Developments in Clinical Immunology

Edited by

M. Ricci, A.S. Fauci, P. Arcangeli and P. Torzuoli

PREFACE

Professor Mario Ricci, Director of the Clinical Immunology Department in the University of Florence, thought it appropriate to organize a post-graduate course in the ambit of the Post-Graduate School of Allergology, by holding a symposium with some of the most active scientists in this field.

Given the rapid accumulation of knowledge in both basic scientific and clinically applicable aspects, it was difficult, if not impossible, to include all appropriate subjects. Hence, it was decided to focus on relatively limited areas with potentially broad applications.

With regard to immunology, the "Identification, Separation and Function of Lymphoid Cell Subpopulations" was chosen as the theme. Discussions in this area comprise the first section of this volume. A broad scope of subjects is discussed ranging from ontogenic and developmental aspects of lymphocyte subpopulations, to the most recent advances in characterization and separation of lymphocyte subsets, to mechanisms of immunoregulation and, finally, to application of these advances to diseases with immunologic implications.

Concerning allergy, "Advances in Diagnosis of Allergy" was the general topic chosen and discussions in this area comprise the second section of the book. Emphasis is placed on recent technological advances in the specific diagnosis of allergic disorders of the immediate hypersensitivity type, and critical comparisons are made between these newer techniques and the more standard diagnostic approaches. In addition, the interrelations between the classic non-immediate cellular and humoral immune systems and immediate hypersensitivity are considered both at the basic mechanistic level and with regard to their relevance to clinical allergy.

Scientists, clinical immunologists and allergists from several countries who are actively engaged in investigations in these areas presented overviews, as well as their most recent data concerned with these topics. This volume represents these presentations. The conference proved to be extremely fruitful both for the contributors and attendants. Hopefully, this volume will reflect the intellectual stimulation which was generated at the conference.

The symposium was sponsored by Bayropharm Italiana S.p.A., in order to participate actively in the scientific development of allergology and immunology with its Hollister-Stier Laboratories Division.

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M. Ricci
A. S. Fauci

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Session One

IDENTIFICATION, SEPARATION AND FUNCTION OF LYMPHOID CELL SUBPOPULATIONS

THE STAGES OF LYMPHOCYTE MATURATION

I. C. M. MacLennan

Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford, England

THE PRINCIPAL TYPES OF LYMPHOCYTES

There are a number of different classes of lymphocytes which can be recognized on functional grounds. Of these the most easily defined comprises those cells which produce antibody and their precursors. These cells are termed B cells. The second major group of lymphocytes (T cells) is far more heterogeneous in its functions but lymphocytes of this class have the generic property of requiring the thymus for their development. T cells include those which assist in triggering antibody responses, those which have specific cytotoxic capacities, cells which are concerned with the homeostasis of immune responses and lymphocytes with the capacity to secrete a variety of soluble factors (lymphokines) which may influence immunological and inflammatory reactions. In addition to these two major classes of lymphocytes it has become necessary to define a third type of lymphocyte which kills target cells sensitized with antibody. These cells do not require the thymus for their development and do not appear to be involved in antibody production. They are now termed K cells.

Although these three classes cover the known functional types of lymphocytes, it is by no means certain that all lymphocytes can be made to fall into one of these categories. This particularly applies when one is considering human lymphocytes where much of the current classification is based on indirect evidence and extrapolation from animal data.

ORIGINS OF LYMPHOCYTES – THE PRIMARY LYMPHOID ORGANS

B Cells

B lymphocyte development has been investigated in detail in birds. These animals have a distinct organ which is essential for B cell maturation. This organ is an outpushing of the hind gut and is termed the bursa of Fabricius. Cells with the

capacity to develop into antibody-producing cells probably migrate from the blood islands of the yolk-sac via the blood to the bursa (Moore and Owen, 1967). Once in the bursa, they undergo successive division. In the chick embryo these cells begin to develop surface immunoglobulin about 14 days after fertilization. Surface immunoglobulin, which is produced by the cell itself, is the most characteristic marker of B cells in all species (Greaves, 1970; Raff, 1970; Rabellino *et al.*, 1971).

There is no obvious equivalent organ to the bursa in mammals and all attempts to implicate any one part of the gut or the bone marrow as an organ exclusively involved in B cell maturation have failed. However, experiments by Owen *et al.* (1974) strongly suggest that the liver may be an important primary lymphoid organ concerned with the production of immunologically competent B cells. These workers set up organ cultures of mouse foetal liver, taken from embryos at times up to 5 days before the physiological date at which surface immunoglobulin positive cells are normally first detectable. They showed that these organ cultures would generate mature B cells. There is some evidence to indicate that B cells can also develop from bone marrow (Osmond and Nossal, 1974).

T Cells

Just as in the case of the bursa in B cell development, T cell progenitors do not develop *ab initio* in the thymus but migrate there from elsewhere. The bone marrow has been shown to be capable of providing prethymic T cells (Davies, 1969). The thymus is the site of intense lymphocyte proliferation in early life and, perhaps surprisingly, only about 10% of the cells produced leave the thymus as mature T cells. In mice T cells are distinguished from other lymphocytes by the presence of a surface antigen theta or Thy-1. While no equivalent antigen is obviously present on human T cells, Brain *et al.* (1970) showed that many human lymphocytes will form spontaneous rosettes with sheep red blood cells. As cells with surface immunoglobulin do not form spontaneous rosettes with sheep red cells, the phenomenon has been taken as a putative T cell marker in man (Jondal *et al.*, 1972). However, the thymus-dependency of all such rosetting cells in man has not been demonstrated.

K Cells

The site of K cell production is not known. It seems unlikely that the bone marrow or thymus are involved in K cell development as these organs are very deficient in K cell activity (MacLennan and Harding, 1970). K cells are probably relatively short-lived cells and K cell activity rapidly regenerates following depletion by irradiation (Pudifin *et al.*, 1971) and cytostatic agents (Campbell *et al.*, 1974).

IMMUNOLOGICALLY COMPETENT CELLS

The previous section dealt with the development of T and B cells to the stage when they become capable of recognizing antigen and participating in immune responses. After leaving the primary lymphoid organs most of these cells, now immunologically competent, enter a stage of constant, non-random migration. This

migration is between the blood and the secondary lymphoid organs. The overall pattern of migration is depicted in Fig. 1. It will be seen from this that only a small proportion of the total recirculating pool is in the blood at any time. Consequently redistribution of lymphoid cells without lymphocyte destruction, such as occurs after steroid treatment, can profoundly alter the intravascular lymphocyte content. Nearly all lymphocytes enter lymph nodes, spleen and Peyers' patches directly from the blood through specialized vascular endothelium. Only a small proportion of cells arrive via the tissues. Hall and Morris (1965) estimated that no more than 10% of cells reaching sheep lymph nodes arrive by the afferent lymph.

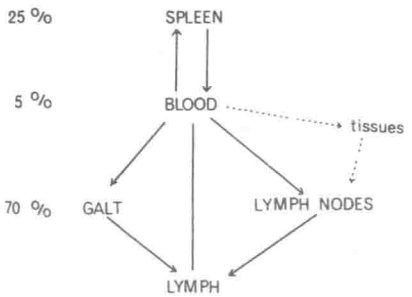

Fig. 1. The direction of migration of lymphocytes of the recirculating pool. The approximate proportions in each compartment in the rat are indicated in the left of the figure. GALT = gut associated lymphoid tissue.

The distribution of different classes of lymphocytes in the various secondary lymphoid organs has been studied in experimental animals in detail. It is clear that B and T cells follow different pathways in their passage through the spleen, lymph nodes and Peyers' patches. However, these pathways cross at various points such as the paracortical area of lymph nodes and the marginal zone in the spleen. The different distribution of lymphocytes in secondary lymphoid organs is an important means of recognizing lymphocyte heterogeneity and understanding of this migration is vital in the interpretation of many aspects of immunopathology. The reader is recommended to study Ford's review (1975) for a detailed account of this subject.

Although most immunologically competent cells recirculate, there is some evidence for static antigen reactive cells. Strober (1972) and Strober and Dilley (1973a, b) in particular, have presented evidence that some B cells, which are relatively rapidly dividing and which have not yet been stimulated by antigen, can remain in secondary lymphoid organs without recirculation.

The sites of secondary lymphoid tissue can be expanded in disease. A good example of this is the synovial membrane in rheumatoid arthritis which develops both primary and secondary lymphoid follicles.

LYMPHOCYTES AFTER ANTIGENIC STIMULATION

So far we have considered lymphocytes before they meet antigen. After appropriate antigenic stimulation, lymphoid cells may enter on a final maturation pathway such as that occurring in the transformation of small B lymphocytes to plasma cells

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(Ellis *et al.*, 1969). Alternatively they may mature to effector cells, as in the case of cytotoxic T cells, and then revert to small recirculating cells which are capable of subsequent restimulation to effector function (MacDonald *et al.*, 1974). Finally some lymphoid cells may not mature directly to effector cells but may proliferate to expand the immunologically competent pool of cells capable of reacting with a given antigen. Figure 2 summarizes the various developmental possibilities which have been discussed.

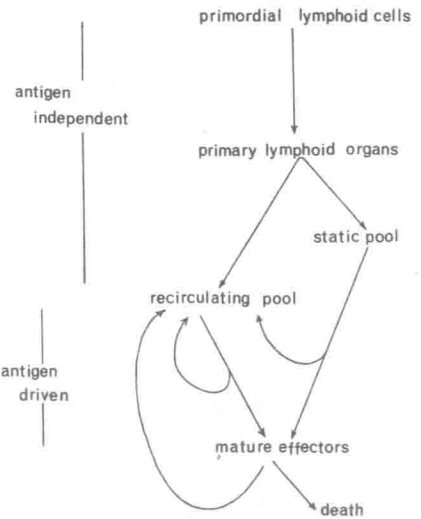

Fig. 2. Diagrammatic representation of the stages of lymphocyte maturation. This figure summarizes the text description.

SITES OF LYMPHOCYTE TRIGGERING

Some lymphoid cells may be activated by antigen where it first appears in the body. However, there is good evidence that lymphocyte triggering occurs to a major extent in secondary lymphoid organs. If antigen is prevented from migrating to secondary lymphoid tissue immune responses can be markedly reduced. For example xenogeneic red cells injected in the portal vein, which consequently have to pass the macrophage beds of the liver, are far less immunogenic than equal doses of red cells injected into the inferior vena cava (Trigger *et al.*, 1974). With our present knowledge of cell co-operation requirements for lymphocyte stimulation it is perhaps not surprising that much of the lymphocyte triggering which is observed during an immune response occurs at the cross-roads of lymphocyte recirculation. Sprent and Miller (1973) showed that antigen-sensitive cells in the recirculating pool become localized in organs which have trapped antigen. There is certainly good evidence that there is a temporary cessation of lymphocyte egress from lymph nodes following triggering of lymphocytes in those nodes by antigen. Hay *et al.* (1973) provide evidence to show that this block of lymphocyte output may be triggered by a soluble factor produced by T cells. Equally lymphocyte traffic from lymph nodes can be non-specifically blocked by corticosteroids (Spry, 1972).

MATURATION OF IMMUNOLOGICALLY COMPETENT CELLS

After antigen is localized in lymph nodes and the spleen, activation of lymphocytes is first seen in the areas of small lymphocyte passage. Histologically the activation becomes apparent by the development of large pyroninophilic cells and many of these cells subsequently enter the lymph (Hall *et al.*, 1967). However, some stay in the secondary lymphoid organ of origin and develop into plasma cells. Local plasma cells are found mainly in the medulla of lymph nodes and the red pulp of the spleen. The greater part of large pyroninophilic cells of B cell origin which enter thoracic-duct lymph migrate to the lamina propria of the gut (Gowans and Knight, 1964). The gut is the largest site of antibody production in the body. This is shown by Crabbé and Heremans (1966) who estimate that the plasma cell content of human lamina propria averages 380 000/mm^3. Reduction in the number of plasma cells in the gut is by no means always reflected by a corresponding loss of small recirculating B cells (Campbell *et al.*, 1974). These authors used rectal biopsy to assess the number of gut plasma cells. This relatively minor procedure is an important clinical tool for investigating abnormalities in immunoglobulin production.

The large pyroninophilic cells which enter the lymph may be either B or T cells. The lineage of these cells cannot be easily distinguished by either light or electron microscopic means. The general feeling that stimulated B cells have well developed endoplasmic reticulum while T cell blasts show only clustered or single ribosomes is probably a serious over-simplification. Hummerler *et al.* (1972) analysed cells in efferent lymph which were forming direct plaques (IgM antibody) against sheep red cells. They found a number of cells with single ribosomes in the cytoplasma which had formed plaques.

CONCLUSION

The purpose of this paper has been to put lymphocyte heterogeneity in the perspective of the several stages of lymphoid cell development from embryogenesis to effector cells. In man we are generally able to study lymphocytes only in the blood. It is important to remember how small this sample is in relation to the lymphocyte pool and to recall that it provides a very poor reflection of the beginning and end stages of lymphocyte development.

SUMMARY

The present chapter discussess lymphocyte heterogeneity in relationship to the various stages of cell development from embryogenesis to effector cells. B cells and their precursors are those lymphocytes which ultimately produce antibody. The second major group of lymphocytes (T cells) is far more heterogeneous but requires the thymus for development. A third type of lymphocyte which kills target cells sensitized with antibody is termed K cell. The origins of lymphocytes are described in the present paper with special reference to primary lymphoid organs. Immunologically competent cells leave the primary lymphoid organs and enter a stage of constant non-random migration between the blood and the

secondary lymphoid organs. The distribution of different classes of lymphocytes in the various secondary lymphoid organs was studied in detail in experimental animals.

After appropriate antigenic stimulation, lymphoid cells may enter a final maturation pathway, such as that occurring in the transformation of small B lymphocytes to plasma cells. The sites of lymphocyte triggering and the maturation of immunologically competent cells are then discussed in the light of the most recent experimental data.

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T-LYMPHOCYTE ONTOGENY

G. Astaldi and Ü. Topuz

The Blood Research Foundation Center, Hospital of Tortona, Italy*

The story of the T-lymphocyte differentiation sequence starts from the CFU_S, as is summarized in Fig. 1. It travels from the bone marrow and goes into the thymus via the stage of the lymphatic committed stem cell and the pre-thymic cell. Owing to the thymic microenvironment (epithelial cells), the above-mentioned stem cell differentiates to the T_0-stage (antigen reactive cell progenitor). This latter cell may either go back to the marrow, or undergo a further maturation within the thymus and then move to the T-structures of the peripheral lymphoid organs (spleen, lymph nodes, etc.). The maturation sequence from the T_0-stage to the T_1-stage is conditioned by the thymic factor(s) present also in the peripheral blood. On the other hand, the shift from the T_1-stage to the T_2-stage is antigen-driven, but this shift probably needs the presence of the thymic factor(s) as well.

Both the T_0- and T_1-lymphocytes have high levels of terminal deoxynucleotidyl transferase (TdT), and the cell may shift respectively from the T_0- to the T_1-stage, or back from the T_1- to the T_0-stage according to the presence or absence of the thymic factor(s). In contrast, the cell in the T_2-stage virtually does not contain any more TdT and has reached an irreversible stage.

The main T-cell features are also shown in Fig. 1.

T_0-lymphocyte

The T_0-lymphocyte has a short life span and a rapid maturation rate. It does not form E-rosettes with sheep erythrocytes, but may form autologous rosettes with erythrocytes of the same species. It does not show any suppressor or helper function. In cell cultures it does not react to mitogenic or antigenic stimulation, not even to allogeneic lymphocytes in the mixed lymphocyte culture (MLC). It

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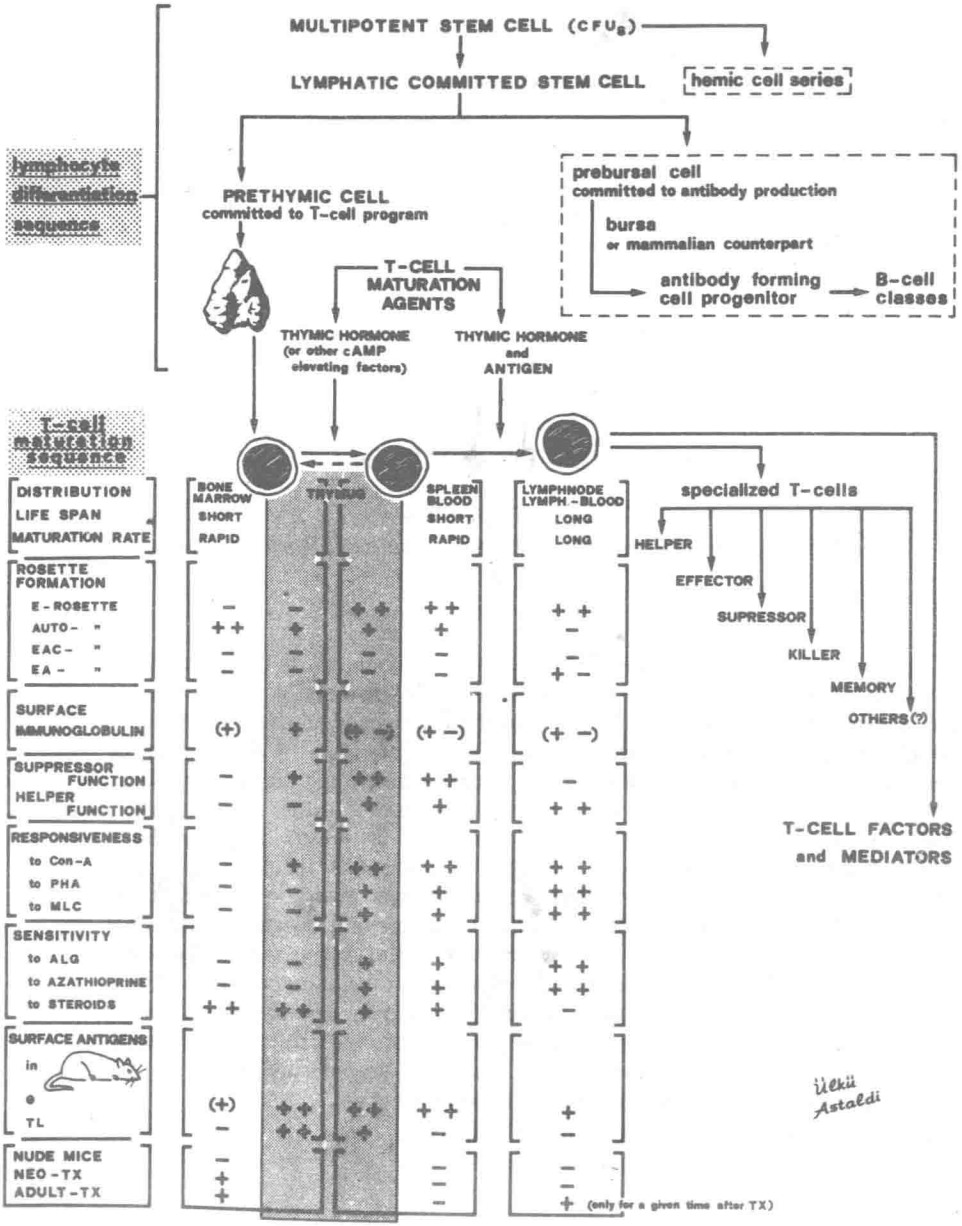

Fig. 1. T-cell ontogenesis and features.

is insensitive to azathioprine (Az), and to the antilymphocytic globulin (ALG), but is sensitive to steroids. In mice, it possesses thymus leukaemia antigens (TL), and probably also low amounts of theta antigens.

The T_0-lymphocyte may be found not only in the intact mouse, but also in both neonatal and adult thymectomized mice. Indeed, during fetal life some T-cell maturation may occur within the thymic microenvironment.

T_1-lymphocyte

The T_1-lymphocyte may be found mainly in the thymus and among the few T-cells present in the spleen; it does not undergo much traffic. It has a short life span and a rapid maturation rate, as does the T_0-lymphocyte. In contrast to the latter, the T_1-cell is markedly E rosette +ve and may also form autologous rosettes. It is intensely responsive to Con A, but only moderately to PHA and MLC stimulation. It is slightly sensitive to ALG and to Az. In mice when investigated within the thymus, it has theta antigens, and TL antigens. It is absent in neo-thymectomized, adult-thymectomized and in nude mice. It shows some functional properties, such as suppressor or helper functions.

T_2-lymphocyte

The T_2-lymphocyte is quite plentiful either in the lymph nodes, or in the lymph and peripheral blood, and undergoes an intense traffic. It is markedly E rosette +ve, but does not form auto-rosettes. It is very sensitive to ALG and to Az, but resistant to steroids. T_2-cells are almost irreversibly differentiated lymphocytes and have a long life-span. After antigen stimulation they may undergo the so-called blastic activation (transformation), and become specialized in function as genetically programmed. In this way T_2-cells may undergo further clonal selection and differentiate into various functional T-lymphocyte subclasses including helper, suppressor, effector and killer cells, as well to produce quite numerous specific T-cell factors and mediators. T_2-cells in mice contain only small amounts of theta and no TL antigens. These cells are not present in nude and neo-thymectomized mice. In adult-thymectomized mice they may be found for a long time after thymectomy, owing to their long life-span, before their final disappearance.

SUMMARY

T-lymphocyte ontogeny is described from its origin in the bone marrow to the thymus. The stem cell differentiates to the T_0-stage, which may undergo a further maturation within the thymus and then move to the T-structures of the peripheral lymphoid organs. The maturation sequence goes on to T_1- or T_2-stages. Finally the features of T_0-, T_1- and T_2-lymphocytes are described.

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