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COMPARATIVE ASSESSMENT OF RADIATION VERSUS NUTRITIONAL AND OTHER FACTORS THAT MAY INFLUENCE IMMUNE STATUS

Report of a Joint IAEA/WHO Advisory Group Meeting

IAEA, Vienna, Austria

3 - 6 May 1994

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SUMMARY

An Advisory Group Meeting was convened jointly by the International Atomic Energy Agency and the World Health Organization in May 1994 to review the role of radiation, nutrition, toxic chemicals and other factors that may influence immune status in human populations. Priorities for future research were proposed, and possibilities for using isotope in such studies were identified. The Group recommended that the IAEA should initiate a broadly based Co-ordinated Research Programme (CRP) focussed mainly on the effects of low-level radiation on immune status in human populations. The main variables of interest are (i) the level of individual radiation exposure, and (ii) the nutritional status. Possible experimental groups include persons living in areas of high radiation background (e.g. in countries where areas of high radiation background are known to occur naturally, or at high altitudes, or in areas affected by the Chernobyl accident). Other possible experimental groups comprise radiation workers and uranium miners. It was also recommended that the contribution of toxic chemical exposure to immune dysfunction in these population groups should be assessed. Such research should be complemented by animal studies, and possibly also by *in vitro* studies with human and animal cells, by some participants in the CRP. This report has been prepared as a source of information for potential participants in the proposed CRP and for other persons associated with related programmes of the IAEA and the WHO.

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1. INTRODUCTION

In recent years — particularly in the aftermath of the Chernobyl nuclear power plant accident — there has been an upsurge of interest in the effects of low level radiation on human health. One of the issues of particular concern is the effect of radiation on the immune system. However, radiation is only one of several factors that may influence the general level of immune status in a population; others include nutrition and toxic environmental chemicals. Most investigations of immune status conducted up to now have only focussed on one factor at a time, generally ignoring the others. In populations exposed to higher-than-normal levels of radiation, it is generally difficult, or impossible, to judge to what extent changes in immune status are associated with the radiation and to what extent they may be associated with other factors.

A Joint IAEA/WHO Advisory Group Meeting (AGM) was convened at the IAEA's Headquarters in Vienna in May 1994 to throw more light on some of these issues. In particular, the Group was requested to review what is known about these topics and about current research priorities, and to advise the IAEA on the purpose and scope of future actions that could be organized within the framework of a Co-ordinated Research Programme (CRP). The agenda of the meeting is given in Annex 1 and the list of participants in Annex 2.

The following report starts by describing briefly the function and structure of the immune system and ways for assessing immune status. It then presents an overview of factors that may affect immunity in human populations. After discussing some interactions between these factors the report concludes with recommendations for future research and other actions that might be supported by the IAEA, including the establishment of a Co-ordinated Research Programme (CRP).

2. THE IMMUNE SYSTEM

The function and structure of the immune system are described in many text books (e.g. [1]). For the purpose of this report, a brief discussion of the structure and function is presented in the following paragraphs.

The immune system functions in the maintenance of homeostasis, supporting the health of the host by serving as the principal defense system against infectious diseases and neoplasia. It does so by responding to new antigens, whether soluble molecules or structural determinants present on pathogens and certain tumour cells or soluble moieties, with a variety of responses designed to control or destroy the foreign cell or inactivate the soluble component. Because of the diversity of antigens present on these cells, the immune system is called upon to recognize a huge repertoire of distinctive antigens. It does so by a process of genomic rearrangements providing a multitude of different genes encoding for the receptors necessary for the system to interact with these antigens, for example T-cell receptors present on the surface of T-lymphocytes and immunoglobulin on B lymphocytes. During the ontogeny of the immune system, and to a lesser extent throughout the entire life span of the individual, selection processes aimed at the deletion of specificities of the host itself are active, in order to reduce the potential for autoimmunity, i.e. host immune responses against host tissues.

The immune system functions as a network of cells that communicate with one another through soluble mediators as well as cell-to-cell contact. The initial encounter of the immune system with antigens, whether soluble or present on foreign cells, is via macrophages. These cells take up the antigens or antigen bearing cell and processes them into a form appropriate for transport to the cell surface for presentation to lymphocytes

in the context of histocompatibility antigens on the cell surface. Antigen presenting cells are specialized macrophage-like cells, for instance Langerhans cells in the skin, interstitial macrophages in lung tissue, or M-cells in the gastrointestinal mucosa. When these cells encounter lymphocytes with the appropriate T-cell receptor, a primary immune response is triggered, characterized by proliferation of these lymphocyte clones and activation of their immunological capacity. The expanded population of sensitized lymphocytes circulates throughout the body into the peripheral tissues and upon renewed encounter with the same antigen a secondary immune response is initiated, generally more intense and more specific that the primary response.

There is a diversity in the nature of the specific immune response, depending in part on the nature of the antigen and the mode of encounter with the immune system. In many instances an inflammatory response is generated, with the production of potentially toxic biologically active mediators, such as cytokines, prostaglandins, leukotrienes, activated complement components, and oxygen and halogen free radicals. These molecules contribute to the killing and ultimate eradication of the pathogens from the host, but at the same time may result in host tissue damage. In order to diminish self destructing events, the responses are under biological regulation such that rapid activation and control of the magnitude of the response can both be achieved. In fact regulation is, in addition to specificity, a hallmark of the organization of the immune system. When the immune system is abnormal, regulation may give way to dysregulation, with serious consequences for the host, ranging from hypersensitivity or autoimmunity to death.

The normal activation and function of the immune system requires extensive communication between many different cell types located in different sites of the body. As noted above, communication is achieved by cell-cell contacts as well as by soluble mediators, including cytokines and growth factors. The soluble messages may engage in cross-talk, and influence the production of one another. Cascades and circular pathways, with or without feedback controls, are known to exist. Besides their presence in lymph nodes, bone marrow, spleen and thymus, lymphocytes are also found within parenchymal organs, skin, and mucosal surfaces, where they have important function, as well as in the circulation, including the blood and lymph, where they are presumably in transit from one site to another. Cells present in circulation may, or may not, be representative of those present in tissues, but are more likely to represent cells derived from immunologically active sites. There are an increasing number of separable lymphocyte subpopulations, detected by the presence of a group of cell differentiation antigens on their surface, which mark both the functional potential of the cell type and, in some instances, their state of activation. T-cells differentiate further within or under the influence of the thymus, whereas B cells are thymus independent. In addition, there are a number of distinctive granulocytes, including neutrophils, eosinophils, mast cells, and various macrophage derived phagocytic cells, such as alveolar macrophages in the lungs or Kupffer cells in the liver. Growth factors secreted by some cells affect the proliferation and differentiation of other cells in an interactive manner.

Most immunologically active cells have their origin from common precursor stem cells in the bone marrow and subsequently undergo differentiation at distant locations, where specific properties may develop. Thus, cells of the macrophage lineage may have a common origin but when terminally differentiated alveolar and peritoneal macrophages are compared, they exhibit remarkable differences in their properties.

Because the function of the immune system depends on so many different cell types, some of which share similar functions, the system may exhibit redundancy. This redundancy underlies a certain reserve capacity of the system, which implies that insults to the immune system by exogenous factors will not always result in hampered function. On the other hand, the diversity of the components and the diversity of locations make the

system potentially sensitive to a variety of deleterious effects of exogenous factors, such as radiation, malnutrition, and toxic chemicals.

3. ASSESSMENT OF IMMUNE STATUS

Immune status assessment in humans must be guided by the nature of the information being sought, and therefore depends on the experimental design of the studies. The immune system of man is amenable to limited sampling, largely due to the inaccessibility of central lymphoid organs, and primarily involves studying circulating cells and soluble factors. The least invasive methods may include analysis of cells and mediators in nasal lavage, followed by blood sampling and in certain circumstances, bronchoalveolar lavage and lymph node biopsies. The level of invasiveness of test that is selected as well as its feasibility will also be dictated by the type of study being done, e.g. field epidemiology versus clinic based investigations.

It is generally recommended to perform screening tests in a hierarchial order, with more general and simpler tests done first, followed by more sophisticated or targeted tests if there are abnormalities detected in the initial screen. In this regard it should be mentioned that tests may differ with respect to sensitivity or specificity. As a general principle, assays that require an active response to a specific challenge provide the most useful information of functional capacity and reserve. Assays commonly used for immunotoxicity assessment in man include: (1) complete blood count with differential; (2) antibody mediated immunity (primary and secondary antibody responses (vaccines), total immunoglobulin levels, proliferation to recall antigens); (3) phenotypic analyses of lymphocytes by flow cytometry; (4) cellular immunity (skin testing, primary DTH to sensitizing or recall antigens, natural immunity to blood group antigens); (5) autoantibodies and inflammation (C-reactive protein, autoantibody titers, IgE to allergen cytokines); and (6) non-specific immunity (NK-activity, phagocytosis). In addition to such tests, in epidemiologic studies incidence and morbidity due to infection or immunologic disorders, and hospitalization due to such phenomena are valuable to study (Table I).

For testing animal species, numerous assays of the capacity of the immune system are available that are fitted for mechanistic studies as well as for the process of risk assessment, e.g. [2] and [3]. In this latter sense host resistance models, in which infectious - or tumor challenges are applied, are most useful (Table II,

4. OVERVIEW OF FACTORS THAT MAY AFFECT IMMUNITY IN HUMAN POPULATIONS

4.1. <u>High dose radiation and immunity</u>

Damage of immune functions following whole body radiation exposure has been extensively studied over the past several decades. These studies have provided important information to understand the mechanisms and process of immune suppression induced by high dose irradiation. However, despite recent rapid advances in methods of analytical assessment of the immune system, there are still difficulties in conducting and interpreting studies in genetically heterogeneous humans whose individual immune responses to foreign antigens are different.

Here results of the immunological studies of A-bomb survivors are summarized as representative of the effects of high dose radiation exposure in humans (Table III). These are classified into two types: acute effects [4], and late effects of radiation ([5], [6] and [7]).

4.1.1 *Acute effects of high dose (A-bomb) radiation on the immune system*

As a result of the acute exposure to A-bomb radiation, the following immunological damage has been observed.

- A rapid reduction in the number of lymphocytes: this results in inadequate production of various factors which regulate the differentiation and activation of immunocompetent cells.

- A decrease in humoral factors, such as antibodies and complement.

- A decrease in the number of phagocytic leukocytes, - including neutrophils and monocytes which reaches a minimum at around 30 days after exposure. Morphologic abnormalities and hypofunction of phagocytosis are found as well.

- The delayed recovery of lymphocytes. This delay of the recovery of the immune system causes (a) a persistent reduction in the activities of antigen recognition and antibody production and in the activation of neutrophil and monocytes, and (b) an impaired ability to recover from infectious diseases. The slow recovery of the lymphocytes may be due to (a) the degradation of the thymus, in which T-cells differentiate and mature, (b) the elimination of the receptor-specific T-cell stimulated by toxins from bacterial infections, and (c) the inactivation of genes which are necessary for the differentiation and proliferation of lymphocytes (T-cell receptor genes, etc.). This may be induced by mutations following radiation exposure.

4.1.2 *Late effects of high dose (A-bomb) radiation on the immune system.*

Cellular immunity

The following late effects of A-bomb radiation have been observed: (a) functional defects in T-cell responses (to PHA and alloantigens, etc), and a reduction of the number of T ($CD3^+$ pan T) cells, particularly $CD4^+CD45RA^+$ naive T-cells, and an increase of a rare T-cell subpopulation, $CD4^-8^-\alpha\beta$T cells, whose differentiation may differ from the conventional $CD4^+$ or $CD8^+$ T-cells, (b) an increase in the numbe. of B ($CD20^+$ pan B and $CD5^{+/-}CD20^+$B) cells, but no effect on B-cell function, (c) no effect on NK cell activity or number ($CD16^+$, $CD57^+$ cells), although NK cells significantly increased with increasing age among the same cohort, and (d) an increase in the frequencies of somatic mutants of immunologically functional molecules (such as T-cell receptor and HLA-class I genes).

The T-cell immune system was affected by radiation to a greater extent among those who were older at the time of bombing. An age-related degradation of the thymus is considered to be responsible for these age-dependent radiation effects. Younger individuals possessed a fully functional thymus at the time of exposure, whereas the thymus of older individuals was already partially involute and hypofunctional at the time. Therefore, it is hypothesized that shortly after exposure to A-bomb radiation, mature lymphocytes were damaged and reduced in number among both younger and older persons, while the recovery process(es) differed between the two groups but in younger survivors, the process of maturation from the precursor cells to mature T-cells in the thymus continued almost normally. In contrast, in older survivors, the replacement process would have been incomplete, resulting in a decreased number of T-cells, even more than 40 years after the radiation exposure.

Humoral Immunity

- Immunoglobulin levels in serum. No significant relationship between radiation dose

and serum immunoglobulin level was noted until the late 1980s. In 1987-89, serum immunoglobulin levels (IgG,-M,-A and -E) were measured again in about 2,000 individuals in the Hiroshima and Nagasaki ABS population. The IgA level in females and the IgM level in both sexes increased as radiation dose increased, but no effect of radiation was found on the levels of IgG and IgE.

- Anti-virus immunity

(1) Influenza virus: the effect of A-bomb radiation on the production of antibody to influenza virus was studied in 1961 *in utero* exposure and their controls. Antibody production to the vaccine was almost completely suppressed in the exposed people.

(2) Hepatitis associated virus: the frequency of positive HBs antigen was significantly higher in persons exposed to 100 rad or more, but not the prevalence of HBs antibody. A stronger relation to radiation dose was noted in the less than 50-year-old group (20 years old ATB) than in the 50 years or older age group.

(3) Epstein-Barr virus: the proportion of persons with high titers ($\geq$1:40) of IgG antibodies to the early antigen (EA) was significantly elevated among the exposed survivors. The results suggest that reactivation of Epstein-Barr virus in the latent stage occurs more frequently in the survivors.

(4) Human T-lymphotropic virus type-I: the HTLV-I antibody positive rate was higher in Nagasaki than Hiroshima survivors and significantly increased with increasing age, but no association with radiation dose was observed.

Autoimmunity

It was predicted that a high rate of autoimmune diseases would be observed in A-bomb survivors as a result of radiation-induced changes in the antigeneity of autologous cells or in the repertoire of the lymphocyte subset.

(1) Autoimmune disease: incidence studies on autoimmune diseases were started in 1958. However, until now, no significant relationship was observed between A-bomb radiation and the prevalence of autoimmune diseases such as rheumatoid arthritis, chronic thyroiditis, systemic lupus erythematosus and scleroderma, diffuse goitre, or hypothyroiditis.

(2) Autoantibody: an age-dependent increase has been observed on the prevalence rate of various autoantibodies. However, an effect of A-bomb radiation was observed only on rheumatoid factor. Rheumatoid factor is not primarily related to autoimmunity but rather to a chronic antigen stimulus, which may cause, and may be associated with, the observed increased level of IgA and IgM.

Granulocyte function

Many studies on the number and function of granulocytes, including bactericidal activity and phagocytosis among survivors have failed to demonstrate significant radiation effects as recovery of the number of granulocytes occurred relatively early after the A-bombing. There are no apparent late effects of radiation on the myeloid-cell lineage.

Systemic bacterial infection

There are no reports yet demonstrating a significantly increased prevalence of bacterial infections.

Haematopoietic disorders

- Monoclonal gammopathy

  The previous epidemiological studies relating multiple myeloma to various types of radiation exposure raised many questions concerning the nature of monoclonal disorders. No overall increase in the relative risk of monoclonal gammopathy was noted and only a suggestive increase in benign monoclonal gammopathy ($p=0.17$) was seer.

- Malignant diseases

  Recently, Preston *et al.* [7] reported an analysis of data on the incidence of leukemia, lymphoma, and myeloma in the Life Span Study cohort of A-bomb survivors during the period from late 1950 through the end of 1987 (93,696 survivors accounting for 2,778,000 person-years). Strong evidence of radiation-induced risks for acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic myelocytic leukemia (CML) exist, but not for adult T-cell leukemia (ATL) and chronic lymphocytic leukemia (CLL). There was some evidence of an increased risk of lymphoma in males (EAR = 0.6 cases per 10^4 PY-Sv) but no evidence of any excess in females. There was no evidence of an excess risk for multiple myeloma in standard analysis.

4.2. Low dose radiation and immunity

The immune system is well known to be sensitive to ionizing radiation. Doses above 0.5 Gy cause immunologic depression in a dose-dependent manner. However, low dose radiation (LDR) below 0.2 Gy may result in an entirely different picture causing stimulation instead of depression of immunity. The clinical significance of this is uncertain. The stimulating effect of LDR on immunity manifests itself in increased response of lymphocytes to antigenic, allogenic and mitogenic stimulation, enhanced phagocytic and digestive activity of macrophages as well as potentiated antitumour cytotoxicity of the killer cells. Immuno-enhancement has been observed both in human and in experimental animals exposed to LDR primarily from *in vitro* studies. T-cell activation has been found to occupy a crucial position in the stimulatory effect of LDR on immunity.

The mechanism of the immuno-enhancement after LDR has been analysed from different levels, mainly in animals. At the cellular level acceleration of thymocyte maturation and differentiation plays an important role. The molecular basis of the potentiation of T-cell function after LDR is closely related to the facilitation of the signal transduction process. The expression of the TCR/CD3 molecules on thymocytes is up-regulated resulting in increased $[Ca^{2+}]$, mobilization and PKC (protein kinase C) activation of T-cells in response to mitogenic or MeAb perturbation. This process is accompanied by a rise in transcription level of the immediate early genes, such as c-fos and c-jun, which control cell cycle progression and cell proliferation. The final outcome is increased expression of functional proteins, such as IFN-γ, IL2 and IL2 receptor. The immunologic changes occurring after whole-body irradiation with low doses are subject to neuro-endocrine modulation. The down-regulation of the hypothalamic-pituitary-adrenocortical axis with lowered serum level of ACTH and corticosterone after LDR may be an important factor facilitating immunologic potentiation.

Experimental studies showed that pre-exposure of mice to LDR suppressed tumour growth after subcutaneous implantation of Lewis lung cancer cells and decreased dissemination of the intravenously injected Lewis lung cancer cells in the lungs.

Examination of the inhabitants in an area of high natural radioactivity (3 times greater than control) in Yangjiang, China, showed increased reactivity of peripheral blood lymphocytes to mitogenic stimulation with increased DNA synthesis and IL2 secretion. A recent epidemiological study reports that the general cancer mortality in that area was significantly lower in the age group of 40-70 years in comparison with the control.

These and other experimental data suggest that there may be clinically detectable effects of LDR on mammals; however, further study in humans is required. Some of these findings are still controversial and need to be confirmed by others. Immuno-enhancement by LDR is not necessarily beneficial, and its significance to human health remains to be determined.

For further background information on these topics, the reader is referred to references [8] and [9].

4.3. Nutrition and immunity

Nutritional status is known to exert considerable influence on the immune system. This conclusion is based on data derived from epidemiological observations in malnourished human populations, experimentally induced malnutrition in both humans and animals, primarily rodents, and *in vitro* studies. While the results are most clearly seen in clinically severe malnutrition and may be difficult to detect in milder states of malnutrition, there is little doubt that defects occur across the entire spectrum of severity. The largest body of data from human studies concerns protein-energy malnutrition, the deficiency state due to inadequate protein and energy in the diet, which is generally accompanied by variable deficiency of several vitamins and minerals. Increasingly in the past several years, attention has been devoted to the deficits, or in some cases excesses, of minerals and vitamins, as well as obesity. Because of the prevalence of these diverse nutritional defects throughout the world, nutritional status is probably the single most important exogenous conditioner of immune responses. However, it is important to note some caveats at the outset. Of particular importance, human malnutrition is not uniform from person to person, even when the same nutrients are involved, because the mosaic of nutritional defects usually differs both qualitatively and quantitatively. This is further conditioned by the duration of malnutrition, variable adaptive changes, and additional influences such as infectious diseases.

Human protein energy malnutrition has been associated with several consistent changes in immune function, particularly in regard to effects on lymphocytes (Table IV). The most consistent finding, initially described a century before the immune system was defined and the significance of lymphocytes as major effector cells was known, is the depletion of lymphocytes from specific regions of thymus, spleen and lymph nodes now known to be populated with T-lymphocytes. As a result of the loss of cells, due at least in part to defects in the hormonal milieu within the thymus gland which impairs the differentiation of T-lymphocytes, these cells are also reduced in number in the circulation. Thus, when circulating lymphocytes are isolated the reduced proportion of mature cells and increased proportion of immature T-cells results in functional defects *in vitro* which are readily detected. Limited data suggests that the T-helper cell population is most dramatically affected, but because of the critical role of these cells in orchestrating and regulating a number of different immune responses, the impact is broad indeed. Although the number of B lymphocytes is relatively normal, antibody response to many antigens is subnormal, presumably due to insufficient T-cell help or possibly secondary to reduced antigen processing and presentation by macrophages, for which there is also some evidence.

Phagocytic cells obtained from malnourished hosts, including neutrophils and

monocyte-macrophages, are similar to cells from normally nourished hosts in their ability to ingest opsonized microorganisms. Although the associated oxidative burst which leads to the production of microbicidal oxygen and halogen free radicals is generally blunted, there is no consistent defect in microbial killing *in vitro*. Moreover, in many studies in which microbicidal defects are observed, the extent of the abnormality is no greater than that present in clinically healthy mothers carrying a single gene for the congenital neutrophil microbicidal disorder, chronic granulomatous disease. However, phagocytosis of microorganisms generally requires the deposition of specific immunoglobulin or complement fragments, a process termed opsonization, which renders the organism recognizable by the phagocytic cell. Deficits in immunoglobulin derived opsonins as well as consistent depression in complement activity are well described in protein energy malnutrition. The latter defect appears most probably to be due to activation and consumption of complement *in vivo* and the inability of the host to increase the synthesis of complement proteins enough during the acute phase protein response to infection.

Thus, all four of the major components of the immune response, T-cell mediated events, phagocytosis, antibody production, and complement activation, are adversely affected by protein energy malnutrition. In contrast to congenital immunodeficiencies of individual components, in which some but not all classes of microbial, viral, protozoan, or helminthic patnogens may be of particular problem, the protein energy malnourished subject is potentially excessively susceptible to all classes.

Some of the immune defects of protein energy may actually be due to abnormal mineral or vitamin status, or be mimicked by defects in micronutrients. These prominently include deficiencies of zinc and or iron. The former, is an essential constituent of several thymic hormones and of enzymes required for proliferation of lymphocytes, the most critical step beyond antigen recognition in immune responses. Similarly iron is a required metal within a number of enzymes regulating oxygen radical metabolism and for the synthesis of DNA. Both metals are transferred from the circulation to the intracellular compartment during the acute inflammatory response, where they presumably can function in these major metabolic events. Both metals may also be toxic to the immune system when in excess or, in the case of iron, present in free ionic form unbound to protein. However, when iron is given to a deficient subject the resulting stimulus to haemoglobin synthesis can favour the replication of malaria parasites, which feed on the globin derived from haemoglobin within the malaria infected erythrocyte. Even less is known about effects of changes in vitamin A, D, C, E or B vitamin status. Contemporary studies to examine the effects of vitamin A on immune function suggest a potentially important role of vitamin A metabolites as transcriptional regulators of multiple immunologically active molecules, and possibly a similar role for vitamin D as well. The specific role of the antioxidant vitamins C and E remains speculative. Most studies have explored the impact of dietary supplements and no controlled studies have demonstrated clear beneficial effects.

The other side of the coin, that is the impact of infection (or non-infectious, inflammatory diseases) on nutritional status, is well documented. A consistent finding in infection is an increase in energy expenditure, which occurs at a time when the host generally becomes anoretic and reduces dietary intake. Infection therefore initiates catabolic events, with consumption of muscle protein leading to the release of amino acids to be used for gluconeogenesis to maintain energy supplies, since concomitant counterregulatory changes in insulin, glucagon and growth hormone levels impede utilization of fat for fuel. In addition, amino acids are used for new protein synthesis of soluble immune factors and proliferating immune cells. These diverse events are co-ordinated by a group of small peptide mediators collectively known as cytokines, which orchestrate not only these stereotyped metabolic changes but activation and the regulation of many of the immune responses described above. Losses of body stores of energy and

protein require a prolonged period in convalescence to restore them to normal.

For further information on these topics, the reader is referred to references [10], [11] and [12]. Figure 1 summarizes what is known about the localization of the specific effects of nutrients on the immunologic network.

4.4. Toxic chemicals and immunity

In recent years, much attention has been placed on the possible adverse effects of exposure to environmental agents on the immunocompetence of exposed individuals [13]. As a result, considerable emphasis has been placed on the development and validation of animal models to measure immunotoxicity and to serve as a mechanism for hazard extrapolation to humans [3]. Studies using such animal models have clearly demonstrated that certain classes of environmental agents adversely affect the immune system leading to impaired host defenses. These agents include polyhalogenated aromatic hydrocarbons (e.g. PCB, PCDFs, PCDDs), certain heavy metals (organo-mercury, organo-tin compounds) and organo-chlorine pesticides (e.g. HCB, DDT, DDE), and oxidant gasses (e.g. NO_2, SO_2, O_3).

Due to the complexity and multi-facetted nature of the cells and tissues of the immune system, no single test can adequately assess immunotoxicity. Implementation of particular tests depends in large part on the existing state of knowledge concerning a particular chemical. Methods to assess damage to the immune system often employ groups of tests and involve a tiered approach to assessing immunocompetence. While there are several proposed tier testing schemes [2, 3], all begin with relatively routine assessment of toxicologic pathology including standard clinical and histopathology measurements. The complexity of testing increases to include antigen challenge studies to assess adoptive immunity, evaluation of specific immune effect cell function and finally host defense response to relevant human pathogens.

While there is considerable evidence that the immune system of laboratory animals can be adversely affected by exposure to environmental agents (under carefully controlled laboratory conditions), epidemiologic evidence linking exposure to environmental agents with a low immune status in humans is less clear. Variability among the selected cohort(s) with regard to a normal response and confounding factors involved in the interpretation of this response complicate the ability of epidemiology studies to identify agents responsible for immune injury. Subtle changes in immune function following exposure to environmental agents may not always result in a measurable health effect. Alternatively, subtle changes in immune function increasing the likelihood of adverse immune related health effects may only manifest themselves during the brief period when the stressor (e.g. infectious challenge) is present. It cannot be ruled out, however, that problems associated with studies of this nature have precluded the detection of evidence for this risk. Problems with animal-human extrapolation (which do not exclusively pertain to toxic chemicals) may be due to one or more of the following:

- The wide variety of what is considered the normal immune response in genetically diverse humans makes it difficult to demonstrate an effect statistically without a large experimental group.

- Diseases associated with immune dysfunction in humans can only be detected after a long period of latency.

- Depending on the chemical, differences in metabolism and pharmacokinetics can render humans less susceptible to exposure than experimental animals.

- Adverse health effects in humans associated with exposure to environmental agents resulting in subclinical immune dysfunction may be difficult to quantitate.

- Exposure levels required to induce immune changes in animals may not have been achieved in humans.

It should be re-emphasized that while these uncertainties related to animal-human extrapolation are valid, they do not rule out the possibility that the human immune system is at risk for damage from inadvertent exposure to environmental agents. Human tissue body burden data clearly demonstrate bioaccumulation of toxic chemicals in fat. Mobilization of fat stores and release of chemicals resulting from malnutrition, coupled with subtle changes in immune responsiveness induced by low level exposure to ionizing radiation or stress may render the host more susceptible to infection and disease.

4.5. Ageing and immunity

It is well documented that persons over 65 years of age are more susceptible to most infectious diseases than their younger counterparts [14]. Morbidity data also shows that ageing is accompanied by various immune-mediated chronic degenerative diseases, such as cancer and inflammatory diseases. Some of these changes can be modified by nutritional supplementation [15].

Ageing is accompanied by many immunological changes (Table V), including an impairment of immunocompetence — especially cell-mediated or T-cell responses. Delayed type hypersensitivity (DTH) responses to common recall antigens are decreased in the aged. Apart from a reduction in T-cell number, several studies have also demonstrated a reduced lymphocyte proliferation response to mitogens and antigens. In comparison with young adults the aged also have a higher number of natural killer cells (NK cells), but with decreased NK cell activity. However, their potential roles in the pathogenesis of immune-mediated diseases have not been elucidated. Serum thymulin (thymic hormone), which has immune enhancement property, has been reported to be markedly decreased in the aged. There are fewer changes in antibody response in the aged. A reduction of serum IgG and an increase of serum IgA concentrations have been reported. Serum autoantibody levels increase in the aged. Immune senescence leads not only to an impaired humoral and cell-mediated immune response to infectious agents but also to decreased efficacy of immunization.

The effects of ageing on immune system need to be interpreted carefully, considering various co-factors, including nutritional status, psychological factors, co-morbidity, polypharmacy, and physical activity, which may compound or potentiate the impairment of immune status in the elderly.

5. INTERACTIONS BETWEEN DIFFERENT FACTORS THAT MAY AFFECT IMMUNITY

Although it is now recognized that many different factors may affect immunity in an individual or population group, virtually nothing has been reported in the open scientific literature about the ways in which these factors interact. On theoretical grounds one would expect to observe different kinds of interaction, depending on the circumstances, e.g. that the effects could be additive or subtractive.

One of the few kinds of interactions that is well documented is the one between nutrition and ageing, as discussed in section 4.5 (i.e. some of the immune deficits associated with ageing can be partially corrected by nutritional supplementation).

In Japanese atomic bomb victims it is reasonable to suppose that there may have been some interaction between radiation injury and nutrition (in respect of immune function) during the years immediately following their exposure. Their nutritional status at that time was sufficiently poor for there to be good grounds to postulate immune deficits for purely nutritional reasons. The extensive health data available for Japanese atomic bomb victims may permit an evaluation of this issue.

The complexity of the immune system, and the fact that its function can be influenced by many different factors, make it very difficult to interpret the outcome of studies on large population groups. These difficulties are particularly evident in studies of populations affected by the Chernobyl accident. In addition to the problem of accurately assessing the radiation doses to which they were exposed, these groups have experienced nutritional problems, including ***changes*** in nutrition and radiation exposure during the period of some of the studies. Some groups may also be exposed to toxic chemicals.

Because of the multifactorial nature of immunity it is important that, in studies of immunity, all relevant factors should be controlled. Thus, in population studies of the effects of radiation, it is essential to select a control group that is similar with respect to ***all*** the other factors that can influence immune function (e.g. age, nutrition, and exposure to toxic chemicals). It is also important that none of these factors be changing in an uncontrolled way during the investigation (e.g. ***changing*** patterns of nutrition complicate the interpretation of the Chernobyl data).

Relevant information on the immune effects of radiation, in combination with toxic chemicals and nutritional factors, is thought to be available from military research programmes carried out one or more decades ago. Reports on some of these studies have only recently been declassified. The Group recommended that IAEA/WHO should commission a review of this material from countries that have conducted such research.

In the context of radiotherapy, it is possible that useful information may also be available from immune studies in such patients, and the effects of nutritional therapies. It would be useful to do a literature review of well conducted studies of immune function in radiotherapy patients in cases where nutritional investigations have also been carried out.

Some interactions between nutrition and radiation, mediated by free radical production and scavenging, may be postulated on theoretical grounds. From the nutritional viewpoint there is considerable interest at the present time in the possible role of antioxidants and free radical scavengers (e.g. vitamins C and E, beta-carotin, selenium) as agents that may protect the body from some chronic diseases, and delay ageing processes. Concerning radiation it is relevant to note that one of the most direct effects of radiation is the production of free radicals and oxidative reactions. Some of these effects almost certainly involve the immune system.

6. APPORTIONING IMMUNOLOGICAL CHANGES TO DIFFERENT POSSIBLE CAUSATIVE AGENTS

From studies of immune parameters it is generally not possible, at the present time, to identify which specific "insult" is responsible for the immune deficit or to apportion the deficit among different kinds of "insult". With few exceptions there are presently no specific "markers" by which any one kind of insult can be identified.

Information on causative agents can only be obtained from studies of the "environment" to which the individual, or population, has been exposed, e.g. by specific

investigations of nutritional status and exposure to radiation and toxic chemicals.

In cases of simultaneous exposure to two or more factors that may affect immunity (e.g. radiation and toxic chemicals) it is generally not possible to quantify *a priori* what their individual effects may be.

Considerable research is now being conducted with a view to identifying biomarkers of immunotoxicology. It is possible that this research may, in the future, reveal specific markers for different kinds of agent that may affect the immune system.

7. PRIORITIES FOR FUTURE RESEARCH

Given the fact, that not much is known yet about interactions between radiation, chemicals and nutrition concerning their effects on the immune system, experimental animal research is at present best suited for systematic investigations to find and characterize such links. From a chemical point of view, those pollutants that are common in the environment and most likely to produce significant immunotoxic effects should have high priority for studying possible interactions with radiation. Examples include polyhalogenated aromatic hydrocarbons, heavy metals and oxidant gasses. Setting up such experimentation is not fundamentally different from how immunotoxicology experiments on one stressor only would be done. Endpoints, including host resistance models, would likewise be similar, the latter being very valuable for risk estimation. It is recommended that studies of potential interactions should mainly aim at single interactions, rather than multiple ones, in order to gain interpretable results.

Studies should also be carried out in man to assess the validity of results from animal experiments and because such studies are inherently of interest in their own right. Although human studies raise a number of practical problems as compared with animal experiments, they are nevertheless worth attempting, and suitable opportunities can certainly be found. In such cases, however, it is of utmost importance to assure adequate dosimetric information. In addition, some of the already-ongoing epidemiologic studies on populations exposed to higher-than-normal levels of radiation (e.g. in areas affected by the Chernobyl accident) and already available data should be evaluated in the light of nutritional status, potential chemical exposure, etc. This would benefit the studies on radiation effects, and may retrospectively yield data on possible interactions.

At present, it is difficult to design prospective epidemiologic studies in human populations aimed at investigating possible interactions between radiation, nutrition, and chemical exposure. In any case, if research protocols are designed to further study radiation effects, information on chemical exposure and nutritional status should always be included.

8. POSSIBILITIES FOR APPLYING ISOTOPE TECHNIQUES IN STUDIES OF IMMUNE STATUS AND FUNCTION

A wide variety of isotope techniques are already well established and widely applicable in studies of immune status and function. Examples are given in table VI, and their advantages and disadvantages are summarized in table VII.

Stable isotopes can potentially make substantial contributions in human studies of immune status and function. The salient advantage of stable isotopes is that they impose no radiological hazard. The possibility that stable isotope methods can be developed for measuring the kinetics of acute phase reactant production merits consideration. Were

such methods available, it would be possible to make repeat measurements in a single individual to assess the kinetics of the response to immunostimulation. Such information would be important in various ways, including understanding the competence of the immune system.

9. RECOMMENDATIONS FOR THE ESTABLISHMENT OF A CO-ORDINATED RESEARCH PROGRAMME (CRP)

The kinds of research discussed in this report require a high level of technical ability, together with substantial resources in terms of equipment, highly trained personnel, and, for human studies, access to large numbers of experimental and control subjects. Such work requires resources that far exceed what can be made available by IAEA or WHO alone. For this reason, it is recommended that opportunities should be exploited for linkages with on-going studies that are already being supported by other organizations. The role of the IAEA (in addition to providing small research grants) would then be mainly in the area of networking, developing common protocols, promoting information exchange and assisting with quality assurance.

In view of the fact that this is a very new subject area, and that no one particular topic stands out as having overwhelming priority, it is recommended that a broadly based CRP be established. Importance should be given to human population studies on the effects of low-level radiation on immune status. The main variables of interest are (i) the level of individual radiation exposure, and (ii) the nutritional status. Appropriate control groups should also be studied.

With respect to radiation, the experimental groups should have significantly elevated levels of exposure. Such exposure may come from any one of several possible sources, e.g. the natural radiation background (in countries where areas of high radiation background are known to occur, or at high altitudes), or fallout radionuclides (e.g. in areas affected by the Chernobyl accident). Other possible experimental groups comprise radiation workers (including the "liquidators" who were involved in cleanup operations after the Chernobyl accident) and uranium miners. In all such cases it is of the utmost importance that the accumulated radiation doses and, where possible also the dose rates be known with acceptable accuracy and precision. In some cases, validation of these doses may be desirable by biological dosimetry.

With respect to nutrition, these studies should exploit already-existing differences in nutritional status between experimental and control groups, or they should involve modifying the nutritional status in a controlled manner, such as by the administration of food and/or micronutrient supplements. Standard methods of nutritional assessment should be applied, including anthropometry, body composition studies, metabolic assessment, and biochemical analysis (possibly including retrospective analysis of stored samples).

With respect to toxic chemicals, as is the case for radiation, defining the magnitude of the exposure and dose is of the utmost importance in characterizing experimental cohorts. At present, analytical chemical and epidemiologic techniques currently exist to define populations where there is documented exposure to environmental pollutants. Furthermore, standardized techniques to assess immune function in these studies are similar to those described in section 3 for both animals and humans. Therefore, with careful design, it is possible — and also desirable — to assess the contribution of toxic chemical exposure to immune dysfunction in populations that are also affected by radiation and nutritional factors.

In all such studies, immune status should be investigated by the methods discussed in section 3. In order to assure the comparability of the results obtained, it is important to agree on the use of common protocols and materials, and to develop quality control procedures (the services of a central reference laboratory may be required for doing this). In appropriate cases, additional information on immune status should be sought by compiling epidemiological data on morbidity and mortality from infectious diseases, neoplasms and immunological disorders (including changes in the incidence of type-I diabetes).

Research of the kind outlined above should be complemented by animal studies by some participants in the CRP. These provide the opportunity to conduct more closely controlled investigations, and also open the possibility for including studies of the effects of toxic chemicals. In order to be better able to extrapolate data from experimental animals to man, *in vitro* studies with animal cells and human cells under the same conditions, using comparable outcomes, offer a valuable approach.

10. SUMMARY OF THE MAIN RECOMMENDATIONS OF THE ADVISORY GROUP

IAEA and WHO are recommended to commission a review of relevant reports on the immune effects of radiation, in combination with toxic chemicals and nutritional factors, that may be available from military research programmes carried out one or more decades ago, and that have recently been declassified. (See section 5.)

The IAEA is also recommended to initiate a broadly based Co-ordinated Research Programme (CRP) focussed mainly on the effects of low-level radiation on immune status in human populations. The main variables of interest are (i) the level of individual radiation exposure, and (ii) the nutritional status. Possible experimental groups include persons living in areas of high radiation background (e.g. in countries where areas of high radiation background are known to occur naturally, or at high altitudes, or in areas affected by the Chernobyl accident). Other possible experimental groups comprise radiation workers and uranium miners. If possible, it is also recommended to assess the contribution of toxic chemical exposure to immune dysfunction in these population groups. This research should be complemented by animal studies, and possibly also by *in vitro* studies with human and animal cells, by some participants in the CRP. (See section 9.)

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TABLE I ASSAYS SUGGESTED FOR IMMUNOTOXIC ASSESSMENT OF HUMANS

	ASSAY
1.	Complete blood count with differential
2.	Antibody-Mediated Immunity (one or more of following): • Primary antibody response to protein antigen (e.g., epitope labelled influenza vaccine) • Immunoglobulin concentrations in serum (IgM, IgG, IgA, IgE) • Secondary antibody response to protein antigen (diphtheria, tetanus or poliomyelitis) • Proliferation to recall antigens
3.	Phenotypic Analysis of Lymphocytes by flow cytometry: • Surface analysis of CD3, CD4, CD8, CD20
4.	Cellular Immunity: • Delayed-type hypersensitivity (DTH) skin testing using multitest Biomerieux • Primary DTH reaction to protein (KLH) • Natural immunity to bloodgroup antigens (e.g., anti-A and anti-B)
5.	Autoantibodies and inflammation: • C-reactive protein • Autoantibody titres to nuclei (ANA), DNA, mitochondria and IgE (rheumatoid factor) • IgE to allergens
6.	Measure of non-specific immunity: • NK cell enumerations (CD56 or CD60) or cytolysis activity against K562 • Phagocytosis (NBT or chemiluminescence)
7.	Clinical chemistry screen

TABLE II METHODS FOR DETECTING IMMUNOTOXIC ALTERATIONS IN THE RAT CURRENTLY BEING EVALUATED AT NIPHEP, BILTHOVEN [2]

PARAMETERS	PROCEDURES
Tier 1	
Non-functional	- Routine haematology, including differential cell counting; - Serum IgM, G, A, and E determination; lymphoid organ weights (spleen, thymus, local and distant lymph nodes); - Histopathology of lymphoid tissues, including mucosa associated lymphoid tissue; - Bone marrow cellularity; - Analysis of lymphocyte subpopulations in spleen by flow cytometry.
Tier 2	
Cell-mediated immunity	- Sensitization to T-cell dependent antigens (e.g. ovalbumin, tuberculin, *listeria),* and skin test challenge; - Lymphoproliferative responses to specific antigens *(Listeria);* - Mitogen responses (Con-A, PHA).
Humoral immunity	- Serum titration of IgM, IgG, IgA, IgE responses to T-dependent antigens (ovalbumin, tetanus toxoid, *Trichinella spiralis,* sheep red blood cells) by ELISA; - Serum titration of T-cell independent IgM response to LPS by ELISA; - Mitogen response to LPS
Macrophage function	- *In vitro* phagocytosis and killing of *Listeria monocytogenes* by adherent spleen and peritoneal cells; - Cytolysis of YAC-1 lymphoma cells by adherent spleen and peritoneal cells
Natural killer function	- Cytolysis of YAC-1 lymphoma cells by non-adherent spleen and peritoneal cells.
Host-resistance	- *Trichinella spiralis* challenge (muscle larvae counts and worm expulsion); - *asteria* monocytogenes challenge (spleen and lung clearance); - Rat cytomegalovirus challenge (clearance from salivary gland); - Endotoxin hypersensitivity; - Autoimmune models (Adjuvant arthritis, experimental allergic encephalomyelitis).

TABLE III DECREASE IN SELF-DEFENSE ABILITY A SHORT TIME AFTER EXPOSURE

TYPE OF DAMAGE (TIME OF MANIFESTATION)	CAUSE	EFFECT ON IMMUNE FUNCTION
Rapid decrease of lymphocyte count (1st day)	Destruction of mature lymphocytes	General decrease
Decrease in humoral factors: antibodies and complements (immediately after exposure)	Decrease in antibody-producing cells (B cells) Loss of body fluids by burns and trauma	Decreased bacteriolysis Decreased phagocytosis by neutrophils and monocytes
Decrease in neutrophil and monocyte count (3rd-50th days)	Insufficient supply due to damaged haematopoeitic function	Decreased phagocytosis and bactericidal function
Delayed recovery of lymphocyte reduction (after 4th week)	Incomplete differentiation and maturation Elimination and non-activation of specific T-lymphocytes due to exposure to bacterial toxins	Delayed recovery from infection Activation of latent viruses Ease of infection by external viruses Decrease in ability to eliminate mutant cells

TABLE IV EFFECTS OF PROTEIN ENERGY MALNUTRITION ON LYMPHOCYTES

EFFECT
Depletion of lymphocytes from T-cell regions of thymus, spleen and lymph nodes
Reduction in mature T-lymphocytes in circulation
Increase in immature T-lymphocytes in circulation
Impact on T_H cells more profound than on T_S
Reduced functional capacity of circulating lymphocyte population
Normal number of B-lymphocytes
Normal or increased levels of circulating immunoglobulins
Reduction *in vivo* antibody response to some, but not all, vaccine antigens

TABLE V SUMMARY OF AGE-RELATED CHANGES IN THE IMMUNE SYSTEM

VARIABLE	DIRECTION
Lymphocyte	
* DTH	Decreased
* T cells	Decreased
* CD4/CD8 ratio	Decreased
* NK cells	Increased
* Proliferation response to mitogens/antigens	Decreased
* NK cell activity	Decreased
* IL-2 production	Decreased
Thymulin (Thymic hormone)	Decreased
Antibody	
* IgG	Decreased
* IgA	Decreased
* Autoantibodies	Increased

TABLE VI EXAMPLES OF RADIOISOTOPE TECHNIQUES FOR ASSESSING IMMUNE FUNCTION

APPLICATION	ISOTOPE/METHOD
cell division mitogenesis/blastogenesis	^{3}H-thymidine
tumour cell killing	^{51}Cr labelling of target cells
phagocytic cell assessment	^{51}Cr labelling of phagocytosed particles
acute phase protein measurement	^{13}C, ^{15}N
assessment of response to *in vivo* antigen challenge	radioimmunoassay
assessment of celayed-type hypersensitivity *in vivo*	^{125}I-uridine
molecular techniques: assessment of translation/transcription polymerase chain reaction, etc.	various isotopes/ blotting (Northern, Western, Southern)

TABLE VII ADVANTAGES/DISADVANTAGES OF USING RADIOISOTOPE TECHNIQUES FOR ASSESSING IMMUNE FUNCTION

ADVANTAGES	DISADVANTAGES
sensitivity/specificity of measurement high	high reagent cost
quantitative measurement possible	unique waste disposal problems
permits analysis of small sample volumes making repeated measurements (kinetics) possible	special handling, tracking, storage and training required
methodology and instrumentation standardized	

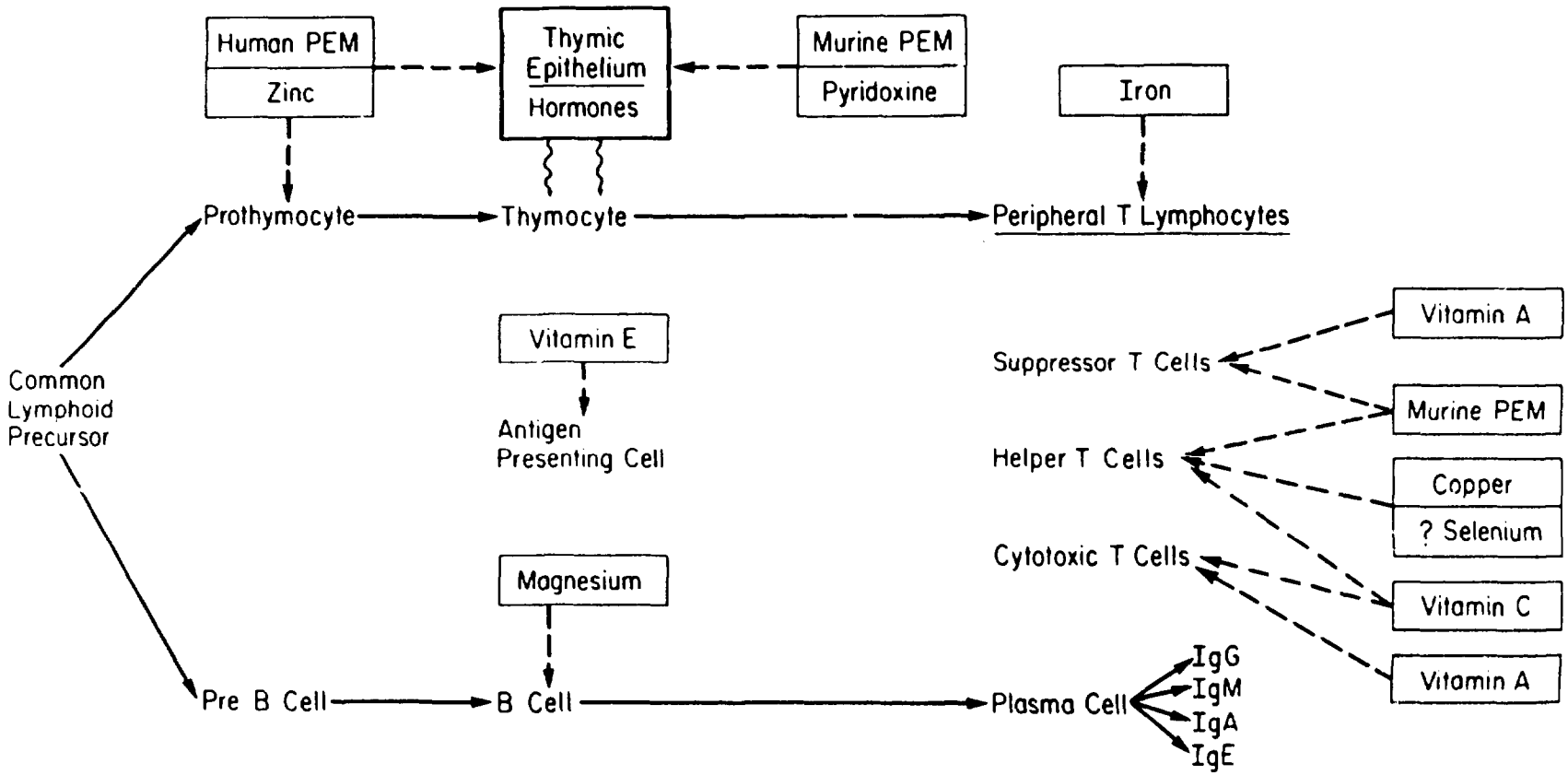

Figure 1 Localization of the specific effects of nutrients on the immunological network. *Dashed arrows*: site or specific cell type affected by the various nutrients listed for effects elicited by nutrient deprivation or supplementation.

Annex 1

AGENDA

TUESDAY, 3 MAY 1994

9:00 - 9:15	REGISTRATION
9:15 - 9:30	OPENING: *Welcome & Introductions*
	IAEA: Dr. A. Cuaron, Director, Division of Human Health WHO: Dr. I. Riaboukhine, Division of Environmental Health
9:30 - 12:30	SESSION 1: *Chair:* Keusch, G.T.
	Adoption of the agenda
	Background and purpose of the meeting (Scientific Secretary)
	PARTICIPANTS' PRESENTATIONS
	Radiation and immunity
Akiyama, M.	Immunological studies on atomic bomb survivors exposed to high radiation doses, and overview of immunological studies on human exposure to low dose radiation
Liu, S.Z.	The present status of research on the stimulatory effect of low level radiation on immunity
14:00 - 17:20	SESSION 2: *Chair:* Akiyama, M.
	PARTICIPANTS' PRESENTATIONS (continuation)
	Radiation and immunity (continuation)
Riaboukhine, I	The role of WHO in studying haematological and immunological effects after the Chernobyl accident
Vorontsova, T.V.	Immune status of children in Belarus after the Chernobyl accident
Osechinsky, I.V.	Epidemiological studies of immunodeficiency diseases as consequences of the Chernobyl accident
Barabanova, A.	Some immunological changes in patients with acute radiation syndrome
Tuschl, H.	Occupational exposure to external radiation and inhalation of tritium: effects on some immune parameters
Mircheva, J.	Basic principles in tumour immunology: immunosurveillance, immunological escape and impaired host defence of cancer patients

Nutrition and immunity

Keusch, G.T. — Effects of nutrition on immune function, and of infection and mediators of immune function on nutrition

17:30 — Cocktail

WEDNESDAY, 4 MAY 1994

9:00 - 12:30 — SESSION 3: *Chair:* Liu, S.Z.

PARTICIPANTS' PRESENTATIONS (continuation)

Nutrition and immunity (continuation)

Lukito, W. — Nutrition and immunity in the aged and in development

Immunotoxicology

Van Loveren, H. — Immunotoxicology: assessment and relevance to man

Thomas, P.T. — Chemically induced immunotoxicology: a scientific and regulatory perspective

GENERAL DISCUSSION - see separate list of discussion topics

WEDNESDAY, 4 MAY 1994

14:00 - 17:30 — SESSION 4: *Chair:* Thomas, P.T.

GENERAL DISCUSSION (continuation)

THURSDAY, 5 MAY 1994

9:00 - 12:30 — SESSION 5: *Chair:* Van Loveren, H.

Discussions and recommendations (continuation)

Preparation of draft report of the meeting

14:00 - 17:30 — SESSION 6: *Chair:* Lukito, W

Preparation of draft report of the meeting

FRIDAY, 6 MAY 1994

9:00 - 12:30 — SESSION 7:

Remaining discussions

Approval of report of the meeting

CLOSING OF THE MEETING

DISCUSSION TOPICS

1. Report of the meeting - possible options

2. Main features and functions of the immune system, and means for assessing immune status

3. Highlights of current knowledge of (1) radiation and immunity, (2) nutrition and immunity, (3) immuno-toxicology, and (4) other factors (if any) that may influence immune status

4. Interactions between different factors that may affect immunity

5. Identification of the means for quantifying immune status and for apportioning immunological changes to different possible causative agents

6. Priorities for future research

   - basic research including animal studies
   - research on human population groups exposed to higher-than-"normal" levels of radiation
   - research on human population groups exposed to "normal" levels of radiation

7. Possibilities for applying isotope techniques in studies of immune status and function

8. Recommendations to the Agency on the establishment of a Co-ordinated Research Programme in this area, and other supporting activities.

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