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# Factors Regulating the Immune Response

## Report of a WHO Scientific Group

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# *WHO Scientific Group on Factors Regulating the Immune Response*

Geneva, 1–6 September 1969

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# Factors Regulating the Immune Response

**Report of a WHO Scientific Group**

The meeting of the Scientific Group on Factors Regulating the Immune Response, which took place in Geneva from 1 to 6 September 1969, was opened on behalf of the Director-General by Dr. A. M.-M. PAYNE, Assistant Director-General

## *1. Introduction*

The immune response comprises all the phenomena that result from the specific interaction of cells of the immune system with antigen. As a consequence of such interaction, cells appear that mediate cellular immune responses (such as delayed sensitivity, or homograft immunity) as well as cells that synthesize and secrete one of several classes of immunoglobulin.

The task of the Group was to outline the present state of knowledge concerning the genetic and physiological factors that regulate these phenomena and possible approaches to manipulating the immune response. It is evident that in many experimental and clinical situations it would be highly desirable to be able to enhance the immune response or some of its manifestations (such as immunization against specific infectious agents or cellular immunity to tumour-specific antigens), while in other situations it would be valuable to be able to suppress the immune response (allergic disorders, autoimmune diseases, homotransplantation).

Methods have been developed to modify the immune response in prophylaxis and in the handling of clinical problems. Thus, the use of

adjuvants in immunization is well established, and the use of specific antibody to suppress immune responses against Rh antigen has met with considerable success. However, effective and safe immunosuppressive agents have not yet been developed, and there is a need for more adjuvants that can be used in man.

As will be shown later, the immune response is a very complex phenomenon. Accordingly regulatory factors may act at various stages and levels and may result in qualitative as well as quantitative modifications.

Experience has shown that in this field increases in basic knowledge are quickly followed by clinical application. It is therefore hoped that this report will contribute to the better understanding and control of clinical problems related to immunology.

### *2. Ontogeny and Turnover of Antigen-Sensitive cells*

Two main categories of cells are involved in the immune response:

1. Cells that specifically recognize antigenic determinants and are often referred to as antigen-sensitive or immunocompetent cells. They are thought to produce specific immunoglobulins without secreting them in detectable amounts. Following antigenic stimulation antigen-sensitive cells may differentiate to produce a progeny of either plasma cells that secrete the known classes of immunoglobulins or 'sensitized lymphocytes' that are the effector cells in delayed hypersensitivity and allograft rejection.

2. Cells that play an accessory role, in the sense that they facilitate the processing or presentation of the antigen or liberate factors that modify the various manifestations of the immune response. Among these are macrophages, fixed tissue cells, and various types of blood cell.

The terminology used in the literature to describe the morphology, physiology, and developmental aspects of these cell lines is particularly confusing. The Group was aware of the fact that the present state of the nomenclature used for the cells involved in the immune response is not satisfactory. This is mainly due to lack of correlation between the names given to cells as seen in light microscopy and their ultrastructural and, above all, functional properties and their origin. There is an urgent need for a revision of the nomenclature and standardization of the terminology.

## 2.1 Progenitors of Antigen-Sensitive Cells

Stem cells are unspecialized ancestral cells with capacities for extensive proliferation, self-renewal, and differentiation to more mature forms. Even in the adult animal there are stem cells that are sufficiently unspecialized to be the common ancestors of lymphoid cells, erythrocytes, granulocytes, and megakaryocytes. The evidence for this comes from experiments with radiation-induced single chromosomal markers in mice. By means of such markers it was first found that after irradiation bone marrow, spleen, and lymph nodes were all spontaneously repopulated with cells displaying the same karyological aberration. This observation not only showed that extensive endogenous repopulation of lymphoid and haematopoietic tissue could occur from a single stem cell but also suggested, though it did not prove, that the various cell types mentioned might all arise from a common ancestor. The possibility that the markers were all in lymphoid cells was not excluded.

The next important stage was the discovery that discrete macroscopic nodules of haematopoietic cells may be formed in the spleen of irradiated mice given intravenous injections of appropriate doses of syngeneic cells from various sources, notably spleen or bone marrow. The direct proportionality between the injected cell dose and the number of nodules or colonies formed was in itself indicative of the fact that each colony arises from a single unit of cells, normally referred to as the CFU (colony-forming unit). This point was proved beyond doubt by means of the T6 chromosomal marker[1]. Histologically, the spleen colonies are mixtures of erythropoietic and granulopoietic cells as well as of megakaryocytes, and the relative proportions of these are subject to extreme variation. The fact that they all arise from a single cell thus proves that at least these three lines of haematopoietic cells have a common stem cell.

The conclusive evidence that the same haematopoietic stem cell can also give rise to lymphoid cells comes from experiments in which other single radiation-induced karyotypes have been found, both in the endogenously repopulated thymus and lymph nodes of the irradiated animal and in the spleen colonies formed by the CFU of the animal on transfer to other mice. The pedigree of all these cell lines is not, how-

[1] This marker first arose in a single mouse after sublethal irradiation and was later transferred to a pure CBA strain by appropriate crosses and inbreeding.

ever, known in detail. It is possible, for example, that specialization of the stem cell descendants making them progenitors of lymphoid cells occurs before differentiation into a CFU. Alternatively, the CFU may give rise to lymphoid lines in addition to haematopoietic lines.

Stem cells are found first in the primitive blood islands and yolk sac of the embryo, later in foetal liver and finally in marrow. They are disseminated via the blood stream into both embryonic myeloid tissues (marrow and spleen) and primary lymphoid tissues (see below), where their differentiation is induced by the embryonic rudiment concerned. Nothing is known concerning the inductive forces but it is probable that humoral influences, such as erythropoietin or thymus humoral factor, play an essential role.

Stem cell migration and differentiation occur not only in the developing animal or in the animal recovering from irradiation exposure but also in the normal adult, though to a limited extent. Under normal physiological conditions, pluripotent stem cells may be used to 'top up' populations of line-progenitive cells when they become depleted as a result of age or the demands placed on them. The pluripotent stem cell pool itself is replenished by its extensive cell renewal capacity, which outlasts the animal's own lifespan. These findings have been made by continued serial passage of bone marrow in syngeneic irradiated mice. It was apparent that lymphoid cells stopped being renewed before haematopoietic cells did. The blood concentration of stem cells is low in the normal adult (about 10 per ml of mouse blood) but is increased whenever there is a demand for stem cells or line-progenitive cells, for instance, during the postirradiation recovery phase and following stimulation by pertussis vaccine.

The morphological appearance of the stem cell is not known for certain. A study of the developing lymphomyeloid tissues of chick embryos has suggested that it may be a blast cell with heavily basophilic cytoplasm and a prominent nucleolus. In the normal steady state in the adult, stem cells may not be continuously replicating as in the embryo and may have the morphological appearance of monocytoid cells or lymphocytes.

## 2.2 Primary Lymphoid Organs

The thymus in all vertebrates and the bursa of Fabricius in birds are primary lymphoepithelial organs derived initially from the

ectoendodermal junction in association with gut epithelium. During the second half of embryogenesis, stem cells migrate into these structures and differentiate in ways dictated by the local inductive influences prevailing in the respective rudiments. Under normal conditions, they differentiate exclusively into lymphoid cells. Once they have differentiated into lymphocytes they can no longer function as stem cells and will not repopulate the depleted primary lymphoepithelial organs of irradiated animals. Thymus-influenced lymphoprogenitive cells mediate cell-mediated immune responses – delayed hypersensitivity and allograft immunity –, while bursa-influenced cells (or their equivalent in mammals) are capable of producing immunoglobulins and humoral antibodies.

There are certain fundamental features that distinguish primary from secondary lymphoid organs:

1. The first organs to become lymphoidal during development are the primary lymphoepithelial tissues, the thymus and bursa of Fabricius. In these tissues lymphocytes differentiate from stem cells that have migrated into the epithelial rudiment. In secondary lymphoid tissues lymphoid development occurs later. Lymphocytes do not make their appearance within epithelial tissue or epithelium-lined follicles (which do not exist in these organs) but originate from lymphoid cells that have already been wholly or partly differentiated in the primary organs.

2. Lymphopoiesis in primary lymphoid organs is intense and antigen-independent. This is evident from the intense mitotic activity characteristic of the lymphoid cells in the thymus and bursa, a phenomenon that can be observed even in the foetus, which is relatively antigen-free, and in germ-free animals. Lymphopoiesis in lymph nodes, splenic white pulp, and Peyer's patches, on the other hand, is low or non-existent before birth and markedly reduced in germ-free as compared to other animals. It is normally antigen-dependent.

The cytological hallmarks of an active immune response (e.g., production of large pyroninophilic cells, plasmacytopoiesis, and germinal centre formation) are generally not apparent in primary lymphoid organs under normal conditions, even after intense antigenic stimulation. They are, of course, characteristic events in secondary lymphoid tissues after antigenic stimulation. The absence of antigen-induced cytological changes in the thymus and bursa does not imply that primary lymphoid organs do not generate cells that, after migration to the appropriate environment of the secondary lymphoid tissues, can

respond to antigens by undergoing transformation to large pyroninophilic cells or plasma cells. Thus, during development, lymphocytes capable of becoming transformed to large pyroninophilic cells under the impact of foreign histocompatibility antigens are detected *first* within the thymus. Mouse neonatal thymus cells of parental strain injected intravenously into lethally irradiated $F_1$ hybrids rapidly undergo transformation within the spleen to large pyroninophilic cells. At that stage of development there are no cells in the spleen or blood capable of producing this response. The bursa is the *first* site of IgM synthesis in the developing chicken and in the chicken raised in either conventional or germ-free conditions. This synthesis is thus unlikely to be antigen-dependent but may reflect the production of an immunoglobulin by immature cells which, upon migration to secondary lymphoid tissues and following antigenic stimulation, can differentiate into mature plasma cells.

3. Repopulation of primary lymphoid organs after irradiation is dependent upon a supply of stem cells. In birds it is not known whether the same stem cell is responsible for populating the thymus and the bursa with their respective lymphoid progenitors. Repopulation cannot be effected by differentiated lymphocytes such as those from the thymus, bursa, thoracic duct, or lymph nodes. On the other hand, repopulation of secondary lymphoid tissues can be achieved by populations of lymphocytes lacking stem cells. For instance, thoracic duct cells can repopulate certain areas of the lymph nodes, spleen, and Peyer's patches in irradiated animals. Peyer's patches and other secondary lymphoid tissues have been repopulated endogenously in animals in which one Peyer's patch was shielded and the rest of the body exposed to heavy doses of irradiation.

4. Extirpation of primary lymphoid tissues under defined conditions leads to characteristic defects in immunological capacity. Removal of these organs *prior* to the development or appearance of the lymphoid cells responsible for specific immunological functions impairs, or prevents the development of, the capacity to carry out these functions. Removal of the same organs in the adult does not influence immunological capacity unless the pool of immunologically competent lymphoid cells is depleted as a result of age or following destruction by irradiation or by antilymphocytic measures. Under these conditions, the maintenance or recovery of normal immunological capacity is dependent upon the existence of primary lymphoid organs.

In the chicken, there is a clear-cut separation between thymus-dependent cellular immunity and bursa-dependent humoral antibody production. Chickens bursectomized *in ovo* are characterized by a deficiency of both 19S and 7S immunoglobulins and by the absence of both primary and secondary antibody synthesis. They exhibit cell-mediated immune responses such as graft rejection and delayed hypersensitivity reactions. Thymectomy at hatching does not depress the capacity for immunoglobulin or antibody production but impairs cell-mediated immunities. In mammals, thymectomy impairs cell-mediated immunities but does not directly influence the generation of cells that produce immunoglobulins and humoral antibody. There must therefore be a non-thymus-dependent system that controls the differentiation of sets of immunoglobulin-producing cells. No single or multifocal organ has been identified that can unequivocally be shown to exert a specific bursa-like function. It is possible, however, that the gut epithelium produces some factor that controls the differentiation of antibody-forming potential in specific sets of lymphoid cells present throughout the secondary lymphoid tissues.

Some humoral antibody responses in mammals are, however, diminished by thymectomy, e.g., the responses to heterologous erythrocytes and serum proteins. The reason for this may be that, in order to produce a normal antibody response to these antigens, there must be collaboration between thymus-dependent and thymus-independent cells (see section 5.5.2).

Extirpation of secondary lymphoid tissues does not generally inhibit the *development* of the capacity to make a response, but it is not possible to carry out this operation quantitatively. Such extirpation may inhibit the response itself if the cells involved are predominantly located within the organ removed. Thus, for instance, splenectomy in the mouse diminishes the response to an intravenous injection of sheep erythrocytes. These observations may be related to the reports that, in several instances, splenectomy in infancy was followed by repeated episodes of septicaemia and meningitis, particularly the pneumococcal form.

Immunological deficiency syndromes have been described in man[2]. Some of these show clear-cut abnormalities. Thus, several of these syndromes, not associated with thymic abnormalities, are characterized by hypogammaglobulinaemia and deficient humoral antibody re-

[2] Wld Hlth Org. techn. Rep. Ser., No. 402 (1968).

sponses, although cellular immunity is normal or only slightly impaired. Others show the reverse phenomenon: they are characterized by absence of the thymus (Di George's syndrome), have deficient cell-mediated immunities, normal levels of peripheral blood lymphocytes, normal immunoglobulin levels, and little impairment of humoral antibody production. There are also a number of syndromes associated with thymus hypoplasia and characterized by severe deficiencies of both cell-mediated immunities and humoral antibody responses.

It is of considerable interest that implantation of thymus tissue alone has resulted in the recovery of immunological capacity in several patients with Di George's syndrome. On the other hand, it has recently been reported that blood transfusion and the grafting of marrow from HLA-compatible siblings (presumably a source of stem cells), without thymus implantation, was beneficial in patients of the thymus hypoplasia group who exhibited combined immunological deficiencies. These clinical observations indicate that the immune system in man is also composed of (1) a thymus-dependent system responsible for the development of small lymphocytes capable of initiating cell-mediated immune responses; (2) a system equivalent to the bursa-dependent system and responsible for the production of plasma cells and lymphoid follicles and the synthesis of immunoglobulins and antibodies; and (3) a population of stem cells responsible for populating the primary lymphoid tissues.

## 2.3 Secondary Lymphoid Organs

The differences between primary and secondary lymphoid organs are given above. The cells that populate the secondary lymphoid tissues are derived originally from stem cells that have come under the influence of the thymus or of the bursa or its equivalent. The secondary lymphoid organs are compound structures made up of mixed populations of 'thymus-dependent' and 'bursa-dependent' lymphocytes. The terms 'thymus-dependent' and 'bursa-dependent' are used to indicate that these cells are dependent upon the existence of the thymus and bursa for their origin; they do not imply a dependence on the primary lymphoid organs for the functioning of these cells once they have been produced. This is evident from the fact that normal immune responses can occur in animals thymectomized or bursectomized as adults – after

the development of 'thymus-dependent' and 'bursa-dependent' cells. The latter in mammalian species will be referred to as 'thymus-independent' cells. Some evidence has been produced to show that these cells or their ancestors originally emigrated from the thymus and bursa and may thus be termed 'thymus-derived' or 'bursa-derived' cells. Other evidence indicates that humoral factors produced by the thymus or bursa may have had an influence at some stage on the differentiation of the stem cell to a lymphoid cell and that this influence may be exerted outside the environment of the thymus or bursa.

*2.3.1 Thymus-Dependent Cells*

These are localized in the paracortical area of the lymph nodes, periarteriolar lymphocyte sheaths of the spleen, and diffuse lymphoid tissue of Peyer's patches. They circulate in blood and lymph and form part of the recirculating pool of small lymphocytes. These cells specifically migrate from the blood through the periarteriolar lymphocyte sheaths of the spleen and back to the blood by some unknown route. In the lymph nodes they pass through the post-capillary venules into the paracortical areas and return to the blood stream via the efferent lymphatics and main lymph ducts. They are responsible for the initiation of some immune responses such as graft-versus-host reactions and host-versus-graft reactions – responses that are diminished by thymectomy and by any measures that deplete the recirculating pool. Among the latter are thoracic duct drainage, irradiation of the spleen or of the blood in an extracorporeal circuit, and intravascular implantation of a radioactive source in some site where extensive movement of recirculating cells occurs.

That thymus-derived cells can become part of the recirculating pool has been shown by injecting chromosome-marked neonatal thymus cells intravenously into mice thymectomized at birth. The pool of recirculating cells was restored to its normal level and contained cells that, upon appropriate antigenic stimulation, devided and exhibited the chromosome markers characteristic of the thymus donor.

Experiments with radioactive markers introduced directly into the thymus have provided evidence that some of the lymphocytes produced by the thymus migrate to the secondary lymphoid tissues, where they become localized in the paracortical areas of the lymph nodes, periarteriolar lymphocyte sheaths of the spleen, and diffuse lymphoid tissue of Peyer's patches.

Thymectomy has been associated with cellular depletion in the paracortical areas of the lymph nodes and periarteriolar lymphocyte sheaths of the spleen. These areas have accordingly been termed 'thymus-dependent areas'. The depletion does not affect the superficial subcortical zone, the lymphoid follicles, germinal centres, or medullary cords that contain plasma cells. In chickens that have been thymectomized and irradiated after hatching, cell depletion is marked in the periarteriolar lymphocyte sheaths of the white pulp of the spleen but not in the sharply circumscribed lymphoid follicles. There is a lack of small lymphocytes but not of plasma cells. In the mouse, neonatal thymectomy or adult thymectomy followed by total body irradiation and marrow protection has led to extreme diminution in the number of recirculating small lymphocytes as determined by prolonged thoracic duct drainage.

The evidence just summarized suggests that the thymus is responsible for exporting some cells, which become part of the recirculating pool. It is evident, however, that in many immunological situations the immunological activity of thymus cells is inferior to that of, for instance, thoracic duct cells. It may be, therefore, that thymus-dependent immunologically competent cells are present in the thymus in a proportion that is much less than that in the thoracic duct and/or that there are one or several maturation steps between the thymus lymphocyte and the recirculating small lymphocyte. Experiments in which $^{51}$Cr-labelled thymus cells were injected intravenously into mice showed that a small proportion of these cells can, in fact, recirculate in the same manner as thoracic duct cells.

It is pertinent to mention here that some degree of correction of the defects caused by neonatal thymectomy can also be achieved by implanting thymus tissue enclosed in a diffusion chamber, the pores of which do not allow the passage of cells to or from the chamber. This suggests that the thymus might exert its effect in immunogenesis partly via a humoral factor. A substance, 'thymosin', has recently been prepared from calf thymus and been found capable of partially reversing the immunological defects of thymectomized mice. The mode of action of thymus humoral factor is unknown. Two alternative possibilities can be envisaged. (1) A humoral factor, produced by thymus epithelial cells, may normally act within the thymus to control the differentiation of stem cells into thymus lymphoid cells. Under artificial conditions which preclude the passage of cells to or from the thymus,