and *TCR* genes from three separate gene segments—the *V*, *D* and *J* genes—during lymphocyte differentiation. In addition to this recombination, which occurs in the central lymphoid tissues, somatic hypermutation may take place during a late phase of the immune response in peripheral lymphoid tissues [G37]. As immature T-cells undergo maturation in the thymus, the TCR and other molecules are responsible for the selective processes involved in creating immunocompetent T-cells [J1]. While  $\alpha\beta$  T-cells only recognize ligands presented within class I or class II MHC (see section I.C.3), most  $\gamma\delta$  T-cells recognize intact proteins, as well as a variety of other types of organic molecule that are fundamentally different from the short peptides seen by  $\alpha\beta$  T-cells in the context of MHC. Most of these  $\gamma\delta$  T-cells have neither CD8 nor CD4 on their surface (they do not recognize class I and class II HLA) [H28].

41. The multichain TCR/CD3 complex is one of the most elaborate cell surface signalling receptors and plays a key role in antigen recognition, T-cell activation and triggering antigen-specific immune response. This process is induced by direct interaction of the TCR with an antigen presented by the MHC on APCs. Upon the structural and functional cooperation of the TCR with the CD3 complex, the activating signal is transmitted through the cell membrane to the nucleus [F10, S2]. The structure of the TCR/CD3 complex is represented schematically in figure III.

42. Engagement of the TCR by the antigen leads to a series of intracellular biochemical events culminating in the transcription of new genes and T-cell activation. One or more tyrosine kinases phosphorylate first the CD3 chains themselves and subsequently other substrates. Subsequent to tyrosine kinase activation, a series of secondary events follow TCR engagement, including activation of serine/threonine kinases, activation of the guanosine triphosphate (GTP)-binding protein p21ras and activation of transcription factors for receptors and growth factors such as the major T-cell growth factor IL-2. The CD4 and CD8 co-receptors bind a tyrosine kinase (p56Lck) by their intracytoplasmic tail, which plays a critical role in T-cell signalling. However, TCR binding is not sufficient to activate T-cells, and a second (co-stimulatory) signal is required. Indeed, as originally envisaged in the “two-signal hypothesis”, T-cell activation required stimulation both by the TCR (signal 1) and through additional co-stimulatory molecules (signal 2). The principal co-stimulatory molecules expressed on APCs belong to the B7 family: B7-1 (CD80) and B7-2 (CD86). T-cells display receptors for these B7 molecules—CD28 and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4)—with similar structures but opposite functions. While CD28 helps to initiate T-cell activation, CTLA-4 down-regulates the response [I14, L30]. Activated T-lymphocytes may be identified by the expression of certain surface markers, e.g. CD25, CD38 and

**Figure III. Structure of the TCR/CD3 complex (adapted from reference [J1]).**



HLA-DR. Only a low percentage of circulating lymphocytes are in an activated state in healthy individuals; these activated lymphocytes can be rapidly mobilized against aggressors and they help in the further recruitment of defending cells [J1].

43. The stages of T-cell development within the thymus are identified by the expression of specific cell surface markers, such as TCR, CD3 (which serves as the signal transduction component of TCR) and CD4/CD8. Direct cell-to-cell interaction between these cells and thymic cells induces their proliferation and also differentiation. Pre-T-cells do not express any of the above-mentioned T-cell markers; at this stage they are referred to as “double-negative” cells (CD4<sup>-</sup> CD8<sup>-</sup>). At this point the  $\beta$ -chain of TCRs undergoes rearrangement. The successful rearrangement of this chain serves as a signal for these cells to undergo further proliferation. During this time, both CD4 and CD8 start to be expressed; thus these cells are referred to as “double-positive” cells (CD4<sup>+</sup> CD8<sup>+</sup>). It is only at this point that the  $\alpha$ -chain of the TCR undergoes rearrangement. At the double-positive stage, a second molecular sensor assembles and controls the transition to the single-positive CD4<sup>+</sup> CD8<sup>-</sup> or CD4<sup>-</sup> CD8<sup>+</sup> stage on the basis of the specificity of the TCR  $\alpha\beta$  heterodimers (table 6). Likewise, thymocytes committed to the  $\gamma\delta$  lineage also find a checkpoint at the penultimate double-negative stage (CD44<sup>-</sup> CD25<sup>+</sup>), which counterbalances the stochastic nature of the concurrent TCR  $\gamma$  and  $\delta$  rearrangements and allows only cells expressing a  $\gamma\delta$  TCR to mature rapidly into CD25<sup>+</sup> CD4<sup>-</sup> CD8<sup>-</sup>  $\gamma\delta$  cells and leave the thymus [N15].

**Table 6 T-cell  $\alpha\beta$ + development and its correlation with CD expression on the cell surface**

Adapted from reference [J1]

Surface molecule	Double-negative (CD4- and CD8-negative)			Double-positive (CD4- and CD8-positive)			
	CD44+ CD25-	CD44+ CD25+	Pre-TCR	Proliferation	CD4/CD8	TCR	Single-positive <sup>a</sup>
CD2				—————→			
C-Kit	—————→						
CD44	—————→						
CD25		—————→					
CD3			—————→	—————→			
CD4						—————→	a
CD8						—————→	a
CD24						—————→	

<sup>a</sup> Single-positive: CD4+ or CD8+.

44. The expression of CD4, CD8, CD3 and TCR chains changes during the different stages of thymocyte development, and this is shown in figure IV. First, immature thymocytes do not express the above-mentioned markers. These double-negative CD4- CD8- cells are precursors of two populations of cells: a minor proportion are CD3+  $\gamma\delta$ + thymocytes and a larger proportion are CD3+  $\alpha\beta$ + thymocytes. CD3+  $\gamma\delta$ + thymocytes express neither CD4 nor CD8, even when they reach maturation and are exported to the periphery. In contrast, CD3+  $\alpha\beta$ + thymocytes go through further stages of development, including changes in CD4 and CD8 expression. Large double-positive thymocytes (CD3+  $\alpha\beta$ + CD4+ CD8+) differentiate into small double-positive cells, and the majority (97%) die by apoptosis within the thymus. The remaining cells (3%) lose the expression of either CD4 or CD8 and become single-positive thymocytes (CD3+ CD4+ CD8- or CD3+ CD4- CD8+), which are exported to the periphery after maturation.

45. The immune system is a site of intense DNA modifications, which result from programmed and specific mechanisms during its maturation, or as a consequence of non-specific injuries inflicted during cellular proliferation and/or cellular activation. The V(D)J recombination is initiated by lymphoid-specific proteins through the introduction of a DNA double-strand break. The terminal maturation of B-lymphocytes, which occurs during an immune response in the germinal centre of secondary lymphoid organs such as the spleen, is characterized by two important modifications of the rearranged immunoglobulin genes. The isotype class switch recombination and the generation of somatic hypermutations ensure the production of efficient antibodies of various isotypes. These two B-cell-specific processes are triggered by the activation-induced cytidine deaminase

protein through DNA modification within immunoglobulin genes. Beside these three DNA-altering mechanisms, B- and T-lymphocytes are also exposed to general DNA injuries known to occur, for example, during DNA replication, as several waves of intense cellular proliferation accompany not only their maturation but also their expansion during immune responses. Lastly, one important aspect of an immune response relies on the inflammatory reaction, during which several soluble factors and/or natural reactive metabolites are produced that can be considered as possible causes of DNA damage. Altogether this demonstrates that the lymphoid tissue is at particular risk for mutagenic events inflicted through defective DNA repair machineries [R20].

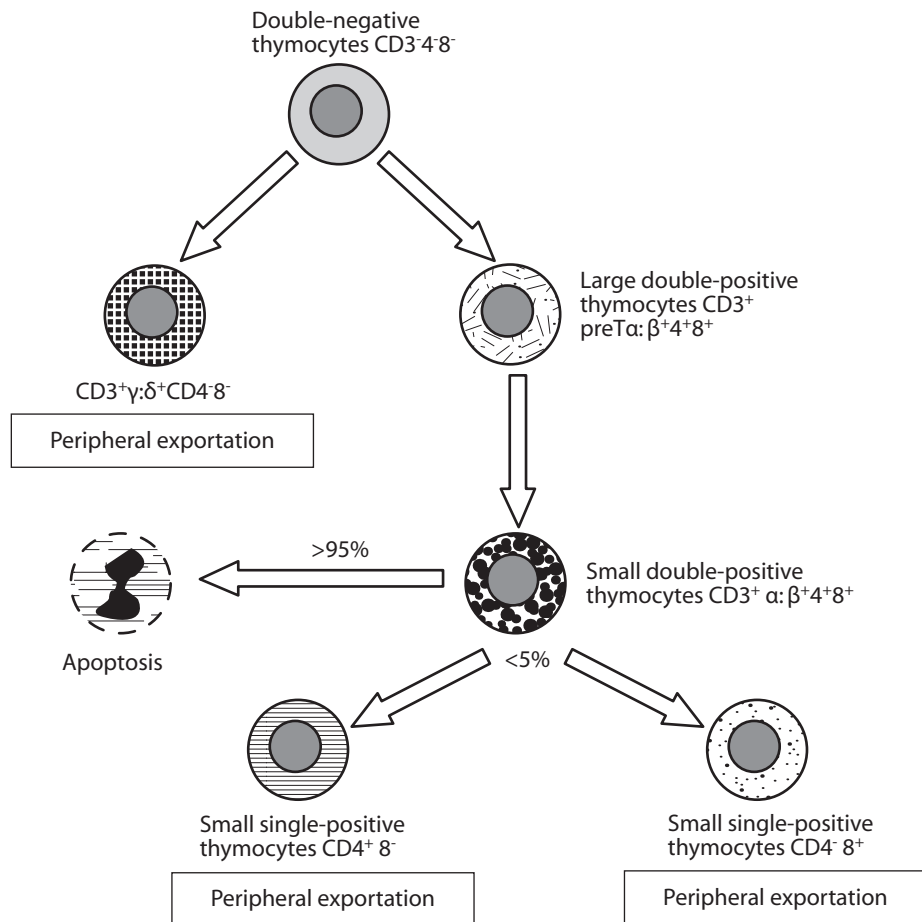
### 3. Human major histocompatibility complex

46. TCRs recognize specific epitopes on the surface of APCs, once these are degraded into small peptides, a form referred to as “processed antigen”. The major histocompatibility complex (MHC), which in humans is called the human leucocyte antigen (HLA), plays a fundamental role in enabling T-cells to recognize antigens by forming complexes with the peptides. Antigen processing and presentation are intracellular processes that result in fragmentation (proteolysis) of proteins, association of the fragments with MHC molecules and expression of the peptide-MHC complexes at the cell surface, where they can be recognized by the TCR on a T-cell (figure V). The peptide-binding cleft of MHC molecules is the location where an antigen is attached for display to a T-cell. However, not the entire antigen is bound to the cleft. Only a few “anchoring” residues of the antigen need actually to attach to the MHC molecule for the antigen to be displayed. This makes it possible for only a few common

residues on antigens to allow binding to one form of MHC. This is important because it allows many different antigens with only a few residues in common to be displayed by one form of an MHC molecule, giving these molecules broad

specificity. Furthermore, the size of the peptide-binding cleft (larger in class II MHC) is proportional to the size of the peptide that it can accommodate (class II MHC molecules bind larger residues).

**Figure IV. Stages of thymocyte development. The expression of CD4, CD8, CD3 and TCR chains changes during the different stages of thymocyte development (adapted from reference [J1]).**

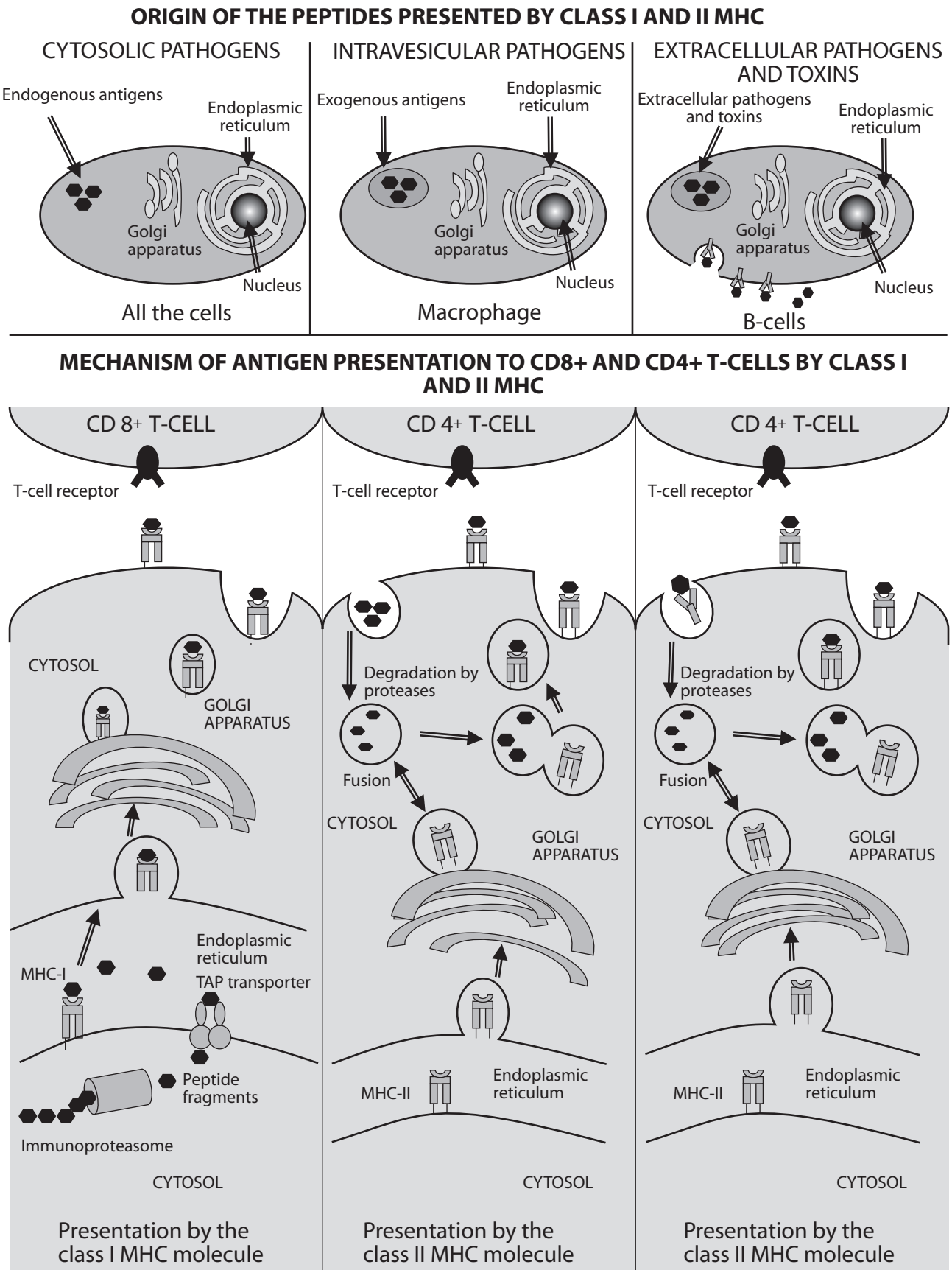


47. The MHC is a complex of closely linked genes that code for two families of glycoproteins (class I and class II MHC). While class I molecules are constitutively expressed by virtually all somatic nucleated cells, class II molecules are expressed only by APCs. The class I MHC family in humans is divided into classical and non-classical sub-families composed of HLA-A, -B and -C, and HLA-E, -F and -G, respectively. The Class II MHC family in humans includes HLA-DR, -DP and -DQ. Classic class I and class II HLA molecules are characterized by polymorphisms; within each class, and even at a single locus, an enormous number of variants (alleles) are found among different individuals in a population. A haplotype is a group of alleles that are inherited together on a single chromosome. Even though the human population is outbred, because the HLA genes are tightly linked, they are frequently inherited together, and patterns of inheritance have been identified among certain groups of HLA antigens. Each individual has only a

very small set of different HLA genes and expresses one (homozygous) or a maximum of two (heterozygous) alleles for each locus.

48. HLA-G is a non-classical class I MHC molecule involved in immunotolerance, with restricted tissue distribution. It was first found in extravillous cytotrophoblast cells [C4] and was subsequently demonstrated in a variety of normal tissues, including T-cells. The class I MHC chain-related molecule MICA belongs to a novel family of heat-shock-induced proteins, by which the immune system can recognize and destroy stress-induced cells. This represents an immune surveillance mechanism for the detection of damaged, infected or transformed cells [D4, S12]. MICA molecules are recognized by TCR γδ. It has been demonstrated that HLA-G mediates a dominant negative signal leading to tumour escape from immune surveillance by counteracting the MICA-activating signal [R9].

**Figure V. Upper panel: origin of the peptides presented by MHC; lower panel: mechanism of antigen presentation to CD8+ and CD4+ T-cells by class I and class II MHC (adapted from reference [J1]).**





49. The central MHC region or human class III encodes, within a ~700 kb sequence located between the centromeric class II MHC and the telomeric class I MHC regions, a heterogeneous collection of more than 60 genes where a few families do emerge. These genes include those involved in the activation cascades of the complement system, steroid hormonal synthesis, inflammation and cell stress (tumour necrosis factor, lymphotoxins, heat shock proteins) and extracellular matrix organization (tenascin), as well as immunoglobulin superfamily (Ig-SF) members. The remainder (the majority) of the loci are involved mainly in more “core” biological functions with no immediate implication for the immune system [H21].

50. T-cells are grouped functionally according to the class of HLA molecules that associate with the peptides they recognize. CD8+ cytotoxic and CD4+ helper T-cells tend to recognize peptides bound to class I and class II HLA molecules, respectively. Endogenous antigens may be viral proteins synthesized within the infected cell or the specific proteins synthesized by tumour cells. These cytosolic pathogens are degraded into peptide fragments that form complexes with class I HLA and are then transported through the endoplasmic reticulum towards the cell surface. Proteasomes are the major non-lysosomal protein degradation machinery in eukaryotic cells. They deal primarily with endogenous proteins. Specialized proteasomes called immunoproteasomes are responsible for the processing of antigens

for presentation by the class I HLA pathway. In mammals, activation of the immune system leads to the release of cytokines, causing the activation of immunoproteasomes and degradation of the antigenic protein into peptides about 10 amino acids in length. These peptides are then transported from the cytosol into the endoplasmic reticulum, where each enters the groove at the surface of a class I HLA molecule. This peptide–HLA complex then moves through the Golgi apparatus and is inserted into the plasma membrane, where it can be recognized by CD8+ T-cells to induce cell death (cytotoxicity) [R6, T3] (figure V). Exogenous antigens are degraded by APCs by endocytosis. The pH of the endosomes containing the engulfed pathogens progressively decreases, activating proteases that reside within these acidified endocytic vesicles to degrade the engulfed material. The resulting peptides are located within class II HLA molecules, which are then exported towards the cell surface. Toxins are extracellular pathogens, for example the majority of bacteria, which mainly reside and replicate extracellularly. They are degraded inside intracellular acidified vesicles and associate with class II HLA molecules to be presented to CD4+ T-cells that can help B-cells to secrete Ig against these bacteria. Other exogenous antigens, for example some bacteria and parasites, grow intracellularly (intravesicular pathogens). Once degraded in acidified vesicles, their peptides are also bound to class II HLA and presented to CD4+ T-cells. Upon recognition of these peptides by the TCR, the presenting cell is activated to kill the pathogens (table 7).

**Table 7 Origin of the peptides presented by class I and II HLA**

<i>Property</i>	<i>Cytosolic pathogen</i>	<i>Intravesicular pathogen</i>	<i>Extracellular pathogen or toxin</i>
Degraded in	Cytoplasm	Acidified vesicles	Acidified vesicles
Peptides bind to	Class I HLA	Class II HLA	Class II HLA
Presented to	CD8+ T-cells	CD4+ T-cells	CD4+ T-cells
Effect on presenting cell	Cell death	Activation to kill intravesicular bacteria and parasites	Activation of B-cells to secrete Ig to eliminate extracellular bacteria or toxin

#### 4. Antigen-presenting cells

51. The immune system contains three types of antigen-presenting cell (APC): macrophages, dendritic cells and B-lymphocytes. These three types of APC present different sets of antigens and may also serve to activate helper T-cells at different points during the immune response. As previously discussed, T-cell activation requires stimulation both by the TCR and through additional co-stimulatory molecules. The most relevant property of APCs is that, in addition to antigen presentation, they provide co-stimulatory signals. APCs (except dendritic cells) do not constitutively express these co-stimulatory molecules. Since these cells potentially phagocytose both self and infectious materials, there has to be some mechanism for the recognition of self and non-self. Upon this recognition, the APCs will up-regulate their

co-stimulatory molecules (namely B7 molecules), and only then activate T-cells, by interacting with B7 receptors.

52. Macrophages are part of the innate response. Unlike T- and B-cells, they do not contain specific receptors but do express TLRs that allow them to recognize differential PAMPs on foreign cells. Stimulated macrophages up-regulate class II HLA and express co-stimulatory molecules (B7 molecules). It is at this point that antigen presentation by class II HLA will activate CD4+ helper T-lymphocytes.

53. Dendritic cells are mostly found in the skin and mucosa epithelium (Langerhans cells). Dendritic cells also possess TLRs that can recognize PAMPs, and they continuously express high levels of co-stimulatory molecules (B7 molecules). Upon recognition of infectious particles, these

cells migrate through the lymphatic system to the nearest lymph node, where they come into close contact with naive T-cells. Unlike macrophages, however, dendritic cells can also recognize viral particles as non-self. In addition, they can present antigen via both class I and class II HLA. Thus they can directly activate both CD8 and CD4 T-cells. Once the T-cells are activated, they leave the lymph nodes and travel to the sites of inflammation. Since dendritic cells present viral particles, these should also activate CD8 cells, the main effector cells for fighting viral infections. Dendritic cells are also very numerous in the thymus, where they act in T-cell selection during development. While a role of dendritic cells in the negative selection in the thymus has been well established [W13], their role in the positive selection is still questionable [W11].

54. The skin is equipped with specialized cells called Langerhans cells (LCs) which play a central role in the skin's immune system as an integral part of the body's total defence system. LCs are epidermal antigen-presenting dendritic cells originating in the bone marrow. They migrate to the epidermis, where they form a regularly ordered network representing 1.86% of all epidermal cells. A constant numerical ratio (1:53) exists between LCs and the other epidermal cells. The surprisingly constant relationship of LCs to other epidermal cells supports the hypothesis of an epidermal LC unit where one LC seems to be responsible for the immune surveillance of 53 epidermal cells [B21].

55. After contact with the corresponding antigens (viruses, contact allergens, skin transplants), LCs migrate from the epidermis to the regional lymph nodes for presentation of antigenic peptides to T-cells. On their journey, LCs undergo a maturation process leading to the presentation of the antigen on the cell surface. The migrating LCs are replaced by a corresponding number of new LCs from the bone marrow. In the lymph nodes the mature LCs activate the helper T-cells that have the matching antigen-specific receptors on their surfaces. In this way they direct the reaction of the immune system [K42].

56. Epidermal LCs have a spectrum of different functions with implications that extend far beyond the skin. They have the potential to internalize particulate agents and macromolecules, and display migratory properties that endow them with the unique capacity to journey between the skin and draining lymph nodes where they encounter antigen-specific T-lymphocytes. In addition, LCs are considered to play a pivotal role in infectious disease, allergy, chronic inflammatory reactions, tumour rejection or transplantation [V10]. Factors influencing the activity of the LCs in the epidermis include cytokines such as IL-10, immunosuppressive drugs such as corticoids, and ultraviolet and ionizing radiation [K39].

57. B-cells are the least efficient APCs. Unlike the other two APCs, they possess specific antigen receptors (surface immunoglobulins). B-cells effectively ingest soluble antigens that bind to cell surface immunoglobulin receptors by

receptor-mediated endocytosis. Thus B-cells can present specific antigens to activated T-cells. However, resting B-cells do not express co-stimulatory molecules; in order to do so, most of the B-cells need to be activated by helper T-lymphocytes. The role of B-cells as APCs in vivo is not very well understood [R21].

## 5. Self tolerance and self-HLA-associated recognition

58. As seen, a diverse and polymorphic T-cell repertoire is generated in the thymus by random recombination of discrete *TCR* gene segments. This repertoire is then shaped by intrathymic selection events to generate a peripheral T-cell pool of self-HLA-restricted, non-autoaggressive T-cells. To ensure that self tolerance is achieved, self antigens are presented to the matured T-cells by APCs in the thymus. At this stage, these cells undergo the processes of positive and negative selection. During positive selection, double-positive T-cells that can recognize self HLAs are selected for proliferation, and those T-cells that do not recognize self HLAs die by apoptosis. Positive selection has been associated mainly with cortical thymic epithelial cells and their associated HLA molecules, both of which are necessary and sufficient for positive selection of double-positive thymocytes and the development of single-positive cells [G15]. By negative selection, those T-cells that are strongly activated by self HLA and self peptides are eliminated in the thymus. This process of clonal deletion prevents lymphocytes from subsequently reacting against self antigens and causing autoimmune diseases. Mature T-cells leave the thymus, go into the circulation and eventually find their way to lymph nodes, mucosa-associated lymphoid tissue or the spleen [J1]. Although the majority of self-reactive T-cells are clonally deleted in the thymus, some mature lymphocytes may remain capable of responding to self antigens. Intrinsic biochemical and gene expression changes, as well as a lack of co-stimulation, can reduce this ability by triggering a process generally termed clonal anergy. Finally, even if the lymphocytes have evaded these controls, mechanisms of extrinsic control, such as active suppression by Treg cells, can prevent the danger of self-reactive receptors [G37].

59. The achievement of immunological self tolerance raises the question of how T-lymphocytes that are reactive to proteins expressed only by non-thymic tissues can be identified and addressed. The clinical relevance of this question is related to the fact that many of these tissue-restricted proteins (e.g. insulin, thyroglobulin, myelin, retinal S-antigen) are associated with organ-specific autoimmune diseases (e.g. type 1 diabetes, thyroiditis, multiple sclerosis, uveitis). A classical explanation for this phenomenon was that, while tolerance to ubiquitously expressed or blood-borne antigens is centrally achieved in the thymus, tolerance to tissue-restricted antigens is secured by peripheral extrathymic mechanisms. However, ectopic synthesis of these peripheral tissue-restricted proteins has recently been demonstrated in thymic medullar epithelia

cells [A22]. A startling discovery for the understanding of this ectopic synthesis is the autoimmune regulator *AIRE* gene that enables a battery of tissue-restricted antigens to be expressed in thymic medullar epithelia cells, thus playing an important role in controlling tolerance induction. In the absence of *AIRE*, autoimmunity, and ultimately overt autoimmune disease, develop [A23, M16]. Although *AIRE* transcripts are by far most abundant in the thymus, they were also detected in peripheral lymphoid organs at significantly lower levels [A22].

60. Although 1–24% of T-cells are alloreactive, i.e. they respond to MHC molecules encoded by a foreign haplotype, it is generally believed that T-cells cannot recognize foreign peptides binding foreign HLA molecules [D21]. The term “MHC restriction” refers to the phenomenon whereby T-cells from one individual recognizing an antigen fail to recognize cells presenting the same antigen unless the presenting cells express one or more HLA alleles identical to those on this individual’s cortical thymic epithelial cells, in which those T-cells matured. This phenomenon derives in part from the requirement to recognize self HLA (self-HLA-associated recognition) and in part from the different peptide-binding specificity of different HLA alleles. Through positive and negative selection in the thymus, self peptides bound to autologous HLA molecules determine the repertoire of peripheral T-cells, which then respond to infection by recognizing foreign peptides bound to those same HLA molecules [P13].

## 6. Cytokines

61. As described above, the immune system has many different types of cell acting together to deal with unwanted infections and altered cells. Cytokines are small proteins produced by the immune cells for signalling and for orchestrating the attack. They generally act over short distances and short time spans and at very low concentrations by binding to specific membrane receptors, which then signal via second messengers, often tyrosine kinases. Responses to cytokines may include increased or decreased expression of membrane proteins, secretion of effector molecules and cell proliferation. By these actions cytokines mediate and regulate immunity, inflammation and haematopoiesis.

62. Many cell populations make cytokines, but the predominant producers are CD4+ helper T-cells and monocytes/macrophages. Cytokines made by lymphocytes are called lymphokines, and cytokines made by monocytes are called monokines. Other groups of cytokines include interferons (IFNs) and chemokines. While IFN- $\alpha$  and IFN- $\beta$  inhibit virus replication in infected cells, IFN- $\gamma$  has the additional property of stimulating HLA expression. Chemokines are cytokines with chemotactic properties that attract leucocytes to infection sites. The general term interleukins (ILs) is used to define cytokines made by one leucocyte and acting on other leucocytes. Interleukins have been numbered in the order in which they were identified. Thus the first IL identified was named IL-1; about 30 different ILs have been identified so far (table 8).

**Table 8 Main cytokines and their functions**

Adapted from reference [J1]

<i>Cytokine<sup>a</sup></i>	<i>Main source<sup>b</sup></i>	<i>Target cells</i>	<i>Function</i>
IL-1 $\alpha$ and $\beta$	APCs	Th cells B-cells NK cells Various	Co-stimulation Maturation and proliferation Activation Haematopoiesis, inflammation, fever, acute phase response
IL-2	Th1 cells	T-, B- and NK cells	Activation, growth, proliferation
IL-3	Th1 cells, NK cells	Stem cells Mast cells	Growth and differentiation Growth and histamine release
IL-4	Th2 cells, mast cells	B-cells T-cells Macrophages	Proliferation, differentiation, IgG and IgE synthesis Proliferation Expression of class II HLA
IL-5	Th2 cells	Eosinophils B-cells	Proliferation Proliferation, differentiation, IgA synthesis
IL-6	APCs, Th2 cells, stromal cells	B-cells Plasma cells Stem cells Various	Differentiation (into plasma cells) Antibody secretion Differentiation Acute phase response



<i>Cytokine<sup>a</sup></i>	<i>Main source<sup>b</sup></i>	<i>Target cells</i>	<i>Function</i>
IL-7	Marrow stroma, thymus stroma	Stem cells	T- and B-lymphopoiesis
IL-8	Macrophages, endothelial cells	Neutrophils T-cells	Chemoattractant
IL-9	T-cells	Stem cells, thymus cells	Haematopoiesis and thymopoiesis
IL-10	Th2 cells, macrophages, Tc cells, B-cells	Macrophages, B-cells, mast cells	Inhibition of cytokine production Proliferation, differentiation, Ig synthesis Inhibition of growth Suppression of cell-mediated immunity
IL-11	Stromal cells	Marrow cells	Haematopoiesis and thrombopoiesis
IL-12	Macrophages, B-cells	Tc cells NK cells	Differentiation, promotion of cell-mediated immunity Activation, proliferation, IFN- $\gamma$ production
IFN- $\alpha$ and - $\beta$	Macrophages, neutrophils, fibroblasts	Various	Inhibition of viral replication Induction of class I HLA expression
IFN- $\gamma$	Th1 cells, NK cells	Various Various B-cells Th2 cells	Inhibition of viral replication Induction of HLA expression Ig switch to IgG2a Inhibition of proliferation
TGF- $\beta$	T-cells, monocytes	Macrophages B-cells Various	Chemotaxis and IL-1 synthesis IgA synthesis Inhibition of proliferation
TNF- $\alpha$	Monocytes, NK cells, mast cells	Macrophages Tumour cells	Cytokine expression Cell death
TNF- $\beta$	Th1 cells, Tc cells	APCs Tumour cells	Phagocytosis, NO production Cell death

<sup>a</sup> IL = interleukin; IFN = interferon; TGF = transforming growth factor;  
TNF = tumour necrosis factor.

<sup>b</sup> Th = CD4+ helper T-cells (Th1- or Th2-type); Tc = CD8+ cytotoxic T-cells.

### 63. Cytokines have several important characteristics:

- The same cytokine may be made by a number of different cells;
- Pleiotropy: the same cytokine may have different effects and/or may act on several different cell types;
- Redundancy: similar functions can be induced by different cytokines;
- Synergy: cytokines often act together and increase one another's effects;
- Antagonism: some cytokines may cause opposing effects;
- Cascade effect: cytokines are often produced in a cascade, as one cytokine stimulates its target cells to make additional cytokines;
- Paracrine actions: cytokines act on cells near to them or that they actually touch;

- Autocrine functions: cytokines may act on the same cells that secrete them;
- Endocrine functions: some cytokines may act in some instances on distant cells.

### 7. Remarks concerning cells and molecules of the immune system

64. The preceding paragraphs concerned cells and molecules of the immune system. Anatomic, physiological, inflammatory and phagocytic barriers constitute the innate immune system. Phagocytes (granulocytes, monocytes/macrophages and dendritic cells) engulf and digest pathogens. They express a special family of receptors (TLRs) capable of recognizing PAMPs. NK cells also belong to the innate arm of the immune response. NK cells can recognize, bind and kill virus-infected and tumour cells. Spontaneous NK cytotoxic activity is regulated through activating and inhibiting membrane receptors. NKT cells are a particular type

of lymphocyte expressing some NK surface markers and TCRs, and that bridge innate and acquired immunity. As part of the innate immunity, mast cells are involved in allergy, anaphylaxis, wound healing and defence against pathogens. The complement system circulates in an inactive form. In response to the recognition of certain molecular patterns of pathogens, complement activation results in the production of biologically active molecules, which may lead to lysis, opsonization and inflammation.

65. Lymphocytes are the predominant cells involved in acquired immunity. B-lymphocytes and T-lymphocytes are the effector cells of the humoral and the cellular response, respectively. B-cells recognize native antigens through the immunoglobulins expressed on their surface as BCRs. T-cells recognize antigens previously processed into small peptides through the TCR/CD3 complex expressed on their surface. B-cell/T-cell interactions involve additional co-stimulatory signalization. Most of T-cells belong to the CD8+ or CD4+ subpopulations. CD8+ cytotoxic T-cells are effectors that, once activated, can remove foreign organisms. CD4+ helper T-cells induce other immune cells to become better effectors.

66. By secreting different cytokines, Th1 and Th2 CD4+ helper T-cells promote cellular or humoral immunity, respectively. A minority of CD4+ T-cells belong to neither Th1 nor Th2 subsets and are called regulatory T-cells that express CD25, the receptor for IL-2. These CD4+ CD25+ Treg cells protect against autoimmunity and prevent severe systemic reactions such as septic shock. Naive (CD45RA+) lymphocytes have never encountered an antigen. When an immune response is triggered, they become activated and proliferate (clonal expansion). Once the response ceases, most of the activated lymphocytes die by apoptosis, but a minority of them remain as memory (CD45RA-) T-cells.

67. TCRs recognize antigenic peptides bound to the MHC (in humans called HLA) on the surface of APCs (dendritic cells, macrophages and B-cells). While CD8+ T-cells recognize endogenous antigens bound to class I HLA, CD4+ T-cells recognize exogenous antigens bound to class II HLA. Through positive and negative selection in the thymus, self antigens bound to autologous HLA molecules determine the repertoire of T-cells to ensure immunological self tolerance. Cytokines are proteins, mainly produced by CD4+ T-cells and monocytes/macrophages, that bind to specific membrane receptors that act as second messengers.

## D. Physiological immunosenescence

### 1. Concept of immunosenescence

68. Immunosenescence can be defined as the progressive decline in immune function observed in the elderly; it results in a higher susceptibility to infections and increased morbidity and mortality [B6, G2, H16]. These age-related changes

in immune function have been well documented [H13, R7]. A reduction with age has been reported in the overall capacity for renewal of haematopoietic stem cells, indicating that some of the deficits of immunosenescence may be initiated at the stem cell level [H1, L1].

### 2. Main features of immunosenescence

69. The immune property most sensitive to ageing is the production and export of T-cells from the thymus, which is manifested by a decrease of naive cells with age [H16]. Naive cells have the greatest diversity of TCR repertoire. With ageing, the thymus involutes, the supply of naive T-cells falls and there is a gradual accumulation of memory T-cells. Thus in elderly persons the T-cell population shifts to a lower naive/memory T-cell ratio, and the TCR repertoire available to respond to new antigens is reduced [H1, V2, V4].

70. There is a progressive increase with age in the proportion of CD8+ T-cells that lack expression of CD28, a critical co-stimulatory molecule [E2, H16]. This results in lower proliferative capacity, decreased IL-2 production, telomere erosion and less response to TCR stimulation. Taking into account the fact that specific effector cells should be able to proliferate sufficiently to fight an infection, the age-related limitation of cell division could have devastating consequences for immune function [E1]. Immunosenescence also involves hyporesponsiveness to mitogens, lowered lytic capacity, decline in transmembrane signalling and higher oxidative stress [B6, B15, V2].

71. As a result of thymic involution, the kinetics of the transition from CD4- CD8- (double-negative) to CD4+ CD8+ (double-positive) thymocytes is altered in old age. It has been demonstrated that thymic levels of p56Lck, a factor involved in the maturation of T-cells from CD4- CD8- double-negative into CD4+ CD8+ double-positive T-cells, are negatively correlated with age, which could lead to the accumulation of CD4- CD8- double-negative T-cells in the elderly [H15].

72. There exists a chronic inflammatory state in the elderly due to an increased release of pro-inflammatory cytokines such as TNF- $\alpha$  and IL-6 [B6, E1]. Dysregulation of TNF- $\alpha$  and IL-6 may be involved in age-related diseases such as osteoporosis, atherosclerosis, Alzheimer's disease, diabetes, cardiovascular disease and cancer [H16]. The balance between Type 1 and Type 2 cytokines, important for the outcome of several infectious diseases, also changes with age. Evidence suggests that, while a Th1-type response predominates in adults, a Th2 response predominates in the elderly. This shift from a Th1 to a Th2 cytokine profile is a possible mechanism for age-associated immune dysfunction [R1, S7].

73. As a result of ageing, the immune system may lose the ability to distinguish self versus non-self antigens.

Age-related changes in humoral immunity involve reduced vaccine responses and increased production of autoantibodies. Although the ability of B-cells to generate antibody responses declines with age, many of the humoral changes observed in the elderly are related to declining T-cell function with dysregulation of T-cell/B-cell interactions [B15, F3, H1, L5]. The B-cell repertoire changes with age, and the altered spectrum of expressed immunoglobulins may affect the quality of the antibody response in the elderly and be highly relevant for health [V4].

74. The innate response is not free from the effects of ageing. The production of reactive oxygen and nitrogen species (ROS/RNS) by neutrophils and macrophages in the elderly is significantly impaired, which diminishes the capacity to destroy bacteria. In contrast to the case with T- and B-cells, the absolute number of NK cells is increased in elderly persons, but their cytotoxic capacity on a per-cell basis is impaired [B15, H1]. NK cells from elderly people show a decreased proliferative response to IL-2 [H16]; NK cells are thus less able to destroy virus-infected and tumour cells in the elderly [P8]. The immune system in older people is also characterized by an increased proportion and number of NKT cells in the peripheral blood [M9].

75. Several aspects of immune response exhibit diurnal and seasonal circadian rhythmicity related to the level of melatonin, which exerts immunoenhancing and antioxidant actions. Such rhythms play an important role in immune homeostasis [S22]. The gradual decline of pineal melatonin synthesis and secretion over the lifespan could cause immunocompetence to deteriorate in the elderly [K6].

### 3. Remarks concerning immunosenescence

76. The main features of immunosenescence are:

- Thymic involution;
- Progressive decrease of the naive cell pool with age (shift to a lower naive/memory ratio);
- Reduced TCR repertoire;
- Increase in the proportion of CD8+ CD28– T-cells;
- Accumulation of CD4– CD8– T-cells;
- Chronic inflammatory status;
- Progressive loss of the ability to distinguish self versus non-self antigens;
- Dysregulation of T-cell/B-cell interaction, resulting in humoral changes;
- Impaired phagocytic activity;

- Increase in the absolute number of NK cells, with a decrease in their cytotoxic capacity;
- Gradual decline in melatonin synthesis.

### E. Summary

77. The general features of the immune system can be summarized as follows:

- The main function of the immune system is to protect against infections and cancer;
- The bone marrow and the thymus are the primary lymphoid tissues where maturation of lymphocytes takes place;
- Mature lymphocytes travel by the bloodstream towards the lymph nodes, spleen and mucosa-associated lymphoid tissue, which are considered the secondary lymphoid tissues;
- The ability of the cutaneous barrier to help defend the body against pathogens relies on both acquired and innate immune responses;
- Monocytes, macrophages, polymorphonuclear leucocytes, dendritic cells, natural killer cells and mast cells are the immune cells involved in the innate immune response;
- Lymphocytes are the predominant cells involved in the acquired immune response. While B-lymphocytes are the effector cells of the humoral response, T-lymphocytes (with their two subsets CD4+ helper T-cells and CD8+ cytotoxic T-cells) are responsible for cell-mediated responses;
- The main features of the acquired immune response are memory, specificity, diversity and self/non-self discrimination;
- There is a crosstalk between the innate and the acquired immune response;
- Cells involved in immune recognition have surface receptors that bind to specific antigens;
- The lymphoid tissue is naturally prone to DNA modifications that occur during the maturation of B- and T-lymphocytes and during immune responses;
- The MHC complex (HLA in humans) plays a fundamental role in enabling T-cells to recognize antigens;
- APCs present antigens and activate helper T-cells by co-stimulatory signals;
- Cytokines are small proteins that mediate and regulate immunity, inflammation and haematopoiesis;
- Immunosenescence is a complex process involving dysregulation, rather than a simple unidirectional decline of the whole immune system function.



## II. RADIATION-INDUCED ALTERATIONS OF THE IMMUNE SYSTEM

### A. Introduction

78. Radiation-induced effects on the immune system have attracted interest from the research community for several decades, and lymphocyte radiosensitivity was one of the earliest subjects of experimental radiobiology [A13, A27, A28, A29, D11, D29, H24, K54, M30, P12, P15, T19]. Immunosuppression is a consequence of whole-body irradiation (WBI) at medium to high doses. Localized radiotherapy can also result in immunosuppression. In contrast, it has been reported that very low doses of ionizing radiation may give rise to immunostimulatory effects, particularly at short times after irradiation. Because of these divergent effects, ionizing radiation is probably better considered as an immunomodulatory rather than as an immunosuppressive agent [M2, U4]. This section summarizes the main alterations induced by ionizing radiation in the immune system and considers the influence of dose, dose rate and radiation quality. Data concerning the radiosensitivity of the different lymphocyte subpopulations are analysed. Particular attention is given to alterations of the developing immune system following pre-natal irradiation. Several human immune pathologies associated with hypersensitivity to ionizing radiation are also reviewed.

79. For the purposes of this annex, low doses are defined as <0.2 Gy to the whole body. Low-dose-rate exposures are considered to be those delivered at <0.1 Gy/h. These are the levels below which the International Commission on

Radiological Protection [I2] deems that the dose and dose-rate effectiveness factor (DDREF) should be applied.

### B. Data concerning low-dose irradiation

80. The effects of low doses of ionizing radiation on the immune system were reviewed by the Committee in 1994, in the context of adaptive response [U4]. At that time, some evidence in animals seemed to indicate that low doses of ionizing radiation may enhance immune response, but the evidence for a similar effect on the human immune system was sparse. It was concluded that further investigations were needed on these effects and their clinical significance.

#### 1. Animal data

81. The effects of external low-dose irradiation upon the blood and the immune system of experimental animals have been studied at several centres. The dose–response relationship of immunological parameters following exposure to ionizing radiation is affected by a number of factors, the most important of which are the target cells under observation, dose range, dose rate and dose spacing, as well as the temporal relationship of the changes and the strain of animal used [L20]. Table 9 summarizes the most commonly observed alterations of immunological parameters in low-dose/low-dose-rate (LD/LDR) irradiated animals.

**Table 9 Effects of ionizing radiation on the immune system of animals irradiated at low doses and low dose rates**

<i>Parameter</i>	<i>Animal species</i>	<i>Dose rate (mGy/d)</i>	<i>Time of detection of effect, or total dose</i>	<i>Trend of change</i>	<i>Reference</i>
Leucocyte score (blood)	Dogs	3–128	0–200 d 200–1700 d	Decrease Accommodative phase	[S40, N23]
Lymphocytes (spleen, thymus)	CBA mice C57BL mice C57BL mice	10–60 50 100	30 d 100–200–800 mGy 2 a	Decrease No change No change	[Y1] [J6] [C16]
Haematopoietic progenitors					
CFU-GM	Dogs	75	0–200 d 200–800 d	Decrease Recovery phase	[S39]
CFU-GM	Dogs	18.8	150 d 700–1200 d	Decrease Partial recovery	[N23]

Parameter	Animal species	Dose rate (mGy/d)	Time of detection of effect, or total dose	Trend of change	Reference
Lymphocyte mitogenic response	Dogs	>20	>300 d	Increase (T-cells)	[Y1]
	C57BL mice	50	100–200–800 mGy	Increase (T-cells)	[J6]
	C57BL mice	40	20 d	Increase (T-cells) No change (B-cells)	[N20]
	C57BL mice	40	200 mGy	Increase (T-cells)	[P11, S16, S17]
	BALB/c mice		75 mGy	Increase (T-cells)	[L18]
Cytokines	Mice		75 mGy	IL-10 decrease IL-12 increase	[L19]
	Mice		75 mGy	IL-1, IL-2 increase	[J8]
	C57BL mice		40 mGy	IL-1 $\beta$ , IFN- $\gamma$ increase	[I6]
	Rats		200 mGy	IFN- $\gamma$ , TNF- $\alpha$ increase TGF- $\beta$ decrease	[H20]
	C57BL mice	40	200 mGy	IFN- $\gamma$ decrease	[P11]
Macrophage phagocytosis and NK activity	CBA mice	0.02 + nuclides	180 d	Decrease	[G18]
	BALB/c mice		100–200 mGy; 48 h	Increase	[C21]
	BALB/c mice		200 mGy; 24–72 h	Increase	[C21, N24]
	C57BL mice	40	200 mGy	Increase	[P11]

82. Yagunov et al. [Y1] reviewed extensively the haematopoietic and immune system effects of LD/LDR irradiation in animal studies. These long-term effects of LD/LDR irradiation seem largely dependent on the capacity of radiosensitive tissues to repair DNA damage induced by a given daily dose. Post-irradiation changes in the peripheral blood counts of experimental animals returned to control levels within several months of the start of irradiation. The precise mechanisms by which these adaptive or accommodative processes occur are largely unknown, although repair, cell cycle and cell selection are considered to play a role [S40]. The effect of low-daily-dose gamma irradiation (3–128 mGy/d) on the blood-forming system of canines was studied by Seed et al. [S40]. Low but significant suppression of blood leucocytes, including granulocytes, monocytes and lymphocytes, occurred at 3 mGy/d. As the dose rate increased from 3 mGy/d to 128 mGy/d, the rate of suppression increased approximately eightfold. The time required to achieve accommodation decreased as the daily dose rate increased. Within the time required to reach a cumulative dose of 700 mGy, none of the dose rates affected blood cells sufficiently to compromise short-term immune function.

83. Clear associations were found between the tissue responses and marrow progenitor responses of chronically irradiated dogs. The granulocyte–macrophage (GM)-committed progenitor marrow numbers showed a suppressive

phase and a later recovery phase preceding the changes in blood leucocyte concentration [N23, S39]. Results also showed that 75 mGy/d represented a threshold below which the haematopoietic system retained either partial or full tri-lineal cell-producing capacity (erythropoiesis, myelopoiesis and megakaryopoiesis) for periods of exposure longer than one year [S41]. One might interpret these observations as evidence of an adaptive effect and acquisition of resistance to radiation exposure. However, at long times after exposure, animal populations experienced a high incidence of myeloid leukaemia and related myeloproliferative disorders [S49]. These animal data also indicated that a high degree of individual variability existed.

84. Post-irradiation recovery of marrow precursors and mature cells was incomplete, as evidenced by a deficient response to challenge stimuli (acute haemorrhage or gamma irradiation). Several factors may account for deficient haematological recovery after chronic irradiation [Y1]:

- Persisting post-irradiation deficiency of haematopoietic stem cells observed after exposures of >10 mGy/d for several months;
- Accelerated cell cycling of marrow precursors in irradiated animals, leading to increased ratios of S-phase populations among stem cells (colony-forming units (CFUs)) and committed marrow precursors;



- Shortened lifespan of immature erythroid and myeloid cells due to decreased cell viability in irradiated animals.

85. Internal irradiation of animals (using tritiated water incorporation) caused much more severe and prolonged immune depression than did external irradiation at the same total doses, thus indicating the higher relative biological effectiveness (RBE) of incorporated radionuclides, even for this low-LET (linear energy transfer) beta emitter [Y1].

86. The experimental findings of studies, mainly conducted in rodents and canines, reviewed by Yagunov et al. [Y1] are summarized as follows:

- Haematopoietic stem cells and blood cell progenitors seem to be the main target of chronic LD/LDR irradiation;
- Direct radiation damage to the blood/immune precursor pool results in a decrease of stem cell fraction;
- Radiation-induced depletion of the stem cell and progenitor pools results in accelerated cycling of bone marrow precursors;
- Decreased viability of mature blood cells results from ineffective haematopoiesis, thus causing restriction of myeloid (and probably lymphoid) cell reserves;
- Disturbances of cellular and humoral immunity are likely to be caused by extreme radiosensitivity of lymphoid tissues and by a restricted progenitor cell pool;
- Post-irradiation recovery is characterized by gradual reconstitution of peripheral blood and bone marrow patterns. However, residual deficiency of haematopoietic and lymphopoietic precursors may be a limiting factor in blood/immune system recovery;
- An increased percentage of S-phase marrow cells well after prolonged radiation treatment presents a typical response of the haematopoietic system to LDR irradiation, since this is not observed after acute or subacute irradiation or following chronic exposure to certain heavy metal ions;
- DNA misrepair following chronic irradiation may result in stable chromosome aberrations, increased incidence of micronuclei, and detectable point mutations in blood/immune populations.

87. Courtade et al. [C16] irradiated C57BL/6 female mice at 100 mGy/a for two years. No changes were found in cellular immunity parameters regarding CD4+ and CD8+ cells in the thymus and the spleen. In this work, cell subsets were evaluated by flow cytometry before and after stimulation with lectins. While the number of B-cells in the spleen also remained unchanged, a significant decrease in IgG1, IgG2a and IgG2b was observed, at 12, 24 and 18 months post-irradiation, respectively. Using mice irradiated with a

single LDR exposure (100 mGy, 10 mGy/min), Sharetskii et al. [S18] observed increased thymus-dependent humoral immune response and polyclonal activation of B-cells. The study of the dynamics of primary immune response showed that the period of radiation-induced elevation was followed by a phase of profound reduction of antibody formation.

88. On the other hand, a large number of studies have described stimulative effects of low-dose irradiation, including stimulation of growth rate, enhancement of survival after lethal high-dose irradiation, prolongation of lifespan, down-regulation of tumour incidence and activation of immune function.

89. Regarding the activation of the immune function, LDR WBI of mice increased the proliferative response of splenic and thymic lymphocytes to mitogens such as concanavalin A (Con A), phytohaemagglutinin (PHA) or anti-CD3, with an acute exposure of 20 or 75 mGy [I5, L16, L18] or fractionated doses of 200–800 mGy, 40 mGy/d [J6, J7, N20, S16, S17]. James and Makinodan [J6] investigated the proliferative capacity of differentiated effector cells in the spleen and its correlation with alterations in thymic precursors and peripheral T-cell subsets. In C57BL/6J mice exposed to 40 mGy/d over 20 d, the increase in spleen cell proliferative response was associated with an increase in the proportion of thymic progenitor cells (L3T4– Lyt2– equivalent to CD4– CD8–) and an increase in the proportion of mature L3T4+ (CD4+) thymocytes. In the spleen, the L3T4+ and Lyt2+ (CD8+) cell proportion was increased and the double-negative cell proportion was decreased. Interestingly, caloric restriction independently altered functional activity and T-cell subpopulations in the same direction as low dose rates. The changes observed are consistent with an increase in proliferative capacity and could reflect adaptive mechanisms operating with LDR irradiation and/or caloric restriction. Interpretation could be complicated by the metabolic status of the irradiated animals. Using the same irradiation protocol, comparable results were obtained by Nogami et al. [N20]. A finding of interest in this last study is the demonstration that LDR exposure can significantly enhance the proliferative activity of splenocytes in response to T-cell mitogens, but the response to LPS, a B-cell mitogen, was not influenced by the LDR treatment. Recently, Pandey et al. demonstrated a preferential activation of CD8+ T-cells as compared with CD4+ T-cells in mice following stimulation with Con A after fractionated 200 mGy exposure [P11].

90. Assays of mitogenic-induced proliferation were also performed using co-cultures of non-irradiated splenolymphocytes and peritoneal macrophages preirradiated with 20 and 40 mGy. The response was increased to 120% and 145% of the control, respectively, suggesting that the enhancement in Con-A-induced proliferation resulting from LDR exposure was caused not by direct activation of splenocytes but by activation of macrophages in the spleen, and that the lymphocytes were activated indirectly [I5]. LDR exposure acts on both the APCs and

the T-lymphocytes, facilitating the intercellular reactions within the immunologic synapse formed between these two categories of immune cells [L20].

91. Data also indicate that enhancement of immune response parameters takes place at a certain very narrow range of dose rate and dose. When WBI with an acute dose was changed from 20 to 200 mGy, the proliferation was inhibited [I5]. Likewise, a change in the dose rate from 40 to 100 mGy/d decreased the proliferative response to PHA or produced no effect [J7, N20].

92. The enhancement of T-cell-dependent immune response as measured by plaque-forming cell counts after immunization with sheep red blood cells (SRBCs) was reported to be stimulated by both single-dose WBI of mice (75 mGy, 12.5 mGy/min) [L15] and continuous 1.2 mGy/h irradiation for up to 140 days [I9, I13]. The *in vivo* T-cell response was also evaluated by delayed-type hypersensitivity (DTH) after immunization with *Mycobacterium vaccae* or dinitrofluorobenzene (DNF). While DTH to *M. vaccae* and DNF was suppressed in C57BL/6 mice, DTH to *M. vaccae* was increased in BALB/c mice, and DTH to DNF was not significantly changed [S17]. In the same study, the authors reported an inverted proliferative response to Con-A, with enhancement in C57BL/6 mice and suppression in BALB/c mice. Thus the outcome may depend on the strain of animal, the type of antigen and the type of response [S17]. Ina and Sakai [I13] examined the effects of continuous LDR irradiation of the whole body of several wild-type mouse strains and observed a significant activation of the immune system both before and after the immunization with antigens. The different strains displayed different levels of response but with the same tendency: significant increases of CD4+ T-cells, CD8 molecule expression and CD40+ B-cells, and significant enhancement in SRBC-antibody-producing cells by immunization. The age at exposure may also determine the intensity of immunoenhancement, since the presence of a non-involuting thymus contributes to this response [P11].

93. Furthermore, the efficiency of the immune response was measured by the NK activity of splenocytes [C21, N24], functional response of macrophages and cytokine secretion [G11, H20, L18, L19, P11, S33]. Enhanced cytotoxic activity of NK cells was found between 24 and 72 h post-irradiation in whole-body-irradiated BALB/c mice with single doses of 100 and 200 mGy [C21, N24]. LDR irradiation also enhanced the phagocytic activity of macrophages from C57BL/6 mice exposed to a total dose of 200 mGy (40 mGy/d) [P11].

94. Regarding the modulation of cytokine expression by LDR irradiation, increased secretion of interleukins that activate T- and NK cells, such as IL-2 by mouse splenocytes, and IL-1 and IL-12 by APCs, has been described, as well as down-regulation of IL-10 synthesis in splenocytes following WBI with 75 mGy [J8, L14, L18, L19]. There have been observations of the increased secretion of TNF- $\alpha$  and IL-1 $\beta$  by macrophages in response to WBI of mice with both low and high doses [I6, S33]. These two pro-inflammatory

cytokines exert a regulatory effect on lymphocytes, promoting their activation. Hashimoto et al. [H20] showed increased expression of the genes coding for TNF- $\alpha$  and IFN- $\gamma$  in splenocytes of tumour-bearing rats given 200 mGy of WBI, while mRNA of transforming growth factor  $\beta$  (TGF- $\beta$ ) had decreased. These findings suggest immune activation, since IFN- $\gamma$  plays a key role in both innate and acquired immune defences and also has antitumour properties, while TGF- $\beta$  is an immunosuppressive cytokine that allows tumour escape from immune destruction. All of these changes might contribute to a shift of the immune response in favour of Th1 differentiation.

95. In contrast to these observations, Gridley et al. found that LDR irradiation (50 mGy at 0.030 mGy/h) enhanced the intracellular expression of IFN- $\gamma$  in CD3+ CD4+ T-cells from C57BL/6 mice. Surprisingly, it did not result in increased levels of secreted IFN- $\gamma$  after protracted irradiation alone or when mice were exposed to a protracted low-dose followed by an acute high-dose irradiation [G11]. In the carefully conducted study of Pandey et al. on immunomodulation induced by LDR irradiation, the secretion of IFN- $\gamma$  by C57BL/6 mouse spleen cells stimulated by Con A was considerably reduced in the group irradiated at 200 mGy. This may appear contradictory to the enhanced cytotoxic T-cell response observed in the same study, but IFN- $\gamma$  may also have other important regulatory roles in the immune system [P11].

96. Evidence for the suppressive effect of LDR irradiation on tumour growth, metastases and carcinogenesis has been presented. Ishii and colleagues reported a decreased incidence of spontaneous thymic lymphoma in AKR mice as a result of chronic fractionated low-dose whole-body X-irradiation [I1]. In another well-recognized model of thymic lymphoma induced in C57BL mice by fractionated WBI (four acute doses of 1.8 Gy, one per week), preirradiation with 75 mGy given before each 1.8 Gy dose decreased the frequency of tumours from 90% to 63%. This level was further lowered to 43% by continuous WBI at 1.2 mGy/h for 450 days starting 35 days before the first 1.8 Gy dose. Interestingly, continuous irradiation to a total dose of 7.2 Gy over 258 days yielded no thymic lymphoma. In parallel, CD4+ T-cells, CD40+ B-cells and plaque-forming-cell counts in the spleen were significantly increased by continuous 1.2 mGy/h irradiation alone, indicating the involvement of immune activation in tumour suppression by LDR irradiation [I9]. However, other modifying factors, such as DNA repair and elimination of injured cells by apoptosis, are involved in the mechanisms for suppression of tumours. In addition, the complex nature of murine thymic lymphoma, with involvement of cell killing in the aetiology of the tumour, makes the interpretation of the data difficult [C34].

97. Significant suppression of the development of pulmonary tumour nodules was reported by Ju et al. [J8] and Cai [C33], who irradiated mice with single doses of X-rays ranging from 50 to 150 mGy, 24 h before injection of B16 melanoma or Lewis lung cancer cells. These results were corroborated later by Cheda et al. [C21], who injected syngeneic



low-immunogenic sarcoma cells into BALB/c mice 2 h after WBI with 100 or 200 mGy. This resulted in significantly reduced pulmonary tumour colonies. The authors associated the effect with stimulation of NK-cell-mediated cytotoxicity detected in splenocyte suspensions obtained from irradiated mice but not from sham exposed mice.

98. A report of the French Academy of Sciences extensively reviewed published data concerning the dose–effect relationship and carcinogenic risk of low doses of ionizing radiation [F11]. A database of cancer induction by LDR irradiation obtained from 472 different animal experiments was analysed [D30]. The meta-analysis showed that the spontaneous cancer rate fell significantly after LDR irradiation in only 40% of those experiments that could potentially have revealed the effect. It was suggested that, together with other mechanisms, the finding could partly be explained by the stimulation of immunological mechanisms. However, the author states that the statistical strength of the overall observations has not yet been determined. Although many observations show a reduction in the cancer rate and a longer life in low-dose-irradiated animals, these studies should act as a focus for further research in order to confirm or disprove the generality of the effects.

99. The adaptive response to radiation is a biological defence mechanism in which low-dose ionizing radiation (a “priming dose”) elicits cellular resistance to the genotoxic effects of subsequent irradiation (the “challenge dose”) [S37]. The adaptive response to radiation in animal and human populations, as well as its effects on the immune system, have been extensively considered in the UNSCEAR 1994 Report, annex B [U4].

100. The adaptive response has been observed after WBI. The experiments show that a priming exposure to chronic irradiation can induce radioresistance in mice. The manifestation of this resistance is reduced mortality (from the haematopoietic syndrome) of pre-exposed mice after a challenge acute irradiation [S43]. Yonezawa et al. have reported that pre-exposure of mice to 500 mGy two weeks before a lethal (7–8 Gy) WBI induced marked radioresistance and survival of the mice [Y7, Y8]. Later, using the same priming dose and a challenge dose of 5 Gy for haematopoietic studies, they investigated whether preirradiation favours recovery of pluripotent haematopoietic stem cells. They found that radiation-induced resistance to lethality appeared to be closely related to the recovery of endogenous colony-forming units of the spleen (CFU-S), with a maximum response when the priming dose was given 14 days before the challenge dose [Y5]. In addition, they demonstrated that the adaptive response at a challenge dose of 5 Gy seemed to be induced through a reduction of p53-dependent apoptosis in haematopoietic stem cells [H29].

101. Gong and co-workers [G28] studied thymocyte apoptosis and cell cycle progression induced by WBI in Kummung mice, using priming doses of 25–200 mGy and challenge doses of 1–2 Gy given 6 h after the priming dose.

Their results indicated that the percentage of thymocyte apoptotic bodies decreased, the arrest of G1 and G2/M phases diminished and the frequency of cells in S-phase increased. However, when the priming dose was 200 mGy, the adaptive response was no longer induced. Furthermore, a dose-dependent increase in thymocyte apoptosis was found with doses of 250 mGy or higher [M27]. The question of threshold dose for the immune-enhancing effects is critical. It depends on the end point tested, but the dependence on other factors such as animal species and the radiation dose rate is still an open question [S34].

102. Selective changes in the expression of proteins are reported to accompany LDR exposure [S17]. Increased levels of stress proteins were observed in mitogen-stimulated splenocytes of mice exposed to LDR radiation. The biological relevance of this was supported by the demonstration that splenocytes that failed to elevate their constitutive levels of heat shock proteins following LDR irradiation also were unable to increase their capacity to proliferate [N20]. Later, Chen et al. isolated a 10 kD protein from thymocytes after LD WBI. This protein, named RIP10, potentiates spontaneous thymocyte and mitogen-induced splenocyte proliferation, and modulates apoptosis [C23]. In addition, changes in cell cycle and apoptosis-related intracellular and extracellular proteins accompanying increased response to Con A in mouse lymphocytes following WBI were reported [S16].

103. Although growing evidence suggests that low-dose WBI can be immunostimulatory, many of the questions about immunoenhancement remain unanswered and require further experimental studies. One important aspect is the molecular basis of the stimulatory effect of LDR irradiation. Experimental data have been accumulating in this field. It has been reported that the intracellular free  $Ca^{2+}$  concentration increases after LDR irradiation and that protein kinase C (PKC) also increases in response to different doses of radiation, leading to the activation of early genes. Another signal pathway involved is the cyclic adenosine monophosphate/cyclic guanosine monophosphate protein kinase A (cAMP/cGMP-PKA) cascade: the cAMP/cGMP ratio falls after LDR irradiation, with PKA responding in the same pattern. A third pathway is phospholipase 2-prostaglandin E2 (PLA2-PGE2), which is also down-regulated. An adaptive response mediated by a feedback signalling pathway involving p38 mitogen-activated protein (p38 MAP) kinase, phospholipase C (PLC) and PKC has been demonstrated [L20].

## 2. Human data

104. Although few data are available on the effects of low-dose exposures on humans, some reports suggest that chronic low-dose radiation exposure can lead to effects on the human immune system. Chang et al. analysed the immune status in residents of buildings constructed using  $^{60}Co$ -contaminated steel rods [C7]. They evaluated CD3+, CD4+, CD8+ and HLA-DR+ markers in lymphocyte subsets in 196 exposed subjects with a mean cumulative excess

dose of 169 mSv (range 8–1,662 mSv) protracted over 2–13 years. These results were compared with those obtained in 55 close relatives considered to be the non-exposed reference population. Their analysis was restricted to individuals with no apparent history of medical conditions that could compromise their immune profile. The mean percentages of CD4+ T-lymphocytes and HLA-DR+ lymphocytes and the CD4+/CD8+ ratio in the exposed individuals were significantly lower than those in the reference population, while total CD8+ cell counts in the exposed individuals were moderately increased compared with the reference population. In addition, changes in the percentages of CD4+ T-cells and HLA-DR+ activated T-cells were significantly associated with radiation dose, while CD4+/CD8+ ratios were only moderately associated with dose [C7]. Low CD4+/CD8+ ratios are observed in primary or secondary immune deficiencies, and this ratio has been proposed as a method for estimation of the cellular immune status [H25]. The results presented by Chang et al. suggest that protracted gamma radiation exposure in a residential environment may induce a dose-dependent decrease of cellular immunity. However, these findings should be interpreted cautiously, taking into account the wide range of cumulative doses and their protraction among the exposed subjects. A new analysis of these results separating subgroups of people with narrower dose ranges could allow higher-quality conclusions to be drawn.

105. Immune status was evaluated by Godekmerdan et al. in 50 radiology workers [G26]. A decrease of CD4+ helper T-cells with diminished levels of immunoglobulins (IgA, IgG and IgM) and complement (C<sub>3</sub> and C<sub>4</sub>) was found, suggesting impairment of cellular and humoral immunity. The authors make reference to the fact that there was no significant difference between subjects exposed for more than or less than 5 years. This study does not provide data concerning either cumulative doses or dose rates. There is no mention of the type of medical procedure performed by the subjects involved in this study or the area of the radiology department in which they worked. Thus it is not possible to establish a relationship between these findings and radiation dose.

106. Rees et al. did not find significant changes in the immune profiles of 325 male workers occupationally exposed to external low-LET radiation at the British Nuclear Fuels facility at Sellafield, United Kingdom. The cumulative exposures were >200 mSv in a period of from 19.1 to 45.7 years in one group and <27.5 mSv in a period of from 15.1 to 32.5 years in the other. No statistically significant differences in circulating T- and B-cell total counts, CD4+ and CD8+ T-cell subsets, CD4+/CD8+ ratio or CD3+/HLA-DR+ were observed [R3]. This study took account of possible confounding factors such as age, sex and cigarette smoking, and the sample size was sufficient to substantiate the conclusion that occupational exposure to low doses does not affect the immune profile of workers.

107. Similar findings were reported by Tuschl et al. in employees working at the research reactor of the Austrian

Research Centre (Seibersdorf), exposed during the preceding 3 months to very low doses of gamma radiation (from 0.2 to 4.9 mSv). The percentages of CD2+, CD4+, CD8+ and NK cells were investigated in peripheral blood lymphocytes. Data were pooled in two groups of individual doses: <0.5 mSv and >0.5 mSv. Except for a slight increase in the relative number of cells expressing CD2 (a marker of T-cell activation), radiation-associated changes were not observed [T8].

108. Previously, the same group of authors had reported that lymphocytes of radiation workers exposed to 0.14–0.98 mGy/month exhibited an enhanced capacity to repair DNA damage inflicted by an *in vitro* challenge dose of ultraviolet (UV) radiation [T17]. More recently, Mohankumar et al. have analysed the UV-induced DNA repair capacity of the lymphocytes of 16 healthy, non-smoking radiation workers of the Indira Gandhi Centre for Atomic Research, Kalpakkam, India, who received whole-body gamma exposures ranging between 1 and 6.3 mGy during a period of 3 months prior to the study [M25]. At very low gamma doses (1–1.9 mGy), they found higher UV-induced unscheduled DNA synthesis (UDS) levels in samples receiving gamma irradiation *in vitro* than in control samples, but there was no such increase in the radiation workers' samples, owing mainly to the large standard deviation values of the means. For doses of over 2 mGy, both *in vitro* and *in vivo* irradiated samples show higher UDS levels. *De novo* synthesis of repair enzymes induced by low-dose ionizing radiation exposure was suggested as an explanation for these results. Particularly in the dose range 3–7 mGy, the UV-induced DNA repair capacity of radiation workers' lymphocytes was higher than that of cells exposed *in vitro*. These authors tried to explain these findings as an adaptive response of lymphocytes to radiation, due to cell renewal mechanisms, that led to a shift in the lymphocyte population in favour of a cell type with greater DNA repair capacity. They also postulated a possible involvement of the endocrinological system. However, the sample size was too small to confirm the observations and to sustain these hypotheses.

109. Tuschl et al. [T9] investigated some immunological parameters in 10 nuclear power plant (NPP) workers exposed during a 4-week period to external radiation (1.4–9.8 mSv) and tritium inhalation (committed effective doses of 1.2–2.8 mSv). Blood samples were taken 25 days after the start of this exposure period for quantification of lymphocyte subsets and evaluation of their mitogenic response to PHA. Data were compared with reference values obtained in healthy donors. CD4+/CD8+ ratios were increased in NPP workers owing mainly to an increase in absolute numbers of CD4+ T-cells. The authors interpreted these findings as a potentiation of the immune response by low radiation doses and suggested selective cell renewal of CD4+ T-cells as a possible underlying mechanism. Although the tritium burden in these workers was very small (0.47–6.3 kBq/24 h in urine), they postulated that the RBE of beta particles from tritium may account for this effect, which was not observed in other studies of occupationally exposed workers. Nevertheless, the

sample under investigation was too small, and further studies are needed to substantiate this hypothesis.

110. Beta particles from tritium are of greater biological effectiveness than gamma rays and X-rays. At low doses or low dose rates, RBE values of 2–3 have been proposed for the oxide form and even higher values when tritium is bound to organic molecules [S47]. However, depending on the end point and the irradiation conditions, RBE values for beta particles from tritium may greatly differ [M26, T10, T14]. The total number and percentage of leucocyte subpopulations were determined in 54 workers exposed to tritium in the workplace. Tritium contamination was well below the annual limit on intake for occupationally exposed subjects (mean tritium activity in urine: 1.9 kBq/litre). The functional status of leucocytes was evaluated by alkaline phosphatase (AP) and myeloperoxidase (MP) activity staining. While total leucocyte counts did not differ from those of the control group, lymphocyte and eosinophil counts were higher in radiation workers. AP and MP activities were lower in exposed workers [M23]. The author interpreted the increase in lymphocyte counts as a stimulation of the immune system by tritium and eosinophilia as a compensatory reaction of the bone marrow, where tritium had entered and disturbed enzyme synthesis in leucocyte precursors. The author stated that the workers had no clinical manifestations of immunity disorders. The selected end points seem inappropriate for evaluation of both innate and acquired immune response. The biological significance of these data cannot be readily discerned, and these results are inconclusive.

111. Few data have been published concerning the impact on the immune system of people living in high-level natural radiation areas (HLNRAs). Comparison of cord blood samples from newborns from the Kerala coast in India (average dose rates of greater than 1.5 mSv/a) with samples from newborns from areas with lower levels of natural radiation (less than 1.5 mSv/a) did not show any significant difference in the frequency of dicentrics, translocations, inversions or other types of aberration known to be associated with radiation exposure [C26]. Ghiassi-Nejad et al. reported in 2002 that no differences were found either in laboratory tests of the immune system or in haematological parameters among people living in Ramsar, an HLNRA in the Islamic Republic of Iran, where the mean dose rate is 260 mSv/a [G1]. Similar results had previously been reported for people living in Yangjiang, an HLNRA (6.4 mSv/a) in China [Z1]. In this preliminary study, Ghiassi-Nejad et al. did not observe significant differences in the basal frequency of chromosome aberrations in peripheral blood lymphocytes between people living in Ramsar and people living in normal background areas. Following a dose of 1.5 Gy of gamma rays *in vitro*, lymphocytes of Ramsar residents showed a significantly reduced frequency of chromosome aberrations. The authors interpreted their findings as an adaptive response induced by chronic radiation exposure.

112. Two years later the same group published new results showing a higher frequency of chromosome aberrations in

Ramsar residents [G20]. Concerning humoral immunity, IgG and IgA levels were not different, whereas a significant increase in IgE levels was observed in Ramsar residents. The authors interpreted this finding as radiation-induced immunostimulation due to a shift from a Th1 to a Th2 response. While no differences were found in the expression of CD69 (a marker of lymphocyte activation) in unstimulated samples, the expression of CD69 was higher in PHA-stimulated CD4+ helper T-cells of Ramsar residents, a finding that the authors considered as an indication of a higher risk of proto-oncogene activation. However, taking into account the lack of consistency between these two papers [G1, G20], the results are not conclusive, and these hypotheses appear rather speculative. The frequency of chromosome aberrations was also higher in residents from other HLNRA in Brazil [B18] and China [C22]. It has been reported that smoking plays a more significant role than natural radiation in the induction rate of stable lymphocyte aberrations in those areas [Z2]. However, this statement should be interpreted with caution since a recent meta-analysis from retrospective biological dosimetry data from seven European laboratories indicated that there was a strong variation of translocation yield with age, but no variation was detectable with sex or smoking habits [W17].

### 3. Remarks concerning low-dose/low-dose-rate irradiation

113. On the basis of the previous paragraphs, the following remarks may be made concerning LD irradiation data. It has been reported in some animal studies that under protracted gamma ray exposure at low dose rates, the normally highly radiosensitive haematopoietic system adapts and becomes radioresistant. Depending on the dose and dose rate, induced the pattern of changes in leucocyte populations can be described as an initial suppressive phase followed by a stable accommodative phase. This pattern of changes is preceded by similar changes in the haematopoietic progenitor cell compartment. However, it was suggested that the recovery of marrow precursors might be incomplete.

114. In animal experiments there is evidence demonstrating that LDR irradiation can produce activation of the immune function. Enhancement of the proliferative response of splenic and thymic lymphocytes to mitogens, enhancement of NK activity and increased secretion of cytokines with regulatory effect on immune cells promoting their activation, *inter alia*, have been reported. Nevertheless, the data are not entirely consistent, and the observed effects are highly dependent on the range of dose and dose rate, and upon the animal and strain of animal studied.

115. Data demonstrating suppressive effects of LDR exposure on tumour growth, metastases and carcinogenesis have been reported. An association of these effects with enhanced NK activity of splenocytes, higher antibody-dependent cellular toxicity and increased levels of CD4+ cells, CD40+ B-cells and plaque-forming cells in the spleen has been found.

116. An adaptive response to radiation is another phenomenon observed in many systems. Interestingly, radiation-induced resistance to lethality after WBI with a high challenge dose in mice appeared to be closely related to the recovery of CFU-S after the priming dose. It was also related to the reduction of apoptosis in the haematopoietic stem cells, giving insight into the possible mechanisms by which these adaptive processes occur.

117. Regarding human data, while some authors have reported evidence for effects after chronic LDR irradiation, others have not found such evidence. A dose-dependent decrease of cellular immunity, mainly evaluated by the CD4+/CD8+ ratio and HLA-DR+ activated T-cells, was described for residents of buildings constructed with <sup>60</sup>Co-contaminated materials, although these findings should be interpreted cautiously. In contrast, no significant changes were observed in the same parameters of workers occupationally exposed to external low-LET radiation in nuclear facilities.

118. Concerning tritium incorporation, it would appear to have a higher RBE than external irradiation at the same doses. This has been observed in both experimental studies and human studies, but the results are inconclusive.

119. In the same way, when the impact on the immune system of living in areas with high levels of natural radiation was analysed, the results were controversial, and the significance of these findings remains unclear.

### C. Data concerning high-dose irradiation

#### 1. High-dose-induced immunosuppression

120. The effects of radiation on the immune system generally intensify with the dose received. Massive cell death, inflammation and infection are the acute effects of high-dose radiation exposure. Human data concerning the effects on the immune system of WBI have been widely studied in victims of radiation accidents and in patients undergoing WBI as a conditioning regime for bone marrow transplantation. The effects of high doses of ionizing radiation upon immune system function were reviewed by the Committee in 1988 [U6].

121. Acute radiation syndrome (ARS) occurs after WBI or substantial partial-body irradiation of greater than 1 Gy, delivered at a relatively high dose rate [G22]. With the exception of the haematopoietic syndrome, the other clinical components of ARS (gastrointestinal and cerebrovascular) are not the subject of this annex, and they are reviewed elsewhere [K45, W9]. Since granulocytes and lymphocytes are an essential part of the immune system, profound abnormalities of immune function are expected as a consequence of high-dose WBI.

122. A patient who receives acute external WBI in the range 0.5–1 Gy is generally asymptomatic, and blood counts

may be normal or minimally depressed below baseline levels 3–5 weeks after exposure [G23]. The haematopoietic syndrome may be seen following doses of >1 Gy. Acute whole-body doses of below 2 Gy induce mild cytopenia without significant bone marrow damage. Laboratory analysis in cases with WBI of greater than 2 Gy can show an initial granulocytosis, with pancytopenia evident within the first month after exposure [G23]. Mitotically active haematopoietic progenitors are unable to divide after a whole-body dose of >2–3 Gy, resulting in haematological crisis in the following weeks [G22]. During this symptom-free period of ARS (latency phase), the blood-producing cells in the bone marrow begin to diminish and are not replaced, leading to a severe shortage of white blood cells, followed by a shortage of platelets and then red blood cells. The shortage of white blood cells can lead to severe immunodeficiency, increasing the risk of infectious complications and impairing wound healing [D1, G23].

123. The rate of decrease for different leucocytes in the blood after WBI is dependent on their particular cell cycle kinetics. Neutrophils have a relatively short lifespan, and thus they have a tendency to be depleted over a matter of days following acute WBI, owing to radiation-induced damage to their progenitor cells. They show an initial increase within the first few days after doses of >2 Gy; this increase is greater after higher doses. This first “abortive rise” may be due to a cytokine-dependent transient mobilization from bone marrow or extramedullary sites and to accelerated maturation of granulocyte precursors. A progressive neutropenia then occurs, the rate and extent of which are dose-dependent; this may be followed by a second abortive rise following doses of <5 Gy as a result of haematopoiesis recovering from precursor cells. This second abortive rise is not seen following doses of >5 Gy, indicating the failure of haematopoiesis to recover permanently after very high doses, a finding that may be clinically helpful as a prognostic indicator [G22]. The duration of neutropenia may be long, requiring prolonged administration of haematopoietic growth factors, blood product support and antibiotics.

124. In non-uniform exposures, evaluation of cytopenia may be somewhat misleading because the cumulative curve of the granulocytes “averages” the actual production by different portions of the bone marrow that have received different doses. Temporal parameters in such cases are more relevant to the magnitude of the dose, and blood counts are more correlated to the volume of damaged bone marrow. Lymphopenia in non-uniform WBI is more prolonged than in uniform WBI [G34]. When the ARS is associated with cutaneous radiation syndrome, the lymphohaematopoietic suppression impairs wound healing and increases the risk of wound bleeding and infection [B16].

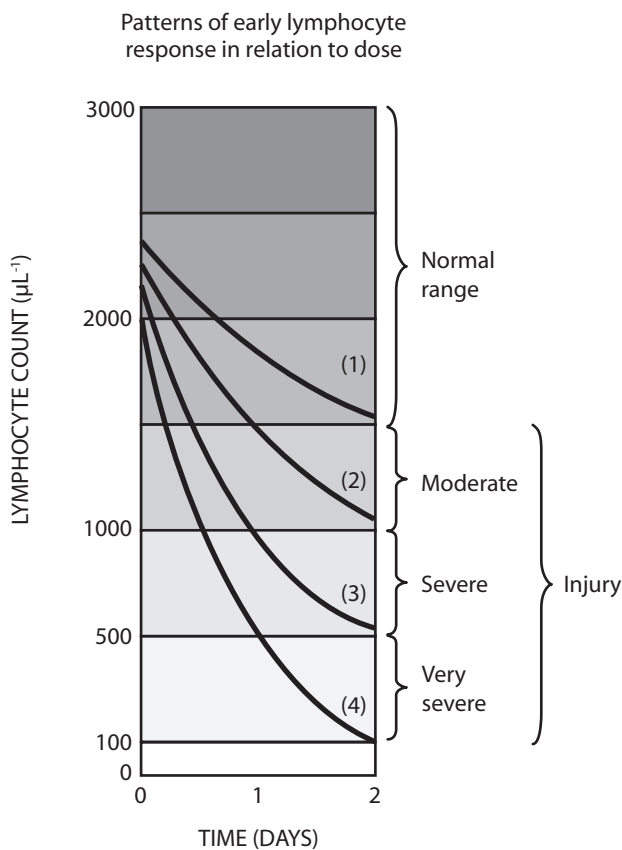
125. A follow-up of neutrophil values for several days post-irradiation was found to correlate well with dose. The time to reach the critical level of 500 granulocytes/mL has been proposed as a dosimetric bioindicator [B30]. However, in many cases of overexposure, an earlier approximation of



dose is required for efficient medical intervention. This can be achieved by counting the decrease of lymphocytes, as their nadir is reached much earlier than for other cell types. The predictability of lymphocyte depletion following high doses of ionizing radiation, which may be recognized within hours of exposure, has allowed the development of biodosimetric models. This approach was originally developed to give a rough categorization of the magnitude of exposure (figure VI).

**Figure VI. Classical Andrews lymphocyte depletion curves and clinical severity ranges.**

Whole-body doses: curve (1), 3.1 Gy; curve (2), 4.4 Gy; curve (3), 5.6 Gy; curve (4), 7.1 Gy [G3].



126. A mathematical biodosimetric method for evaluating uniform WBI by peripheral blood lymphocyte and neutrophil counts was widely used and validated following the Chernobyl accident [B31, U6]. The doses evaluated by peripheral blood cell counts, chromosome aberrations and electron spin resonance of tooth enamel were highly correlated [B30].

127. A mathematical model for lymphocyte depletion following gamma irradiation, intended only for providing a first approximation of dose, was developed on the basis of accident cases with recorded haematological data and physically reconstructed doses. During the first 8 hours after exposure, the decrease in lymphocytes followed a single-term exponential curve, and the rate constant for this decrease correlated well with dose estimates obtained from other sources

of dosimetry [G3]. This technique was further extended to include analysis of various types of criticality accident. Lymphocyte depletion in high-level mixed gamma-neutron accidents was found to be approximately equal, at a given effective dose, to that for gamma ray accidents. This finding indicates that, in terms of lymphocyte depression, the RBE of neutrons could be close to unity [G24].

## 2. Immune reconstitution

128. Reconstitution of the immune system after radiation-induced bone marrow aplasia has been widely studied in patients undergoing bone marrow grafting. Bone marrow transplantation is characterized by a subsequent period of immunodeficiency, the duration and severity of which vary according to graft manipulation, choice of graft type (donor and source), development of graft-versus-host disease and level of residual thymic activity.

129. During the first month, T-lymphocytes reconstitute by peripheral expansion of the T-cells present in the graft. Thereafter, starting from 100 days after transplantation, the production of substantial numbers of new naive T-cells by the thymus can be detected [D9]. Rapid early expansion of transferred or residual mature T-lymphocytes of extrathymic origin results in inversion of the CD4/CD8 ratio, which persists primarily as a result of delayed CD4+ T-cell recovery. Normalization of the CD8+ subset after 60 days was reported, while persistent CD4+ reduction has been observed after 2 years, and normalization of the CD4+ subset was achieved only after 6 years in a long-term study [L4]. The different behaviour in this immune reconstitution may be explained by an extrathymic origin of CD8+ cells, while the CD4+ subset recovery, which is thymus-dependent, is impaired in the adult population. In children, who are characterized by having greater amounts of active thymic tissue and more effective thymopoiesis than adults, there is a more rapid recovery of CD4+ T-cells, which express a naive phenotype (CD45RA+). This finding may have significant implications for attempts to generate protective immunity against pathogens and clinically relevant antitumour responses by vaccination strategies in the post-transplantation period [P7].

130. Oligoclonal B-lymphocyte repopulation can be demonstrated in the early post-transplantation period, achieving normal values after 90–120 days [L4]. Serum immunoglobulin levels usually decrease post-transplantation, followed by a gradual increase and normalization in a sequential pattern similar to that identified in neonates. Recovery initially occurs in IgM levels (2–6 months), followed by IgG levels (3–18 months) and finally IgA levels (6–36 months), the time depending to some degree on conditioning and graft characteristics [P7]. Cooperation between T-cells and B-cells is necessary for antibody production. Thus, although B-cells transferred with the graft may confer temporary protection against pathogens, antigen-specific T-cells must be regenerated in order to ensure sustained B-cell competence [L4].

131. Reconstitution of innate immunity following transplantation is characteristically more rapid than for acquired immunity. Most studies have shown early normalization or even rebound increases in NK cell numbers in the early months [P7]. NK cells might provide an efficient defence against pathogens and residual tumour cells, especially immediately after transplantation, where the lack of cooperation between T-cells and B-cells results in an inability to produce antibodies.

132. The results presented all support the conclusion that normalization of immunological function is achieved within several years after transplantation. The functional capacity of the recipient thymus appears to be the dominant influence on thymus-dependent reconstitution. Thymus-independent reconstitution, which accounts for the majority of the early reconstitution, might be predicted to depend more heavily on stem cell sources.

### 3. Remarks concerning high-dose irradiation

133. As discussed in the preceding paragraphs, acute WBI in the range 0.5–1 Gy is generally asymptomatic, and leucocyte counts may be minimally depressed or even normal. Acute radiation syndrome (ARS) occurs after acute whole-body or substantial partial-body exposure of >1 Gy. While mild cytopenia is observed within the range 1–2 Gy, initial granulocytosis (first abortive rise) followed by pancytopenia within the first month result from whole-body doses of greater than 2 Gy. A second granulocyte abortive rise may be seen when the whole-body dose is below 5 Gy. In non-uniform exposures, temporal blood cell parameters are more relevant for dose estimation than the magnitude of cytopenia. Mathematical models have been developed for correlating the dose with the kinetics of lymphocyte depletion during the first hours after exposure.

134. Acquired immune reconstitution after radiation-induced bone marrow aplasia includes both thymus-dependent and thymus-independent pathways. T-lymphocytes reconstitute during the first month by peripheral expansion, and, during the following months, new naive T-cells begin to appear from the thymus. However, an inversion of the CD4/CD8 ratio persists for several years. While CD8+ T-cells normalize within two months, the recovery of CD4+ T-cells may be achieved within 6 years. Central T-cell recovery in adults is delayed relative to that in children, probably owing to differences in thymic function. B-lymphocyte repopulation takes place during the first 4 months. Following an initial decrease of immunoglobulin levels, recovery gradually occurs in IgM levels (2–6 months), IgG levels (3–18 months) and Ig A levels (6–36 months). Innate immune reconstitution is more rapid than reconstitution of acquired immunity. NK cells normalize or even increase in the early months.

## D. Influence of dose rate and radiation quality on immune response

135. Several studies have examined the immunomodulating effects of whole-body exposure to different qualities of radiation. The driving forces for these investigations are the challenges created by exposures in the space flight environment (which are dominated by charged particles [S21, S28]), clinical use of proton radiation in the management of cancer [L9, S23] and exposures due to radon and its progeny [N1].

### 1. Low-LET exposures

136. The effects on the immune system of radiation, such as gamma rays or X-rays, have been extensively investigated and reviewed [A13, S1, U8, U10]. Variability in the biological response may reflect differences in the total dose, dose rate, end points measured and time of evaluation post-exposure. Analysis of the influence of each of these variables is of importance not only in radiation therapy but also for environmental or occupational radiation exposure [G8].

137. Pecaut et al. [P6] investigated early effects on mice of gamma ray doses of up to 3 Gy at low and high dose rates. They observed a significant dose-dependent loss of spleen and thymus mass, which was somewhat independent of dose rate. Decreasing lymphocyte and leucocyte numbers in the blood and the spleen with increasing dose, as well as dose-dependent decreases in lymphocyte subpopulations (CD4+ helper T-cells, CD8+ cytotoxic T-cells and CD19 B-cells) were observed at both dose rates. While the percentages of CD4+ increased with increasing dose (with some differences between blood and spleen), the percentages of the CD8+ population remained stable in both compartments, and CD19+ cells declined markedly with high or low dose rate. The number and proportion of NK cells remained stable. Because of the differences in phenotypic radiosensitivities, a decrease in lymphocyte counts sometimes results in a proportional enrichment of specific cell types. Overall, these data indicate that the changes observed were highly dependent on dose but not on dose rate, and that cells in the spleen are affected more by dose rate than those in the blood.

138. Similar data have been reported for high-dose treatment under comparable experimental conditions [H11, W5]. A more pronounced dose-rate effect was seen in spleen mass, probably owing to a change in homing receptors in circulating populations of splenic endothelial cells [P6]. It has been demonstrated that ionizing radiation can up-regulate the expression of ICAM-1 and E-selectin, both of which are important in leucocyte trafficking. CD44, a molecule involved in the migration and homing of immune cells, was altered in irradiated lymphoid cells [H3, N1].

139. Using the same animal model, the functional characteristics of splenocytes and cytokine expression was evaluated after WBI at various total doses and at low and high dose rates [G8]. An assay of spontaneous blastogenesis in

leucocytes is sometimes performed in order to determine the proliferative capacity and activation of lymphocytes. Mitogen-induced cell proliferation is employed extensively to assess the general responsiveness of lymphocytes to stimulating agents. The data showed that at 4 days post-irradiation, increasing doses resulted in a rise in spontaneous blastogenesis in blood and spleen leucocytes. This finding was correlated with a reduction in white blood cells [P6], and suggested that cell regeneration was in progress. Splenocyte response to LPS (bacterial lipopolysaccharide), a potent non-specific stimulator of B-cells, was inversely related to radiation dose. This finding is consistent with the demonstration that B-cells are the most radiosensitive lymphocyte subpopulation [C6, P6, W14]. Lymphocyte responsiveness to T-cell mitogens (PHA and Con A) was shown to depend on experimental conditions. A decreased ability of splenocyte mononuclear cells to respond to mitogens after WBI (0–7 Gy) was described by Harrington et al. [H5].

140. Acute doses of some centigrays, as well as chronic WBI, can enhance mouse splenocyte response to these mitogens [L15, N13]. Furthermore, WBI induced changes in expression of CD25 (IL-2 receptor) and CD71 (transferring receptor) activation markers [M7]. IL-2 secretion by PHA-activated splenocytes was reduced in a dose-dependent manner. Plasma levels of transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) and splenocyte secretion of tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) were not affected by either the dose or the dose rate of radiation [G8]. The data demonstrate that the responses of the blood and the spleen for the immune parameters studied were largely dependent upon the total dose of radiation and that dose rate was not a significant factor.

141. Even though the quantity of energy deposited is minimal, a repetitive irradiation inflicts a series of small insults on the tissue. Each delivered fraction contributes to the development of inflammation. For a repetitive radiation exposure with low to medium doses, the amount of the cumulative dose is more important than the number of fractionated doses. Gremy et al. [G32] showed a modulation of expression of inflammation mediators during fractionated gamma radiation restricted to a colorectal zone in the rat (5 Gy 3 times a week, maximum dose 45 Gy). The IL-1 $\beta$  mRNA showed an overexpression from a cumulative dose of 20 Gy. The level of IL-10 mRNA was highly repressed at 45 Gy. The monocyte chemoattractant protein 1 (MCP-1) chemokine expression progressively increased with the cumulative dose, while IL-8, less overexpressed, went to a maximum expression at 20 Gy. Some signalling pathways were altered, exacerbated or suppressed, triggering a neutrophil recruitment to the inflammatory site at the end of the irradiation protocol.

## 2. High-LET exposures

### (a) Neutrons

142. Neutrons have been shown to have high values of relative biological effectiveness (RBE) for some biological

end points, including chromosome aberration induction in human lymphocytes. Immediate early death of lymphocytes and thymocytes after neutron irradiation seems to be comparable to that for low-LET gamma rays and X-rays when assayed by apoptosis, suggesting that the neutron RBE is close to unity for this end point. Moreover, the route to cell death is independent of dose rate. These observations are in marked contrast to the much larger RBE values and dose-rate effects typically seen in many systems of clonogenic survival, replicative death and chromosome aberration formation.

143. Warenius and Down compared apoptosis in mouse thymocytes following exposure to low doses of fast neutrons (62.5 MeV) and photons (4 MeV). Both the time course and the radiation dose-response curves were similar for high- and low-LET radiation modalities [W16]. Vral et al. examined the effectiveness of fast neutrons compared with  $^{60}\text{Co}$  gamma rays for inducing apoptosis in human lymphocytes. Doses ranging from 0.05 to 5 Gy were applied at 0.2 Gy/min (fast neutrons) or 1.5 Gy/min (gamma rays). To investigate the role of DNA repair in apoptosis induction, they also performed irradiations at low dose rates (0.006 Gy/min). They found that apoptosis induction was independent of LET and dose rate (the calculated RBE for fast neutrons was close to unity), suggesting that the initial DNA damage, as opposed to DNA repair, dominates the induction of apoptosis in resting lymphocytes [V7]. Ryan et al. examined apoptosis in human lymphocytes following doses of between 0.25 Gy and 5 Gy of low-energy fast neutrons (280 keV mean energy) and of  $^{137}\text{Cs}$  gamma radiation. They found that neutrons are equally as effective as gamma radiation at inducing apoptosis in lymphocytes [R23]. Goans et al. analysed lymphocyte depletion kinetics in various types of criticality accident (high-level mixed gamma/neutron fields). The results were approximately equal, at a given effective dose, to those for gamma accidents, suggesting that the RBE of neutrons could be close to unity for this end point, regardless of the structure of their energy spectra [G24].

144. Huiskamp et al. compared the effect of WBI with fission neutrons (1 MeV mean energy) and 300 kVp X-rays on the supportive role in T-cell differentiation of the thymic stroma of neonatal CBA-H mice, after transplantation to athymic nude mice. Doses varied from 2.75 Gy to 6.88 Gy (0.1 Gy/min) for neutrons and from 6 to 15 Gy (0.3 Gy min) for X-rays. Although irradiation had no effect on the stromal and T-cell composition of grafts, the graft size decreased in a dose-dependent manner with an RBE of 2.1 for fission neutrons [H32]. Holl et al. evaluated the effect of WBI with 65 MeV neutrons or 15 MeV X-rays on spleen cells of BALB/c mice following doses ranging from 0.2 to 3 Gy. Their results indicated that the RBE of neutrons differs depending on the end point selected, since they found that the level of apoptosis was equal for high- and low-LET radiation (RBE = 1), whereas spleen weight and cellularity were reduced to a greater extent by fast neutrons (RBE = 2) [H31].

*(b) Protons*

145. Since cells of the immune system are among the most radiosensitive in the body, an investigation of the effect of proton radiation on these cells would support more realistic estimates of the risks associated with space flight. Furthermore, a better understanding is needed of the biological interactions following exposure to protons, in view of the increasing number of patients undergoing proton radiotherapy.

146. Both early and long-term effects on the immune system of mice undergoing whole-body proton irradiation have been reported [G9, K2, P4]. Gridley et al. drew attention to lymphoid organs and specific leucocyte populations of mice receiving WBI with 0.5–3 Gy of 250 MeV protons at two dose rates: 1 cGy/min and 800 mGy/min [G9]. Four days post-irradiation, highly significant dose-dependent reductions were observed in the mass of the thymus and the spleen, and in the numbers of leucocytes and CD3+ T-cells, CD4+ helper T-cells, CD8+ cytotoxic T-cells and CD19+ B-cells in both blood and spleen. A less pronounced dose effect was noted for NK cells in the spleen. Monocyte (but not granulocyte) counts in blood were highly dose-dependent; the numbers for each population tended to be lower with high-dose-rate than with low-dose-rate irradiation. Increases in the percentage of CD3+, CD4+ and NK cells, and in the CD4+/CD8+ ratio, were noted with increasing doses. A significant dose-rate effect was found in the percentages of T- and B-cell monocytes and granulocytes, and in the CD4+/CD8+ ratio. The majority of the dose-related decreases in the spleen were highly linear. Results from gamma- and proton-irradiated groups were similar, although proton irradiation gave consistently lower values in some measurements. Very rough calculations of RBE for protons ranged from 0.82 (based on percentage of B-cells in the spleen) to 1.55 (based on percentage of NK cells in the spleen) [G9]. RBE values for CD4+ T-cells ranging from 0.9 to 1.4 and for CD8+ T-cells from 0.7 to 0.9 were reported by Radojic and Crompton, who compared the induction of apoptosis in human peripheral lymphocytes following irradiation with 300 kVp X-rays or 138 MeV protons [R18].

147. In other studies [K2, K3] that also directly compared the effects of these two forms of radiation at the same dose (dose: 3 Gy; proton dose rate: 0.4 Gy/min and 1 Gy/min; photon dose rate: 0.3 Gy/min), CD19+ B-cells were the most radiosensitive, although recovery back to normal levels was observed by day 15. T-cell (CD3+) and CD4+ helper T-cell recovery was evident by day 29, while CD8+ cytotoxic T-cell counts remained significantly below normal. Plasma TGF- $\beta$ 1 was elevated on day 7 in the  $^{60}\text{Co}$ -irradiated but not in the proton-irradiated mice. However, few differences in assay results were seen between animals exposed to protons versus photons.

148. Pecaut et al. [P5] assessed the functional characteristics of mouse leucocytes following whole-body proton irradiation at doses of between 0.5 and 3 Gy (dose rates

of 10 mGy/min or 800 mGy/min). Treatment with protons caused a significant dose-dependent increase in spontaneous blastogenesis in the blood and the spleen, suggesting that acute cell death had stabilized by day 4 post-irradiation, and that DNA synthesis and regenerative mechanisms were increasingly stimulated by an increasing magnitude of cell depletion. The authors also observed a greater effect of irradiation by protons than by gamma rays at the same doses. A dose-dependent decrease in the response to T- and B-cell mitogens was observed, and the splenocyte response to Con A was significantly lower than that of the gamma-irradiated group. The underlying mechanism for this effect is not known. The authors suggest that it may have been due to greater impairment of DNA synthesis in the T-cells capable of responding to Con A. Although the dose rates employed represented a range within which dose-rate-dependent changes in sublethal damage do occur, no dose-rate effects were observed.

149. Although a large number of studies have provided experimental results of long-term effects of radiation on the immune response [C16, V1], there are few data on effects following proton irradiation. Pecaut et al. [P4] studied the immune status of mice exposed to 3 or 4 Gy of 250 MeV protons and sacrificed at day 122 post-irradiation. The main points that emerged are the following:

- There were no effects on thymus, spleen or liver mass;
- There were significant dose-dependent decreases in macrophage, CD3+ CD8+ cytotoxic T-cell, NK cell, platelet and red blood cell populations;
- In contrast, dose-dependent increases were observed in spontaneous, but not in mitogen-induced, blastogenesis;
- Effects of radiation were more evident in leucocytes in the spleen than in the peripheral circulation.

*(c) Densely ionizing particles*

150. Laboratory studies with mice have provided evidence for acute and chronic effects of high-LET particles on the immune system. Utilizing carbon ions, Erofeeva et al. [E4] and Grigorenko et al. [G12] reported changes in the cell architecture and distribution of lymphoid cells in both the spleen and the thymus that persisted at 60 days post-irradiation. Gridley et al. [G10] evaluated the effects of  $^{56}\text{Fe}$  and  $^{28}\text{Si}$  irradiation on the lymphoid cells and organs of mice at days 4 and 113 after whole-body exposure. In the short term, dose-dependent decreases were noted in spleen and thymus masses, but there was a greater dependence on dose for thymus mass than for spleen mass. With low-LET radiation, in contrast, the spleen mass is more linearly dependent on dose than is the thymus mass [G10]. This suggests that the response of different lymphoid organs may be dependent on radiation quality. Irradiation resulted in a marked reduction in the number of lymphocytes and granulocytes on day 4



post-irradiation. Interestingly, it was observed that high-LET radiation might shift the nadir of peripheral neutropenia to an earlier point. The radiosensitivities of lymphocyte subpopulations were consistent with those obtained using gamma rays or protons. There were no significant differences in the response to T- and B-cell mitogens or secretion of IL-2 and TNF- $\alpha$  by PHA-stimulated splenocytes. In contrast, proton irradiation induced significant depression in the response to all three mitogens [P5]. Overall, the radiation-induced changes on day 4 were significantly more pronounced in the blood than in the spleen.

151. By day 113 post-irradiation, most of these radiation-induced changes were no longer evident. However, B-lymphocyte numbers and percentages in blood were significantly increased. The percentages of total T-cells and CD8+ T-cells were low in both blood and spleen. According to the authors [G10], these findings suggest that  $^{56}\text{Fe}$  irradiation may have compromised cell-mediated or acquired immune responses. These long-term effects seen with 2 Gy  $^{56}\text{Fe}$  irradiation were not observed following exposure to 2 Gy  $^{28}\text{Si}$  ions. Exposure to  $^{28}\text{Si}$  ions did, however, result in increased responsiveness to PHA and LPS, and in lower numbers and percentages of NK cells in both blood and spleen. Thus it appears that exposure to  $^{28}\text{Si}$  ions, which have a depth-dose profile considered to be optimal for maximizing high-LET particle effects, may result in chronic immune dysfunction.

152. The above observations differ somewhat from those reported from examination of the long-term effects of exposures to 250 MeV monoenergetic protons, where irradiated animals had significantly increased basal DNA synthesis and no differences in mitogen-induced blastogenesis. These data suggest that different immunomodulatory consequences may be induced by densely ionizing particles.

#### (d) Radon

153. Data concerning health effects of radon exposure are reviewed in annex E, "Sources-to-effects assessment for radon in homes and workplaces". Only some data concerning the effects of radon exposure on the immune system will be reviewed here. Since previous studies have demonstrated that exposure to radon can trigger genotoxic damage in rat macrophages and deep-lung fibroblasts [K10], it is possible that lymphocytes migrating through the blood or the lymphatic circulatory systems of the lung may be exposed to alpha particles from radon progeny.

154. The effect of radon on the immune response was studied by Nagarkatti et al. [N1]. Mice were exposed 18 h/d for 10 (or 25) days to a concentration of radon and its progeny giving a total cumulative exposure of 1,000 (or 2,500) working level months (WLMs)<sup>1</sup>. A marked decrease in the

total cellularity of most lymphoid organs (such as the thymus, spleen, peripheral lymphoid nodes and lung-associated lymph nodes) was observed compared with controls. The percentage of T-cells increased while that of non-T-cells decreased in peripheral lymphoid organs at both doses. It was interesting to note that in the thymus at 2,500 WLMs, a dramatic decrease in the number of CD4+ CD8+ T-cells and an increase in CD4- CD8- T-cells were observed.

155. The exact mechanism by which radon affects T-cell differentiation in the thymus is not clear. Radon exposure led to differential expression of CD44 in lung-associated lymph nodes. CD44 is an adhesion/homing receptor involved in lymphocyte and macrophage homing to lymphoid and other organs. These data suggest that lymphocytes and macrophages may migrate in different patterns to other organs. In the lung-associated lymph nodes, where one would predict the largest number of damaged cells to be present, there was a significant decrease in T-cell responsiveness to mitogens, while the B-cell response was not affected. This may be due to the fact that in lung-associated lymph nodes there may be a marked loss of macrophages, the accessory functions of which are essential for T-cells to respond to mitogens. Interestingly, radon exposure caused an increase in the T- and B-cell responsiveness to mitogens in the spleen and peripheral lymph nodes.

### 3. Remarks concerning the influence of dose rate and radiation quality

156. Overall, the data presented in the preceding paragraphs show that both high and low dose rates of sublethal gamma radiation induce significant depression in the majority of the parameters evaluated at early times after irradiation. The changes observed were highly dependent on total dose but not on dose rate.

157. The RBE of neutrons differs depending on the end point considered. The level of lymphocyte apoptosis is almost equal for neutron and photon exposures, whereas immune organ weight and cellularity are reduced to a greater extent by neutron irradiation.

158. The response of mononuclear cells following proton irradiation is highly dependent on the total dose but not on dose rate, at least in the range of doses and dose rates analysed. Some cell types exhibited differences in the response to proton versus photon radiation, proton irradiation giving consistently lower values. Significant depressions in some lymphocyte subpopulations were observed after long-

litre of air that results in the ultimate release of  $1.3 \times 10^5$  MeV of potential alpha energy. Exposure of a worker to this concentration for 170 hours (a working month) results in an exposure of one working level month (WLM). However, while the cumulative exposure expressed in working level months provides an estimate of the exposure to radon and its decay products (primarily of the bronchial epithelial tissues of the lung), it does not provide a direct measure of the dose to lymphoid tissues. (See annex E for further discussion of the dose due to radon and its decay products.)

<sup>1</sup>A working level (WL) is a unit of concentration in air of the potential alpha energy released from the decay of radon and its daughter products. The WL is defined as any combination of the short-lived radon daughters in one

term evaluation. Increased spontaneous blastogenesis is a relatively persistent phenomenon throughout short-term to long-term evaluations.

159. Lymphoid cells and tissues are markedly affected by high-LET radiation at relatively low doses, and some rearrangements persist long after exposure. Depression in the percentage of cytotoxic T-cells, and enhancement in the total number of lymphocytes and B-cells, were observed at long times post-irradiation. Increased basal DNA synthesis is a persistent phenomenon.

160. Radon exposure induces marked changes in thymus subpopulations and alters the expression of CD44, a molecule involved in the migration and homing of immune cells. A significant decrease has been observed in T-cell responsiveness to mitogens in lung-associated lymph nodes.

## E. Radiosensitivity of lymphocyte subsets

### 1. General considerations

161. The dose required to induce a defined percentage of cell death in a given cell population defines the level of radiosensitivity of that cell population [N19]. Although the sensitivity of different lymphocyte subsets to ionizing radiation has been extensively studied, there are still controversial results in the literature. The radiosensitivity of lymphocytes is related to the population under study. For B-lymphocytes it depends on their degree of differentiation, and for each subset of T-lymphocytes it depends on their state of activation. Activated lymphocytes have long been known to be more resistant to ionizing radiation than their resting (non-activated) counterparts [A13]. The radiosensitivity of lymphocyte subsets is higher when irradiation is performed on sorted purified lymphocyte subpopulations rather than on unsorted peripheral blood lymphocytes irradiated as a whole [M29].

162. Radiation-induced apoptosis in lymphocytes is triggered by two pathways: the mitochondrial pathway (intrinsic pathway) and the death receptor pathway (extrinsic pathway). Apoptosis via both pathways is mediated by the activation of a series of cysteine proteases, the caspases. Although both pathways of apoptosis involve activation of common effector or executioner caspases, they differ in the activation of apical or initiator caspases. The first one, through Bax protein, induces cell death by acting on mitochondria and accounts for the differential radiosensitivity among lymphocyte subpopulations. The second one is mediated by plasma membrane signals via interaction of the tumour necrosis factor receptor (TNFR) with its ligand (tumour-necrosis-factor-related apoptosis-inducing ligand—TRAIL) and does not seem to be related to intrinsic radiosensitivity of lymphocyte subsets [M29]. Interactions between TRAIL and ceramide signalling pathways in regulating apoptotic death have been reported [L25]. Apoptosis of lymphocytes through the death receptor pathway also occurs in physiological conditions.

Activation-induced cell death is a form of apoptosis in which activation of T-cells occurs through proper engagement of TCRs by specific antigen bound to an HLA molecule, and influenced by antigen concentration and co-stimulatory signals. Activation-induced cell death plays an essential role in both central and peripheral deletion (clonal deletion) events involved in self tolerance and homeostasis [Z4].

### 2. Review of published data

163. In 30 patients who were treated with 12 Gy (fractionated) of WBI, it has been reported that T- and B-lymphocytes showed a sharp radiation-induced decrease, with B-lymphocytes being the most sensitive population [C11]. CD3+ CD4+ CD45RO+ (memory helper T-cells), CD3+ CD4+ CD45RA+ (naive helper T-cells), CD4+ and CD8+ cells appeared equally sensitive. The CD34+ cell subset (progenitor/stem cells) remained basically unchanged, and the CD3- CD56+ CD16+ (an NK cell subset) was relatively radioresistant compared with the other lymphocyte subsets. This study provides evidence that T- and B-cell subsets seem to be highly radiosensitive in vivo, while progenitor/stem cells and NK cells seem to be more radioresistant. Similar findings were reported in eight patients undergoing external beam radiotherapy to the pelvis [L21]. NK cells were the most radioresistant and B-cells the most radiosensitive lymphocytes. No significant differences between helper T-cells and suppressor/cytotoxic T-cells were observed.

164. Data concerning the radiosensitivity of CD4+ helper-inducer and CD8+ cytotoxic T-cells are controversial. Several factors (e.g. the radiation dose, the end point selected and the time period over which this end point is evaluated) could modify the findings. The tendency to spontaneous and radiation-induced apoptosis of lymphocyte subpopulations differs among individuals. In addition, age and sex are factors that may influence the apoptotic response [S8]. Nakamura et al. did not find any difference in the radiosensitivity of CD4+ and CD8+ cells [N4]. By using an in vitro lymphocyte colony assay, they demonstrated that  $D_{10}$  (the dose required to reduce the surviving fraction to 10%) was similar for these two types of cell:  $3.13 \pm 0.10$  Gy (mean  $\pm$  SD) for CD4+,  $3.34 \pm 0.50$  Gy (mean  $\pm$  SD) for CD8+ and  $3.14 \pm 0.17$  Gy (mean  $\pm$  SD) for unsorted cells.

165. Several studies have considered B-cells to be more radiosensitive than T-cells [P12, R5, S8, V1, W14]. However, taking into account the spontaneous apoptosis of each different lymphocyte subpopulation, Wilkins et al. [W4] arrived at a different conclusion. Using the modified neutral comet assay (MNCA) they demonstrated that, following 1 Gy of low-LET radiation, CD8+ T-cells had the highest radiation-induced apoptotic fraction, followed by CD4+ T-cells.

166. Crompton and Ozsahin [C17], using a method based on assessment of DNA internucleosomal degradation through flow cytometry, demonstrated that CD8+ T-cells

were the most radiosensitive population, followed by CD19+ B-cells. For interpreting these results, it should be taken into account that the frequency of radiation-induced apoptosis was calculated by subtracting the fraction of apoptotic cells at 0 Gy (spontaneous apoptosis) from the fraction induced by the radiation treatment of samples. Although the results were identical to those reported by Stewart et al. [S30], it is important to keep in mind that the latter studies were performed using high-dose-rate irradiation.

167. Reports concerning the effects of ionizing radiation on NK cells have been contradictory. As NK cells exhibit so large a degree of interindividual variability, it is difficult to reach firm conclusions concerning the effect of ionizing radiation on this lymphocyte subpopulation. Seki et al. [S11] reported that NK cells were the most radiosensitive in vitro and that CD8 cells and B-cells showed lower susceptibility to radiation, whereas CD4+ cells were relatively radioresistant. On the other hand, Mori and Desaintes reported that, among peripheral blood lymphocyte subpopulations, NK cells are more highly resistant in vitro to ionizing radiation than are T- and B-lymphocytes [M28]. In exploring the potential radioprotective effect of different types of cytokine on radiation-induced apoptosis, it was shown that IL-2 inhibited the apoptosis of NK and that IL-2, IL-4 and IL-7 were able to rescue both CD4+ and CD8+ T-cells from radiation-induced cell death [S11]. The viability of B-cells in culture was maintained by the presence of IL-4 but not by other cytokines. The authors speculated that the protective effect by each cytokine might be attributed in part to an enhancement of cellular induction of the expression of the bcl-2 protein family. However, while overexpression of bcl-2 leads to the inhibition of cell death [V5], it is important to remember that there exist in the immune system apoptotic pathways unaffected by bcl-2 expression, such as the CD-95-mediated pathway [S31].

168. Fuggetta et al. have shown impaired NK activity in vitro after gamma irradiation (20 Gy) [F6]. IFN- $\beta$  (200 IU/mL) was able to completely reverse this inhibitory radiation-induced effect, but was not able to modify the number of CD16+ and CD56+ cells that died by apoptosis after irradiation. Concerning in vivo radiation exposure, no changes in NK cell activity were found in 1,341 atomic bombing survivors residing in Hiroshima [B5].

169. The molecular basis of the differential radiation sensitivity among lymphocyte subpopulations remains unclear [M29]. It has been proposed that the higher radiosensitivity of B-lymphocytes is due to a lower activity of DNA-dependent protein kinase (DNA-PK) in these cells [M14]. Also, the radiosensitivity of lymphocyte subsets has been related to intrinsic differences in basal expression level of specific genes, particularly those related to *Bcl-2* family genes such as *Bax*, *Bcl-2* and *Bcl-X* [I11].

170. However, a recent study using microarray analysis demonstrated that basal gene expression does not

account for differential lymphocyte radiosensitivity. Mori et al. [M29] investigated the profile of gene expression in peripheral lymphocytes 8 hours after in vitro X-ray irradiation. Cell suspensions of magnetically purified lymphocyte subpopulations were irradiated with a dose of 1 Gy at a dose rate of 0.3 Gy/min. A total of 18,433 unique sequences were screened for their transcriptional response to ionizing radiation. The authors found 102 genes whose expression was modulated by radiation exposure: 75 were up-regulated and 27 were down-regulated. The most strongly activated genes belonged to apoptosis, cell cycle and DNA repair functional classes, with a clear predominance of the p53 pathway. The authors reported no difference in the basal level of expression of the proapoptotic genes among lymphocyte subsets. In contrast, their levels of activation following exposure to X-rays differed among cell subtypes. For example, *Bax* expression levels increased in lymphocytes in the following order: NK cells < CD4+ T-cells < CD8+ T-cells < CD19+ B-cells, reflecting their differential radiosensitivity [M29]. On the other hand, the expression level of the *TNFR* gene in irradiated cells did not differ among these lymphocyte subsets.

### 3. Remarks concerning the radiosensitivity of lymphocyte subsets

171. The data presented in the preceding paragraphs indicate that the radiosensitivity of lymphocyte subsets, in terms of the radiation dose required to induce a defined percentage of cell death, depends on several factors:

- Lymphocyte subset under study;
- Irradiation performed on resting or activated lymphocytes;
- Degree of differentiation;
- Irradiation performed on sorted or unsorted subpopulations;
- Influence of cytokines;
- Spontaneous apoptosis exhibited by the subset under study;
- Age and sex of the donors

172. B-lymphocytes (CD19+) seem to be the most radiosensitive subset, both in vivo and in vitro. However, when estimation of radiation-induced apoptosis is performed by subtracting spontaneous apoptosis, CD8+ T-cells exhibit higher radiosensitivity. Most of the data show no difference in radiosensitivity between CD4+ and CD8+ T-cells. While NK cells are relatively radioresistant in vivo, particularly the CD56+ CD16+ NK subset, data concerning in vitro irradiations are more controversial. The level of radiation-induced activation of apoptosis-related genes differs among these subsets, probably reflecting their differential radiosensitivity, as follows: NK cells < CD4+ T-cells < CD8+ T-cells < CD19+ B-cells.

## F. Alterations of the immune response after prenatal irradiation

173. Radiation-induced impairment of both humoral antibody and cell-mediated responses have been reported in numerous experimental and epidemiological studies. However, very few data are yet available concerning radiation effects on the immune system following in utero exposure. These data mainly concern numerical or structural changes, and thus they cannot be directly related to effects on the immune function. The developing haematopoietic system is very sensitive to ionizing radiation. The mammalian embryonic haematopoietic system becomes functional in the yolk sac during organogenesis; in parallel, additional haematopoietic activity takes place in the aorta, gonads and mesonephros. Later the function shifts to the foetal liver and spleen, and finally to the bone marrow [T11, U16]. Foetal liver haematopoiesis is first detectable at about the sixth week of gestation in humans. Although the spleen has largely ceased haematopoiesis in humans by the time of birth, it may regain haematopoietic function in abnormal situations [H27].

### 1. Animal data

174. Grande and Bueren irradiated mice at different stages of development with a single dose of 500 mGy of X-rays. Mice irradiated on days 13 and 17 post-conception showed a significant reduction in the proportion of femoral bone marrow granulocyte-macrophage colony-forming units (CFU-GM) one year after irradiation. This effect was manifested neither in mice irradiated on day 4 post-conception nor in those irradiated in early post-natal life (2 days, 8 days and 12 weeks old) [G6]. The effects of ionizing radiation on the embryo/foetus may differ according to the developmental stage at which the exposure takes place. In this study, the impairment in the CFU-GM population was observed when mice were irradiated after midgestation. As seen in figure VI, this is the period of haematopoietic progenitor cell expansion (day 13) and bone marrow and thymus colonization (day 17) in rodents. This study indicates that, for most stages of development in the mouse, a single acute dose of X-irradiation of 500 mGy is below the threshold dose capable of inducing deterministic effects in the mouse haematopoietic system, although it reveals the induction of a significant impairment in the CFU-GM population when irradiation is given at the late stages of embryonic development.

175. Haematopoiesis is the product of two components: the haematopoietic tissue and the regulatory stromal microenvironment in which it resides. The role of both components in radiation-induced haematopoietic effects remains controversial. Yang et al. [Y3] proposed that irradiation at midterm gestation damages the developing regulatory microenvironment but not the haematopoietic stem cell population that it hosts. To demonstrate this, the authors used an experimental model of prenatal irradiation in combination with cross-transplantation experiments. They found that 1.8 Gy of gamma irradiation given to mice at midgestation caused

a 40% reduction in the haematopoietic stem cell population (CFU-S), which persisted up to at least 6 months of age. Spleen colony formation after sublethal doses of gamma rays reflected this reduced complement of endogenous stem cells. The regulatory haematopoietic microenvironment, measured as fibroblastoid colony-forming cells (CFU-F), was similarly depleted. The quality of the stem cell population in the offspring of irradiated mothers was not affected, since normal growth of the CFU-S population was observed after transplantation into standard recipients. In contrast, when used as recipients of a bone marrow transplant from either normal or irradiated offspring, the offspring of irradiated mothers were unable to support normal growth. Compared with normal offspring, there were 70% fewer CFU-F in the femur 1 month after bone marrow transplantation when the offspring of irradiated mothers were used as transplant recipients. This confirmed a reduced capacity to host normal stem cells and also indicated that CFU-F in the transplant were unable to compensate for the poor microenvironment in irradiated offspring hosts.

176. The developing haematopoietic tissues are very sensitive to  $^{239}\text{Pu}$  contamination. Mason et al. [M1] studied post-natal haematopoietic function in the offspring of pregnant mice injected with 30 kBq/kg of  $^{239}\text{Pu}$  at 13 days of gestation. The maximum dose (10–14 mGy) was absorbed in the liver. A long-term deficit in the number of haematopoietic colony-forming cells (CFU-S) in the spleen, liver and bone marrow was observed. The development of the stromal microenvironment was also deficient, suggesting sublethal damage in those cells destined to become the precursors of the supportive haematopoietic microenvironment.

177. Platteau et al. [P9] looked for medium-term effects in the immune system of rats following 0–2 Gy prenatal or early post-natal WBI. They did not find changes in the histology of the spleen, profiles of lymphocyte subpopulations or serum immunoglobulin levels. At an age of 10 weeks, rats were immunized with a T-dependent or a T-independent dinitrophenylated carrier antigen. T-dependent response was higher in rats irradiated between days 6 and 20 of gestation; however, this increase was significant only for IgM and IgG1 responses.

178. Nold et al. [N14] demonstrated that dogs irradiated prenatally with 1.5 Gy ( $^{60}\text{Co}$  gamma irradiation) on day 35 of gestation exhibited lower primary humoral antibody responses to a T-dependent antigen (sheep red blood cells), with a decrease in helper T-lymphocyte subpopulations in peripheral blood. Moreover, they found defects in epithelios-tromal development of the thymus and concluded that the observed immune alterations could be related to radiation-induced prenatal thymic injury.

179. Thymocytes are either negatively selected as potentially autoreactive and deleted, or positively selected to become mature cells. In addition to the signal mediated by the T-cell receptor (TCR), other signalling pathways also regulate this developmental selection. It has been demonstrated [N12] that the CD28 receptor, which plays a role in



enhancing the survival and expansion of peripheral T-cells, is also involved in negative selection in the thymus. Developing thymocytes of CD28-deficient and wild-type mice displayed similar radiosensitivity in terms of apoptosis, but negative selection was significantly reduced in the former, thus suggesting that CD28 receptor involvement in thymus development is not related to regulation of cell survival.

180. Miller and Benjamin [M6] have studied radiation-induced alterations in prenatal thymic development in the beagle dog. They found that injury to both thymic cortices and medullas was greater following exposures earlier in gestation. Damage to medullas was relatively more severe than in cortices following exposure at any given age. The degree of reduction of medullar volume reflected thymic epithelial injury, which is surprising since the thymic epithelium is considered in the adult to be radioresistant. Such injury may have serious post-natal consequences, as normal differentiation of T-cell subpopulations is dependent upon the integrity of the thymic microenvironment. Damage to the thymic microenvironment could result in immunodeficiencies and defects in immunological regulation.

## 2. Human data

181. Human foetuses are thought to be highly sensitive to ionizing radiation. However, human data concerning the effects of prenatal irradiation on the immune system are scarce. In humans, haematopoietic activity begins in the liver by six weeks of gestation and continues until one week of post-natal life. The spleen also plays a major role

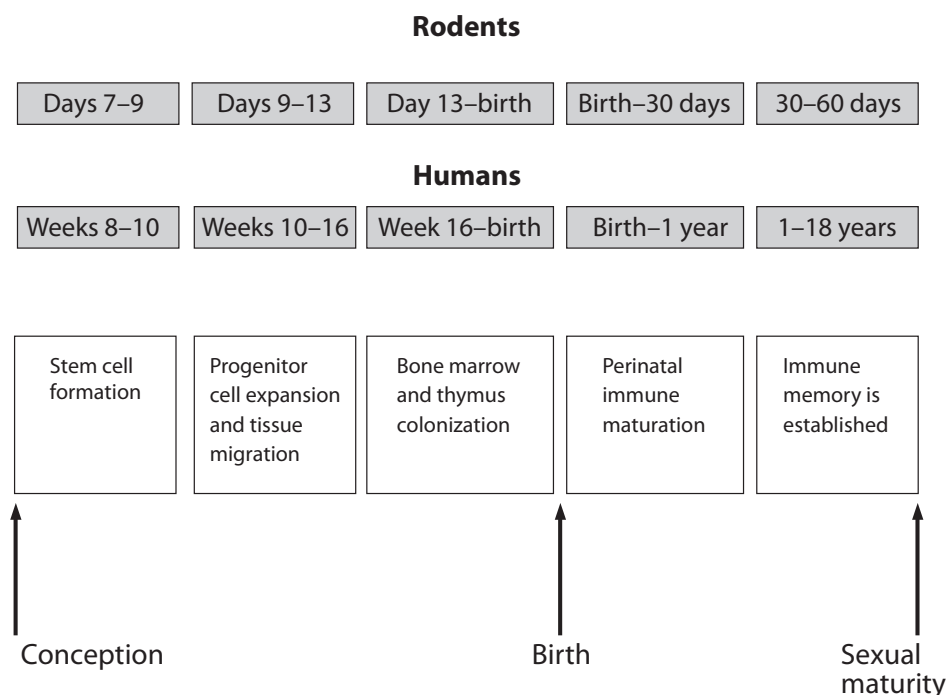
in haematopoiesis in the growing foetus. Post-natal, the bone marrow becomes the main haematopoietic organ; the spleen and liver have ceased their function by the time of birth or soon after.

182. During the intrauterine ontogenesis of the immune system, apoptosis plays a central role in thymus development. A distinction should be made between developmental and radiation-induced lymphocyte apoptosis. While apoptosis during T-cell maturation is p53-independent, p53 protein is a critical mediator of apoptosis in response to genotoxic agents such as ionizing radiation. Immature cortical thymocytes are characterized by a double-positive immunophenotype, CD4<sup>+</sup> CD8<sup>+</sup> TCR<sup>low</sup>. The interaction between immature thymocytes and thymic reticuloendothelial cells induces positive selection of T-lymphocytes that are not activated by self HLA molecules, thus promoting tolerance induction. T-lymphocytes that do not undergo positive selection are killed by apoptosis. Immature thymocytes also undergo negative selection when thymic stromal cells eliminate self-reactive T-lymphocytes by apoptosis [K1].

183. As seen in animals, the effects of ionizing radiation on the human embryo/foetus may differ according to the developmental stage at which the exposure takes place. The definition of critical windows in the development of the immune system may allow identification of periods of special vulnerability. On the basis of known developmental changes occurring within the immune system, Dietert et al. proposed a relative timeline for critical windows in the developing human immune system [D5]. A parallel approach for the development of rodent and human immune systems is shown in figure VII.

**Figure VII. Critical windows of toxic exposure for the developing immune system in rodents and humans.**

Gestation: 21 days for rodents, 38 weeks for humans (adapted from reference [D5]).



184. Prenatal events can affect the immune response at birth and, depending on gestational timing, can result in widely varied outcomes. The potential outcomes of toxic exposures to the developing immune system may derive from suppression, perturbation or up-regulation of the immune response, leading to immunodeficiencies, autoimmunity or hypersensitivity reactions, respectively. Moreover, prenatal damage to the thymus would have a more profound effect than post-maturational damage. Similarly, prenatal destruction of abundant pluripotent stem cells may have a more harmful outcome than destruction of single lineages or differentiated cells, which predominate in adults [H14, H27].

185. Ohtaki et al. evaluated the frequency of stable aberrations (translocations) in blood samples from 150 survivors of the atomic bombings at Hiroshima and Nagasaki who were exposed in utero to doses of  $\geq 5$  mSv and from 181 controls (dose  $< 5$  mSv). Foetal doses were approximated by the dose to the maternal uterus. The mean age of subjects at the time of sampling was  $40 \pm 0.7$  years. The investigators did not find a dose response but did find a small increase in translocation frequency at doses of  $< 100$  mSv, with a peak response at  $\sim 30$  mSv, at which the net increase in translocation frequency was  $\sim 0.4\%$ . There was no evidence for an effect of gestational age in the estimated coefficients. These findings were explained with the hypothesis that the human foetal lymphoid progenitor pool comprises two subpopulations with different radiosensitivity. A major fraction exhibits radiation resistance. The other subpopulation, small in number, was considered sensitive to the induction of both translocations and cell killing, and diminished rapidly following doses of  $> 50$  mSv [O9].

### 3. Remarks concerning the effects of prenatal irradiation

186. On the basis of the preceding paragraphs, the following remarks may be made concerning the effects of prenatal irradiation on the immune system:

- The developing immune system is very sensitive to ionizing radiation;
- There are critical windows of special vulnerability of the prenatal immune system;
- The effects differ according to the developmental stage at which the irradiation takes place; for example, a dose of 500 mGy given to mice in early pregnancy has no effect, whereas the same dose given after midgestation results in significant depletion of bone marrow precursors 1 year later;
- Developing haematopoietic tissues are very sensitive to  $^{239}\text{Pu}$  contamination: even doses of around 10 mGy are capable of inducing a long-term haematopoietic deficit;
- Radiation-induced impairment of the developing stromal microenvironment accounts, at least in part, for these long-lasting effects;
- Prenatal thymic injury is greater following irradiation in early pregnancy;
- Prenatal radiation-induced damage in the thymus is more severe for the medulla than for the cortex;
- Human foetal lymphoid progenitors include a major subpopulation that is relatively radioresistant and a minor subpopulation that is very radiosensitive to the induction of both translocations and cell killing.

### G. Immune dysfunction and hypersensitivity to ionizing radiation

187. As seen, DNA recombination processes not only account for immune diversification but also constitute a major checkpoint in the development of lymphocytes [D26]. Defects in V(D)J recombination lead to immunodeficiency, and a deregulation of this process may result in higher sensitivity to genotoxic agents and increase the risk of malignancies.

188. The understanding of the mechanisms involved in the development of human pathologies exhibiting immune dysfunction and higher radiosensitivity may be relevant in the context of the effects of ionizing radiation on the immune system. There exist a number of human genetic disorders characterized by chromosomal instability that are associated with a higher incidence of cancer. Both the chromosomal instabilities and the neoplastic outcomes are related to abnormalities of DNA metabolism, DNA repair, cell cycle regulation or control of apoptosis. Among them there are several disorders associated with defects in the immune system and increased susceptibility to radiation, including UV and ionizing radiation [B9]. These observations are especially relevant because they are at the crossroads of basic cellular mechanisms, combining immunodeficiency, increased rate of cancer, and defects in DNA repair and pathways known to be associated with hypersensitivity to radiation. Other pathological conditions involving immune dysfunction, such as autoimmune diseases and acquired immunodeficiency syndrome (AIDS), could be also associated with higher radiosensitivity. Because of the double condition of hypersensitivity to radiation and immunodeficiency, the radiation effects on the immune system may be more severe in these patients.

#### 1. Human genetic disorders

189. Abnormal DNA repair and cell death regulation may result in higher vulnerability to ionizing radiation. Several human genetic disorders associated with defective DNA repair present functional abnormalities in the immune system. Some of them exhibit increased susceptibility to radiation. If individuals with such disorders undergo radiotherapy, they may develop adverse side effects in normal tissues, such as acute effects appearing during or shortly after treatment, or late effects developed months or years later. Some of these genetic disorders are reviewed below and are summarized in table 10.



**Table 10 Human genetic disorders with immune system defects and increased susceptibility to radiation**

Disease	Defective mechanism <sup>a</sup>	Mutated gene	Sensitivity to <sup>b</sup>	
			UV	Ionizing radiation
Xeroderma pigmentosum	NER (+/- TCR)	<i>XP-A, XP-B, XP-C, XP-D, XP-E, XP-F, XP-F/ERCC1, XP-G</i>	(+++)	?
Trichothiodystrophy	NER/TCR	TFIIH-related <i>XP-D</i> and <i>XP-B</i>	(++)	?
Xeroderma pigmentosum variant	TLS	<i>hRAD30</i>	(+)	(+)
Ataxia telangiectasia homozygote	DSB cell signalling	<i>ATM (-/-)</i>		(++++)
Ataxia telangiectasia heterozygote	DSB cell signalling	<i>ATM (+/-)</i>		(+)
Ataxia-telangiectasia-like disorder	DSB cell signalling	<i>hMRE11</i>		(++++)
Nijmegen breakage syndrome	DSB cell signalling	<i>NBS1 (nibrin)</i>		(++++)
Fanconi anaemia	Cross-linking repair and HR	<i>FANC-A, FANC-B, FANC-C, FANC-D1/BRCA2, FANC-D2, FANC-E, FANC-F, FANC-G</i>		(++)
Bloom's syndrome	HR/TLS	<i>BLM</i> RecQ helicase	(+)	?
Wiedemann–Rautenstrauch syndrome	Neonatal progeria	<i>WRS</i>		?
Hutchinson–Gilford syndrome	Progeria infantum	<i>HGPS</i> (lamin A)	(++)	(++)
Cockayne syndrome	NER/TCR (youthful progeria)	<i>CS-A</i> and <i>CS-B</i>	(++)	(?)
Werner's syndrome	HR/TLS (adult progeria)	<i>WRN</i> RecQ helicase	(+)	(+)
Severe combined immunodeficiency (SCID)	V(D)J/NHEJ	<i>Artemis</i>		(+++) (SCID with increased radiosensitivity)
Immunodeficiency with microcephaly	NHEJ	<i>Ligase IV</i> <i>Cernunnos/XLF</i>		(++++)
Seckel syndrome	DSB cell signalling	<i>ATR</i>		(+++)
Dyskeratosis congenita	Telomere metabolism	<i>Dyskerin/hTR</i>		(++)
Hyper-IgM syndromes	Class switch recombination	<i>Uracil-N-glycosylase (UNG)</i>		(++)

<sup>a</sup> NER = nucleotide excision repair; TCR = transcription-coupled repair; DSB = double-strand break; HR = homologous recombination; TLS = translesion synthesis; NHEJ = non-homologous end joining.

<sup>b</sup> Increasing number of + symbols means increasing degree of radiosensitivity. Query symbol (?) means probably radiosensitive but radiosensitivity not demonstrated.

190. *Xeroderma pigmentosum* (XP) is an autosomal recessive disease with defective nucleotide excision repair (NER) characterized by severe sensitivity to all types of UV radiation. The NER pathway recognizes and removes UV-induced DNA lesions. XP is characterized by cutaneous and ocular abnormalities, predominantly on sites exposed to sunlight, and in some cases by neurological features resulting from progressive neuronal loss. Expression in skin includes disposition for sunburn, pigmentary abnormalities, telangiectasia, dryness, scarring, and susceptibility to multiple benign and malignant neoplasms, including basal and squamous cell carcinomas and melanomas [L7]. A variety of immune abnormalities have been described in XP patients: decreased

delayed-type hypersensitivity reactions, decreased T-cell proliferative responses to mitogens, decreased CD4/CD8 ratio and impairment of NK cell activity [G19, L6, N21, N22, W15]. XP is categorized in at least eight genetic subtypes labelled A to G, plus the F/ERCC1 group. While both XP-A and XP-C can be attributed entirely to defects in NER, the other complementation groups involve defects in other DNA repair mechanisms [L6]. Some XP patients in complementation group E (XP-E) lack UV-damaged-DNA-binding protein (UV-DDB), which is present in healthy individuals and in patients in other XP complementation groups. UV-DDB protein is composed of two subunits, DDB1 and DDB2. In vivo, UV-DDB protein plays an important role

in the p53-dependent response of mammalian cells to DNA damage. When cells are exposed to UV, the resulting accumulation of p53 activates DDB2 transcription, which leads to increased levels of UV-DDB. A linear dose response for activation of *DDB2* and *XPC* genes has been demonstrated following exposure of peripheral blood lymphocytes to doses of gamma rays as low as 0.2 Gy [A21].

191. The cells of *xeroderma pigmentosum variant* (XP-V) patients are only slightly more sensitive than normal cells to the killing action of UV radiation. The XP-V cells are NER-proficient but present a deficit in translesion DNA synthesis, with an increased probability of being blocked at a DNA lesion [C31]. Defects in translesion synthesis (TLS) due to mutations in the damage-specific polymerase hRAD30 (polymerase  $\eta$ ) are responsible for the UV-hypermotability of XP-V cells leading to skin cancers [K38]. It has been proposed that sunlight carcinogenesis in XP-V patients may be associated with increased genomic rearrangements that result from double-strand breaks and rejoining in cells of the skin in which p53 is inactivated by UV-induced mutations [L12].

192. *Trichothiodystrophy* (TTD) refers to several autosomal recessive diseases whose diagnostic hallmark is short, brittle hair low in sulphur and cysteine because of impaired synthesis of high-sulphur matrix protein [L7, S38]. The clinical symptoms associated with TTD, such as ichthyotic skin, neurodegeneration and developmental disorders, represent a variable range of abnormalities in organs derived from the ectoderm [L7]. The majority of TTD patients exhibit photosensitivity because their NER pathway does not remove UV-induced DNA lesions efficiently. Although their deficiencies result mainly from mutations in either XP-B or XP-D, TTD patients do not present skin cancer susceptibility [C27, S38] and also do not exhibit hypersensitivity to ionizing radiation. Although some abnormal immune parameters have been found in TTD patients [C19, N22, R10], immune deficiency is not a common feature of TTD. However, the finding of additional TTD patients combining features of XP and Cockayne syndrome indicates that there may be considerable clinical heterogeneity with phenotypic overlap.

193. *Bloom's syndrome* belongs to a family of hereditary diseases caused by defects in genes belonging to the RecQ family of DNA helicases, which play a role in regulating homologous recombination. Bloom's syndrome patients, who exhibit a mutation in the *BLM* gene, are hypersensitive to UV and exhibit abnormal replication patterns following DNA damage. They present sun-induced rash, immunodeficiency, subfertility and cancer predisposition [K4]. One of the defining features of cells from Bloom's syndrome individuals is chromosomal instability, characterized by chromosomal breaks, deletions and rearrangements, as well as elevated levels of sister chromatid exchange.

194. *Ataxia telangiectasia* (AT) is an autosomal recessive multisystem disorder with early-onset cerebellar ataxia, conjunctival and cutaneous telangiectasia, and immunodeficiency

as its main clinical features. AT patients are prone to cancer development and are unusually sensitive to ionizing radiation. They carry a mutation in a gene involved in DNA damage signalling [J9], called the ataxia-telangiectasia-mutated (ATM) gene. Only homozygous AT patients present the typical clinical features described above. Homozygous AT is the most common cerebellar ataxia in children under 5 years of age, with a prevalence of 1 in 100,000 live births [S48]. Progressive cerebellar ataxia becomes apparent as early as the first year of life. Oculomotor abnormalities may also be seen. Telangiectases appear after onset of the neurological syndrome and are progressive.

195. AT affects both TCR and Ig genes, thus explaining the immunodeficiency observed in AT patients. Moderate cellular and humoral immunodeficiency, with low levels of certain Ig classes, in conjunction with difficulties in swallowing, lead to frequent pulmonary infections in AT patients. Peripheral lymphocytes from AT homozygotes show elevated chromosomal rearrangements, which preferentially involve chromosomal breakpoints within *TCR* genes, mainly at 14q11 (*TCR- $\alpha$*  and *- $\delta$* ), 7q14 (*TCR- $\gamma$* ) and 7q35 (*TCR- $\beta$* ) [T2]. Telomere shortening and fusions, with normal telomerase activity, have been observed in peripheral blood lymphocytes of these patients [T2]. AT patients show progeroid features, such as strands of grey hair or keratoses, which have been related to accelerated telomere shortening [P2]. Endocrine defects typically result in gonadal abnormalities and retardation in somatic growth. Some patients develop insulin-resistant diabetes, which has been attributed to antibodies directed against insulin receptors [S48]. Many homozygous AT patients develop cancer, mostly lymphoid malignancies of both B-cell and T-cell origin, including Hodgkin's lymphoma, non-Hodgkin's lymphoma and leukaemias [B8].

196. Solid tumours, mainly breast and stomach cancer, occur more commonly as the AT patient matures, and are being seen in greater numbers as these patients are living longer [H23]. Although plausible, the hypothesis of a higher risk of radiogenic cancer in homozygous AT patients is not supported by solid data. In contrast, the hypersensitivity to deterministic effects from ionizing radiation, around three to fourfold compared with the general population, is well established. Indeed, the treatment of cancer in AT patients with conventional doses of ionizing radiation may induce extremely high radiotoxicity, resulting in life-threatening sequelae. The frequency of heterozygous *ATM* mutations among the general population is around 1%. Heterozygous carriers are neurologically normal. Although some authors have not found any evidence of abnormal radiotoxicity in heterozygous AT patients [W12], other epidemiological studies have suggested that heterozygous AT women have a predisposition to breast cancer and may exhibit higher clinical radiosensitivity [A24].

197. *Ataxia-telangiectasia-like disorder* (ATLD) is due to mutations in the *hMre11a* gene, involved in DNA double-strand-break repair. ATLD shares many clinical features with AT. While abnormalities in B-lymphocytes have not been

reported in ATLD, T-lymphocytes show an increased level of translocations involving the TCR. Both lymphocytes and fibroblasts from these patients exhibit higher radiosensitivity *in vitro* [T2].

198. *Nijmegen breakage syndrome* (NBS) is a rare autosomal recessive condition characterized by chromosomal instability, growth retardation, microcephaly, characteristic facial features, radiosensitivity, immunodeficiency, gonadal dysgenesis and cancer predisposition. A high proportion of NBS patients develop lymphoid malignancies, most of B-cell origin. Fibroblasts and lymphocytes from NBS patients are 3–5 times more sensitive to ionizing radiation and radiomimetic drugs than normal cells, and display radioresistant DNA synthesis due to an inability to retard S-phase progression after exposure to ionizing radiation. NBS also involves inversions and translocations in peripheral T-cells affecting the genes for the heavy chain of immunoglobulins and the TCR, thus accounting for the immunodeficiency characteristic of this syndrome [D6]. NBS patients carry a mutation in the *NBS1* gene, which codes for a protein called NBS-1/Nibrin, involved in DNA double-strand-break repair.

199. *Fanconi anaemia* (FA) is a recessive disorder characterized by bone marrow failure, multiple congenital abnormalities and predisposition to cancer, particularly to acute myeloid leukaemia and squamous cell carcinoma [R8, T2]. Although FA is considered a genetically and clinically heterogeneous disorder, the genes mutated in FA are all involved in recruiting and organizing other proteins to cope with DNA damage and participate in the regulation of homologous recombination. FA patients are unusually sensitive to a variety of clastogens, most prominently DNA cross-linking agents such as mitomycin C. Moderate to high sensitivity to ionizing radiation and other oxidative stress inducers has been reported in FA patients [D10]. Djuzenova et al. [D8] observed high initial and residual DNA damage rates and elevated repair half-time in peripheral blood mononuclear cells from FA patients exposed to X-rays.

200. *Human progeria syndromes* are rare disorders characterized by premature ageing. Some of these syndromes are associated with defects in DNA repair pathways and with a higher susceptibility to DNA damage agents, such as ionizing radiation. According to the age at the onset of symptoms, human progeria syndromes may be classified as neonatal, infantum, youth and adult progeria.

201. *Neonatal progeria* (*Wiedemann–Rautenstrauch syndrome*) is a rare autosomal recessive disorder usually lethal by 7 months. It is characterized by a triangular, aged-looking face with prominent veins and sparse scalp hair, and nearly total absence of subcutaneous fat. These features are apparent at birth and therefore differ from congenital generalized lipodystrophy.

202. *Progeria infantum* (*Hutchinson–Gilford progeria syndrome—HGPS*) is a rare genetic disorder characterized by prominent scalp veins, the absence of hair, maxillary

hypoplasia, delayed tooth eruption, facial cyanosis, insulin-dependent diabetes, accelerated ageing and early death, frequently from coronary artery disease. HGPS is caused by mutations in the *lamin A* gene, placing this syndrome within the group of “laminopathies” [B29]. The main functions of A-type lamins include high-order chromatin organization, nuclear structure maintenance and gene expression control. Hypersensitivity to gamma radiation has been described in these patients [W7]. Concerning immune dysfunction, a decreased CD4/CD8 ratio and decreased T-cell response have been associated with HGPS [H19].

203. *Youth progeria* (*Cockayne syndrome—CS*) patients show many developmental defects, including mental retardation, microcephaly, long limbs, birdlike face, pigmented retinopathy, gait defects and sun sensitivity due to a failure of RNA synthesis to recover following UV irradiation [L16], but without an increased incidence of skin cancer. Two complementation groups (A and B) have been identified in CS depending on which gene is mutated (*CS-A* or *CS-B*); these genes encode proteins involved in the transcription-coupled repair pathway of NER [L16]. Cells from CS patients accumulate oxidative-induced DNA lesions after exposure to ionizing radiation [T16]. Decreased T-cell proliferative response and decreased serum levels of thymic hormone have been observed in these patients [B22].

204. *Adult progeria* (*Werner’s syndrome—WS*) patients display age-related disorders, including greying and thinning of the hair, bilateral cataracts, type II diabetes, atherosclerosis, osteoporosis and increased incidence of sarcomas. The incidence of epithelial cancer and mesenchymal sarcoma is 10 times that of the general population. The incidence of WS is extremely high in Japan compared with other countries [Y6]. WS patients carry a mutation in the *WRN* gene, which encodes a protein belonging to the RecQ family of DNA helicases. WS cells exhibit genomic instability, premature senescence, defects in telomere metabolism, and hypersensitivity to DNA cross-linkers and to ionizing radiation [C25, M17]. The WRN protein is recruited in the radiation-induced DNA double-strand breaks and colocalizes with the Mre11 and  $\gamma$ H2AX complex via binding to NBS1. *NBS1* is the gene mutated in the Nijmegen breakage syndrome, thus suggesting a functional link between these two genetic syndromes with partially overlapping phenotypes [C24, C25]. Immune dysfunction has been found in WS patients [G29, G30, N16].

205. *Severe combined immune deficiency* (SCID) is the most severe inherited immunological disorder and is caused by a variety of molecular defects, all affecting at least the T-lymphocyte population [F16]. Human SCID is characterized by the absence of the T- and B-lymphocytes owing to a defect in V(D)J rearrangement of TCR and Ig genes [D26]. Mutations in the *Artemis* gene, which encodes a protein essential for V(D)J recombination and DNA double-strand-break repair, cause a variant of human SCID with increased radiosensitivity (RS-SCID) [L28, M19]. Hypomorphic *Artemis* mutations are associated with the development of B-cell lymphomas [M13].

206. *Immunodeficiency with microcephaly* patients, presenting with various degrees of immunodeficiency and growth delay, display mutations in the *DNA ligase IV* gene, which encodes a protein essential for DNA repair through non-homologous end-joining (NHEJ) and V(D)J recombination [B33, O2]. This clinical phenotype closely resembles Nijmegen breakage syndrome, and cells from the patients show pronounced radiosensitivity [O2]. A new NHEJ factor, Cernunnos, was identified through the survey of similar patients with SCID and microcephaly [B34]. This resemblance is not surprising, given the interactions of Cernunnos and DNA ligase IV in a same protein complex [A26].

207. *Hyper-IgM syndromes* are primary immunodeficiencies characterized by normal or increased serum IgM levels contrasting with low or absent IgG, IgA, and IgE. They are caused by a defect in the class switch recombination [D31]. A subset of these patients display defects in DNA repair genes such as *Uracil-N-glycosylase* [I12].

208. *Seckel syndrome* shares features with the above disorders involving impaired DNA damage repair and elevated radiosensitivity. Seckel syndrome patients have a mutation in *ATR*, the gene encoding ataxia telangiectasia and Rad3-related protein, resulting in failed checkpoint arrest following exposure to genotoxic agents [A9, O3].

209. *Dyskeratosis congenita* (DC) is a rare syndrome caused by defective telomere maintenance characterized by atrophy and reticular pigmentation of the skin, nail dystrophy, leucoplasia of mucosa membranes, hypotricosis, dysphagia, skeletal abnormalities, mental retardation, bone marrow failure and predisposition to malignancies [C29]. The major X-linked form of DC is due to mutations in a nucleolar protein, dyskerin, found in the telomerase complex [D23]. An autosomal dominant form is due to mutations in the RNA component of telomerase (hTR) [S46]. Patients with this form of the disease are more severely affected in later generations that carry the mutations, possibly owing to the inheritance of shortened telomeres, disguising the inherited nature of the disease, which in some cases is classified as aplastic anaemia. Increased sensitivity of DC cells to ionizing radiation and alkylating agents has been described [D24]. It was demonstrated that fibroblasts from DC patients are highly susceptible to X-irradiation-induced chromatid breakage [D18], and hypersensitivity to radiation therapy has been described in these patients [C20]. A severe infantile variant of X-linked DC called Hoyeraal-Hreidarsson syndrome exhibits increased in vitro sensitivity to radiation and alkylating agents in circulating lymphocytes and fibroblasts. Accelerated telomere shortening was also reported in these patients [M24]. Severe B-lymphopenia with decreased IgM levels and moderate T-lymphopenia were observed in autosomal dominant form, probably as a consequence of replicative failure and premature senescence of lymphocytes, supporting a role of telomerase in immune homeostasis [K41].

## 2. Autoimmune diseases and radiation response

210. DNA damage and its defective repair could be important in the pathogenesis of autoimmune diseases. Impaired response to oxidative stress has been postulated as another condition found in autoimmune diseases [H17, K51]. Bhushate et al. demonstrated that the spontaneous number of DNA strand breaks in peripheral blood mononuclear cells from patients with rheumatoid arthritis is significantly increased compared with those from healthy donors [B24]. Neutrophils from systemic lupus erythematosus (SLE) patients display increased spontaneous DNA damage and defective repair of oxidative DNA damage [M20]. Higher sensitivity to oxidative stress has been also described in lymphocytes from patients with SLE, vasculitis and Behcet's disease [B20].

211. McCurdy et al. studied DNA repair of peripheral blood lymphocytes from patients with autoimmune diseases, irradiated with 1.5 Gy. By using single-cell alkaline gel electrophoresis (comet assay) they observed that lymphocytes from patients with systemic lupus erythematosus, juvenile rheumatoid arthritis and systemic sclerosis exhibited delayed repair of radiation-induced DNA damage compared with lymphocytes from healthy donors [M21]. These findings were in accordance with a previous study by Harris et al., who evaluated the lymphocyte responsiveness to mitogens (Con A) following irradiation and found that lymphocytes from patients with rheumatoid arthritis, systemic lupus erythematosus and polymyositis were more radiosensitive than those from healthy volunteers or patients with conditions not associated with autoimmunity [H6].

212. Cossu et al. evaluated the proliferative responsiveness following irradiation with doses of between 0 and 10 Gy in peripheral blood lymphocytes from patients affected by systemic lupus erythematosus in the active and remissive phases. They compared their results with those found in a group of normal subjects. Patients in the clinically active phase of the disease exhibited a decreased responsiveness of the total lymphocyte population as well as of the subpopulations of T- and B-cells. Responsiveness from patients in the remissive phase was similar to that of normal subjects. They concluded that the lymphocytes of patients affected by this autoimmune disease in the active phase are more radiosensitive than those of patients in the remissive phase of these diseases [C15]. Greater toxicity from radiation therapy has been described in patients with collagen diseases, such as systemic lupus erythematosus, polymyositis, dermatomyositis and scleroderma, manifested as significant acute toxicity and severe late effects [M12].

## 3. Ionizing radiation and acquired immune deficiency syndrome

213. A higher incidence of several cancers, e.g. Kaposi's sarcoma, non-Hodgkin's lymphoma, conjunctival squamous carcinoma and Hodgkin's disease, has been observed in patients with acquired immunodeficiency syndrome (AIDS).



Since there is accumulating evidence that AIDS patients exhibit higher radiosensitivity, the effects of ionizing radiation on the immune system may be relevant in medical practice when radiotherapy is administered to AIDS patients with cancer [S53].

214. Enhancement of the human immunodeficiency virus type 1 (HIV-1) by heterologous viral, chemical and physical agents has been shown both in vitro and in vivo. Exposure of AIDS patients to ionizing radiation (for example during radiation therapy) could play a role in activating HIV-1 in vivo. Faure et al. demonstrated in lymphoid cell lines that HIV long-terminal-repeat-directed gene expression is activated by X-irradiation in a dose- and time-dependent manner [F2].

215. Stress agents such as ionizing radiation could directly activate HIV-1 virus replication or reporter gene expression. The kappa B regulatory elements seem to be involved in this up-regulation [F1]. Antioxidant drugs inhibit this effect, which suggests that reactive oxygen species (ROS) are involved. Taher et al. found that ionizing radiation, at both low and high doses, stimulates HIV gene expression to a lesser extent and with different kinetics than does UV radiation [T1]. While UV activation was completely blocked by p38 MAP kinase inhibition, activation by ionizing radiation was reversed by MAP kinase/MEK inhibition. This finding suggests that ionizing radiation modulates HIV gene expression by this latter signalling pathway.

216. Integrase-induced host DNA damage associated with HIV-1 infection elicits DNA damage signalling. Waninger et al. have established that the DNA-PK is involved in HIV-1 replication, suggesting that the NHEJ pathway, involved in repair of radiation-induced DNA double-strand breakage, is required to support efficient retroviral infection [W8]. In addition to DNA-PK, Lau et al. have recently presented evidence that ATM kinase activity has an important role in HIV-1 infection. By using a specific ATM inhibitor (KU-55933), they demonstrated that ATM is required for HIV-1 replication in NHEJ-proficient cells [D14, L24]. A direct correlation exists between apoptosis and HIV gene expression in T-cells in response to ionizing radiation exposure. Caspases seem to be involved in ionizing-radiation-induced activation of HIV, since caspase inhibitors abolish both apoptosis and HIV gene expression [O1]. However, triggering of apoptosis is not sufficient to induce HIV gene expression, since in other cell types (e.g. carcinoma cells), ionizing radiation induces apoptosis but does not enhance HIV expression.

217. Higher morbidity associated with radiation therapy has been reported in HIV-1+ patients [S25], although the mechanisms involved in this response remain controversial. It has been postulated that the pattern of immune dysregulation present in these patients could play a role in their abnormal response to ionizing radiation. Cells infected with HIV-1 exhibit down-regulation of the DNA repair gene *DNA-PKcs* and other cell-cycle-related genes, suggesting that impairment of DNA repair and dysregulation of cell cycle

checkpoints may be involved in the higher radiosensitivity of AIDS patients [S53]. Decreased levels of major endogenous antioxidant systems have been seen in HIV-1 disease, including lower levels of glutathione and decreased levels of superoxide dismutase and catalase [B13]. The state of chronic immune activation secondary to HIV-1 infection and the frequent secondary or opportunistic infections (as well as reactions to other antigenic products) lead to a constant state of oxidative stress. ROS generated by ionizing radiation might trigger additional oxidative stress in these patients.

#### 4. Remarks concerning human pathologies with immune dysfunction and hypersensitivity to ionizing radiation

218. As described in the preceding paragraphs, the identification of human pathologies in which immunodeficiency is associated with higher radiosensitivity is relevant, because the effects of ionizing radiation on the immune system may be more severe in these patients. Table 10 summarized the main human genetic disorders in which immune dysfunction is associated with hypersensitivity to ionizing radiation. The mutated genes and the defective mechanisms that account for these disorders are also presented in this table. The data indicate that ataxia telangiectasia, ataxia telangiectasia-like-disorder, Nijmegen breakage syndrome, severe combined immune deficiency, ligase IV and Cerunnos deficiencies, and Seckel syndrome are the disorders exhibiting a very high radiosensitivity. To a lesser extent, higher radiosensitivity has been proven for xeroderma pigmentosum variant, Fanconi anaemia, progeria infantum and adult, and dyskeratosis congenita. Concerning the other conditions described, their probable hypersensitivity to ionizing radiation has not yet been demonstrated.

219. Defective DNA damage repair and impaired response to oxidative stress seem to be involved in the pathogenesis of autoimmune diseases. Lymphocytes of patients in the active phase are more radiosensitive than those of patients in the remissive phase of these diseases.

220. Concerning AIDS, ionizing radiation activates human immunodeficiency virus (HIV-1) replication. Higher radio-toxicity is observed in AIDS patients, probably owing not only to immune dysregulation but also to impairment of DNA repair combined with a chronic state of oxidative stress.

## H. Summary

221. This section has summarized the influence of dose, dose rate and radiation quality on radiation effects on the immune system. Data concerning the radiosensitivity of lymphocyte subsets and the immune effects of prenatal irradiation were reviewed. Human immune pathologies associated with radiosensitivity were summarized.

222. Activation of the immune function by low-dose irradiation has been mainly demonstrated in splenic and



thymic lymphocytes from some strains of animals. Low-dose enhancement of the immune response is mainly seen in T-lymphocytes, particularly in CD8+ T-cells. This effect, which has not yet been demonstrated in humans, seems to take place within a very narrow dose range. While a dose of 20 mGy (acute exposure) induces immunoenhancement in rodents, changing the dose to 200 mGy induces a shift towards immunosuppression. The same dose (200 mGy) administered using a protracted schedule exhibits immuno-enhancing properties. However, this effect may vary according to the age and strain of the animal. The suppressive effect of low-dose irradiation on tumour growth has been reported in animal models. An adaptive response to radiation in the immune system seems to be related to a reduction of apoptosis and an enhanced capacity to repair DNA damage. Even at very low doses, tritium incorporation appears to have a higher RBE. The long-term impact of low doses on the immune functions related to human health remains to be determined.

223. Concerning high doses, minimal depression of leucocyte counts may be observed following acute WBI in the range 0.5–1 Gy. Acute radiation syndrome occurs after acute whole-body or significant partial-body exposure of >1 Gy. The kinetics and severity of immunosuppression depend on the whole-body dose. In non-uniform exposures, temporal blood cell parameters are more relevant for dose estimation than the magnitude of cytopenia. Reconstitution of innate immunity is more rapid than that of acquired immunity. Humoral recovery precedes T-cell reconstitution, which includes both thymus-dependent and thymus-independent pathways.

224. Radiation-induced changes in immune parameters seem to be more dependent on total dose than on dose rate.

The RBE of high-LET radiation differs, depending on the end point considered. Some cell types exhibited differences in the response to proton versus photon radiation, proton irradiation inducing more significant immunosuppression. Lymphoid cells and tissues are markedly affected by high-LET radiation at relatively low doses in terms of cell depletion and organ size, whereas neutron RBE is close to unity for the induction of lymphocyte apoptosis. Radon exposure induces marked changes in thymus subpopulations and alters molecules involved in the migration and homing of immune cells.

225. Although the radiosensitivity of lymphocyte subsets depends on several factors related to the experimental conditions, the data reviewed indicate that B-lymphocytes are the most radiosensitive subset and that NK cells are more radioresistant.

226. There are critical windows of special vulnerability for prenatal irradiation of the immune system, and the effects differ according to the developmental stage at which the irradiation takes place. Radiation-induced damage to the developing thymus and haematopoietic stromal microenvironment account, at least in part, for the observed effects.

227. The identification of human pathologies in which immunodeficiency is associated with higher radiosensitivity is relevant, because the effects of ionizing radiation on the immune system may be more severe in patients with these disorders. Delayed repair of radiation-induced DNA damage and increased lymphocyte radiosensitivity have been found in patients with autoimmune diseases. AIDS patients exhibit higher radiotoxicity. Ionizing radiation activates HIV-1 replication, and bystander effects involving reactive oxygen species seem to be involved in this activation.

### III. POSSIBLE MECHANISMS INVOLVED AND IMPLICATIONS

228. In order to better understand the findings described in section IV (Epidemiological studies), this section summarizes some mechanisms that are probably involved in radiation-induced alterations of the immune system. Mechanisms of cancer development and the possible roles of the immune system are also discussed.

229. The pathways activated by ionizing radiation that have been investigated in the greatest depth are those dealing with the responses to DNA damage. These pathways involve sensor molecules linked to primary downstream components that integrate and process the signals. As a result of this response to DNA damage, the cell cycle arrests, DNA repair takes place and the cell may survive or die. However, not all the effects of ionizing radiation on the immune system can be ascribed to cytotoxicity. An additional outcome of radiation exposure, which is not often considered, concerns the production of “danger signals” that may also influence the classical cellular responses normally related to DNA damage [M2]. The following possible mechanisms involved in radiation-induced alterations of the immune system will be summarized:

- Lymphocyte apoptosis;
- TCR mutations;
- Modification of Th1 and Th2 balance;
- Bystander effects and genomic instability;
- Shift towards an inflammatory profile;
- Acceleration of immunological ageing;
- Modification of antigen presentation;
- Autoimmune reactions;
- Perturbation of the immunological homeostasis;
- Other possible mechanisms involved.

#### A. Lymphocyte apoptosis

##### 1. Review of published data

230. Apoptosis plays a crucial role within the immune system, in particular in negative regulation, and is a key mechanism in the response to ionizing radiation. Lymphocytes die by apoptosis immediately after exposure (interphase death) [J5] or by reproductive cell death [M22]. The apoptotic process can be divided into three phases: a death-stimulus-dependent induction phase; an effector phase during which the “decision to die” is taken; and a degradation phase,

during which the morphological and biochemical features of apoptosis become apparent [K20].

231. There are at least two pathways for radiation-induced apoptosis: one is mediated by mitochondrial factors and is p53-dependent, and the other is mediated by cell surface receptors (Fas/CD95) [S16]. The latter pathway may be acidic-sphingomyelinase-dependent and ceramide-mediated [K46]. ROS generated by ionizing radiation result in oxidative damage to the cell membrane, and lymphocytes are known to be sensitive to oxidative stress because of their high mitotic potential and the content of polyunsaturated fatty acids in their cell membranes [S51]. In recent years, evidence has accumulated suggesting that the damage to the cell membrane contributes to radiation cell killing [R14]. It has been demonstrated that activation of membrane-bound sphingomyelinase after irradiation produces ceramide, which strongly induces expression of Fas ligand (FasL/CD95L), cleavage of caspases and apoptosis [D22]. These data demonstrate that ceramide links cellular stress responses induced by gamma irradiation or anticancer drugs to the Fas pathway of apoptosis.

232. Radiation-induced apoptosis in lymphocytes may be initiated by the Fas/FasL system. Radiation induces the synthesis of the death ligand FasL. Ligand-mediated cross-linking of the Fas receptor initiates signalling pathways to apoptosis. Anti-Fas or anti-FasL antibodies reduced the level of radiation-mediated cell killing [B3]. Furthermore, ionizing radiation up-regulates the surface expression of Fas, thus increasing the sensitivity of those cells to FasL. The Fas receptor stimulates a variety of molecules, including several members of the caspase family and the acidic sphingomyelinase. Brenner et al. [B10] described a signalling cascade from the Fas receptor via caspases to acidic sphingomyelinase, release of ceramide and activation of Jun N-terminal kinase (JNK) and p38-K kinases (phospho-p38 mitogen-activated protein kinase). Fas-mediated apoptosis could be prevented in CD4+ or CD8+ T-cells by several protease antagonists, suggesting the involvement of the interleukin-1 $\beta$ -converting-enzyme-related cysteine protease in CD4+ T-cell death, and of both a CPP32-related cysteine protease and a calpain protease in CD8+ T-cell death triggered by Fas [E5].

233. In contrast, Ogawa et al. [O4] demonstrated that peripheral blood mononuclear cells irradiated with 5 or 10 Gy of gamma radiation showed positivity to apoptosis markers but displayed no increase in surface Fas expression or caspase-3 activity relative to non-irradiated

cells, suggesting a Fas-independent mechanism. A Fas-independent mechanism was also reported by Kuida et al. [K35] in thymocytes from ICE mice that were sensitive to apoptosis induced by dexamethasone or ionizing radiation but resistant to apoptosis induced by Fas antibody. In addition, TCR stimulation of CD8+ T-cells led to a different Fas-independent death process [E5].

234. A p53-dependent, mitochondria-mediated apoptotic pathway was proposed. The p53 molecule is a key modulator of radiation-induced apoptosis in several cell types [L22]; however, it has also been shown that ionizing radiation can induce apoptosis in human lymphocytes independently of p53 status. Radiation-induced up-regulation of FasL protein defines a p53-independent pathway to apoptosis, since apoptosis induced by FasL does not require p53. In fact, radiation-induced p53-dependent and p53-independent apoptotic pathways are both present in different cell types of the immune system. Seki et al. [S10] demonstrated that p53 protein was inducible in TCR  $\alpha\beta$  T-cells (CD4 and CD8 cells) and B-cells, but not in TCR  $\gamma\delta$  T- and NK cells after gamma irradiation. Cycloheximide was able to inhibit radiation-induced cell death in TCR  $\alpha\beta$  T-cells and B-cells, indicating a requirement for protein synthesis, including p53 protein.

235. Mitochondria serve to integrate and amplify upstream cell death signals, clarifying the cellular reaction to a go/no-go response. These signals include proteins, reactive species and divalent cations. The protein signals include the proapoptotic members of the Bcl-2 family, such as Bax, Bad and Bak. Anti-apoptotic members of the Bcl-2 family include bcl-2 and bcl-x<sub>L</sub>. Reactive compounds include reactive oxygen species (ROS) and reactive nitrogen species (RNS). If the stress is sufficient, mitochondria respond by releasing a series of protein factors. These factors include cytochrome c and Smac/DIABLO, which act to initiate and facilitate the downstream stages of the caspase-dependent cascade. Other factors released include apoptosis-inducing factors and endonuclease G, which then initiate the downstream stages of the caspase-independent cascade [K20, S29].

236. Cui et al. [C13, C18] found an increased expression of Bax protein in thymic lymphocytes of mice 3 hours after exposure to lethal doses of gamma radiation. On the other hand, the expression of bcl-2 and bcl-x<sub>L</sub> proteins was reduced at 3 hours after irradiation, reaching their lowest level at 24 hours. However, a Fas-mediated pathway unaffected by bcl-2 has been described [S31].

237. The role of ROS in radiation-induced apoptosis of human peripheral T-cells was studied by Ogawa et al. [O5]. They found that ROS formation occurred immediately after irradiation, continued for several hours and resulted in oxidative DNA damage. Early (13 hours post-irradiation) and late (23 hours post-irradiation) apoptotic changes were correlated. Therefore the origin of the hyper radiosensitivity of T-lymphocytes seems to be the high production of ROS in the mitochondrial DNA following irradiation. Ogawa et al.

recently described the possible existence of a new apoptotic cascade involving early lysosomal membrane destabilization. Therefore possible involvement of lysosomal protease leakage caused by hydroxyl radical formation in lysosomes (possibly resulting in mitochondrial membrane dysfunction) is considered to play an important role in radiation-induced T-cell apoptosis [O6]. Moreover, in a recent study, Sharma et al. [S51] investigated the immunomodulatory effect of chlorophyllin (CHL), a water-soluble mixture of salts of chlorophyll that had earlier been shown to reduce the level of intracellular ROS. CHL significantly inhibited apoptosis in Con-A-stimulated spleen cells from BALB/c mice, whereas the expression of anti-apoptotic genes *bcl-2* and *bcl-xL* was up-regulated in lymphocytes of CHL-treated mice compared to controls.

238. Down-regulation of mitochondrial transmembrane potential by inhibitors of electron transport and adenosine triphosphate (ATP) synthesis prevented stress-induced p53 protein accumulation and eliminated p53-dependent apoptosis in a wild-type p53 leukaemia cell line (MOLT-3) and in normal T-lymphocytes, identifying mitochondrial activity and ROS levels as critical intracellular determinants of the p53 activity [K7]. On the other hand, Chen et al. [C8] demonstrated that nitric oxide, a key RNS, protects thymocytes from gamma-irradiation-induced apoptosis. The mechanism may involve inhibition of p53 up-regulation and reduction of mitochondrial damage, with subsequent inhibition of downstream caspase activation.

239. The apoptotic response of lymphocytes may differ following low-dose irradiation. Data recently communicated by Shankar and Sainis in whole-body gamma-irradiated mice (cumulative dose 200 mGy at 40 mGy/day) demonstrated that such very low doses enhanced the mitogen responsiveness to Con A of spleen lymphocytes, reduced expression of p53 and down-regulated apoptosis. This anti-apoptotic effect was associated with a higher expression of cyclin D1 and proliferating cell nuclear antigen (PCNA), two critical proteins for cell cycle transition and cell proliferation. Interestingly, these authors also demonstrated that down-regulation of apoptosis was not mediated by the Fas/FasL pathway but rather through the mitochondrial pathway [S16]. These findings suggest that those apparently opposite effects of ionizing radiation on some immune responses observed following moderate to high doses compared with low doses, e.g. immunosuppression versus immunostimulation, may be related to signal transduction pathways involving cell cycle regulatory proteins such as p53, cyclins and PCNA.

## 2. Remarks concerning lymphocyte apoptosis

240. The preceding paragraphs indicate that apoptosis plays a crucial role within the immune system, in particular in negative regulation, and is also a key mechanism in the response to ionizing radiation. It is accepted that there are at least two pathways for radiation-induced apoptosis: DNA-damage-mediated and p53-dependent, and ceramide-mediated.

241. Radiation-induced apoptosis in lymphocytes may be initiated by the Fas/FasL system. Radiation induces the synthesis of the death ligand FasL and up-regulates the surface expression of the Fas receptor, which stimulates the release of ceramides and members of the caspase family. Radiation-induced up-regulation of FasL protein is a p53-independent pathway to apoptosis.

242. A p53-dependent, mitochondria-mediated apoptotic pathway has been proposed. Radiation-induced p53 apoptosis and p53-independent pathways are both present in different cell types of the immune system. Mitochondria integrate and amplify upstream cell death signals. These signals include proteins, reactive species and divalent cations. The proteins include proapoptotic and anti-apoptotic members of the Bcl-2 family, and mitochondria respond by releasing protein factors that initiate the caspase-dependent cascade. Reactive compounds include ROS and RNS, and divalent cations are mainly  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ .

243. The apoptotic response may differ following low-dose irradiation. Protracted exposure to radiations at low dose rate reduces the expression of p53 and down-regulates apoptosis. This effect was associated with a higher expression of cyclin D1 and PCNA, two critical proteins for cell cycle transition and cell proliferation. These findings suggest that the apparently opposite effects observed in the immune system at high and low doses might be related to signalling pathways involving cell cycle regulatory proteins such as p53, cyclins and PCNA.

## B. TCR mutations

### 1. Review of published data

244. It has been well established that ionizing radiation induces somatic mutations in a dose-dependent manner both *in vivo* and *in vitro*. Radiation-induced mutations in TCR genes could result in the phenotypic expression of TCR-defective T-cells. Since the TCR/CD3 complex is involved in the first step of a variety of other T-cell-dependent immune functions, loss or alteration of TCR expression in surviving cells may contribute to radiation-induced impairment of the T-cell response [K34].

245. A considerable volume of literature is available describing the defective expression of TCR gene  $\alpha$  or  $\beta$ . A marginally significant ( $p = 0.051$ ) dose-related increase in the TCR mutation frequency has been detected among survivors of the atomic bombings in Japan. The presence of TCR mutation is dependent on the *in vivo* selection that occurred soon after exposure, and TCR mutants were almost completely eliminated *in vivo* during the first decades following the exposure to radiation from the atomic bombings. The half-life of TCR mutants is quite short, between two and three years. Similar results were found in patients treated with Thorotrast and with  $^{131}\text{I}$  [A7, K34, U19]. An increased frequency of TCR mutations was found among persons

exposed to effluents of the Techa River in the Russian Federation [A17].

246. On the other hand, a positive correlation has been found between TCR mutation frequencies and dicentric chromosome frequencies in lymphocytes from patients who had previously received a full course of radiation therapy for gynaecological disorders. By comparing mutation frequencies of hypoxanthine-guanine phosphoribosyltransferase (HPRT) and TCR using flow cytometry, it was possible to demonstrate that the frequency of TCR mutants correlated well with that of dicentric chromosomes [I4].

247. Although the TCR *in vivo* somatic mutation assay has been proposed as a sensitive indicator of ionizing radiation exposure, this assay cannot be applied immediately, since the mutant phenotype may require as long as several months to express. Ishioka et al. [I3], using IL-2 after PHA pulse stimulation, demonstrated a dose-dependent increase of mutation frequency in CD4+ cells during the first seven days post-irradiation.

248. In 2002, Smirnova et al. described the mutation frequency of TCR genes in lymphocytes from 165 individuals exposed to ionizing radiation [S24]. The cohort was divided into three groups depending on the type of irradiation and time elapsed since exposure: group 1, analysis performed 16–40 years after acute irradiation; group 2, 13 years after acute irradiation; and group 3, 9–13 years after prolonged irradiation. Elevated frequencies of TCR mutant cells were detected in all three groups: 36%, 25% and 15% in the first, second and third group, respectively. The same authors have recently found elevated frequencies of TCR mutant cells in nuclear chemical plant workers chronically exposed to low doses of external and internal irradiation [S44]. In both studies, the frequency of mutant cells was significantly higher than in control groups.

249. Although several papers have provided strong evidence for mutation of the TCR, the question concerning the lifespan of human memory cells in the absence of TCR signalling remained open. The long-term kinetics of TCR/CD3 mutation between CD4+ CD45RA+ naive and CD4+ CD45RA– memory T-cell fractions was studied in cancer patients receiving radiotherapy [U17]. Both the proportion and the number of mutant cells decayed exponentially with time following radiotherapy. The results indicated that the lifespan of mature CD4+ T-cells is limited regardless of their memory or naive phenotype, suggesting that continued cell receptor signalling is required for lifetime maintenance of human memory cells.

250. Moreover, *in vivo* models to establish the spontaneous and radiation-induced TCR mutation frequency showed that the TCR mutation frequency dose response fitted a linear–quadratic or quadratic function. The general trend was that radiation-induced TCR mutation frequency started to increase 3 days after WBI with X-rays, reached a peak at 2–3 weeks and then gradually decreased, with a half-life



of about 2 weeks. The coefficients of the quadratic term in BALB/c mice were significantly higher than for C57BL/6 or C3H/He mice, suggesting that genetic factors may control the susceptibility of somatic genes to both spontaneous and radiation-induced mutagenesis [U18].

251. Studies involving TCR mutation frequencies in p53-deficient mice have clarified the important role of the p53 protein in the repair of radiation-induced mutagenic damage to the immune system. In p53 (-/-) mice, the TCR mutation frequency did not decline significantly with time. It was concluded that complete repair of mutagenic damage in irradiated tissues requires the integration of DNA repair and p53-dependent apoptotic mechanisms [K8, S32].

252. In order to explore the ability of ionizing radiation to induce rearrangements in TCR leading to TCR  $\beta\gamma$  variants (hybrids) in human lymphocytes, peripheral blood lymphocytes from healthy donors were exposed in vitro to 3 Gy of either X- or gamma rays. The TCR  $\beta\gamma$  frequency was not significantly different in irradiated versus control samples up to 55 days after PHA stimulation, suggesting that low-LET radiation is not able to induce this type of TCR rearrangement in vitro [M4].

## 2. Remarks concerning TCR mutations

253. Some remarks may be made on the basis of the information presented in the preceding paragraphs. Radiation-induced mutations in TCR genes could result in the phenotypic expression of TCR-defective T-cells and thus contribute to radiation-induced impairment of the T-cell response. A significantly increased frequency of TCR mutation has been detected among survivors of the atomic bombings, in patients treated with Thorotrast and <sup>131</sup>I, and among the Techa River cohort. It must be emphasized that the presence of mutation is dependent on the in vivo selection, and that the half-life of TCR mutants is between two and three years. Elevated frequencies of TCR mutant cells were also observed in chronically exposed workers at nuclear plants.

254. In vivo models to establish the spontaneous and radiation-induced TCR mutation frequency showed that the dose-response function fitted a linear-quadratic or quadratic function. The coefficients were dependent on the strain of mouse studied, suggesting that genetic factors may control the susceptibility of somatic genes to both spontaneous and radiation-induced mutagenesis. Likewise, in a p53 (-/-) mouse model it was observed that the TCR mutation frequency did not decline significantly with time, leading to the conclusion that repair of mutagenic damage in irradiated tissues requires the integration of DNA repair and p53-dependent apoptotic mechanisms.

255. To date, there are no definitive data demonstrating that low levels of TCR mutation frequency have induced immunodeficiency.

## C. Modification of Th1/Th2 balance

### 1. Review of published data

256. As described earlier, two distinct functional cytokine secretion patterns, designated Th1 and Th2, have been defined for helper T-cells. While Th1 cytokines promote cell-mediated immunity, Th2 cytokines favour humoral immunity, providing B-cell assistance for antibody production. Controversial results have been published concerning the effects of ionizing radiation on the Th1/Th2 balance and its impact on human health. Th1 and Th2 helper T-cells are cross-regulatory in vitro, and the balance of these cells in vivo determines the character of the cell-mediated immune and inflammatory response [N10]. An imbalance between Th1 and Th2 may be responsible both for the progression of several diseases and their resultant complications. Patients with advanced cancer often have impaired cell-mediated immunity associated with a switch from Th1 to Th2. While organ-specific autoimmune diseases have been related to an overreactive Th1 pathway, the Th2 pathway may underlie allergy and systemic autoimmune diseases [G13, K11]. On the other hand, shifting from one cytokine pattern to another may be highly beneficial in certain physiological conditions; for instance, IL-10 (a Th2-type cytokine) may play a role in pregnancy-associated immune tolerance through the establishment of a Th2 cytokine bias at the maternal-foetal interface [S20].

257. The impairment of cell-mediated immunity associated with the increase in the B-cell component and humoral immunity observed in atomic bombing survivors led to the hypothesis that radiation exposure could induce an imbalance towards a Th2 profile. The observed increase in percentage of CD4<sup>+</sup> CD8<sup>-</sup>  $\alpha\beta$ <sup>+</sup> double-negative T-cells, known to produce primarily Th2-type cytokines, supported the idea that ionizing radiation could induce a shift from a Th1 to a Th2 response [K23]. Reduced IL-2 production in response to Con A in survivors was reported to be caused by a reduced number of naive CD4<sup>+</sup> T-cells [K31]. However, a dose-dependent increase in TNF- $\alpha$  and IFN- $\gamma$  production was observed in atomic bombing survivors, suggesting that Th2 does not dominate over Th1 [N3].

258. Interaction between cytokines and their receptors leads to the activation of multiple signalling molecules, including the family of "signal transducer and activator of transcription" (STAT) proteins. Different STAT proteins are capable of regulating the activity of diverse types of cytokine. It has been reported that mice with a disrupted *STAT* gene have impaired IL-12 responsiveness of NK and T-cells, a lack of Th1 responsiveness and enhanced Th2 function [K5]. Gamma radiation has been shown to reduce STAT1 phosphorylation. In contrast, mRNA levels for IL-5 were only slightly increased by gamma radiation compared with non-irradiated samples, suggesting that ionizing radiation induces a polarized Th2 response by interfering with STAT signals, thereby causing suppression of the Th1 response [H4].



259. In addition to the STAT protein family, the transcription factor NF- $\kappa$ B is one of the key regulators of the genes implicated in the immune inflammatory response. In a rat abdominal gamma irradiation model, Linard et al. [L26] have shown that genesis of the inflammatory process involved the translocation/activation of the NF- $\kappa$ B/Rel p65 subunit in the intestine. This activation was inhibited by a specific NF- $\kappa$ B inhibitor (caffeic acid phenethyl ester) that contributed to a reduction in the expression of TNF- $\alpha$ , IL-6 and IL-6 receptors. The cytokine signalling is under negative feedback regulation by intracellular proteins such as the suppressor of cytokine signalling (SOCS) gene. The differentiation into Th1 and Th2 is accompanied by a preferential expression of distinct SOCS (SOCS1 is highly expressed by Th1, whereas Th2 expresses high levels of SOCS3). Reports indicated that Th1 responses are likely to be negatively regulated by SOCS3 in vivo, rather than Th2 responses being attenuated by SOCS1 [17]. Irradiation induced an intestinal overexpression of SOCS3 and a repression of SOCS1 in the first week. The inhibition of NF- $\kappa$ B activation reduced the SOCS3 expression [L27]. These data suggested that the polarized Th2 response induced by ionizing radiation involved the transcription factor NF- $\kappa$ B.

260. Bass et al. [B2] studied the ratio of Th1 and Th2 clones in the spleens of mice 4–6 weeks following total lymphoid irradiation by high-dose X-rays (5.5 and 8.5 Gy). The Th1/Th2 ratio was 1/0.6 in control mice, whereas the ratio in total lymphoid irradiated mice was approximately 1:7, supporting a conclusion that radiation exposure enhances Th2-type cytokine production [B2]. In contrast, it has been shown that low-dose WBI with 0.075 Gy X-rays is able to generate changes in IL-12 p35/p40 mRNA and IL-12 p70 protein levels, while IL-10 decreased significantly in splenocytes, and mRNA levels for both IL-12 p35 and p40 subunits increased in macrophages following WBI. The suppression of IL-10 expression and stimulation of IL-12 expression were interpreted as representing a shift of the immune response in favour of Th1 differentiation [L19].

261. The prevalence of a radiation-induced Th2 response has been verified in irradiated lung. Enhanced lymphocyte reactivity, dominated by Th2 cells, was shown in radiation-induced pneumonitis and subsequent pulmonary fibrosis. The kinetics was studied in T-cell lymphocytes isolated from lungs irradiated with 20 Gy. A selective increase of CD4+ T-cells was observed, peaking four weeks after irradiation of the lungs. When the rats were depleted in CD4+ T-cells, post-irradiation thickening of the parenchyma was significantly reduced, as determined by morphometric analysis. The CD4+ cell subtype of the T-lymphocyte population was analysed by measuring different cytokine mRNAs by RT-PCR (reverse transcription polymerase chain reaction). It was found that IL-4 mRNA was selectively increased in the CD4+ cells isolated from irradiated lungs, which indicates a lymphocyte reactivity by Th2 cells. The authors suggested a critical role for Th2 CD4+ cells in the pathogenesis of radiation-induced pneumonitis preceding lung fibrosis [W3].

262. Pharmacological improvement of the impaired Th1 function after irradiation has been used as an indirect way to investigate the radiation-induced enhancement of Th2 response. Ginsan (an acidic polysaccharide from *Panax ginseng*) is able to induce proliferation of lymphokine-activated killer cells, to increase the mitogen activity in different systems and to induce the production of several cytokines (such as IL-1, IL-6, IFN- $\gamma$  and IL-12) that are required for haematopoietic recovery. In an experimental model of WBI, ginsan was injected in vivo and its action was evaluated by measuring its effect on CFU-S bone marrow cells and cytokine generation. Ginsan was shown to enhance Th1 function while interfering with the radiation-induced Th2 response [S27].

263. Interestingly, the cytokines characteristically expressed in Th1 cells seem to be regulated by cell-mediated suppression. The induction of TNF- $\beta$  mRNA in lymphoid cells is greatly enhanced by gamma radiation. However, the level of TNF- $\beta$  mRNA expressed in response to radiation and other stimuli, whether by mitogen or antigen, is strongly reduced by concomitant activation of suppressive cell subsets. Removal of CD8+ or CD11b+ cells leads to a substantial induction of TNF- $\beta$  mRNA in the depleted cell population; this induction precedes the appearance of suppressive cell activity, allowing for temporary suppression. TNF- $\beta$ , as well as other Th1 cytokines such as IFN- $\gamma$  and IL-2, is suppressed by CD8+ or CD11b+ cells [A1].

264. IL-4 and IL-5 synthesis in lymph node cells primed by keyhole limpet haemocyanin (KLH) was greatly diminished after irradiation. In addition, the capacity of irradiated KLH-primed lymph node cells to induce IgG, IgM and IgE synthesis in hapten-primed cells was studied. Irradiation was not able to modify IgG synthesis in these cells, but their capacity to induce IgE was significantly reduced. Thus irradiation greatly inhibited the capacity of Th2 clones, but only minimally inhibited the capacity of Th1 clones, to induce IgG synthesis in primed B-cells. By adding IL-4 and IL-5, the capacity of Th2 cells to produce IgE was completely restored [D2].

## 2. Remarks concerning modification of Th1/Th2 balance

265. As discussed in the preceding paragraphs, two distinct functional cytokine secretion patterns have been defined for helper T-cells: Th1 and Th2. While Th1 cytokines promote cell-mediated immunity, Th2 cytokines favour humoral immunity. The balance of Th1 and Th2 helper cells in vivo determines the character of cell-mediated immunity and inflammatory response, the imbalance being responsible for the progression of several diseases and their resultant complications.

266. The results concerning the effects of ionizing radiation on Th1/Th2 balance are controversial. In survivors of the atomic bombings, the impairment of cell-mediated immunity associated with the increase in the B-cell component and humoral immunity suggests an imbalance towards a

Th2 profile induced by the radiation exposure. The observed increase in the percentage of CD4<sup>+</sup> CD8<sup>-</sup> αβ<sup>+</sup> T-cells, known to produce mainly Th2-type cytokines, supports the hypothesis of a shift from Th1 to Th2. Nevertheless, a dose-dependent increase in TNF-α and IFN-γ secretion suggests that Th2 does not dominate over Th1.

267. The prevalence of a radiation-induced Th2 response has been verified in experimental studies, for example of the spleens of mice irradiated with high-dose X-rays and the lungs of rats irradiated with 20 Gy, where it was found that Th2CD4<sup>+</sup> cells might play a critical role in the pathogenesis of radiation-induced pneumonitis. In contrast, after low-dose WBI, 0.075 Gy, the changes observed might contribute to a shift in favour of Th1 differentiation.

268. STAT proteins are key molecules in the regulation of the activity of different types of cytokine, and mice with disrupted STAT genes have a lack of Th1 responsiveness and enhanced Th2 function. It has been shown that radiation reduces STAT phosphorylation, inducing suppression of Th1 response. The transcription factor NFκB is another key regulator of the genes implicated in the immune inflammatory response, and the action of specific NFκB inhibitors after irradiation in a rat model contributed to a reduction of Th2 cytokine expression. In addition, the cytokines expressed in Th1 cells seem to be regulated by cell-mediated suppression.

## D. Bystander effects and genomic instability

### 1. Review of published data

269. Bystander effects and genomic instability are two general mechanisms possibly involved in the effects of ionizing radiation on the immune system. The general features of these two mechanisms are reviewed in annex C, "Non targeted and delayed effects of exposure to ionizing radiation". Thus this section reviews only observations of these non-targeted and delayed effects that relate to the immune system.

270. Irradiation has been shown to induce leukaemic transformation of non-irradiated stem cells transplanted into syngeneic mice [D13]. These findings may reflect the altered characteristics of the stem cell microenvironment after irradiation, since irradiated haematopoietic stromal cells release mutagenic ROS, produce different sets of adhesion molecules and growth factors, and alter the overall growth and phenotypic characteristics of co-cultured non-irradiated stem cells [G7].

271. Haematopoietic tissues exposed to ionizing radiation have been shown to exhibit increased macrophage activation [L8]. Activated macrophages are able to induce apoptosis in neighbouring cells [B12, D12] and produce gene mutations [W2], DNA base modifications [D7], DNA strand breaks [S14] and cytogenetic damage [W1] in neighbouring cells.

These various end points have all been demonstrated as non-targeted effects of ionizing radiation. Many properties of activated macrophages are consistent with *in vitro* studies that implicate free radical generation in non-targeted radiation effects [N5], and with other studies in which oxidative processes and nitric oxide have been implicated as having roles in the mechanisms [C12, G4, L11]. Nitric oxide can be either proapoptotic or anti-apoptotic, can either down-regulate or up-regulate p53 activity [B11], and can be either pro-inflammatory or weakly anti-inflammatory [G13, N6], depending on context.

272. A stimulatory bystander effect can be induced in immune cells by low-dose irradiation. When a mouse macrophage cell line (J774A.1) was exposed to a low dose (0.075 Gy) and co-cultured with a non-irradiated mouse T-lymphocyte cell line (EL-4), the irradiated macrophages exerted a stimulatory effect on the EL-4 cells, as shown by increased proliferation. At a high dose (2 Gy), irradiated J774A-1 cells exerted an inhibitory effect on the proliferation of the non-irradiated EL-4 cells. Preliminary mechanistic studies show that changes in CD48 expression and nitric oxide production by the J774A.1 cells after high- and low-dose irradiation might be important factors underlying the differential bystander effects elicited by different doses of radiation [L13].

273. There are few data on bystander effects in whole animals. However, prior to the recent interest in non-targeted effects, there were numerous reports that a transferable clastogenic activity capable of causing chromosome breaks in non-irradiated lymphocytes was present in the plasma of patients after radiotherapy, though with considerable inter-individual variation in both production and response [M15]. Clastogenic factors in plasma have also been obtained from atomic bombing survivors and Chernobyl liquidators [P3, W6], and from patients with a variety of chromosomal instability syndromes and inflammatory disorders. The chromosome-damaging effects of clastogenic factors are mediated by the superoxide anion. Their clastogenic activity may be related to the formation of lipid peroxidation products and cytotoxic cytokines, which are possible agents for mediating radiation-induced bystander effects. A body of clinical and experimental radiotherapy data exists concerning the "abscopal effects" of radiation, where responses are noted in unrelated organs or tissues that had not been irradiated [C2]. However, it is far from clear whether bystander killing contributes to the curative potential of radiotherapy and whether inducible instability is an important component of late adverse effects.

274. Xu et al. demonstrated that low doses of radiation (0.25–10 mGy) stimulate expression of IL-2 receptors (CD25) on the surface of peripheral blood lymphocytes taken from normal human donors [X1]. Clastogenic factors can also stimulate CD25 surface expression in non-irradiated cells, suggesting that this radiation-stimulated surface expression is a bystander effect resulting from the secretion into the medium of a soluble factor from the irradiated cells.

Stimulation of CD25 expression by ionizing radiation shows a triggered-type response rather than being proportional to dose.

275. Persistent subclinical inflammation has recently been reported among survivors of the atomic bombings in Japan [N7], and it was suggested that radiation-induced enhancement of inflammatory reactions might contribute, as an epigenetic and/or bystander effect, to the development of several radiation-induced disorders.

276. Thymic stromal cell cultures are able to support T-cell precursor proliferation and differentiation in the presence of IL-7 and stem cell factor. By exposing thymic stromal cell cultures to 10 Gy of gamma irradiation before the seeding of T-cell precursors, it was possible to demonstrate a reduction of these T-cell precursors without changes in their differentiation. The effect could be reproduced by the addition of supernatants from irradiated stromal cell cultures on to sham-irradiated cultures, which suggests that gamma irradiation induces the production of soluble factors by thymic stromal cells, which in turn modify their ability to support proliferation of T-cell precursors [B4].

277. Radiation-induced overexpression of IL-7 from thymic stromal cells is key to understanding the radiation-induced differentiation of CD8+ TCR  $\gamma\delta$  T-cells. When double-negative foetal thymocytes were co-cultured with foetal thymus irradiated with 25 Gy of low-LET radiation in the absence of direct contact or mitogen stimulation, induction of TCR  $\gamma\delta$  T-cells was observed, reaching 50% after 4 days of co-culture. Supernatants of the irradiated foetal thymus were also able to induce the differentiation from double-negative thymocytes to CD8+ TCR  $\gamma\delta$  T-cells after 3 days of culture, suggesting a radiation-induced production of soluble factors by thymic cells. It was possible using RT-PCR to detect an increased expression of IL-7 mRNA in the foetal thymus 24 hours after irradiation, and antibodies against IL-7 inhibited the radiation-induced differentiation [T7].

278. Shankar et al. studied bystander effects and adaptive response induced by gamma radiation in murine lymphocytes, using irradiated conditioned medium (ICM) from lymphocytes exposed to 0.1 Gy, 0.5 Gy and 1 Gy. They found that ICM enhanced the proliferation response of non-irradiated lymphocytes to Con A, with increased expression of IL-2 receptor and cyclin D, two proteins that drive progression through the cell cycle. ICM also enhanced intracellular ROS content and nitric oxide generation in non-irradiated lymphocytes. Apoptosis was significantly lower in lymphocytes exposed to a challenge dose of 1 Gy when they were preincubated with ICM. The results of these authors suggest that soluble factors released by irradiated lymphocytes trigger signalling pathways that result in increased response to mitogens and resistance to radiation exposure in non-irradiated lymphocytes [S50].

## 2. Remarks concerning bystander effects and genomic instability

279. Some remarks may be made on the basis of the data presented above. Delayed effects and genomic instability in the immune system have been demonstrated in vitro and in vivo after exposure to ionizing radiation. Chromosomal instability in haematopoietic cells can be induced by a bystander-type mechanism, providing a link between these two untargeted effects, as well as other radiation responses that are consistent with the microenvironment contributing to cell damage as a consequence of an inflammatory response to radiation-induced injury.

280. Activated macrophages after irradiation are able to induce apoptosis, gene mutations, DNA base modifications, DNA strand breaks and chromosome-damaging effects in neighbouring cells. Intercellular signalling and free radical generation are implicated in these non-targeted effects of ionizing radiation. Nitric oxide emerges as a key mediator in the bystander effects elicited by high- and low-dose irradiation. Likewise, the superoxide anion has been described as a mediator of clastogenic factors in chromosome-damaging effects.

281. Long-lasting inflammation has been reported among the atomic bombing survivors, and it was suggested that radiation-induced enhancement of inflammatory reactions might contribute as an epigenetic and/or bystander effect to the development of several disorders. However, the potential impact of such delayed effects in humans is not known.

## E. Shift towards an inflammatory profile

### 1. Review of published data

282. Ionizing radiation may induce a persistent inflammatory status that could increase the risks of both cancer and non-cancer diseases [N3]. Higher risks of hepatic, cardiovascular and thyroid pathologies have been observed among atomic bombing survivors, and several authors have investigated the relationship between these chronic diseases and impairment of the immune system.

283. One of the main functions of cytokines is to mediate interactions between the immune and the inflammatory response. Chronic immune inflammatory diseases might be caused in part by dysregulation of cytokine production [B14]. TNF- $\alpha$ , IFN- $\gamma$ , IL-6 and IL-10 coordinate the inflammatory response [H10]. The stimulus for production of acute phase proteins in response to tissue injury is likely to be mediated by these inflammatory cytokines. Thus the cytokine profile as well as the level of acute phase proteins may be useful markers of inflammation.

284. Exposure to high doses of ionizing radiation, such as those delivered during radiotherapy or in cases of accidental irradiation, can induce fibrosis in many tissues as a late effect.

Formation of the fibrotic tissue requires chronic activation of several cell types, including myofibroblasts, that secrete the collagenous matrix. The origin of the chronic activation of these cells is still a matter of debate. TGF- $\beta$ 1 has been proposed as a master switch for the fibrotic programme [M18]. This cytokine can be secreted by the inflammatory cells that chronically invade the irradiated tissue and locally by the myofibroblasts. Antioxidant treatment that reduces established fibrotic tissues in patients can act on both of these cell populations [D19, D20].

285. Neriishi et al. [N7] investigated the status of several inflammation parameters in atomic bombing survivors. They demonstrated a positive association between radiation dose and erythrocyte sedimentation rate, total leucocyte counts, alpha-1 and alpha-2 globulin, and sialic acid.

286. Hayashi et al. [H8] found increased plasma levels of C-reactive protein (CRP) and IL-6 in atomic bombing survivors. This increase was significantly related to radiation dose, by about 30% Gy<sup>-1</sup> for CRP and 10% Gy<sup>-1</sup> for IL-6, and was associated with a decrease in the percentage of peripheral CD4+ T-cells.

287. Inflammatory parameters were analysed in blood samples from atomic bombing survivors, including 180 non-exposed subjects and 90 subjects from each of the following dose groups: low dose (0.0005–0.7 Gy), medium dose (0.7–1.5 Gy) and high dose (>1.5 Gy). The levels of TNF- $\alpha$ , IFN- $\gamma$  and IL-10 were significantly increased with radiation dose, as was the erythrocyte sedimentation rate. There was a radiation-dose-dependent increase in plasma levels of IL-6 and CRP. IL-6 stimulates the synthesis of acute phase proteins (such as CRP) involved in complement activation. A dose-dependent increase was also observed in total immunoglobulin levels. While the levels of IgA and IgM increased significantly with radiation dose, those of IgG and IgE did not [H10]. Increased serum levels of TNF- $\alpha$  [S13] and IgM [K21, S13] with higher functional complement activity [K21] were reported in Chernobyl emergency and clean-up workers. Higher serum levels of both IgG and IgM were found in children resident in contaminated areas around the Chernobyl nuclear power plant [K15, T5].

288. These observations provide evidence of a persistent inflammatory profile as a long-term effect after radiation exposure. Preclinical inflammatory status is linked in

some way to a decrease in CD4+ T-cells, suggesting that radiation-associated immunological changes may cause long-lasting inflammation [K36]. This T-cell impairment may generate age-associated chronic inflammation, which may be responsible for an increased risk of various lifestyle-associated diseases, such as atherosclerosis, coronary heart disease, diabetes and several cancers [K36, N3].

## 2. Remarks concerning the shift towards an inflammatory profile

289. As reviewed in the paragraphs above, a persistent inflammatory status induced by ionizing radiation has been associated with impairment of the immune system and with cancer and non-cancer diseases. Since TNF- $\alpha$ , IFN- $\gamma$ , IL-6 and IL-10 coordinate the inflammatory response, immune inflammatory diseases might be attributed in part to dysregulation of cytokine production.

290. Significant dose-dependent increases of TNF- $\alpha$ , IFN- $\gamma$  and IL-10 in parallel with the erythrocyte sedimentation rate as a biomarker of inflammation were observed from blood analysis of atomic bombing survivors. Total immunoglobulin levels were also enhanced in a dose-dependent manner. On the other hand, increased serum levels of TNF- $\alpha$  and IgM with higher functional complement activity were reported in Chernobyl emergency and clean-up workers.

291. Preclinical inflammatory status may be linked to impairment of cellular immunity, e.g. a decrease in CD4+ T-cells observed after radiation exposure, suggesting that radiation-associated immunological changes could account for long-lasting inflammation.

## F. Acceleration of immunological ageing

### 1. Review of published data

292. It has been proposed that acceleration of immunological ageing may be associated with radiation effects in humans. Immunosenescence has been described in section II above. As illustrated in table 11, comparison of the main features of immunosenescence with the experimental and epidemiological findings concerning radiation effects on the immune system supports this hypothesis.

**Table 11 Comparison between the main features of normal immunosenescence and observed radiation effects on the immune system**

<i>Parameter</i>	<i>Normal ageing</i>	<i>Irradiation</i>
Renewal capacity of stem cells and haematopoietic progenitor cells	Decreased [H1, L1]	Decreased [G5]
Total CD4+ T-cells	Decreased, about 4% per 10 years [K29]	Decreased [C7, C9, G9, K21, K33, P5, S8, T6, Y4], about 2% Gy <sup>-1</sup> [K29] Increased [T9, V9]



<i>Parameter</i>	<i>Normal ageing</i>	<i>Irradiation</i>
CD4+ naive T-cells	Decreased [H16], about 7.5% per 10 years [K36]	Decreased [K25, K29, K37, Y2], about 4.5% Gy <sup>-1</sup> [K36]
CD4+ memory T-cells	No significant changes [K36]	No significant changes [K36, Y2]
CD4+ naive/memory T-cell ratio	Decreased [H1, L5, V2, V4]	Decreased [K24, K25, K37, Y2]
Total CD8+ T-cells	No significant changes [K36]	No significant changes [K36]
CD8+ naive T-cells	Decreased, more than 40% per 10 years [K36]	Decreased [Y2], about 7.7% Gy <sup>-1</sup> [K36]
CD8+ memory T-cells	Increased, about 7.3% per 10 years [K36]	Increased [Y2], about 5.6% Gy <sup>-1</sup> [K36]
CD8+ naive/memory T-cell ratio	Decreased [U21]	Decreased [Y2]
Double-negative CD4 <sup>-</sup> CD8 <sup>-</sup> αβ <sup>+</sup> T-cells	Increased [H15]	Increased [A6, K37, N1]
Available TCR repertoire	Reduced [H1, V2, V4]	Reduced [K25]
CD8+ CD28 <sup>-</sup> (effector) T-cells	Increased proportion of CD8+ CD28 <sup>-</sup> T-cells [E2, H16]	CD28 expression: up-regulation (low doses) and down-regulation (high doses) [L14]
T-cell responsiveness to mitogens	Lower [B6, B15, V2]	High doses: lower [A4, A5, K26, K31, P5] Low doses: higher [J6, L17, L18, N20, P11, S16, S17, Y1]
Thymus mass and cellularity	Decreased [H16]	Decreased [G8, N1, P6]
B-lymphocytes	Decreased, about 7.3% per 10 years [K36]; less ability to generate antibody responses [B15, F3, H1, L5]	Increased [K29, Y1], about 8.5% Gy <sup>-1</sup> [K36] Decreased [G9, K24, P6] Hyporesponsiveness to LPS [C10, P6]
Lymphocyte oxidative status	Oxidative stress [V2]	Oxidative stress [C10]
IL-2 production	Decreased [E1, H16]	High doses: decreased [A2, B5, G8, K31] Low doses: increased [J8, L18]
TNF-α release	Increased [B6, E1], about 15% per 10 years [N3]	Increased [S13], about 7% Gy <sup>-1</sup> [H10, N3]
IL-10	Increased, about 8% per 10 years [N3]	Increased, about 6% Gy <sup>-1</sup> [H10, N3]
IL-6 release	Increased [B6, E1], about 24% per 10 years [H10, N3]	Increased [H5], about 13% Gy <sup>-1</sup> [H10, N3]
C-reactive protein level	Increased, about 25% per 10 years [H10, N3]	Increased [H5], about 39% Gy <sup>-1</sup> [H10, N3]
IgA	Increased, about 5% per 10 years [H10, N3]	Increased [T5], about 8% Gy <sup>-1</sup> [H10, N3] Increased in females [F4] No change [K21]
IgG	–	Decreased [K21] Increased [K15, T5] No change [F4, H10]
IgM	–	Increased [F4, H10, K15, K21, T5]
IgE	–	Increased [K15] No change [F4, H10]
Total Ig	Increased, about 3% per 10 years [H10]	Increased [K14], about 3% Gy <sup>-1</sup> [H10]
Erythrocyte sedimentation rate	Increased, about 15% per 10 years [H10, N3]	Increased [N7], about 17% Gy <sup>-1</sup> [H10, N3]
Th1/Th2 imbalance	Shift from Th1 to Th2 [R1, S7]	Shift to Th2 [A1, B2, H4, K23, S27, T5, W3] Shift to Th1 [D2, L19, N3]



<i>Parameter</i>	<i>Normal ageing</i>	<i>Irradiation</i>
Production of autoantibodies	Increased [H13]	Increased [T6, Y1, Y4] No change [F4]
Neutrophils	Impaired function [G25]	Impaired function [K21]
Monocytes/macrophages	Impaired function [B15, H1]	Impaired function [G18, K33] Macrophage activation [C21, I6, L8, N24, P11] Increased proportion of peripheral monocytes [S13]
Absolute number of NK cells	Increased [B15], about 20% per 10 years [K36]	Increased [K24, K33] No significant change [K29, K36]
NK cell function	Impaired [H1]	Impaired [K15] Enhanced [C21, K18, Y1] No significant change [B5, P6]
CD3+ CD56+ CD16+ (NKT) cells	Increased [M9]	Increased [K33]
Immunoproteasome function	Impaired [M8]	Impaired [L10]

293. Kusunoki et al. [K29] tested the hypothesis that ionizing radiation may accelerate ageing by using the proportion of CD4+ T-cells as an index. They reported that the normal age-related decrease of CD4+ T-cells was about 4% per 10 years and that the radiation-induced decrease of CD4+ T-cells was about 2% Gy<sup>-1</sup>. These findings suggest that exposure to 1 Gy is equivalent to about a 5-year increase in age.

294. In 442 atomic bombing survivors without a history of cancer or inflammatory diseases, Nakachi's group

[H10, N3] found that both age and radiation exposure were associated with increases in selected plasma inflammation markers. Their findings indicated that the radiation effect might be estimated in terms of acceleration of ageing by applying a multivariate model (table 12). The per cent increments in TNF- $\alpha$ , IL-10, IL-6, CRP, IgA and erythrocyte sedimentation rate observed in atomic bombing survivors ranged from 6% Gy<sup>-1</sup> to 39% Gy<sup>-1</sup>, corresponding on average to an increase in age of 10 a/Gy.

**Table 12 Multivariable model of the effects of age at time of irradiation and of radiation dose on inflammatory biomarkers and ageing<sup>a</sup>**

Adapted from reference [N3]

<i>Variable<sup>b</sup></i>	<i>Change in per cent (95% CI)</i>					
	<i>TNF-<math>\alpha</math></i>	<i>IL-10</i>	<i>IL-6</i>	<i>CRP</i>	<i>ESR</i>	<i>IgA</i>
Age at time of irradiation (per cent per 10 years)	15 (9, 20)	8 (4, 13)	24 (19, 30)	25 (13, 38)	15 (9, 20)	5 (2, 9)
Radiation dose (% Gy <sup>-1</sup> )	7 (1, 15)	6 (0, 12)	13 (6, 20)	39 (20, 62)	17 (9, 24)	8 (3, 13)
Estimated ageing effect of radiation (a Gy <sup>-1</sup> )	5 (0, 10)	6 (-1, 14) <sup>c</sup>	5 (2, 8)	14 (4, 24)	11 (5, 17)	15 (1, 29)

<sup>a</sup> Subjects were a total of 442 atomic bombing survivors who did not have a history of cancer or inflammatory-associated disease (e.g. chronic bronchitis, collagen disease, arthritis, myocardial infarction).

<sup>b</sup> TNF- $\alpha$  = tumour necrosis factor  $\alpha$ ; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate.

<sup>c</sup> Estimated by the  $\delta$ -method.

## 2. Remarks concerning the acceleration of immunological ageing

295. The preceding paragraphs indicate that comparison of the main features of immunosenescence with the epidemiological

and experimental findings on radiation effects on the immune system supports the hypothesis that acceleration of immunological ageing may be involved in radiation effects in humans. No demonstrable relationship with cancer or inflammatory diseases in atomic bombing survivors has been found.

## G. Modification of antigen presentation

### 1. Review of published data

296. “Danger” signals affect antigen-presenting cells (APCs), and the dendritic cells are the most powerful. Functional antigen presentation by dendritic cells requires that the cells mature under the influence of different danger signals. However, in addition to the maturation of dendritic cells being essential for lymphocyte activation, immature dendritic cells themselves maintain a state of immunological tolerance. Cells dying by necrosis are more potent at inducing dendritic cell maturation than cells dying by apoptosis (the latter may even block maturation). However, cells that have undergone apoptosis in response to stressors such as heat or ionizing radiation may be more effective for inducing dendritic cell maturation than cells that have undergone natural physiological apoptosis.

297. Although dendritic cells are relatively radioresistant, ionizing radiation affects their functions. Liao et al. demonstrated that non-cytotoxic effects of ionizing radiation might account for the impairment of antigen processing and presentation following irradiation of dendritic cells. Cell viability was not significantly affected over a 24 h culture period following 10 Gy of gamma irradiation at 4.5 Gy/min. However, immunoproteasome activity was down-regulated in irradiated cells by 50–60% [L10].

298. It has been reported that ionizing radiation may cause a generalized decrease in proteasome functions in mammalian cells [M3]. Immunoproteasomes are responsible for the processing of antigens for presentation by the class I HLA pathway. Alterations of immunoproteasome function have been proposed as a signal of immunosenescence [M8]. Proteasomes are direct redox-sensitive targets for the action of ionizing radiation [M2]. Using microarray analysis, Snyder [S26] has studied gene expression profiles of cells exhibiting radiation-induced genomic instability. Two of the genes that were underexpressed belonged to the proteasome/ubiquitin pathway. These findings suggest that ionizing radiation could impair immune function by altering antigen processing at the immunoproteasome level.

299. Findings concerning the effect of ionizing radiation on HLA molecules are controversial. Liao et al. did not find significant changes in the expression of class I and class II HLA molecules on dendritic cells following gamma irradiation with a single dose of 10 Gy [L10]. Reits et al. found that cell surface expression of class I HLA molecules increased in a radiation-dose-dependent manner on murine colon adenocarcinoma cells [R19]. Radiation-induced enhancement in the expression of class I HLA molecules has been reported by Hauser et al. in a murine melanoma cell line following fractionated gamma irradiation (50 Gy in 25 fractions) [H7]. In contrast, a decrease in the expression of class I HLA molecules was found in a human melanoma cell line following gamma irradiation with a single dose of 20 Gy [G35]. These discrepancies may be due to the pathways

activated by ionizing radiation in different cell types as well as to different dose protraction and total doses.

300. HLA mutant lymphocytes are induced by radiation exposure and eliminated by NK cells [K30]. Autologous NK cells are responsible for the elimination of mutant lymphocytes that have lost the ability to express self class I HLA molecules in vivo [K27], and therefore may explain why it has not been possible to detect increased frequencies of HLA mutants in blood samples from atomic bombing survivors.

### 2. Remarks concerning modification of antigen presentation

301. The data reviewed indicate that dendritic cells, the most powerful APCs, are relatively radioresistant, but that radiation may affect their function. Down-regulation of immunoproteasome activity has been reported. Immunoproteasomes are responsible for the processing of antigens for presentation by the class I HLA pathway; consequently, ionizing radiation could impair immune function by modification of antigen processing at the immunoproteasome level. This was also proposed as a sign of immunosenescence.

302. Concerning the effects of ionizing radiation on HLA molecules, controversial results are presented. Non-significant changes were found in the expression of class I and class II HLA molecules following irradiation of dendritic cells. Enhancement in the expression of class I HLA molecules after fractionated irradiation of a murine melanoma cell line, as well as down-regulation of their expression following acute irradiation of a human melanoma cell line, have been reported. Different dose protraction and total doses, as well as different cell types, may account for the discrepancies.

## H. Autoimmune reactions

### 1. Review of published data

303. Ionizing radiation can produce functional alteration the immune system and break self tolerance. Autoimmune diseases are characterized by the activity of autoreactive lymphocytes that produce antibodies targeting self tissues or organs for destruction. Autoimmunity may be seen as a case of “mistaken identity”, in which the immune system mistakes part of the body for a foreign invader. One explanation for why the immune system attacks self tissues in some people is molecular mimicry, which means that a part of a molecule of a given protein closely resembles a part of another, totally different protein. It has been shown that peptides in various infectious agents resemble parts of various self proteins. Therefore, if these protein fragments are presented to T-cells, the activated immune system will not only attack all foreign invaders with the same pattern but could also attack a very similar pattern in a self protein [O10]. The significant dose-dependent impairment of the immune system demonstrated in irradiated populations [K14, K21, K29, K31, V9] could lead to many of these persons being less responsive to

infectious agents. These individuals are at a higher risk for viral or microbial infection, and are therefore more prone to developing autoimmune diseases by molecular mimicry.

304. It was not proved that autoimmune reactions were involved in the pathogenesis of thyroid diseases in atomic bombing survivors [18]. However, the immune system has been demonstrated to be involved in the pathogenesis of thyroid diseases in victims of the Chernobyl accident. These apparently contradictory results may be due to the different thyroid exposure conditions (acute external irradiation versus internal exposure from radioiodine). It is possible that thyroid damage induced by internal exposure from radioiodine sets into motion antigenic mechanisms that lead to autoimmune responses. Epidemiological data from children exposed to radioiodine during the Chernobyl accident indicate signs of autoimmune thyroid disorder and impaired NK-related elimination of tumour cells, both of which may be contributing to the promotion of thyroid neoplasia in this population.

305. Sakaguchi et al. [S6] demonstrated that high-dose fractionated total lymphoid irradiation (42.5 Gy in 17 fractions) caused various organ-specific autoimmune diseases in mice. Irradiation of the target organs alone failed to elicit the autoimmunity, and shielding the organs from irradiation failed to prevent it, suggesting that radiation-induced tissue damage is not the primary cause of the autoimmune disease. A significant decrease in mature thymocytes and peripheral T-cells was observed for one month post-irradiation. These findings suggest that high-dose fractionated total lymphoid irradiation can cause autoimmune disease by affecting the T-cell immune system (rather than the target self antigens), presumably by altering T-cell-dependent control of self-reactive T-cells.

306. In a further paper, Sakaguchi [S3] demonstrated that elimination of CD4+ CD25+ T-regulatory cells (Treg) led to the development of various organ-specific autoimmune diseases in mice. Reconstitution of the Treg population prevented the development of autoimmunity. Moreover, elimination or reduction of the Treg population by environmental agents also induced autoimmune diseases in normal mice, suggesting that radiation-induced CD4+ T-cell alterations may cause autoimmunity without altering the antigenicity of the host organs concerned [K23].

## 2. Remarks concerning autoimmune reactions

307. As discussed in the preceding paragraphs, self tolerance breaking may be among the alterations on the immune system induced by radiation. No radiation-induced autoimmune reactions were involved in the pathogenesis of thyroid diseases observed in atomic bombing survivors. In contrast, the immune system was involved in the pathogenesis of thyroid diseases in people exposed to radiation from the Chernobyl accident. This may be due to the different thyroid exposure conditions. Experimental data obtained

from fractionated total lymphoid irradiation showed that the development of autoimmunity was related to the alteration of T-cell-dependent control of self-reactive T-cells.

## I. Perturbation of immunological homeostasis

### 1. Review of published data

308. Immunological homeostasis is the mechanism by which the immune system responds to foreign antigens (e.g. an infectious organism) and then returns to its original state, although retaining memory cells that will protect the host against subsequent infection by the same organism. This homeostasis is achieved in the T-cell system by the balance between renewal and death of naive and memory T-cells. The ability to maintain both naive and memory T-cell pools, which declines with age, is critical for immune function. An immunostatic mechanism exists that controls the proportions of immune cell types, the production of cytokines and the level of expression of functional immune cell molecules.

309. Exposure to ionizing radiation is thought to affect T-cell homeostasis. Both experimental and epidemiological data have demonstrated that ionizing radiation may perturb T-cell homeostasis by reducing the ability of the immune system to produce new naive T-cells and by disturbing the regulation and maintenance of memory T-cell pools. A diverse pool of naive T-cells is necessary to produce immune responses to new antigens. Exposed atomic bombing survivors showed lower numbers of naive CD4+ and CD8+ T-cells. Although memory T-cell pools were either normal (CD4+) or even larger (CD8+) in size [Y2], their TCR repertoire was significantly reduced with radiation dose [K25], probably due to clonal expansion of memory T-cells. Alterations in T-cell subpopulations were also described in Chernobyl clean-up workers [T6, Y4].

310. Radiation-induced perturbation of T-cell homeostasis may have important health implications. The reduction of the naive T-cell pool may lead to reduced ability of the host to defend against new pathogens, and the clonal expansion of memory T-cells associated with TCR repertoire deviation may compromise the ability of the host to control recurrent and latent infections [K36].

### 2. Remarks concerning perturbation of immunological homeostasis

311. The data reviewed indicate that immunological homeostasis is the mechanism by which the immune system responds to foreign antigens and returns to its original state, but retaining memory cells to protect against subsequent infection by the same agent. This homeostasis is achieved by the balance between renewal and death of naive and memory T-cells. Experimental and epidemiological data have demonstrated that radiation alters T-cell homeostasis by reducing the ability of the immune system to produce new

naïve T-cells and by disturbing the maintenance of memory T-cells. The perturbation of T-cell homeostasis may have health implications in terms of decreased defences against new pathogens and compromised ability to control recurrent and latent infections.

## J. Other possible mechanisms involved

### 1. Review of published data

312. Liu et al. [L16] proposed that catecholamines mediate the increase in proliferative reactivity of splenic and thymic lymphocytes that has been observed in mice irradiated at a low dose (75 mGy). In a similar model, they demonstrated the involvement of intracellular calcium and protein kinase C in the facilitation of signal transduction in lymphocytes and suggested that this facilitation was implicated in the mechanism of immune enhancement after low-dose irradiation [L17].

313. Modulation of oxidative status has been postulated as another mechanism for radiation-induced immune stimulation following low-dose irradiation. Kojima et al. [K16] found an increase of immune function in mouse splenocytes that correlated with endogenous glutathione (GSH) accumulation within the first six hours after irradiation. This effect was enhanced by exogenous addition of precursors of GSH synthesis and was completely blocked by inhibition of GSH synthesis, suggesting that low-dose exposure to ionizing radiation enhances immune function through the induction of GSH [K16, K17, K18].

314. Indirect effects of local radiotherapy on tumour cells outside the radiation field have been reported in many malignancies [A14, C2, E3, K13, N11, O7, R2, S15, U20]. It has been proposed that this phenomenon, originally described as the “abscopal effect”, may be related to radiation-induced effects on the immune system. Several factors could influence this effect, such as the immunological state of the tumour-bearing host and the immunogenicity of the tumour cells, as well as the schedule and overall dose of radiotherapy.

315. In an experimental mammary carcinoma model, Demaría et al. [D3] demonstrated that the growth of tumours outside the radiotherapy field was impaired by the combination of radiotherapy and Flt3 ligand, a haematopoietic cytokine that has been shown to facilitate the expansion of dendritic cells and the generation of an antitumour immune response. Importantly, in this experimental model Flt3 ligand alone had no effect on the growth of primary or secondary tumours, indicating that ionizing radiation was involved in the abscopal phenomenon. In addition, this abscopal effect was absent in nude mice (i.e. in mice lacking T-cells). In contrast to immunocompetent mice, the addition of Flt3 ligand did not result in any abscopal effect in nude mice. These findings indicate that the abscopal effect triggered by local irradiation was T-cell dependent [D3].

316. In a recent paper, Van der Meeren et al. [V3] investigated the radiation-induced inflammatory response after total-abdominal or whole-body irradiation of mice at a dose of 15 Gy. A comparison with WBI was used to take into account haematopoietic involvement in the inflammatory process. The authors found a systemic inflammatory reaction after both abdominal irradiation and WBI, with an increased cytokine and chemokine production at the intestinal and lung levels, indicating a possible abscopal effect of radiation. They postulated that the effects observed in the lungs after irradiation of the abdominopelvic region may be caused by circulating inflammatory mediators due to the gut inflammatory response.

317. One question that remains unanswered is whether the immunogenetic background may be involved in disease risks of irradiated subjects. There are large individual variations in the level of immunological parameters and inflammatory markers. Only some individuals with reduced immune function and/or elevated inflammatory biomarkers develop particular diseases [K36]. Thus it may be postulated that individual immunogenetic background may determine individual susceptibility to certain diseases. One particularly important genetic factor that can affect host immune response appears to be the *HLA* gene. Higher risks of type 2 diabetes were found between heavily exposed atomic bombing survivors with different class II *HLA DQA1* and *DRB1* alleles [H12], suggesting that certain class II *HLA* genes regulate one or more components of the immune system that are related with the risk of diabetes development in irradiated people.

318. Ionizing radiation exhibits immunomodulatory properties. The “danger” model of immunity describes antigen-specific cellular immunity engendered by an inflammatory milieu, where an important role is played by dendritic cells. Ionizing radiation may create an inflammatory setting via induction of apoptosis, necrosis, cell surface molecules and secretory molecules. Radiation may influence the expression of immunomodulatory surface molecules (MHC, co-stimulatory molecules, adhesion molecules, death receptors, heat shock proteins) as well as secretory molecules (cytokines, inflammatory mediators), in both tumour and normal cells. Experimental data indicate possible radiation-mediated modulation of tumour antigen-specific immunity [F5]. Radiation-mediated immunomodulation currently remains unquantified and poorly understood. A major research effort will be required to better elucidate the mechanisms involved.

### 2. Remarks concerning other possible mechanisms involved

319. Other possible mechanisms involved are:

- Involvement of catecholamines in the proliferative activity of splenic and thymic lymphocytes, as well as intracellular calcium and protein kinase C in the signalling of immunoenhancement after low-dose irradiation;



- Modulation of oxidative status following low-dose irradiation;
- Indirect effects of local radiotherapy, outside the radiation field, described originally as “abscopal effects”;
- Possible involvement of immunogenetic background in disease risks of irradiated subjects.

## K. Immune mechanisms and cancer

### 1. General considerations

320. That cancer may result as a stochastic effect from exposure to ionizing radiations has been well known for a long time, and mechanisms of radiation carcinogenesis have been extensively reviewed [U2, U4, U5, U6]. In the context of this annex, it is most important to understand the mechanisms of cancer development and especially to investigate the possible roles of the immune system.

321. Cancer is a multifactorial disease for which a genetic susceptibility and environmental factors—chemical, physical or viral—can be responsible. It is worth noting that some specific cancers are usually linked to specific exposures to environmental or infectious factors [N3, W18]. Some evidence has been recorded of a possible connection between haematological malignancies and exposure to ionizing radiation, although nitrates, pesticides, HTLV1 or Epstein–Barr virus infections, and immunodeficiency are other possible risk factors [D27]. The increased cancer incidence observed in organ transplant recipients has been related to immunosuppressive treatments that must be maintained to prevent and treat acute rejection [A11, A30, O11].

322. Cancers are not merely autonomous masses of mutant cells, but are composed of multiple cell types such as fibroblast and epithelial cells, cells that form blood and lymphatic vasculature, specialized mesenchymal cells that are unique to each tissue environment, and indeed innate and acquired immune cells [C35]. While tissue homeostasis is maintained by collaborative interactions between these diverse cell types, cancer development is enhanced when mutant cells neutralize homeostatic growth constraints and hijack the normal physiological processes to favour their own survival. Furthermore, tumours can develop an angiogenic phenotype which gives them a potential of growth and metastatic migration [F14].

323. It is recognized that each stage of cancer development can be exquisitely susceptible to modulation by immune cells. In essence, there is a complex relationship between immune cells and developing tumours with the following paradox: full activation of immune cells in response to the tumour may result in eradication of malignant cells and conversely an inefficient immune response may leave tumour cells with the possibility to grow, whereas chronic activation of various types of innate immune cell in or around

pre-malignant tissues may actually promote tumour development. In cancers, the abundance of infiltrating lymphocytes, which are the predominant cells involved in the acquired response, correlates with a favourable prognosis. On the other hand, an abundance of infiltrating innate immune cells, such as macrophages, mast cells and neutrophils, correlates with increased angiogenesis and/or a poor prognosis [D17], although clusters of macrophages around tumours are often associated with tumour regression [G27].

324. Therefore, with regard to the role of the immune system in cancer development, it could be inferred that ionizing radiation might modify cancer risk not only by acting as a carcinogen per se but also by modulating host immune response. Genotoxic stress and stalled DNA replication forks induce the expression of ligands for the NKG2D receptor found in NK and certain T-cells, cell types that are able to attack tumour cells [G36]. This activation depends on proteins involved in DNA damage-sensing pathways and cell cycle regulation. This might explain how DNA damage response participates in altering the immune response to the presence of potentially dangerous cells.

### 2. Immune surveillance theory

325. The fact that so many persons die each year of cancer suggests that their immune response to tumour cells is inefficient. Indeed the immune system may respond only if novel antigens are expressed on the cell surface by tumour cells and are subsequently recognized as non-self neoantigens [L29]. A number of alterations occur in the cell during tumorigenesis: depression of some genes, expression of others or alteration of genes via mutations. Thus genetic changes related with carcinogenesis may result in the expression of “aberrant” molecules by transformed cells (reappearance of embryonic proteins not expressed in adult life, expression of unique antigens not expressed by normal cells). However, the prevalence of an antigen expression can vary, meaning that not all tumours of a particular type may express the antigen at all. Many antigens have heterogeneous expression, with the result that the proportion of cells that express the antigen within each tumour may vary from patient to patient. Indeed, antigen expression depends on the status of the cellular machinery; for example, antigen expression would be reduced in the case of proteasome deficiency [D28]. It is worth noting that most tumours induced by physical, chemical or viral agents express neoantigens [S52], while in contrast, spontaneously occurring tumours are only often weakly immunogenic or are non-immunogenic.

326. In the immune surveillance theory of cancer, tumours can develop only when cancer cells can escape from the immune surveillance either by reducing the expression of tumour antigens or by modifying immune recognition and activation [Z3]. As an example, the down-regulation of the synthesis of class I HLA molecules in tumours and metastases is a potential mechanism by which cancer cells can escape from class I HLA restricted lysis by cytotoxic T-cells.



In addition, the expression of the non-classical class I HLA molecule HLA-G on the cell surfaces of various cancers (melanomas, kidney cancers, breast cancers) inhibits both the cytotoxic activity of NK lymphocytes and the antigen-specific cytotoxic lymphocyte response [R22].

327. In support of the immune surveillance theory is the fact that the incidence of cancers is significantly increased in immunodepressed patients whatever the cause of their immunodepression. This is well documented in patients with AIDS due to viruses of the HIV family. These patients, who present with an unusually high frequency of Kaposi sarcoma, are also more sensitive to radiation. Unlike the classical Kaposi sarcoma, where radiation therapy is associated with minimal toxicity, radiotherapy treatment of this tumour in AIDS patients is associated with very high morbidity [C32, H30, R11].

328. A higher incidence of cancers is also observed in transplant patients who receive immunosuppressive treatments [A11, O11]. This higher incidence concerns not only tumours associated with latent infections [N3], but also tumours commonly observed in the general population, such as digestive, respiratory, endocrine and breast cancers. The increased incidence of these tumours can be associated with the immunosuppressive treatment [L3], although some confounding factors such as tobacco and alcohol certainly play a role [C28, F13, H26].

329. The immune system can produce significant anti-tumour effects, for example after allogeneic bone marrow grafting. T-cells from the donor recognize the tumour as non-self and develop impressive antitumour effects (graft-against-tumour effects).

330. Natural antibodies are not very efficient at destroying tumour cells. However, it has been suggested that the immune system may eliminate tumour cells that carry tumour-specific antigens, leaving room for the tumour to grow cells with a low level of these antigens or with antigens that differ only very slightly from those of normal cells [G27]. On the other hand, monoclonal antibodies can be successful in controlling tumours when they have a high affinity for the tumour and can be used in large quantities. The most successful examples are the treatments of B-lymphoma with anti-CD20 monoclonal antibodies and of some breast cancers with anti-human epidermal growth factor receptor 2 (HER2) monoclonal antibodies.

331. Against the immune surveillance theory, it has been observed that the relative risk for some common non-virus-associated solid tumours of epithelial origin (breast, prostate and bladder) is decreased in some immune-suppressed patients [F12, G17]. This finding has not yet been completely explained, although it has been suggested that immunosuppressive drugs could have a direct antitumour effect.

332. In order to test the validity of the immune surveillance theory, experiments can be carried out in animals.

This is the case in nude mice with no thymus, which do not develop more cancers than normal mice though this would be expected given the lack of T-cells. On the other hand, it has been observed in some tumour grafting experiments that the tumour does not develop after injection of intermediate doses of cancer cells, while tumour development is observed after injection of low and high doses of cancer cells; this finding is difficult to reconcile with the immune surveillance theory.

### 3. Immune response against tumours

333. Cancer immune surveillance involves innate and acquired responses. Innate responses for transformed cells are associated mainly with NK cells, and the function is balanced between activating and inhibitory receptors. This balance significantly influences the efficacy of the immune response and consequently of tumour progression [S12]. Activating NK receptors may respond to stress-inducible proteins overexpressed by tumour cells. Conversely, the lost or down-regulated expression of class I HLA molecules in transformed cells ("missing self") may suppress the inhibitory signalling in other NK receptors. NK cells can protect against experimental tumour growth, in part by producing mediators with anti-angiogenic properties [H22, S45]. NK cell deficiency as observed in the Chediak-Higashi syndrome in humans and in Beige mice results in some circumstances in an excess of cancers.

334. Tumour cells may express HLA-G, a non-classical class I HLA molecule involved in immunotolerance. The expression of HLA-G by malignant cells prevents their elimination, and constitutes a newly described mechanism by which tumour cells may evade immune surveillance [C4]. Through the interaction with specific inhibitory receptors, HLA-G can protect tumour cells lacking classical class I HLA expression from cytotoxicity mediated by NK and T-cells [S12]. Through the inhibition of MICA signals, HLA-G may lead to tumour escape from immune surveillance [R9].

335. Genotoxic stress and stalled DNA replication forks induce the expression of ligands for the NKG2D receptor found in NK and certain T-cells, cell types that are able to attack tumour cells [G36]. This activation depends on proteins involved in DNA damage-sensing pathways and cell cycle regulation. This may explain how DNA damage response participates in altering the immune response to potentially dangerous cells.

336. Activated macrophages can play a significant role in the immune response against tumours. Their antitumour activity is probably linked to lytic enzymes and the generation of free radicals. Furthermore, macrophages produce TNF- $\alpha$ , a cytotoxin with a powerful antitumour activity.

337. Acquired immunity involves the recognition by T-cells of the products of mutated genes, oncogenic virus

products or normal proteins aberrantly expressed. Moreover, interactions between T- and B-cells mediated by cytokines, as well as minor T-cell subsets such as NKT cells and  $\gamma\delta$  T-cells, may act in eliminating transformed cells.

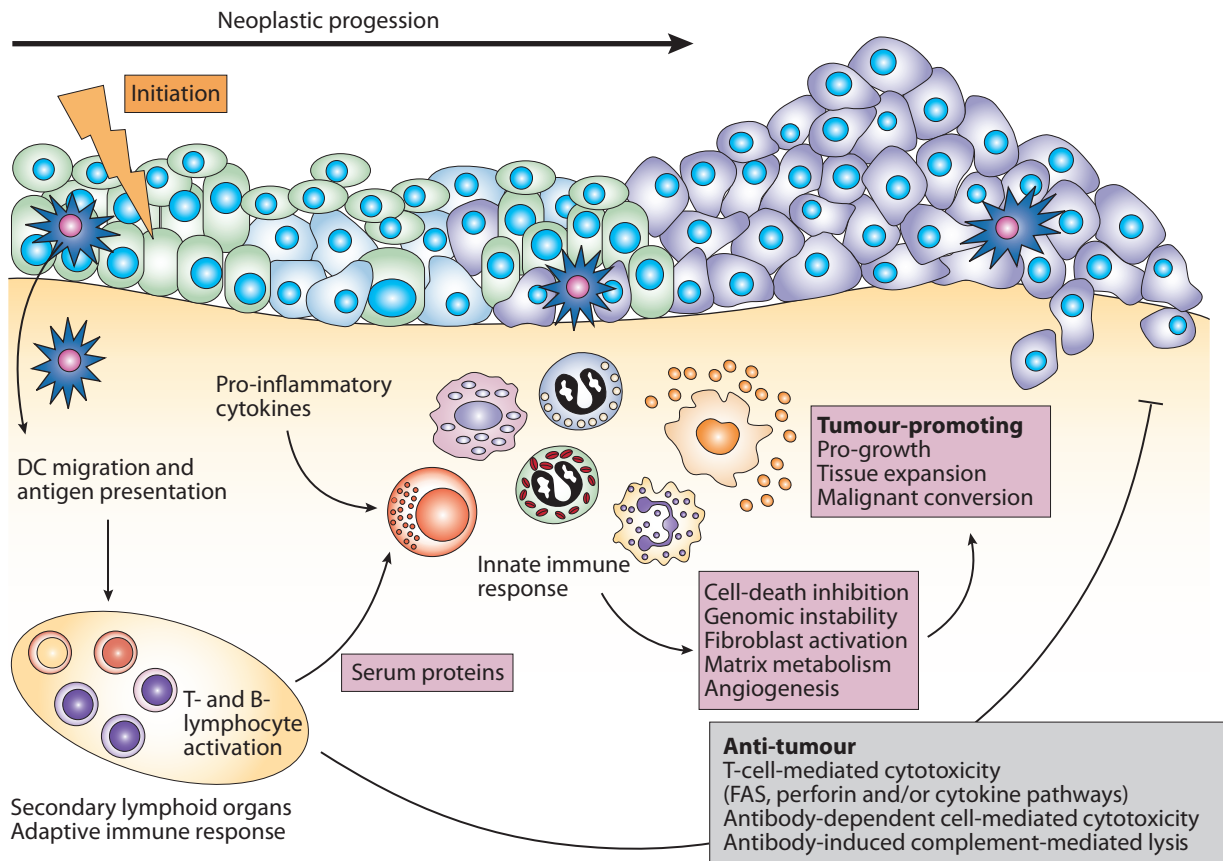
#### 4. Immunologic promotion of tumours

338. Exposure to ionizing radiation may impair the immune system and, on some occasions, result in low-grade chronic inflammation. It has been proposed that persistent inflammation could play a role in promoting the proliferation of initiated cells through the generation of ROS, the production of inflammatory cytokines and the

induction of genetic instability. During chronic inflammatory processes there is an excess production of free radicals, which deregulate cellular homeostasis and can drive normal cells to malignancy [B1]. Recent findings have provided evidence suggesting that persistent inflammation involving repeated infection could be a key step in carcinogenesis. Indeed, the long-term inhibition of chronic inflammation by aspirin and selective cyclooxygenase-2 (COX-2) inhibitors in patients with premalignant disease, or who are predisposed to cancer development, has significantly reduced cancer risk [D15]. Figure VIII shows a model proposed by de Visser et al. [D17] for explaining innate and acquired immune cell functions during inflammation-associated cancer.

**Figure VIII. Model of innate and acquired immune cell functions during inflammation-associated cancer.**

Tumour antigens are presented by dendritic cells (DCs) to activate acquired immune responses, which may result in both antitumour (direct effects and tumour-promoting effects (via innate immune response) [D17].



339. Chronically activated innate immune cells can also contribute indirectly to cancer development through suppression of antitumour escape from immune surveillance. For example, myeloid suppressor GR+CD11b+ cells, e.g. a subset of innate immune cells, induce T-lymphocyte dysfunction by direct cell-cell contact and by production of immunosuppressive mediators, and therefore actively inhibit antitumour acquired immunity [G16, S42, Z3].

340. Immunological activation of tumour growth by tumour cells has been observed in experimental animals. In many circumstances, attempts to protect animals against tumour growth by active immunization by tumour-specific antigens or by passive immunization by specific tumour antibodies have, surprisingly, yielded tumour growth. This is interpreted as being due to the existence of blocking factors. either the antibody itself, which after binding the antigen hides it from

cytotoxic T-cells, or the antibody–antigen complex, which inhibits the antibody-dependent cell-mediated cytotoxicity by binding to the Fc receptors on the surface of NK cells or macrophages and blocking their activity.

#### 5. Remarks concerning immune mechanisms and cancer

341. On the basis of the preceding paragraphs, some remarks may be made concerning immune mechanisms and cancer. A complex relationship giving rise to a paradox is observed between immune cells and developing tumours: full activation of immune cells in response to the tumour may result in the elimination of tumour cells, whereas an inefficient immune response allows their growth. In addition, chronic activation of various types of innate immune cells in or around premalignant tissues may actually promote tumour development.

342. The immune surveillance theory suggests that tumours can develop only when cancer cells can escape from the immune surveillance either by reducing the tumour antigens or by modifying the immune response to them. A large body of evidence in favour of the immune surveillance theory comes from immunodepressed patients, who present a higher incidence of cancer. Evidence has also been seen in transplant patients who receive an immunosuppressive treatment. In contrast to these observations, it has been reported that the relative risk for some common non-virus-associated solid tumours of epithelial origin is decreased in some immunodepressed patients. An antitumour effect of the immunosuppressive drugs was suggested in these cases.

343. The capability of the immune system to develop antitumour effects involves innate and acquired responses. The innate response is mainly associated with NK cells. Its function is balanced between activating and inhibitory receptors. NK cell deficiency as observed in human and mice syndromes results in some circumstances in an excess of cancers. A newly described mechanism by which malignant cells avoid their elimination is through the expression of HLA-G, a non-classical class I HLA molecule that can protect tumour cells lacking classical class I HLA expression from cytotoxicity mediated by NK and T-cells. Similarly, activated macrophages play an antitumour role linked to lytic enzymes and the generation of free radicals along with the release of cytokines with a powerful antitumour activity.

344. Acquired immunity involves the recognition by T-cells of the products of mutated genes, oncogenic virus products and normal proteins aberrantly expressed.

345. Finally, long-lasting inflammation could play a role in promoting the proliferation of initiated cells through the generation of free radicals, the release of inflammatory cytokines and the induction of genetic instability, because of alterations of immune cells.

#### L. Summary

346. There are many mechanisms potentially involved in radiation-induced alterations of the immune system:

- Radiation-induced apoptosis is a key mechanism, well established for blood-circulating white cells, mostly lymphocytes.
- Mutations of TCR genes is a radiation-dose-dependent mechanism which can produce defective TCRs and alter the discrimination between “self” and “non-self”.
- There is still some controversy regarding the functional cytokine secretion pattern of helper T-cells following exposure to ionizing radiation, although it is likely that the homeostatic balance between Th1 pattern (cell-mediated immunity) and Th2 pattern (humoral immunity) is shifted towards a pro-inflammatory profile.
- Delayed effects, e.g. bystander effects and genomic instability, have been demonstrated after exposure of the immune system to ionizing radiation. However, the potential impact of such delayed effects in humans is not known.
- Inflammation resulting from the effects of ionizing radiation can be observed at the microscopic level and involves immune cells within and around tumours, but inflammation may be large enough to produce significant alterations of parameters in blood samples. Inflammation may be associated with chronic diseases.
- There is a vast literature showing that immune cells after exposure to ionizing radiation show abnormalities that are quite similar to those observed in normal ageing. These observations at the biological level have so far not been linked to diseases.
- Alterations of the process of antigen presentation at the level of the immunoproteasome have been demonstrated and are possibly a signal of immunosenescence after exposure to ionizing radiation.
- Ionizing radiation can contribute to a disturbance of self tolerance and consequently can pave the way towards autoimmunity.
- Finally, the immunological response against foreign antigens implies T-cell homeostasis, which is disturbed after exposure to ionizing radiation.

347. Besides apoptosis, which is a key mechanism within the immune system, it is rather difficult to classify the other mechanisms according to their importance after exposure to ionizing radiation. It is likely that these mechanisms are interlinked, e.g. microscopic inflammation, propagated by a type of bystander mechanism and contributing to the ageing of tissues and promotion of cancer. Three hypotheses to further explore the mechanisms involved in the effects of

ionizing radiation on the immune system and their impact in human health have been postulated by the Radiation Effects Research Foundation (RERF) (see section IV):

- Ionizing radiation may accelerate immunological ageing by perturbing T-cell homeostasis;
- Ionizing radiation may induce long-lasting inflammation that may lead to disease development;
- Individual immunogenetic background may determine individual susceptibility to succumbing to disease.

348. Immune surveillance is different for the various cancer entities and has not been reported for all of them. The immune surveillance theory of cancer development remains controversial. Although the immune system has the capability to develop impressive antitumour effects, it is not very clear that cancer results from a deficiency of the immune system, and tumours can be promoted through low-level chronic inflammation because of alterations of immune cells. The potential effects of low doses of ionizing radiation on the critical balance existing in the immunological network (promoting or suppressing antitumour immunological response arising in the tumour microenvironment) has been insufficiently studied.

## IV. EPIDEMIOLOGICAL STUDIES

### A. Atomic bombing survivors

#### 1. General considerations

349. Epidemiological studies of the survivors of the atomic bombings in Japan are currently the most important single source of radiation risk estimates for humans. Most of the information on the health effects of ionizing radiation available to date comes from long-term studies of survivors of the atomic bombings in Hiroshima and Nagasaki. Almost sixty years after exposure to radiation, survivors of the atomic bombings still exhibit increased risks of developing solid tumours. Immune mechanisms have consistently been associated with either resistance to or development of numerous tumours. Also, an association between non-cancer mortality and radiation dose has been observed among survivors, cardiovascular, thyroid and liver diseases being the more frequently reported causes. Long-lasting inflammation may be considered an important contributory factor for the development of some of these diseases [K36]. Knowledge of the impact of radiation on the immune system is therefore critical to assessing radiation-induced effects on the long-term health of survivors [K23, K36].

#### 2. Short-term effects

350. It has been estimated that around 114,000 people in Hiroshima (an additional 20,000 military personnel not included) and 74,000 people in Nagasaki died before the end of 1945, in total about 210,000 deaths, as a direct result of the bombings. These deaths are referred to as “acute deaths”. The short-term effects of the bombings have been extensively described, and include thermal, mechanical and radiation injuries (in particular radiation-induced bone marrow depletion).

351. At the time of the bombings, the haematopoietic systems of survivors underwent a level of damage for which the severity and persistence were dose-dependent. The most serious effects were those related to radiation-induced cell death resulting in the development of symptoms of acute radiation syndrome. Several months after exposure, the haematolymphoid function of many survivors had almost completely recovered [O8]. Studies initiated soon after the bombings showed little evident dose-dependent effects on the immune system [A6]. However, even several decades later, it was still possible to detect long-term alterations in the immune system of exposed survivors.

#### 3. Long-term effects

352. In an early paper investigating T-cell immunity among the atomic bombing survivors, Akiyama et al. [A5] looked at the responsiveness of peripheral blood lymphocytes to allogenic antigens in mixed lymphocyte cultures from 139 atomic bombing survivors. This study revealed a significant decrease in mixed lymphocyte culture response with increasing radiation dose. The decline was most marked in the survivors who were more than 15 years old at the time of the initial exposure. These results were interpreted as an impaired thymic function.

353. In 1983 Akiyama et al. [A4] described functional defects in T-cell response to mitogens such as PHA. IL-2 is known to have different actions regarding T-cell proliferation and T-cell development. CD4+ T-cells are those primarily responsible for producing IL-2 in response to mitogens such as concanavalin A (Con A). Decreased production of IL-2 has been implicated as a potential factor in radiation-induced impaired immunity [B5]. To elucidate the biological significance of the T-cell abnormalities observed in atomic bombing survivors long after exposure, Kusunoki et al. investigated the percentage of T-cells capable of responding to PHA or Con A or that could produce IL-2. The study used a limiting dilution assay method to evaluate the responsiveness of the T-cell population to these mitogens. The subjects in this study were 251 atomic bombing survivors exposed to <0.005 Gy and 159 survivors exposed to >1.5 Gy. The percentage of CD2+ cells (activated T-lymphocytes) capable of proliferating in response to PHA in the presence of exogenous IL-2 did not differ substantially between distally and more heavily exposed survivors. In contrast, T-cell capacity in response to Con A was lower in the more exposed individuals. Moreover, heavily exposed survivors possessed fewer T-cells with the capability of producing IL-2. It was concluded that peripheral blood samples from heavily exposed survivors contained significantly fewer IL-2-producing CD4+ T-cells than did similar samples from those distally exposed to radiation from the atomic bombings. Radiation might have a long-lasting negative effect on the capacity of the CD4+ T-cell populations involved in IL-2 production [K31].

354. A decreased proportion of mature CD3+ T-cells was found in peripheral blood lymphocytes among the atomic bombing survivors exposed to >1.5 Gy, particularly in the proportion of the CD4+ CD45RA+ naive T-cell subset. The frequency of a rare T-lymphocyte subpopulation bearing CD3 surface antigen and TCR ( $\alpha$  and  $\beta$  chains), but lacking



both CD4 and CD8 (double-negative CD4<sup>-</sup>CD8<sup>-</sup>  $\alpha\beta$ ), was studied in 409 atomic bombing survivors (160 who had been exposed to  $\geq 1.5$  Gy and 249 controls). The frequency of CD4<sup>-</sup>CD8<sup>-</sup>  $\alpha\beta$  T-cells was significantly elevated in individuals exposed to  $>1.5$  Gy [K37]. The authors interpreted this finding as a result of altered differentiation and development of T-cells. This rare T-cell population may be differentiated through a pathway different from that of conventional CD4<sup>+</sup> or CD8<sup>+</sup>  $\alpha\beta$  T-cells [K32].

355. Alterations of T-cell population subsets were confirmed in further studies. The proportions of subsets of T-, B- and NK cells in peripheral blood lymphocytes of atomic bombing survivors were studied by flow cytometry analysis [K29]. Blood samples from 159 survivors estimated to have received  $>1.5$  Gy and from 252 controls were evaluated using multiple combinations of monoclonal antibodies to lymphocyte differentiation antigens. The findings revealed that the proportion of CD4<sup>+</sup> T-cells was decreased significantly in the heavily exposed survivors and that a similar tendency was apparent for the CD4<sup>+</sup> CD45RA<sup>+</sup> naive T-cell subset. No significant changes were found in the proportion of CD8<sup>+</sup> T-cell subsets between exposed individuals and controls. Also, a dose-dependent increase in the frequency of PHA-stimulated lymphocytes bearing chromosome aberrations was reported in 1975 [A15].

356. The high sensitivity of CD4<sup>+</sup> CD45RA<sup>+</sup> naive T-cells to ionizing radiation in long-term studies appears to involve functional defects in T-cell response or demonstrable impairment in CD4<sup>+</sup> T-cell immunity. In T-cells from 723 atomic bombing survivors, almost uniformly distributed with respect to age, sex and dose, the ability of T-cells to proliferate in vitro was tested after a challenge by each of the *Staphylococcus aureus* toxins SEB, SEC-2, SEC-3, SEE and TSST-1. The results revealed that the proliferative responses of the T-cells of the atomic bombing survivors became progressively weaker as the radiation dose increased, and that they did so in a manner that correlated with the decrease in the percentage of CD4<sup>+</sup> CD45RA<sup>+</sup> (naive) T-cells, but not with that of CD4<sup>+</sup> CD45RA<sup>-</sup> (memory) T-cells. These findings indicated that irradiation from the atomic bombings led to impairment of the ability of exposed individuals to maintain their naive T-cell pools, explaining why they responded poorly to toxins encoded by common pathogens [K26].

357. A dose-dependent increase in the relative risk of myocardial infarction has been observed in RERF's Adult Health Study cohort of atomic bombing survivors [K43]. The effects of ionizing radiation on conditions other than cancer have previously been reviewed in the UNSCEAR 1982 [U8] and 1993 [U5] Reports. This subject is now extensively reviewed in annex B, "Epidemiological evaluation of cardiovascular disease and other non-cancer diseases following radiation exposure". However, some data will be discussed here concerning the hypothesis of a causal relationship between immune dysfunction and myocardial infarction in atomic bombing survivors.

358. The T-cells of survivors with a history of myocardial infarction responded poorly to *Staphylococcus aureus* toxins, and these individuals had proportionally fewer CD4<sup>+</sup> CD45RA<sup>+</sup> (naive) T-cell populations than survivors with no myocardial infarction in their history [K26]. It had previously been reported that among 1,006 survivors uniformly distributed with respect to age, sex and dose, 18 persons had a history of myocardial infarction; the proportion of CD4<sup>+</sup> cells was significantly decreased with increased dose and history of this disease. Further, the prevalence of myocardial infarction was significantly greater in those individuals who had a lower proportion of CD4<sup>+</sup> helper T-cells [K28].

359. Kusunoki et al. suggested that myocardial infarction in atomic bombing survivors may be due at least in part to their having diminished ability to mount an immune response against certain infections that may be implicated in the aetiology of cardiovascular disease [K36]. However, this inference may be premature. Blood samples for determination of the proportion of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells were taken between 1992 and 1995, while histories of myocardial infarction were recorded from 1958 to 1990. Although Kusunoki et al. assumed that measurements of T-cell subsets before disease onset would have shown similar values to those obtained in their study, in order to demonstrate a causal relationship they had to carry out a prospective study to record newly diagnosed cases and compare the incidence between subjects exhibiting low and normal CD4<sup>+</sup> T-cell proportions. Changes in the proportion of CD4<sup>+</sup> T-cells have been described in patients with acute myocardial infarction and post-myocardial-infarction syndrome without antecedents of radiation exposure [A19, B26, K53, T15]. Although some of these changes were exhibited only temporarily, long-term effects may also exist. It is thus possible that CD4<sup>+</sup> T-cell deficiencies in survivors with myocardial infarction history are, at least in part, of a fundamental nature rather than being totally attributable to radiation exposure.

360. Kusunoki's observation is reminiscent of an earlier report by Roberts-Thomson et al. [R7], who examined the number of positive delayed-type hypersensitivity reactions and the number of deaths among study participants aged over 80 years. They found that those who manifested fewer than two positive reactions had significantly greater mortality over the two-year period of study than those who showed two to five positive reactions. Bronchopneumonia, cerebrovascular accidents and cardiac failure were the most commonly recorded causes of death; no deaths were attributed to cancer, suggesting that reduced T-cell-mediated immune responsiveness resulting from earlier exposure to radiation could cause diseases other than cancer related to old age by as yet unknown mechanisms.

361. No dose-response relationship was found between anti-hepatitis-C-virus (HCV) seropositivity and radiation dose in atomic bombing survivors. The relative risk of chronic liver diseases among anti-HCV-positive individuals was marginally increased with radiation dose. The authors interpreted their findings with the hypothesis that radiation

exposure may accelerate the progress of chronic liver disease associated with HCV infection [F8]. The rates of seropositivity for hepatitis B surface antigen (HBsAg), which indicates current hepatitis B virus (HBV) infections, and anti-HBV core antibody, which indicates either cured or current infections, increased with radiation dose among 6,121 atomic bombing survivors. However, no relationship was observed between radiation and anti-HBV surface antibody, indicating cured infection, suggesting a lower likelihood of clearance after HBV infection among those who were more likely to have been infected with HBV as adults after irradiation from the atomic bombings [F7]. Taking into account that Th1 responses are able to clear hepatitis virus infections very efficiently, these findings could be interpreted to be the result of a persisting radiation-induced Th1/Th2 imbalance promoting chronic infection [K23].

362. As discussed earlier, a large and diverse TCR repertoire, necessary for recognition of the many antigenic peptides possible, is a critical feature of T-cell populations. Kusunoki et al. [K25] evaluated whether the recovery of CD4+ T-cell populations in atomic bombing survivors was associated with a long-term reduction in the diversity of the TCR repertoire. Using a panel of monoclonal antibodies against 13 TCR V  $\beta$  families, they employed flow cytometry to analyse peripheral blood samples from 710 survivors, distributed almost uniformly with respect to age, sex and dose (controls, <0.0005 Gy; exposed,  $\geq$ 0.0005 Gy). They defined a parameter referred to as “repertoire deviation”, which expressed the extent to which the TCR V  $\beta$  repertoires of T-cells from a given individual deviated from the mean for the whole population. The naive helper T-cell pools (CD4+ CD45RA+) of exposed individuals showed a dose-dependent decline without changes in their TCR V  $\beta$  repertoires. In contrast, the percentages of memory helper T-cells (CD4+ CD45RA-) did not decline, but their TCR V  $\beta$  repertoires were skewed in a dose-dependent manner among individuals who were at least 20 years of age at the time of the bombings.

363. Normally, since fewer new T-cells emerge from the thymus in the elderly, naive T-cell pools gradually become smaller with age. In contrast, memory T-cells that are lost tend to be replaced through clonal expansion. Thus the memory T-cell pool remains almost constant in size but gradually loses the diversity of TCR repertoire with age. Recovery of T-cell populations after radiation-induced depletion involves two different pathways: the production of new T-cells from thymus stem cells and the proliferation of peripheral mature cells that have managed to survive. The observations by Kusunoki et al. [K25] lead to the conclusion that restoration of the peripheral T-cell pools of atomic bombing survivors involved these two different pathways, and suggest that ionizing radiation accelerated the normal processes of immunological ageing.

364. Radiation exposure from the atomic bombings was demonstrated to be associated with long-lasting deficits in both naive CD4+ and CD8+ T-cell populations. Statistically

significant dose-dependent decreases in the percentages of naive CD4+ and CD8+ T-cells were found in the peripheral blood lymphocyte populations of 533 Hiroshima atomic bombing survivors. In contrast, while the percentages of memory CD8+ T-cell subsets were found to increase with radiation dose, no changes were observed in the percentages of memory CD4+ T-cell subsets [Y2].

365. The numbers of peripheral blood lymphocytes belonging to different subsets were studied in 1,328 atomic bombing survivors using immunocytochemistry (fluorescent antibodies) [K24]. A decreasing trend in the numbers of CD4+ and CD8+ T-cells and in CD19+ B-cells was observed with increasing age. The CD5 molecule has an important role in T-cell/B-cell interactions [B25]. The number of CD5+ B-lymphocytes was significantly lower in those persons exposed to >1 Gy within the group exposed at the age of 30 years or later. A similar tendency towards decreased numbers of CD4+, CD8+ and CD19+ cells was observed in these older survivors, although the differences were not statistically significant. These results suggest that ageing of the T-cell-related immune system is accelerated in people irradiated at an advanced age. Owing to the age-related decrease in thymic function, subjects who were older at the time of the bombing may have decreased functional capability of the immune system for recovery after radiation injury.

366. In a further paper reporting flow cytometric analyses of the lymphocyte subsets from atomic bombing survivors, Kusunoki et al. [K29] demonstrated a significant increase in the proportion of B-lymphocytes in heavily exposed survivors. The increase was evident in both positive and negative cells for CD5 antigen (a marker of mature B-cells), as well as in both positive and negative cells for CD23 antigen (a marker of stimulated B-cells). The discrepancy between these results and those from earlier studies [K24] may be due to a difference in the measurement method.

367. NK cell numbers were studied in atomic bombing survivors using immunocytochemistry. The NK cell population was found to be increased significantly in the older compared with the younger age group, but there was little dependence on dose [K24]. These results are in good agreement with a further study carried out using flow cytometry that did not find any effect of radiation dose on the proportion of NK cell subsets [K29]. Concerning NK cell activity, no significant changes were observed in a study of 1,341 atomic bombing survivors [B5].

368. Several studies have been carried out since 1968 concerning serum immunoglobulin levels. Data published by Hall et al. [H2] and by King et al. [K12] in 1973 showed no relationship between radiation dose and serum immunoglobulin levels in the cohort of atomic bombing survivors at that time. Fujiwara et al. [F4] determined the levels of autoantibodies and immunoglobulins among 2,061 individuals exposed to radiation from the atomic bombings in Hiroshima and Nagasaki for whom dose estimates ranged from 0 to 5.6 Gy. They found: a significant increase in IgA levels

in females, a significant increase in IgM levels in both sexes, and no changes in the prevalence of antinuclear antibody, antithyroglobulin antibody and antithyroid microsomal antibody, or in levels of IgG and IgE. These results were recently confirmed by Hayashi et al. [H10].

369. In 1994 Nagataki et al. reported an increase in the prevalence of antibody-positive hypothyroidism among atomic bombing survivors in Nagasaki [N2]. In contrast, the Adult Health Study by the Atomic Bomb Casualty Commission/RERF reported a lack of dose-effect relationship on the prevalence of autoantibodies [F4]. Imaizumi et al. have recently re-evaluated these observations and found that the prevalence of hypothyroidism with autoantibodies marginally increased among moderately exposed survivors but not among highly exposed survivors in Nagasaki. They concluded that there is no statistically significant dose response in the prevalence of antithyroid autoantibodies or hypothyroidism with autoantibodies in atomic bombing survivors [I8].

370. In their studies of somatic mutations, Kushiuro et al. [K22] used a flow cytometric assay to identify both gene mutation and somatic recombination. In 168 Adult Health Study participants and 58 employees of RERF, the frequency of variant lymphocytes lacking expression of HLA-A2 or HLA-A24 allele products was about  $10^{-4}$  in heterozygous donors and increased with donor age. Possible mutant cells lacking expression of HLA-A2 or HLA-A24 were isolated and clonally propagated. Molecular analysis of 164 clones derived from six donors revealed that 71–100% were defective in expression of the selected HLA-A alleles. On the other hand, 80% of the clones consisted of CD4+ T-cells, and the remainder were CD8+ T-cells. The effect of exposure to radiation from the atomic bombings was not statistically significant. However, while 1 out of 50 mutants in vivo derived showed a possible small deletion at the *HLA*-locus without any chromosomal abnormality, the majority of mutants derived from in vitro X-irradiated peripheral blood mononuclear cells bore important deletions [K22].

371. As already discussed, the *HLA* gene seems to be a particularly important genetic factor that can affect host immune response. Significant differences in type 2 diabetes prevalence were found between heavily exposed (>1.5 Gy) and low-dose or non-exposed Hiroshima atomic bombing survivors with different class II *HLA DQA1* and *DRB1* alleles [H12]. The prevalence was higher for heavily exposed individuals who were less than 20 years old at the time of the bombing and who presented *DQA1\*0401* and *DRB1\*08* alleles or *DQA1\*0301* and *DRB1\*09*. These results suggest that certain class II *HLA* genes regulate one or more components of the immune system related with the risk of diabetes development among the younger and more heavily exposed survivors. This was the first report suggesting that the development of a particular disease can be affected by radiation exposure in individuals with different genetic backgrounds.

372. The possibility of various degrees of radiation-associated immune suppression being dependent on HLA

type has been addressed. To investigate the possibility of differing frequency distributions of HLA type in the Hiroshima cohorts, *HLA-DQA1* alleles and *HLA-DR* antigens were typed for 201 survivors exposed to >1.5 Gy, 339 exposed to between 0.005 and 1.5 Gy, and 388 in a distally exposed group (<0.005 Gy). Although no dose-related differences were found, when the subjects were grouped by the presence of a specific allele or antigen, males carrying *DQA1\*0103* in at least one of their two *HLA-DQA1* loci exhibited frequency distributions that decreased as radiation dose increased [H9].

373. In earlier work, Kusunoki et al. [K27] had demonstrated that mutant lymphocytes lacking expression of class I HLA molecules were eliminated by autologous NK cells. In an attempt to explain the inability to detect any increase in HLA-A2 negative cell number in HLA-A2 heterozygous individuals exposed to irradiation from the atomic bombings, the hypothesis was tested that HLA mutant lymphocytes might well have been induced by radiation exposure but eliminated by strong negative selection associated with their almost inevitable exposure to autologous NK cells [K30]. The results strongly supported the hypothesis that autologous NK cells are responsible for the elimination of mutant lymphocytes that have lost the ability to express self class I HLA molecules in vivo, and therefore might explain why it has not been possible to detect increased frequencies of HLA-A2 mutants in samples from any of the 164 atomic bombing survivors whose HLA-A2 heterozygote status made their lymphocytes suitable for such a test.

374. Kodama et al. wanted to investigate whether radiation exposure had induced chromosomal instability in peripheral lymphocytes. Ordinary cytogenetics was not expected to help them solve this problem, as lymphocyte stable aberrations (mainly translocations) induced at the time of the bombings would not be distinguishable from those that may have arisen later as a result of the instability. Therefore they studied clonally expanded T-cell populations, e.g. cells bearing identical translocations, because they are descendants of a single progenitor cell which acquired aberrations as a result of radiation exposure. By determining the frequency of additional translocations among clonal cells, the authors found that clonally expanded T-cell populations of atomic bombing survivors do not exhibit increased chromosomal instability [K44].

375. It has been argued that chronic low-level inflammatory responses induced by radiation could be a significant risk factor in the well-documented increase of non-cancer disease occurring in atomic bombing survivors. Hayashi et al. investigated the long-term effects of ionizing radiation on the levels of two markers of inflammatory response, C-reactive protein (CRP) and IL-6, in blood samples from 453 participants in an epidemiological cohort of atomic bombing survivors [H8]. Blood lymphocyte subpopulations were identified by flow cytometry, using monoclonal antibodies to CD3, CD4 and CD8. CRP levels were significantly increased, by about 35% Gy<sup>-1</sup> ( $p = 0.0001$ ). After adjustment

for confounding factors (sex, age, etc.), CRP levels were still increased significantly with dose, by 28% Gy<sup>-1</sup> ( $p = 0.0002$ ). IL-6 levels also increased with radiation dose, by 9.3% Gy<sup>-1</sup> ( $p = 0.0003$ ) and, after multiple adjustments, by 9.8% at 1 Gy ( $p = 0.0007$ ). Elevated CRP and IL-6 levels were associated with decreases in the percentages of CD4+ T-cells in the peripheral blood lymphocyte population [H8]. Hayashi et al. recently reported long-term effects of radiation dose on inflammatory markers in atomic bombing survivors. They found that erythrocyte sedimentation rate, IFN- $\gamma$ , TNF- $\alpha$  and IL-10 increased significantly with radiation dose [H10].

#### 4. Remarks concerning data on survivors of the atomic bombings

376. The preceding review of data concerning atomic bombing survivors shows that short-term effects of radiation on the immune system were expressed mainly as dose-dependent acute bone marrow depletion due to radiation-induced cell death. These effects were reversed over several months, and studies initiated shortly after the bombings showed few dose-dependent effects on the immune system [A6].

377. Studies of the late effects of radiation on the immune system commenced about 20 years after the atomic bombings. The most remarkable late effects of radiation were functional and quantitative abnormalities of T- and B-cells in survivors exposed to high doses ( $\geq 1$  Gy). The main findings observed up to 1995 have been reviewed and summarized by Akiyama [A6]. With respect to data published thereafter, the most remarkable late effects of radiation on the immune system of atomic bombing survivors are summarized in the following paragraphs.

#### 378. Effects on T-cell immunity:

- Decreased proportion of CD3+ CD4+ TCR $\alpha\beta$  + T-cells;
- Decreased proportion of CD3+ CD8+ TCR $\alpha\beta$  + T-cells;
- Decreased proportion of CD3+ CD4+ CD45RA+ naive T-cells;
- Non-significant changes in the proportion of CD3+ CD4+ CD45RA- memory T-cells;
- Skewed TCR repertoires of CD3+ CD4+ CD45RA- memory T-cells in individuals exposed in adult life;
- Decreased proportion of CD3+ CD8+ CD45RA+ naive T-cells;
- Increased proportion of CD3+ CD8+ CD45RA- memory T-cells;
- Increased frequency of CD4- and CD8- (double-negative)  $\alpha\beta$ + T-cells;
- No change in the proportion of CD3+ TCR  $\gamma\delta$ + T-cells;

- Functional defects in T-cell responses to mitogens and alloantigens.

#### 379. Effects on B-cell immunity:

- Significant increase in the proportion of B-cells;
- Increase in serum IgA levels in females;
- Increase in IgM levels in both sexes;
- No changes in IgG and IgE levels.

#### 380. Effects on innate immunity:

- In contrast with acquired immunity, significant dose effects were not observed on the number and function of NK cells. However, some studies showed an increase in the proportion of NK cells in the peripheral blood of atomic bombing survivors.

#### 381. Other findings:

- Increased frequencies of somatic mutations (TCR and HLA) and chromosome aberrations;
- Marginal increase with radiation dose in the prevalence of chronic liver diseases and hepatocellular carcinoma among anti-HCV-positive individuals;
- Decreased cellular immunity and enhanced humoral immunity may have led to long-term imbalance in Th1/Th2 responses, resulting in altered cytokine expression profiles;
- Qualitative and quantitative changes suggesting a radiation-induced acceleration of the normal process of immunological ageing;
- Radiation-associated chronic inflammatory responses;
- Decreased proportion of CD4+ cells with increased dose and history of myocardial infarction, and higher prevalence of myocardial infarction in those survivors who had a lower proportion of CD4+ cells;
- Differences in type 2 diabetes prevalence in heavily exposed individuals with two particular class II HLA *DQA1* and *DRB1* alleles.

## B. Chernobyl workers and residents

### 1. General considerations

382. The accident at the Chernobyl nuclear power plant (NPP) on 26 April 1986 resulted in both acute and long-lasting health effects. Early and late immune system changes were among the key points studied after the accident. Acute radiation effects in victims were extensively described in an appendix to the UNSCEAR 1988 Report [U6]. Further information about the immunological effects of exposure to radiation from the Chernobyl accident as they were known up to



the year 2000 was provided by the Committee in reference [U2]. The Committee is currently updating its assessment of the health consequences of the accident. However, some data concerning immunological issues will be discussed here. A broad spectrum of immune abnormalities had been reported among Chernobyl victims; however, it has not been possible to interpret these results, since it was unclear whether all possible confounding factors (such as heavy metal contamination, infections and diet) had been taken into account.

383. Early changes in immune parameters were characterized by alteration of the amount or function of peripheral lymphocytes and changes in serum immunoglobulins. The data indicate that radiation-induced effects on the immune system remained detectable for a considerable period after the accident. The available data vary widely, according to the characteristics of the populations studied, the dose received and its protraction in time, the mode of exposure (external irradiation and/or internal contamination) and the time elapsed since the accident.

384. Immunological monitoring of persons affected by the Chernobyl accident comprised two main groups:

- Individuals who worked at the NPP during the emergency and/or participated in further clean-up activities;
- Residents of contaminated areas, particularly children living in several settlements around the NPP, who were included in the Children of Chernobyl Project, as requested by the Ukrainian Government.

Different parameters have been evaluated and several phases may be identified in these two groups concerning the temporal behaviour of the immunological effects.

## 2. Emergency and clean-up workers

385. The workers involved in the recovery and clean-up after the Chernobyl accident were subjected not only to external and internal radiation exposure but also to other non-radiation factors that may have affected their health. As a consequence of the accident, different heavy metals were deposited at the reactor site, with a significant amount being vaporized and distributed in rain clouds. Higher blood concentrations of iron, zinc and lead have been found in workers who took part in cleaning up after the accident [N9]. It could be hypothesized that some of the observed changes in the immune status of clean-up workers might be due to a combined action of ionizing radiation and heavy metal contamination. [B19, G10]. Moreover, these workers suffered strong psychological stress, which may significantly affect the immune system [G21].

386. Yarilin et al. [Y4] evaluated disorders in T-cell subpopulations 5 years after the accident in two groups of workers from the Chernobyl NPP accident, the first being

workers without manifestations of acute radiation syndrome (ARS), for which total doses from external irradiation were 0.1–0.5 Gy (group 1), and the second being individuals who survived ARS, for which total doses from external irradiation were 0.5–9 Gy (group 2). Decreases in the percentage and absolute number of CD3+ T-cells were observed in both groups. A decrease in the percentage and absolute number of CD8+ T-cells was observed only in group 1 (which had lower doses). A decrease in the percentage and absolute number of CD4+ T-cells was evident only in heavily exposed people from group 2.

387. Titova et al. found that the mean number of CD8+ T-cells was decreased in personnel working in the 30 km control zone around the NPP [T6]. The absolute number of CD4+ T-cells was also decreased, but their percentage remained higher. Kurjane et al. characterized the immune status of a group of Latvian workers who received external radiation doses of 0.01–0.5 Gy [K21]; this study was performed 10–14 years after the accident. A significant decrease was observed in the number of CD3+ T-cells. This study also demonstrated that both CD4+ and CD8+ T-cells were decreased. A study performed by Kuzmenok et al. [K33] 11–14 years after the accident did not, however, find significant changes in the phenotypic characterization of the main subpopulations of peripheral lymphocytes in a group of Belarussian clean-up workers who received mean doses of 0.15–0.5 Gy.

388. Other lines of evidence point to the role of serum inhibitory factors that down-regulate T-cell surface antigen expression and that could regulate T-cell differentiation *in vitro*. It has been reported that the decreased percentage of CD4+ helper-inducer T-lymphocytes of recovery operations workers returned to normal values after 3 days of culture without activation, suggesting that the phenotype may be under the control of suppressive soluble factors in the blood of these individuals [K33]. The fact that the percentage of CD4+ T-cells in control samples did not change after culture reinforces this hypothesis.

389. A decline in the number of CD3+ and CD4+ T-cells, with augmentation of the percentages of CD8+ T-cells and CD16+ CD56+/NK cells, was observed after 3 days of culture with the polyclonal activator PHA at optimal concentration ( $10 \mu\text{g mL}^{-1}$ ). This activation-induced deviation in the maturation of T-cell subpopulations may be a consistent characteristic of impaired T-cell immunity in clean-up workers [K33].

390. The functional status of T-cells plays a key role in the immune regulation of human pathology. The proliferative response of lymphoid cells to mitogens was also altered in recovery operations workers. Kuzmenok et al. [K33] reported a significant decrease of the response of peripheral blood mononuclear cells to PHA and the phorbol ester PMA. Neither the TCR-restricted proliferative response of T-cells nor the level of Con-A-induced proliferation was significantly decreased.



391. The proliferative response of peripheral blood mononuclear cells to exogenous IL-2 was higher in recovery operations workers, which may indicate an up-regulation of the IL-2 receptor CD25 in their cells [K33]. Interleukin-2 (IL-2) is a cytokine responsible for a variety of immune stimulatory and regulatory functions, including activation and stimulation of cytotoxic cells able to recognize and kill human tumour cells, and T-cell proliferation and differentiation. Similar results had previously been obtained by Xu et al. in an experimental model of low-dose (0.25–10 mGy) *in vitro* irradiation [X1].

392. Changes in cell subpopulations have been reported as evidence of radiation-induced disturbance of the T-cell system [W5]. An increase in peripheral blood CD3+ CD16+ CD56+ NKT cells, a small subpopulation of lymphocytes that exhibits certain characteristics of both T-cells and NK cells and that can be the source of an abnormal pattern of cytokines, was observed in Belarussian clean-up workers [K33].

393. One of the best described effects of the Chernobyl accident on the immune system was the increased level of monocytes in peripheral blood. Senyuk et al. evaluated the long-term effects of ionizing radiation in Chernobyl recovery operations workers [S13]. In many cases, the cumulative gamma radiation dose was >0.5 Gy. The authors found a high number of monocytes with an increase in plasma levels of cytokines IFN- $\alpha$  and TNF.

394. Functional changes have been reported in the monocytes and macrophages of clean-up workers. T-cell activation implies cooperation between the APCs and T-cells. The functions of monocytes as APCs were compared in clean-up workers and control individuals. Impaired function of monocytes as APCs was found in the T-cell proliferation assay. Indeed, allogenic monocytes purified from clean-up workers significantly augmented the proliferative response to mitogens of T-cells from control individuals but inhibited proliferation of T-cells from clean-up workers. In contrast, allogenic monocytes purified from healthy donors marginally augmented the proliferative response to mitogens from both clean-up workers and control individuals [K33].

395. Yarilin et al. evaluated the effect of ionizing radiation on the thymus and its role in radiation-induced T-cell disorders in victims of the Chernobyl NPP accident. A decrease in serum concentration of thymosin alpha-1 and serum thymic activity (STA) level was found with increased titres of autoantibodies to epithelial cells of the thymus [Y4]. This titre was higher in persons with lower levels of thymosin alpha-1. The dynamics of post-irradiation recovery of CD4+ and CD8+ cells were different. It had been suggested previously that preferential CD4+ cell deficiency might result from radiation-induced damage to the thymus [A12]. The good correlation observed by Yarilin et al. between thymic hormone and CD4+ cell levels would confirm this hypothesis [Y4].

396. Yarilin's results are in good agreement with those of Titova et al. [T6], who also found lower levels of thymosin alpha-1 and an increased serum level of antithymic epithelium autoantibodies in personnel from the 30 km control zone around the Chernobyl NPP. The dose-dependent decrease of the serum level of thymosin alpha-1 and STA observed 5 years after the accident indicates late impairment of thymic function, which could be a result of a disturbance of thymic epithelial cell renewal. Thymus dysfunction after low doses could be due to the action of anti-epithelial autoantibodies induced by the release of antigenic material from the cells damaged, even minimally, by ionizing radiation.

397. Thomas et al. [T4] performed a multiple end point study comparing hypoxanthine phosphoribosyltransferase (HPRT) mutation frequency with chromosome translocations in peripheral blood lymphocytes of clean-up workers. When adjusted for age, smoking status and year of sampling, the authors demonstrated a significant increase in HPRT mutation frequency in clean-up workers, with a dependence on time elapsed since radiation exposure (a decline of 4.4% per year). They found little difference in the overall deletion spectra. However, they observed a decline in the average size of deletions of clean-up workers as time after exposure increased from 6 to 13 years. Jones et al. found an increase, more than 5 years after the accident, of chromosome translocations and in HPRT mutation frequency in peripheral lymphocytes of clean-up workers with absorbed doses of below 0.25 Gy [J4].

398. Chumak et al. demonstrated an accumulation of autoxidized lipoxygenase products of polyunsaturated fatty acids in the peripheral blood mononuclear cells of 23 clean-up workers with absorbed doses of below 0.3 Gy [C10]. They observed higher levels of free and esterified fatty acids, with a positive correlation between the absolute number of CD4+ T-cells and the amount of 15-hydroxyeicosatetraenoic acid (15-HETE) in the phospholipid fraction. The percentage of CD4+ T-cells was higher in heavily irradiated workers, and the percentage of CD8+ T-cells tended to decrease with dose.

### 3. Residents of contaminated areas

399. Titov et al. investigated the production of immunoglobulins in children living around the Chernobyl NPP. They found a decrease in B-cell numbers, a transient decrease of IgM and IgG and an increase of IgA levels (in both serum and saliva) during the first months following the accident. Over a six-year period of living in contaminated areas, children exhibited increasing production of IgG and IgM. A correlation was found between the changes in B-system immunity and the levels of <sup>137</sup>Cs contamination. There was also a strong correlation between the production of natural (heterophilic) antibodies and dose to the thyroid due to incorporation of <sup>131</sup>I (in the range 0.1–1 Gy). Higher accumulation of <sup>131</sup>I resulted in decreased titres of these antibodies. High levels of heterophilic antibodies correlated with high levels of IgE. Altered production of subclasses

of IgG associated with the increasing biosynthesis of IgE suggests that T-cells may have been driven towards the Th2 profile [T5]. However, trying to find a causal association between radiation exposure and these changes in Th2 profile would be speculative, because no data about confounding factors such as parasite infections in these children were taken into account.

400. Chernyshov et al. examined peripheral blood lymphocyte subsets eight years after the accident in children living around the Chernobyl NPP [C9]. They evaluated children living in 15 contaminated settlements in Ukraine, with and without recurrent respiratory disease (RRD), and a control group of children living in non-contaminated areas, again with and without RRD. The average dose (internal plus external contributions: 0.57–3.09 mSv) was calculated on the basis of the average density of contamination with  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$ ; no data were included concerning thyroid doses due to  $^{131}\text{I}$  incorporation. Lower percentages of CD3+ T-cells were observed in RRD children from contaminated areas than in control RRD children. Lower percentages of CD3+ CD4+ (helper) T-cells were observed in RRD children from contaminated areas than in control RRD children. RRD children from contaminated areas exhibited lower percentages of CD3+ T-cells and CD3+ CD4+ (helper) T-cells than healthy children. Healthy children (no RRD) from contaminated areas had the same mean values for lymphocyte subsets as control healthy children. However, a wider range of percentage levels of CD3+ CD4+ T-cells was found among healthy children from contaminated areas, which allowed them to be divided into three subpopulations: children with very low, normal or very high percentage levels of CD3+ CD4+ T-cells. The other lymphocyte subsets studied did not differ among groups.

401. Children from contaminated areas had a healthy population (non-RRD) without major lymphocytic manifestations of immune disorders and an RRD population that exhibited lower percentages of total T-cells and helper T-cells than RRD controls. This decrease was more marked in RRD children with higher doses [C9]. This dose dependence provided strong evidence for a radiation-induced effect. The question is what accounts for the difference in immune response observed between exposed children who are healthy and those with RRD. It may be that repeated exposure to pathogenic or antigenic stimuli is necessary for the development of radiation-induced immune disturbances. Moreover, individual features (including genetic factors) are involved in the sensitivity of immunocompetent cells to ionizing radiation. It could be proposed that children predisposed to RRD have an impaired immune response and may exhibit greater radiosensitivity. Although this study did not consider thyroid doses, it is well known that the region where the study was performed (northern Ukraine) had a high level of  $^{131}\text{I}$  contamination. It could be hypothesized that healthy children from contaminated areas exhibited a higher percentage of CD3+ CD4+ T-cells owing to clinical and subclinical autoimmune disorders related to radioiodine incorporation.

402. The same group of researchers published further results that confirmed previous findings and showed in addition that children with RRD from contaminated areas had higher levels of CD3– CD56+ CD16+ NK cells than did RRD children living in non-contaminated regions [V8]. It is interesting to note that children examined 8–10 years after the accident exhibited a more marked decrease in CD4+ cells than those with the same dose but who were examined at 5 years after the accident. This finding shows that long-term exposure to low doses (i.e. a time effect) rather than low-dose radiation exposure itself (i.e. a dose effect) altered the composition of peripheral blood lymphocyte subsets in children with RRD living in contaminated areas. Although it has been suggested that CD4+ T-cells are relatively radioresistant in vitro [S8], it may be that the combination of repeated exposure to antigenic stimuli together with long-term exposure to low doses of ionizing radiation leads to CD4+ T-cell depletion. Considering that NK cells have a lower in vivo radiosensitivity [C11, L21], their relative increase in children with RRD from contaminated areas could also be an effect of long-term low-dose exposure. The different responses of blood lymphocyte subsets could contribute to the lower risk of developing autoimmune thyroid abnormalities in children with RRD living in contaminated areas.

403. Koike et al. compared NK activity in children living in Gomel, a highly contaminated area, with that of children living in non-contaminated areas [K15]. While children living in non-contaminated areas exhibited a narrow range of NK cell cytotoxicity percentages, a wider range of NK cell cytotoxicity (from 8.9% to 76%) was found in children from contaminated areas. The NK cell cytotoxicity of these children was correlated neither with NK cell number nor with the amount of internal contamination by  $^{137}\text{Cs}$ . The authors interpreted these findings as a loss of the normal regulatory mechanisms that maintain a correlation between cytotoxic activity and NK cell number. It seems a rather speculative conclusion. Even if such dysregulation exists, it cannot be attributed to ionizing radiation. The lack of correlation between these abnormalities and the levels of internal contamination suggests that related factors, other than direct internal exposure to  $^{137}\text{Cs}$ , may be responsible. Diet and/or environmental exposure to some agent (such as heavy metals) might be proposed. The effect of external ionizing radiation from contaminated ground and the effect from radionuclides other than  $^{137}\text{Cs}$  remain to be determined.

404. Mikhalevich et al. investigated cytogenetic and mutational effects in the lymphocytes of children living in a contaminated region of Belarus nine years after the accident [M5]. Their results indicated a doubling of the percentage of micronuclei in the mononucleated lymphocytes of exposed children, while the same parameter studied in binucleated lymphocytes showed no differences. No evidence was found for induction of HPRT mutations.

405. An adaptive response of lymphocytes to radiation has been suggested by a number of in vitro studies indicating that cells can become less susceptible to radiation-induced

damage when a “challenge” exposure to ionizing radiation is preceded by a very low “priming” dose. Padovani et al. [P1] administered a challenge dose of 1.5 Gy to stimulated peripheral lymphocytes of children chronically exposed in contaminated areas around Chernobyl. They did not find any decreased susceptibility for the two end points examined (chromosome and chromatid aberrations). An important consideration relates to the dose rate at which the priming dose is delivered. Assuming a constant intake for 1 year, the average value of committed effective dose equivalent was 450  $\mu$ Sv (range 50–2,000  $\mu$ Sv); this dose rate is lower than that used in most published studies. Another point of concern is that the priming dose in this study took place in resting lymphocytes, and it has been reported that a radio-adaptive response cannot be induced in the G<sub>0</sub> stage of the cell cycle.

#### 4. Radioiodine contamination, immune status and thyroid diseases

406. A higher incidence of goitre has been reported among the Chernobyl clean-up workers. Kurjane et al. [K21] analysed several parameters of the immune system in 385 male Latvian residents who participated in the clean-up work at the Chernobyl site. The results were compared with those from 47 healthy age- and sex-matched controls. This group of clean-up workers received external doses of 10–500 mGy. No data were provided in this paper concerning thyroid doses due to radioiodine incorporation. Workers were exposed during 2–6 months, while working at Chernobyl, and then they lived in uncontaminated territories after they returned to Latvia. The prevalence of non-cancer thyroid diseases as determined in January 2000 was higher among clean-up workers (47 cases among 385 workers; 121/1,000), than in a non-exposed Latvian population (30/100,000), with goitre being the most frequent disorder. Diminished acquired cellular immunity (total CD3+ cells, CD4+ and CD8+ T-cells) was found among the Latvian clean-up workers. The phagocyte activity of neutrophils was significantly decreased. Lower levels of IgG and higher levels of IgM, without changes in IgA were also found in this group. The observed decrease in the percentage of CD16+ NK cells contrasts with previous reports suggesting that NK cells display radioresistance. It is important to note that blood lead concentration was six times higher in clean-up workers [K21].

407. Kurjane et al. [K21] also reported that some immune parameters of workers with thyroid diseases differed from those observed in workers without thyroid disease: a lower number of NK cells, higher IgG plasma concentrations and higher activation of the classical pathway of complement.<sup>2</sup>

<sup>2</sup>Note: These results are presented here as discussed by Kurjane et al. [K21] in the text of their paper. However, the Committee found a lack of correlation between these comments and the values presented by the authors in table 2 of their paper, which seem to be contradictory: a *higher* number of NK cells, *lower* IgG plasma concentrations and *lower* functional activity of the classical pathway of complement in clean-up workers with thyroid diseases. The Committee considered that this discrepancy might be due to a mistake in typing column headings.

Complement split product C3d was higher in both groups (i.e. with and without thyroid disease) of clean-up workers. Thyroid follicular cells are protected from lysis by locally activated complement. Although the underlying mechanism of the complement activation in clean-up workers is unclear, it may reflect a secondary radiation-induced inflammatory response. The question of whether this mechanism is also involved in the development of thyroid abnormalities among these workers remains unanswered.

408. Considering that the immune system is a vulnerable target for the effect of lead contamination [B19], the results presented for Kurjane et al. should be interpreted cautiously. It has been reported that CD16+ NK cells and CD4+ T-cells are vulnerable targets for the effects of lead [G10]. Elevated blood and urine lead concentrations were found in Kurjane’s group of clean-up workers. It could be concluded that even 10–14 years after exposure, a combined impact of both radiological and non-radiological factors could be observed on the immune system of Latvian workers: impairment of phagocytic activity, reduction of cell-mediated immunity parameters and a shift towards an inflammatory profile.

409. Kiseleva et al. reported an increase in serum levels of antithyroglobulin and microsomal fraction autoantibodies, with higher levels of circulating immune complexes in liquidators 11 years after the Chernobyl accident [K14]. Vykhoanets et al. investigated the involvement of auto-immune mechanisms in the development of thyroid abnormalities in the context of radioiodine exposure of children living around the Chernobyl NPP [V9]. The study, which was carried out 8 years after the accident, included children living in 15 contaminated settlements and control children living in non-contaminated areas. Individual absorbed doses to the thyroid due to radioiodine (<1 Gy, 1–2 Gy and >2 Gy) and average doses (internal plus external; range 0.57–3.09 mSv) due to <sup>137</sup>Cs and <sup>90</sup>Sr were estimated. A positive correlation was found between thyroid <sup>131</sup>I dose and serum AbTg levels, content of CD4+ T and CD4+/CD8+ ratio. In contrast, a negative correlation was observed between thyroid <sup>131</sup>I dose and CD8+ T-cells and NK cells. The lack of correlation between thyroid-stimulating hormone (TSH) levels and thyroid dose suggests that the higher levels of TSH found among children living in contaminated areas may be due to iodine deprivation in areas of endemic goitre. Children with individual absorbed doses of >2 Gy due to radioiodine have several signs of autoimmune disorder: abnormal thyroid echogenicity, positive sera for AbTg, higher levels of CD4+ T-cells (which play a central role in immune response and are able to increase immunoglobulin production by B-cells), lower levels of CD8+ T-cells and higher CD4+/CD8+ T-cell ratios, a frequent finding in autoimmune thyroid diseases [V9].

410. Two further studies [P10, V12] also reported a higher prevalence of antithyroglobulin or antithyroperoxidase antibodies in children living in contaminated areas; this was already apparent in individuals who were in utero or newborn at the time of the accident. Autoimmune phenomena were limited to an increased prevalence of circulating

thyroid autoantibodies, without evidence of significant thyroid dysfunction. It should be taken into account that children involved in these geographical correlation (“ecological”) studies may present a combined effect of iodine deficiency and internal contamination with short-lived iodine isotopes.

411. Koike et al. evaluated the immune status in children with goitre living in the highly contaminated area of Gomel. They found increased serum levels of IgG, IgM and IgE, and depressed NK cell activity [K15]. Although IFN- $\gamma$  and IL-2 enhanced the cytotoxicity of these NK cells, the response to IFN- $\gamma$  was still below control values. The other parameters were within normal values.

#### 5. Remarks concerning data on Chernobyl workers and residents

412. Some remarks may be made on the basis of the publications on the Chernobyl accident reviewed above. Even many years after the accident, the impacts of both radiological and non-radiological factors on the immune system were observed in Chernobyl recovery operations (clean-up) workers. Ionizing radiation accelerated the natural ageing of the immune system due to a progressively declining thymic function. The dynamics of post-irradiation recovery are different for CD4+ and CD8+ cells, and it has been suggested that preferential CD4+ cell deficiency may result from radiation-induced damage to the thymus.

413. Short-term as well as long-term effects were detectable in B-cell and T-cell function profiles, as well as in the biosynthesis of immunoglobulins in both serum and saliva, of children living in contaminated areas around the Chernobyl NPP. Some of these changes were dose-dependent and were characterized by phases. Indeed, immune profiles were different several months as opposed to several years after the accident. The NK cell system of these children may have lost the normal regulatory mechanisms that maintain a correlation between cytotoxic activity and NK cell number. Repeated exposure to pathogenic or antigenic stimuli seems

to be necessary for the development of radiation-induced immune disturbances. Individual features, including genetic factors, are involved in the sensitivity of immunocompetent cells to ionizing radiation. Illnesses associated with both radiation exposure and genetic factors could be determinants of the immune status after the accident.

414. The immunological effects of exposure to ionizing radiation from the Chernobyl accident were mainly related to changes in the amounts or function of peripheral lymphocytes and serum immunoglobulin levels. These effects were detectable long after the accident. The immune system seems to be involved in the pathogenesis of thyroid diseases in victims of the Chernobyl accident, probably owing to antigenic mechanisms being triggered by radiation-induced thyroid damage, leading to autoimmune responses. Neuroendocrine and other stress-related factors, respiratory diseases, chronic infections, chemical contamination and autoimmune dysbalance could also be factors in some of the immune disorders found in this population.

### C. Techa River study

#### 1. General considerations

415. The Techa River study is seen as an excellent opportunity to obtain more reliable risk estimates for a general population exposed over an extended period to low-dose-rate gamma rays. More than 25,000 inhabitants of Techa riverside villages were exposed, predominantly during the early 1950s, to external gamma radiation from fission products associated with discharges of high- and medium-level wastes into the river from the Mayak nuclear facility. In addition, residents incorporated, via drinking water and through the food chain, large activities of short-lived fission products such as  $^{89}\text{Sr}$ , and subsequently long-lived activity, especially from  $^{90}\text{Sr}$  and  $^{137}\text{Cs}$ . Mean and median doses to soft tissue and bone marrow according to the earlier Techa River Dosimetry System (TRDS-1996) and the revised dosimetry system (TRDS-2000) are shown in table 13.

**Table 13 Mean and median doses to soft tissue and bone marrow in inhabitants residing near the Techa River [K9]**

<i>Dosimetry system</i>		<i>Soft tissue dose (mGy)</i>	<i>Bone marrow dose (mGy)</i>
TRDS-1996	Mean	99	405
	Median	17	267
TRDS-2000	Mean	35	353
	Median	7	253

#### 2. Epidemiological data

416. In the early period, cases of chronic radiation sickness (CRS; 940 cases in total) were diagnosed. The diagnosis of

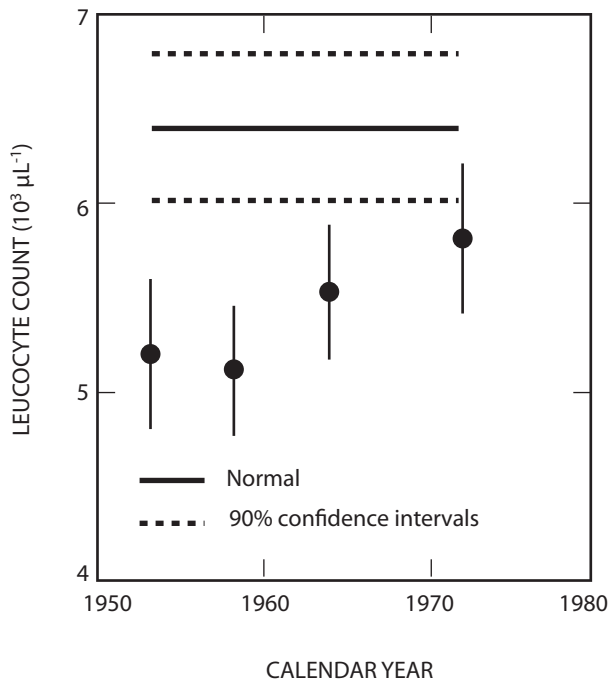
CRS was based on the occurrence of the following signs: changes in blood parameters (leucopenia, thrombocytopenia, granulocytopenia); nervous system disorders; ostealgia; cardiovascular syndrome; and changes in immunity



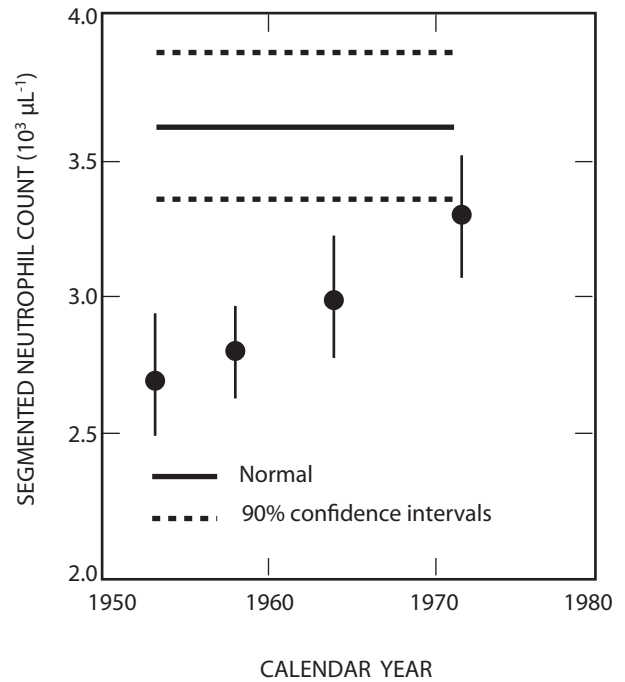
(inhibition of innate immunity, autoimmunity). At dose rates in excess of 300–500 mSv/a to red bone marrow, a portion of the irradiated population developed post-irradiation reactions of the haematopoietic and immunological systems [A8]. Some of the residents exposed did not develop CRS, but in the early years they manifested isolated reactions most commonly represented by haematological changes in peripheral blood studies. The dynamics of blood parameters clearly manifested a reduction in the number of cellular elements at the highest dose rates and a subsequent normalization with a decrease in dose rate. The average leucocyte counts for CRS patients persisted at lower than 90% confidence intervals of the reference value for three decades after the beginning of exposure. Only after 1970 did the difference in leucocyte counts between followed-up patients and reference

values disappear (figure IX). The dynamics of segmented neutrophils correlated with leucocyte dynamics, indirectly corroborating the decrease in leucocyte counts as associated with the decreased number of granulocytes (figure X) [K19]. According to the data for individuals with CRS, there was an increase in the fractions of myelocytes and metamyelocytes in the bone marrow, corresponding to the occurrence of leucopenia and granulocytopenia in peripheral blood. Such findings can be interpreted as delayed maturation and differentiation of granulocytes at the final stage of cell development. The subjects who were initially exposed in utero or at age 1–2 years showed the greatest changes in the immune system parameters already mentioned. Haematopoietic disturbances developed almost at the same time as the signs of immune insufficiency [K19].

**Figure IX. Mean leucocyte counts at different times after the beginning of exposure [K19].**



**Figure X. Mean neutrophil counts at different times after the beginning of exposure [K19].**



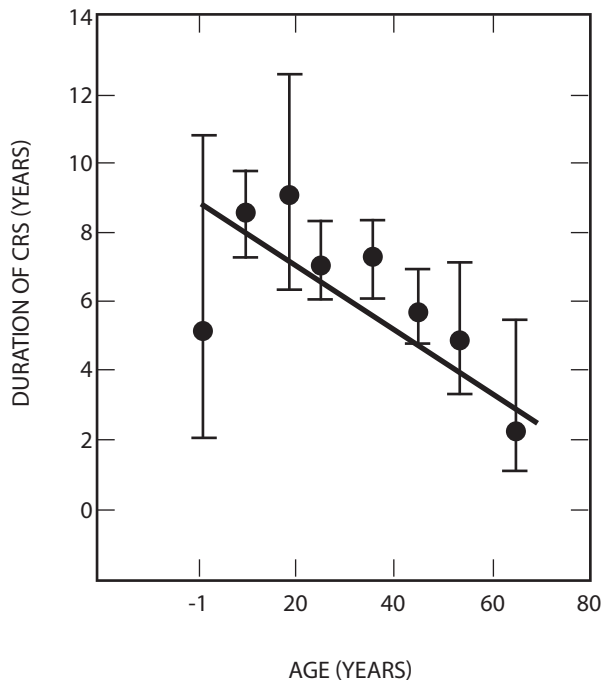
417. Recovery from these symptoms depended on exposure rates, with greater exposure leading to longer recovery times. Even after the cessation of external exposure and intakes of radionuclides, leucopenia and neutropenia persisted for a long period; this may have been due to incorporation of long-lived strontium, which contributed to irradiation and maintained a certain level of dose rate. The average duration of the disease was 7.35 years, but at doses in excess of 700 mSv to the red bone marrow, the repair process lasted for more than 9 years [K19]. Complete recovery from the haematological and neurological effects occurred within 13–16 and 14–20 years, respectively, following the beginning of exposure. The duration of the disease was presumably dependent on the patient's age at exposure. Recovery

processes developed more slowly in children and teenagers who received the highest doses, since a close dependence of exposure dose on age was observed. The age dependence of the duration of CRS is shown in figure XI [K19]. Immunity disorders persisted for 30 years and longer after the exposures began. Long-term immunity changes involved cellular immunity (decreased expression of differentiating antigens of T-lymphocytes, T-lymphocyte blast transformation), natural toxicity (reduced counts of NK cells) and signs of immunological imbalance. At long times (43–48 years) after the beginning of exposure, the status of haematopoiesis and immunity was normal among most of the exposed subjects. However, the proportions of the exposed persons still showed an increased frequency of chromosomal aberrations



and CD3-CD4+ mutant T-lymphocytes in the peripheral blood [A18]. An increased frequency of TCR mutant lymphocytes was also noted [A17].

**Figure XI. Dependence of CRS duration on age at time of exposure [K19].**



418. CRS diagnosed in a number of residents of Techa riverside villages was found to be associated with higher death rates from cancer and leukaemia. The Extended Techa River Cohort (ETRC) includes 29,873 people born before 1950 who lived near the river sometime between 1950 and 1960. Between the years 1950 and 1999, 1,842 solid cancer deaths and 61 leukaemia deaths occurred. The maximum incidence of leukaemia was observed 15–19 years after the exposure began. The excess relative risk per unit dose for solid cancer was 0.92 (95% CI: 0.2, 1.7) Gy<sup>-1</sup>, while the values for leukaemia, including and excluding chronic lymphocytic leukaemia, were 4.2 (95% CI: 1.2, 13) Gy<sup>-1</sup> and 6.5 (95% CI: 1.8, 24) Gy<sup>-1</sup>, respectively. It is estimated that about 2.5% of the solid cancer deaths and 63% of the leukaemia deaths were associated with the radiation exposure [K52]. Clinical manifestations of immune insufficiency with respect to certain infectious diseases (such as chronic pneumonia, pulmonary tuberculosis and non-traumatic osteomyelitis) occurred with higher frequency among exposed patients with tumours than in exposed individuals without tumours [A8].

419. The Techa River Offspring Cohort (TROC) comprises 10,459 children at least one of whose parents lived along the Techa River during the period 1950–1992. Of these children, 3,897 were born during the period of highest release, i.e. between 1950 and 1956, and might thus have been exposed in utero or during childhood. A total of 1,103 individuals

have since died, mainly owing to infectious and respiratory diseases and trauma [K49]. A total of 75 cases of cancer were detected. The most frequent cancer types were found to be respiratory tract, malignant lymphoma and leukaemia. The overall cancer incidence rate was 24.3 per 100,000, this comparatively low morbidity rate being attributed to the fact that the highest age attained was 45 years, with only 26% of the cohort being over 40 years of age [K50].

### 3. Remarks on the Techa River study

420. Some remarks may be made concerning the information reviewed above. Residents of Techa riverside villages were exposed to external gamma radiation and internal contamination with both short-lived and long-lived fission products. Early effects on the immune system included leucopenia, neutropenia, inhibition of innate immunity and autoimmune disorders. Long-term effects include impaired cellular immunity and a decrease of NK cells. A long-lasting delayed maturation and differentiation of granulocytes was observed. Leucopenia and neutropenia persisted for around three decades after the beginning of the exposure. Continuous exposure from long-lived incorporated radionuclides may account for this long-lasting effect. Greater exposures were correlated with longer recovery times. Subjects exposed in utero or during the first two years of post-natal life presented more severe effects. Five decades after the beginning of the exposure, immunological parameters had normalized, but an increase in the frequency of chromosomal aberrations and mutations was found in the peripheral lymphocytes of residents of Techa riverside villages. Manifestations of immunodeficiency occurred more frequently among residents who developed tumours.

## D. Hanford nuclear site

### 1. General considerations

421. As previously described, there is no statistically significant dose response in the prevalence of autoimmune hypothyroidism or thyroid autoantibodies in atomic bombing survivors. On the other hand, it was suggested that the immune system might be involved in the pathogenesis of thyroid diseases in Chernobyl accident victims. It is thus interesting to present here another epidemiological study developed for evaluating thyroid diseases in people exposed to ionizing radiation.

422. Approximately  $2.73 \times 10^{16}$  Bq of <sup>131</sup>I were released to the atmosphere from the Hanford nuclear site between the years 1944 and 1957. This facility manufactured plutonium for early nuclear weapons. This production process caused the release of a variety of radioisotopes, which drifted on the wind and the river, settled on vegetation and were consumed by grazing animals. The vast majority of radioactive releases came in a single year. A study was recommended to estimate radiation doses to area residents and another to examine the

feasibility of potential health effects of  $^{131}\text{I}$ , the radioisotope that accounted for most of the exposures [R12].

## 2. Hanford Thyroid Disease Study

423. The Hanford Thyroid Disease Study was conducted as a retrospective cohort study (1992–1997) to determine if thyroid disease had increased among persons exposed as children to these atmospheric releases of  $^{131}\text{I}$ . The cohort included a sample of all births between the years 1940 and 1946 to mothers whose usual residence was in one of seven counties near the Hanford site. The thyroid doses ranged from 0.0029 to 2,823 mGy (mean 174 mGy, median 97 mGy) [K47]. Assessments of thyroid disease, including a thyroid ultrasound, a physical examination, and a fine needle biopsy if required to evaluate thyroid nodularity, were carried out in 3,440 individuals.

424. There was no evidence of a relationship between radiation dose and the cumulative incidence of any of the following outcomes: total neoplasia, thyroid cancer, benign thyroid nodules, autoimmune thyroiditis and hypothyroidism. Although ultrasound abnormalities were observed in 55.5% of women and 37.4% of men, they were not significantly associated with the dose. The Hanford Thyroid Disease Study has sufficient statistical power to test for dose–response relationships between thyroid outcomes and radiation exposure [D16, K48, R12].

## 3. Remarks concerning the Hanford nuclear site

425. As seen in the preceding paragraphs,  $^{131}\text{I}$  was the radioisotope that accounted for most of the dose received by residents near the Hanford nuclear facility. Fifty years after exposure, the Hanford Thyroid Disease Study did not find evidence of a relationship between radiation dose and thyroid pathologies, including autoimmune thyroiditis. The results of this study support the hypothesis that exposure during infancy and childhood to  $^{131}\text{I}$  at these dose levels and in these exposure circumstances does not increase the risk of these forms of thyroid disease.

## E. Patients undergoing radiotherapy

### 1. General considerations

426. Ionizing radiation is an important and often indispensable strategy for cancer treatment. More than 50% of people with cancer undergo radiotherapy at some time during their illness [F9]. Although more alternatives for fractionation are now available, conventional fractionation schedules for local external radiotherapy (9–10 Gy/week delivered at dose rates of approximately 50 Gy/h over 5–6 weeks) continue to be the main modality. The potential for increased tumour control with protocols combining low-dose-rate with high-dose-rate irradiation is now under discussion [G11]. The rapid

expansion of the number of elderly individuals in the world population will lead to a substantial increase in the prevalence of cancer and hence in the number of individuals undergoing radiotherapy. Second primary malignancies among cancer patients account for 16% of all cancer incidences [T13]. An extensive body of literature concerning second cancers in patients undergoing radiotherapy has recently been published [A20, D25, G33, R15, R16, R17]. However, papers concerning changes of immunological parameters after radiotherapy are scarce, and the actual impact of these changes on health has not been well established.

### 2. Review of published data

427. Nakayama et al. investigated changes in peripheral blood lymphocyte subsets of 15 lung cancer patients who had undergone thoracic irradiation [N17]. After radiation therapy, the percentage and the absolute number of CD4+CD45RA+ cells (naive T-cells) and CD56+ and/or CD16+ cells (NK cells) decreased. The percentage of HLA-DR+ CD4+ cells (activated CD4+ T-cells) and HLA-DR+ CD8+ cells (activated CD8+ T-cells) increased, although the absolute number did not change significantly. Changes in local inflammatory cells in bronchoalveolar lavage fluid were analysed by the same authors in a similar group of patients [N18]. The percentage of lymphocytes and eosinophils, the percentage of HLA-DR+ CD4+ and CD8+ cells (activated CD4+ and CD8+ T-cells, respectively) and the incidence of ICAM-1+ T-cells was higher in lung cancer patients who had undergone thoracic irradiation than in controls (lung cancer non-irradiated patients). Naive T-cells seem to be more selectively damaged than memory T-cells by thoracic irradiation. The reduction of NK cells may be disadvantageous for antitumour immunity. On the other hand, thoracic irradiation enhanced both peripheral and local T-cell activation, which may promote antitumour effects. Nakayama's results are in good agreement with the findings of Ishida et al. in patients who underwent thymectomy and post-operative radiation therapy, which also indicated that irradiation was associated with a higher percentage of activated T-cell subsets [I10].

428. Van Mook et al. studied 24 B-cell chronic lymphocytic leukaemia patients after splenic irradiation. Radiation treatment consisted of a weekly dose of 1 Gy up to a total dose of 10 Gy to the spleen. Six weeks after splenic irradiation, total leucocytes decreased significantly, with a decrease in the fraction of lymphocytes and an increase in the neutrophil and platelet counts. The number of CD4+ and CD8+ cells decreased significantly without significantly changing the CD4+/CD8+ ratio. No significant changes in immunoglobulin levels were observed [V11].

429. A long-term deficit in total CD4+ T-cell counts after radiation treatment for Hodgkin's disease (HD) was reported many years ago [P14]. In patients who received mediastinal irradiation for HD, Watanabe et al. found a marked depletion in both CD4+ and CD8+ naive T-cell counts that persisted for up to 30 years after completion of treatment.

In contrast, CD4+ and CD8+ memory T-cell subsets and total CD8+ T-cells recovered to normal or above normal levels by five years post-treatment, with different kinetics (early expansion of CD8+ memory T-cells versus the gradual recovery of the others). Thus the long-term deficit in total CD4 T-cell counts in irradiated HD patients was due to specific depletion of the naive T-cell subset. Similarly, total CD8+ T-cell counts returned to normal values by 5 years post-treatment, particularly because CD8+ memory T-cells expanded to higher than normal levels. As the thymus is the main source of naive T-cells, these findings suggest that mediastinal irradiation results in a long-term depletion of the CD4+ naive cell pool, probably owing to thymus impairment. This dysregulation of T-cell subset homeostasis may explain the altered T-cell function observed in treated HD patients, including the poor response to immunization after treatment. An extrathymic (peripheral) expansion of mature T-cells may partially compensate for the loss of thymus-derived T-cells, but this expansion is primarily restricted to the memory population, thus resulting in a selective expansion of memory T-cells, while naive T-cell numbers remain low [W10].

430. Safwat et al. [S35] studied immunological parameters in 35 non-Hodgkin's lymphoma patients undergoing WBI consisting of two cycles of four daily fractions of 0.2 Gy separated by 2 weeks of rest (total dose of 1.6 Gy over four weeks). WBI was associated with a significant decrease in the percentage of lymphocytes and a significant increase in the percentage of CD4+ T-cells, with a consequent significant increase in the CD4+/CD8+ ratio. In terms of absolute values, WBI leads to a significant reduction in the absolute number of all the lymphocyte subsets. The significant increase in the percentage of CD4+ cells in the peripheral blood was interpreted as indicating a higher radiosensitivity of the CD8+ T-cell subset. This contrasts with previous data published by Clave et al. [C11] for patients given WBI (total dose 12 Gy) before bone marrow transplantation, which revealed that all major T-lymphocyte subsets appeared equally radiosensitive, while the NK cells were relatively radioresistant. In that study, however, blood samples were collected 6 h after a single dose of 2 Gy, while in Safwat's study the samples were collected 24 h after a total dose of 1.6 Gy delivered over 4 weeks.

### 3. Remarks concerning data on patients undergoing radiotherapy

431. As presented in the preceding paragraphs, the effects on the immune system observed in cancer patients undergoing local radiotherapy include decreases in the absolute number of total leucocytes, total lymphocytes, CD8+ and/or CD4+ T-cells, and NK cells. Naive T-cells are more selectively damaged than memory T-cells. While CD4+ and CD8+ memory T-cell subsets and total CD8+ T-cells return to normal levels within 5 years after irradiation, CD4+ naive T-cell depletion may be long-lasting (recovery several decades after radiotherapy). This is particularly evident

following mediastinal irradiation, probably owing to thymus impairment. Extrathymic (peripheral) expansion of memory T-cells results in a lower naive/memory cell ratio.

432. Although reduction in the absolute number of all lymphocyte subsets is observed after therapeutic WBI, the effects differ according to the fractionation schedule. While an early increase of the CD4+/CD8+ ratio is observed after a total whole-body dose of 1.6 Gy protracted over four weeks (low-dose fractions of 0.2 Gy), indicating a higher radiosensitivity of CD8+ T-cells, this effect was not observed after a total whole-body dose of 12 Gy given over three days (high-dose fractions of 2 Gy).

## F. Summary

433. This section reviewed the effects observed on the immune system of human populations exposed to ionizing radiation in very different conditions. Diverse immunological parameters were evaluated in these populations, at different times after exposure. There are similarities and differences among the results reported by different authors. The analysis of similarities might aid identification of the predominant effects of ionizing radiation on the human immune system. However, findings should be interpreted taking account of the specific characteristics of each population.

434. The detonation of the atomic bombs in Hiroshima and Nagasaki in 1945 resulted in a short burst of external neutron and gamma irradiation. Radiation-induced cell death accounts for the short-term effects observed in the immune system, mainly associated with the development of acute radiation syndrome. An almost complete recovery of the haematopoietic system of atomic bombing survivors took place within the first year after the exposure. Little evidence of dose-dependent effects on the immune system of the survivors was found soon after the bombings. Studies of the long-term effects of ionizing radiation on the immune system began about 20 years after the atomic bombings.

435. The Chernobyl accident released large amounts of radionuclides into the environment over a period of around 10 days. External irradiation was predominant among emergency and recovery operations workers. Doses to residents of contaminated areas resulted from external irradiation from radionuclides deposited on the ground and from internal irradiation mainly due to ingestion of short-lived (e.g. <sup>131</sup>I) and long-lived (e.g. <sup>137</sup>Cs) radionuclides present in foodstuffs. Large quantities of radioiodine were internalized during the early period. Ingested and inhaled radioiodine was preferentially incorporated into the thyroid, resulting in higher levels of exposure to this gland compared with the rest of the body. As seen in the atomic bombing survivors, short-term effects on the immune system were mainly associated with the symptoms of acute radiation syndrome.

436. The residents of Techa riverside villages were exposed predominantly during the early 1950s to external

gamma irradiation from fission products and internal incorporation of short-lived (mainly  $^{89}\text{Sr}$ ) and long-lived (mainly  $^{90}\text{Sr}$  and  $^{137}\text{Cs}$ ) radionuclides via drinking water and food-stuffs. In the early period, chronic radiation sickness (CRS) was diagnosed, including leucopenia, neutropenia, thrombocytopenia, impaired innate response and autoimmunity. The effects were more severe in children exposed in utero or at 1–2 years old. Normalization of haematological parameters in CRS patients was observed 30 years after the beginning of the exposure. Long-lived radionuclides contributing to chronic irradiation may explain why immunity disorders persisted for such a long period, even after cessation of external exposure and intakes. Long-term effects included decreased cellular immunity and reduced counts of NK cells.

437. Major radioactive releases into the air, water and soil occurred at the Hanford nuclear site between 1944 and 1957. Thyroid diseases were selected for the study of health effects because the release of  $^{131}\text{I}$  caused the highest exposures in the population. There is no evidence of a relationship between radiation dose and the incidence of total neoplasia, thyroid cancer, non-cancer thyroid diseases, hypothyroidism and autoimmune thyroiditis.

438. Most cancer patients undergoing radiotherapy receive a very localized high-dose irradiation protracted over several weeks. Most of the published data concern mainly short-term effects on the immune system: total leucocyte decrease, total lymphocyte decrease, selective damage to naive T-cells compared with memory T-cells, enhancement of local and peripheral T-cell activation and decrease of NK cells. Long-term effects described in Hodgkin's disease patients show long-lasting (30 years) depletion of naive T-cells with a more rapid (5 years) recovery of memory T-cells. Studies concerning short-term effects of ionizing radiation on the immune system of patients receiving WBI indicate that the fractionation regime influences the resulting effects. Although a decrease in total lymphocytes is a common feature, the behaviour of lymphocyte subsets differs according to the WBI schedule.

439. The following findings may be considered as similar effects on the immune system observed in human populations exposed to ionizing radiation:

- Impairment of T-cell immunity: decreased total T-cells and CD4+ T-cells, lower T-cell responsiveness to mitogens and alloantigens;

- Increased humoral immunity: higher levels of total Ig, IgA and IgM;
- Shift towards an inflammatory profile: inflammatory cytokines, activated complement.

440. Atomic bombing survivors presented with a reduction of naive CD8+ and CD4+ T-cell pool, and clonal expansion of memory CD8+ T-cells associated with TCR repertoire deviation. This kind of perturbation of T-cell homeostasis was not evident in Chernobyl workers and residents. While total CD4+ T-cells were diminished in both populations, total CD8+ T-cells were decreased only in the Chernobyl population.

441. Findings concerning NK cells also differ: while significant changes were found in neither their number nor their cytotoxic activity among atomic bombing survivors, a decrease in both parameters was found in the Chernobyl population. Many workers who took part in recovery operations after the Chernobyl accident were contaminated with heavy metals, which may account, at least in part, for the observed changes in their immune status. An increase in the NKT lymphocyte subset was observed in Belarussian recovery operations workers, an observation that was not reported in atomic bombing survivors.

442. The development of autoimmunity is a relevant finding among Chernobyl workers and residents. There were regions with iodine deficiency in most affected territories of Belarus, the Russian Federation and Ukraine. Ionizing radiation might have induced thyroid gland changes affecting the expression of endemic goitre. These factors should be considered in interpreting the increase in thyroid autoantibody levels and the development of autoimmune thyroid diseases, which were not observed in the atomic bombing survivors or in residents near the Hanford site.

443. Studies of the long-term effects of ionizing radiation on the immune system of atomic bombing survivors began about 20 years after the bombings and continue to be carried out. Because the Chernobyl accident occurred in 1986, data concerning long-term effects of ionizing radiation on the immune system are today limited to the first 20 years after the event. These should be considered for cross-comparison of the long-term effects observed in the two populations.





## V. FINAL SUMMARY

444. The immune system is certainly one of the most complex systems of the body. It is composed of a large variety of cells spread widely throughout the body and of different organs where stem cells can differentiate into one of the major lineages. Immune cells communicate via cytokines, which are soluble molecules that stimulate immune cell proliferation and/or differentiation. Consequently the immune cells can differentiate towards specific cell types. Clusters of differentiation (CD) are cell surface glycoproteins associated with specific functions; their expression may change depending on cell environment, e.g. the effect of ionizing radiation.

445. One of the main functions of the immune system is the recognition of foreign antigens and the development of subsequent actions of protection, for example against infection and cancer. Autoimmune disease may result from the alteration of self tolerance mechanisms.

446. For protecting the body, the immune system can use two different but interrelated forms of immunity, i.e. innate and acquired. While innate immunity provides a rapid defence because it is always ready for use, acquired immunity develops only after a pathogen has entered the body. Acquired immunity is very antigen-specific and keeps the memory of a previous exposure, yielding a stronger response at the time of a subsequent exposure to the same antigen. Acquired immunity can respond to the diversity of foreign antigens, those antigens being processed by APCs. The major histocompatibility complex, in humans called human leucocyte antigen (HLA), plays a fundamental role in the processing of antigens by APCs and their presentation for recognition by T-cells via specific receptors. HLA-G is a family of particular molecules involved in immunotolerance; cells expressing HLA-G can escape from immune surveillance.

447. The effects of ionizing radiation on each component of the immune system (e.g. organs of the immune system, cell populations, expression of CDs) have been documented. Although significant changes occur, the results of the publications on these changes are difficult to compare, because the circumstances and the protocols of exposure to ionizing radiation (dose, dose rate, quality of radiation, cell type) differ considerably.

448. The data reviewed in this annex indicate that exposure to ionizing radiation often leads to immunosuppression, particularly following high-dose irradiation. Immunosuppression is most often ascribed to lymphocytes being highly radiosensitive, owing to their proclivity to undergo

radiation-induced apoptosis. In addition to these cytotoxic effects, ionizing radiation may induce “danger signals”, which may in turn influence cell responses in the immune system. Such evidence has led to the emerging notion that ionizing radiation has much more to offer than its qualities as a powerful cytotoxic agent, and because of this, ionizing radiation is probably better considered an immunomodulatory agent rather than an immunosuppressive one.

449. Although many questions remain open, the role of the immune system with regard to cancer development is better understood. In the classical immune surveillance theory, tumours may develop when cancer cells escape from immune surveillance either by reducing the expression of tumour antigens or by modifying the immune response to them. Although a strong antitumour activity can be efficiently developed as a result of the activation of both innate and acquired immune responses, some immunological promotion of tumours may result from low-grade persistent inflammation, chronic activation of innate immune cells or the blocking of cell-mediated cytotoxicity by antibodies.

450. The effects of ionizing radiation on the immune system at low doses (<200 mGy) and low dose rates (<100 mGy/h) remain controversial. In animals, although depletion of different categories of immune cells is observed, as well as changes in lymphocyte subsets, there is some evidence that low-dose WBI can be immunostimulatory. Data concerning suppressive effects of low doses on tumour growth have been reported. Such data have been obtained after low-LET radiation exposure, they are dependent on a number of factors and they are very variable. In humans, although a decrease of CD4+ T-lymphocytes, a decrease of HLA-DR+ lymphocytes and decrease of the CD4+/CD8+ ratio due to an increase in CD8+ are frequently reported, these numerical findings could not be directly related to a decrease of immune function. Some studies regarding people living in areas with high levels of natural radiation suggest the existence of an adaptive response induced by chronic radiation exposure.

451. In atomic bombing survivors and the residents of Techa riverside villages, the haematolymphoid system was damaged in a dose-dependent manner, although the groups underwent different types of radiation exposure. Several months or even years later, their systems regenerated, and haematolymphoid function recovered almost completely. However, even after several decades, with different effects in the two populations, significant effects have been observed in the haematolymphoid systems.

452. In atomic bombing survivors, long-lasting effects are still observed more than a half-century after their radiation exposure. These effects include an increase in the frequency of somatic mutations and chromosome aberrations in lymphocytes, as well as significant changes in lymphoid cell composition and function. TCR-defective cells could result in the impairment of the immune function. Although low frequencies of somatic mutations or chromosome aberrations would not influence the regeneration or homeostasis of the immune system, the extensive proliferation of a single cell bearing a radiation-induced mutation may result in clonal expansion, particularly in haematopoietic stem cells, committed lymphoid precursor cells and memory T-lymphocytes. With regard to changes of lymphoid cell composition and function, the main kinds of damage observed among atomic bombing survivors include: impairment of T-cell immunity, especially owing to a decreased proportion of CD4+ helper, CD4+ naive and CD8+ cytotoxic T-cells; a dose-dependent increase in the proportion of B-cells and of immunoglobulin production; and impairment of viral immunity and other T-cell functions, such as PHA-dependent proliferation, ability to produce IL-2 and alloantigen responses.

453. In Techa riverside village populations chronically exposed to radiation, long-term immunity changes involved decreased expression of differentiating antigens of T-lymphocytes, reduced counts of cells involved in natural cytotoxicity and signs of immunological imbalance. As in the atomic bombing survivors, a preferential CD4+ cell deficiency was observed many years later in persons affected by the Chernobyl accident. Proliferative response to mitogens was also altered. The dynamics of post-irradiation recovery of CD4+ and CD8+ were different, suggesting that radiation may induce damage to the thymus, accelerating the natural ageing of the immune system by a progressive decline in thymic function. Both short-term and long-term effects were detectable in the B- and T-cell function profiles, as well as in the synthesis of immunoglobulins, depending on the population studied. Some of these changes were dose-dependent and characterized by phases. The immune system is involved in the pathogenesis of the thyroid diseases observed in victims of the Chernobyl accident, probably owing to antigenic mechanisms leading to autoimmune damage.

454. Animal data involving low-dose irradiation reinforced some of these results, for example the gradual reconstitution of peripheral blood and bone marrow patterns with partial deficiency of haematopoietic and lymphopoietic precursors, suggesting that ineffective haematopoiesis could cause restriction of myeloid and lymphoid cell reserves and consequent disturbances of cellular and humoral immunity. Enhancement of immunity may be observed under certain circumstances, in particular following low-dose irradiation, and modulation of oxidative status seems to be involved in this effect.

455. Recent developments in immunology have contributed to our understanding of how human diseases may be related to abnormalities in the immune system. The study

of these disorders from an immunological viewpoint could therefore provide further insight into the mechanisms involved in certain radiation-associated diseases. Radiation-induced perturbation of T-cell homeostasis may have important implications for human health, not only by reducing the ability of the immune system to fight against new pathogens but also by compromising the control of recurrent and latent infections. Increasing evidence indicates that persistent exposure to infections leads to more rapid senescence of the immune system. An association between non-cancer diseases and radiation dose has recently emerged among atomic bombing survivors. This association has led to the hypothesis that radiation-induced effects on the immune system may account at least in part for this phenomenon, although the mechanisms involved remain incompletely understood.

456. Epidemiological findings suggest that the radiation-induced impairment of immunocompetence may increase the risk of diseases that normally occur in elderly people. The data reviewed in this annex reinforce the hypothesis that ionizing radiation may accelerate immunosenescence by perturbing T-cell homeostasis in the same direction that ageing does. Immunological homeostasis has critical implications for human health, for example with respect to the relationship between the immune system and disease susceptibility, and the possible interaction between hereditary and environmental factors such as ionizing radiation.

457. A statistically significant dose-response relationship has been found for several inflammatory biomarkers in irradiated subjects. The persistent inflammatory status induced by ionizing radiation may increase the risks of both cancer and non-cancer diseases. The negative correlation between plasma levels of inflammatory biomarkers and the percentage of CD4+ helper T-cells in peripheral blood indicates an association between radiation-induced impairment of cell-mediated immunity and a preclinical inflammatory status that could further promote the development of various diseases.

458. The proportion of CD4+ cells was significantly decreased among atomic bombing survivors with increased dose and history of myocardial infarction. It has been suggested that a diminished immune response against certain infections implicated in the development of cardiovascular diseases could account for this finding. Moreover, inflammatory biomarkers were significantly elevated in these patients, indicating that inflammatory responses may be playing a role in the development of cardiovascular diseases, such as myocardial infarction, in irradiated people.

459. In order to explain the possible links between radiation-induced perturbation of immunological homeostasis and human diseases, the "Th1/Th2 paradigm" has been invoked. Experimental data, further reinforced by human data, seem to sustain the hypothesis that ionizing radiation reduces cellular responses controlled by Th1 cells and increases humoral responses controlled by Th2 cells by triggering a shift from Th1 towards Th2 (Th1/Th2 imbalance). Recent findings demonstrated that radiation-dose-dependent

increases observed in irradiated individuals involved not only Th2-related cytokines but also Th1-related cytokines. This evidence leads to the postulate that ionizing radiation might better be considered an agent that modulates the production of cytokines towards a pro-inflammatory response rather than towards a Th2 response.

460. Programmed cell death (apoptosis) is essential for the development and maintenance of cellular homeostasis of the immune system. The functional balance of proapoptotic versus anti-apoptotic influences determines whether a lymphocyte will live or die. Multiple molecules, often working in concert, control serial stages of lymphocyte development and homeostasis. Radiation-induced apoptosis is one of the mechanisms by which ionizing radiation alters the homeostasis of the immune system.

461. Evaluations of the human health risks associated with radiation exposure have been based primarily on the assumption that the effects of radiation occur in irradiated cells. Non-targeted cellular responses to ionizing radiation, such as bystander effects and genomic instability as well as adaptive responses, have also been demonstrated in the immune system, although their implications for human health are still poorly understood. These effects, which predominate at the low doses of relevance to radiation protection, need to be fully characterized; they pose new challenges to evaluating the risks associated with radiation exposure. In contrast, local radiotherapy may facilitate the expansion of dendritic cells and the generation of antitumour immune responses outside the radiation field (abscopal effect).

462. The idea that the immune system functions by discriminating “self” from “non-self” has a long history in immunology. The “self/non-self theory” proposes that lymphocytes with reactivity against host constituents are destroyed during development, and only those tolerant lymphocytes that are not self reactive are left to engage foreign antigens. Although the notion that the immune system has evolved to recognize (dangerous) pathogens is not new, recent discussions have emerged concerning the notion that antigenicity in the immune system may be seen as a question of degree, where “self” evokes one kind of response (tolerance) and “foreign” evokes another kind of response (destruction) based not on intrinsic foreignness but rather on the immune system’s recognition of foreign antigens in

the context of “danger signals”. Whichever theory is correct, ionizing radiation has emerged as an agent capable of disturbing the ability of the immune system to deal with this sort of recognition. As a result of radiation-induced damage, the immune system may tolerate what should be destroyed and, conversely, it may destroy what should be tolerated. Examples of these two opposing consequences are the diminished ability to mount an immune response against some infections and the development of autoimmune reactions as a consequence of radiation exposure, both of which have been demonstrated by experimental and human data.

463. Individual genetic susceptibility to ionizing radiation has been clearly demonstrated in patients. Some of the molecular and cellular mechanisms that determine sensitivity to ionizing radiation have been elucidated, most of them representing a defect in the response to DNA damage. Research on this subject will be relevant, since most of the human genetic disorders involving such defects are also associated with alterations of immune system functioning.

464. Finally, the question of how radiation-induced effects on the immune system may impact on human health remains unanswered. There are many issues that need to be more thoroughly investigated before firm conclusions can be reached. Possible future directions for research concerning the effects of ionizing radiation on the immune system that should provide new insights about the underlying mechanisms may include:

- Effects of low-dose and low-dose-rate irradiation versus intermediate- and high-dose irradiation;
- Combined effects of ionizing radiation and other agents;
- Differential effects of external and/or internal irradiation;
- Immunomodulation and cancer;
- Perturbation of T-cell homeostasis;
- Immune function and disease development;
- Immunogenetic background and disease susceptibility;
- Immunological ageing and inflammatory response;
- Effects on the skin immune system.



## VII. CONCLUDING REMARKS

465. This annex reviews data related to radiation-induced alterations of immune response, considers the possible mechanisms involved and reviews epidemiological studies of the effects of ionizing radiation on the human immune system.

466. The effects of ionizing radiation on the immune system can be assessed by estimating changes in cell numbers or by using a variety of functional assays. The impact of such alterations in immune response depends on factors such as the dose of radiation, its temporal relationship with immune system challenge and individual genetic constitution.

- High doses of radiation produce immunosuppression mainly through the destruction of cells.

Lymphocytes are very radiosensitive, and their reduction is currently used as an early indicator of the level of an accidental acute exposure. Radiation-induced changes in immune parameters seem to be more dependent on total dose than on dose rate. Persisting effects on the immune system have been observed after exposure to ionizing radiation.

- At low doses and dose rates, the effects of ionizing radiation on the immune system may be suppressive or stimulatory. The long-term impact of low radiation doses on the immune function in relation to human health needs to be further evaluated.





## Abbreviations

<i>AIDS</i>	acquired immunodeficiency syndrome
<i>AIRE</i>	autoimmune regulator
<i>AP</i>	alkaline phosphatase
<i>APC</i>	antigen-presenting cell
<i>ARS</i>	acute radiation syndrome
<i>AT</i>	ataxia telangiectasia
<i>ATLD</i>	ataxia-telangiectasia-like disorder
<i>ATM</i>	ataxia-telangiectasia-mutated gene
<i>ATP</i>	adenosine triphosphate
<i>BCR</i>	B-cell receptor
<i>C</i>	constant gene segment
<i>cAMP</i>	cyclic adenosine monophosphate
<i>CD</i>	cluster of differentiation
<i>CFU</i>	colony-forming unit
<i>CFU-F</i>	fibroblastoid colony-forming units
<i>CFU-GM</i>	granulocyte–macrophage colony-forming units
<i>CFU-S</i>	stem cell colony-forming units
<i>cGMP</i>	cyclic guanosine monophosphate
<i>CH</i>	heavy chain constant domain
<i>CL</i>	light chain constant domain
<i>Con A</i>	concanavalin A
<i>COX-2</i>	cyclooxygenase-2
<i>CRP</i>	C-reactive protein
<i>CRS</i>	chronic radiation sickness
<i>CS</i>	Cockayne syndrome
<i>CTLA-4</i>	cytotoxic T-lymphocyte-associated antigen-4
<i>D</i>	diversity
<i>DC</i>	dyskeratosis congenita
<i>DNA</i>	deoxyribonucleic acid
<i>DNA-PK</i>	DNA-dependent protein kinase
<i>DNF</i>	dinitrofluorobenzene
<i>DSB</i>	double-strand break
<i>DTH</i>	delayed type hypersensitivity
<i>ESR</i>	erythrocyte sedimentation rate
<i>ETRC</i>	Extended Techa River Cohort
<i>FA</i>	Fanconi anaemia
<i>Fas/CD95</i>	Fas death receptor
<i>FasL/CD95L</i>	ligand for Fas death receptor
<i>GM-CSF</i>	granulocyte–macrophage colony-stimulating factor
<i>GSH</i>	glutathione
<i>Gy</i>	gray
<i>HBsAg</i>	hepatitis B surface antigen
<i>HBV</i>	hepatitis B virus

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<i>HD</i>	Hodgkin's disease
<i>HER2</i>	human epidermal growth factor receptor 2
<i>HETE</i>	hydroxyeicosatetraenoic acid
<i>HGPS</i>	Hutchinson–Gilford progeria syndrome
<i>HIV</i>	human immunodeficiency virus
<i>HLA</i>	human leucocyte antigen
<i>HLNRA</i>	high-level natural radiation area
<i>HPRT</i>	hypoxanthine-guanine phosphoribosyltransferase
<i>HR</i>	homologous recombination
<i>HVC</i>	hepatitis C virus
<i>ICE</i>	interleukin-converting enzyme
<i>IFN</i>	interferon
<i>IFN-<math>\gamma</math></i>	interferon gamma
<i>Ig</i>	immunoglobulin
<i>IL</i>	interleukin
<i>ILT</i>	immunoglobulin-like transcript
<i>J</i>	joining
<i>JNK</i>	Jun N-terminal kinase
<i>KIR</i>	killer cell immunoglobulin-like receptor
<i>KLH</i>	keyhole limpet haemocyanin
<i>LC</i>	Langerhans cell
<i>LD</i>	low dose
<i>LDR</i>	low dose rate
<i>LET</i>	linear energy transfer
<i>LPB</i>	lipopolysaccharide-binding protein
<i>LPS</i>	lipopolysaccharide
<i>MALT</i>	mucosa-associated lymphoid tissue
<i>MAP kinase</i>	mitogen-activated protein kinase
<i>MBP</i>	mannan-binding protein
<i>MCP-1</i>	monocyte chemoattractant protein 1
<i>MHC</i>	major histocompatibility complex
<i>MICA</i>	class I MHC chain-related A molecule
<i>MNCA</i>	modified neutral comet assay
<i>MP</i>	myeloperoxidase
<i>mRNA</i>	messenger ribonucleic acid
<i>NBS</i>	Nijmegen breakage syndrome
<i>NCR</i>	natural cytotoxicity receptor
<i>NER</i>	nucleotide excision repair
<i>NHEJ</i>	non-homologous end-joining
<i>NK</i>	natural killer (cell)
<i>NKG2</i>	lectin-like receptor
<i>NKT</i>	lymphocyte with certain characteristics of both T- and NK cells
<i>NPP</i>	nuclear power plant
<i>p38 MAP</i>	p38 mitogen-activated protein
<i>PAMP</i>	pathogen-associated molecular pattern

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<i>PCNA</i>	proliferating cell nuclear antigen
<i>PGE2</i>	prostaglandin E2
<i>PHA</i>	phytohaemagglutinin
<i>PKA</i>	protein kinase A
<i>PKC</i>	protein kinase C
<i>PLA2</i>	phospholipase 2
<i>PLC</i>	phospholipase C
<i>PRRs</i>	pattern recognition receptors
<i>RBE</i>	relative biological effectiveness
<i>ROS</i>	reactive oxygen species
<i>RRD</i>	recurrent respiratory disease
<i>RT-PCR</i>	reverse transcription polymerase chain reaction
<i>SCID</i>	severe combined immune deficiency
<i>SIS</i>	skin immune system
<i>SLE</i>	systemic lupus erythematosus
<i>SOCS</i>	suppressor of cytokine signalling gene
<i>STAT proteins</i>	signal transducer and activator of transcription proteins
<i>TCR</i>	T-cell receptor
<i>TCR</i>	transcription-coupled repair
<i>TCR/CD3</i>	T-cell receptor/CD3 complex
<i>TGF-<math>\beta</math></i>	transforming growth factor $\beta$
<i>Th</i>	lymphocyte T-helper
<i>Th1</i>	lymphocyte T-helper subset or subclass 1
<i>Th2</i>	lymphocyte T-helper subset or subclass 2
<i>TLR</i>	Toll-like receptor
<i>TLS</i>	translesion synthesis
<i>TNF</i>	tumour necrosis factor
<i>TNFR</i>	tumour necrosis factor receptor
<i>TRAIL</i>	tumour-necrosis-factor-related apoptosis-inducing ligand
<i>TRDS</i>	Techa River Dosimetry System
<i>Treg</i>	T-regulatory cells
<i>TROC</i>	Techa River Offspring Cohort
<i>TSH</i>	thyroid-stimulating hormone
<i>TTD</i>	trichothiodystrophy
<i>UDS</i>	unscheduled DNA synthesis
<i>UV</i>	ultraviolet
<i>UV-DDB</i>	UV-damaged-DNA-binding protein
<i>V</i>	variable
<i>VH</i>	heavy chain variable domain
<i>VL</i>	light chain variable domain
<i>WBI</i>	whole-body irradiation
<i>WLM</i>	working level month
<i>WS</i>	Werner's syndrome
<i>XP</i>	xeroderma pigmentosum
<i>XP-V</i>	xeroderma pigmentosum variant





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