the lymphocyte

structure and function

part I

John J. Marchalonis

THE LYMPHOCYTE

STRUCTURE AND FUNCTION

PART I

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PREFACE

Lymphocytes are small round cells of undistinguished appearance which are found in the blood of all vertebrates. Although various activities have been proposed for these cells, it is now clear that they function in immune recognition and facilitate the elimination of infectious agents, grafts, and tumors. These cells, which are morphologically so homogeneous, can be functionally differentiated into two broad categories, thymus-derived lymphocytes (T cells) and bone-marrow derived lymphocytes (B cells), and these categories can be further subdivided on the basis of biological properties and surface markers. Lymphocyte biology is an exciting and rapidly growing area of research in which immunologists, cell biologists, membrane biochemists, and molecular biologists are making important contributions. In assembling this volume I invited young scientists who are all actively engaged "at the bench" in order to capture the excitement of this challenging area of biology as well as to delineate general principles of lymphocyte structure and function.

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SECTION IV: IMMUNE FUNCTIONS OF LYMPHOCYTES

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Introduction: Lymphocytes—Past, Present, and Future

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In the last 15 years, our knowledge of the origin, structure, and function of lymphocytes has increased considerably. This increase is certainly evident from the subject matter described in the chapters in this book.

We owe to Gowans the first clear demonstration of the immunologic function of lymphocytes. Contrary to what had been held for 50 years, small lymphocytes were shown not to be short-lived "end" cells but rather to be mobile and recirculating cells, able to transform to larger cells and to initiate immune responses after appropriate antigenic stimulation [1]. Other investigators rejected the idea, prevalent among immunologists prior to 1960, that the thymus had become redundant during evolution. On the contrary, this organ was shown to be essential for the production of those lymphocytes, now called T cells, responsible for cellular immunity (cellular resistance to infection, delayed-type hypersensitivity, allograft rejection, and other properties) [2]. Other lymphocytes (B cells) were found to be the precursors of antibody-forming cells and to arise independently of the thymus, being derived from the bursa in birds and from the bone marrow or fetal liver in other species (chapter 3). Research is now being directed toward finding when and where during ontogeny the stem cell becomes committed to the T- or B-cell pathway and what humoral influences might play a role in these differentiation processes.

Two lines of experimentation have added strong support for Burnet's clonal selection theory. One was the demonstration that immunoglobulin molecules are incorporated in the cell membrane of B lymphocytes and act as receptors for antigen. The other was the finding that a particular B cell can

bind, via such molecules, only one of many antigenic determinants available [3]. The identity of the analogous antigen-binding receptor on T lymphocytes is still the subject of much controversy. Simplicity and logic dictate that the T-cell receptor for antigen should be similar to the surface immunoglobulin of B cells. There is, in fact, experimental evidence for an IgM-like molecule on normal T lymphocytes and on some T lymphomas (chapter 10). Some investigators, however, have failed to find immunoglobulin molecules on normal T cells and have suggested that the antigen-binding receptor on these cells is quite different from immunoglobulin. Since uncertainty prevails, it is premature to be dogmatic about this issue. It is hoped that immunochemists will standardize their reagents and techniques of cell purification and of biochemical extraction to solve this fundamental problem.

Although lymphocytes are very heterogeneous in their origin, function, life-span and immunologic task, in vivo and in vitro methods are now available to achieve enrichment of selected cell populations. These methods have been made possible by our increased knowledge of the physiology of lymphocytes (e.g., their different homing properties) (chapter 2) and of their physicochemical characteristics. Cells can be separated according to density, charge (chapter 5), and surface components (chapter 11). Many surface antigens have been identified by immunologic methods, and these antigens have given us yet another means of approaching the identification and purification of lymphocyte subsets, whether these represent cells of the same lineage but at different stages of differentiation or cells that belong to separate populations with different immunologic functions. The task of the future is to identify the exact function of these surface antigens and their possible role as binding sites for specific molecules, as homing devices, or as recognition structures involved in cell interactions.

Operationally, receptors may be defined as structures able to recognize specific molecules and to "translate," through defined connections, the information received from such a recognition event to other cellular components responsible for initiating a biologically significant effect. The plasma membrane, the boundary between the cell's internal and external milieu, holds the key to the understanding of how the cell "interprets" the various molecular influences that impinge upon it. Investigations of membrane structure and function are thus of immense importance if we are to gain an insight into the processes that control various facets of cell behavior, differentiation, and development. Many chapters in this book clearly illustrate this and describe the new technology now available for the functional study of cell membrane components, e.g., electron microscope investigations with freeze-fracture techniques (chapter 1), physicochemical procedures of isolating membrane fragments and relevant membrane components (chapter 10), the use of membrane-binding molecules, such as lectins, to study changes in conformational states

(chapter 12) and so on (see sec. V). It has already become clear that receptors are not fixed but are, instead, mobile in the plane of the lymphocyte surface membrane (chapter 15). When cross linked by antigens or by mitogens, such as lectins (chapter 12), varying degrees of aggregation, patch formation, capping, and even shedding of receptors may occur. Such perturbations were found to be associated with alterations in membrane permeability and in intracellular levels of constituents, such as Ca^{2+} and cyclic nucleotides. This association, in turn, has been related to alterations in metabolic activity of the cell. Investigations of this type may enable us to determine what signals are essential to activate the cells and the early biochemical events that follow. The difference between a state of tolerance and immunity should soon be explicable in molecular terms at the level of the individual cell.

Investigations of the structure and function of the many constituents of the lymphocyte membrane are likely to yield information useful in other cellular systems. Methods derived from such studies should be applicable in such areas as parasitology, pregnancy, and cancer. For example, the mechanism by which parasites interact with host cells prior to penetration might be explored on a molecular scale at the level of the membranes involved, by use of the tools developed for studies of lymphocyte membrane receptors. Likewise, similar techniques might provide some insight into the interactions that occur between spermatozoa and ovum during the early stages of fertilization. Furthermore, since there is considerable evidence that changes in cell membrane behavior accompany the origin of malignancy, cancer research may benefit from the introduction of methods derived from lymphocyte membrane studies.

Genetic factors have a profound influence in immune responses [4]. Two groups of unigenic, autosomal, dominant control systems have been identified in the last 10 years (chapter 9). One is the allotype-linked specific immune response (Ir) gene group. These genes are expressed via B cells and control the structure of the specific immunoglobulin receptor and therefore its ability to effectively bind a particular antigenic determinant. The other is a series of genes linked to the species' major histocompatibility complex (H-linked Ir genes). These genes are expressed either at the level of some structure required for antigen to trigger T cells or at the level of some component involved in the cooperation that occurs between T and B cells (or T cells and macrophages). It is known that many T-cell functions in mice are restricted by the H-2 gene complex [5], and there is evidence that H-2 gene complex-associated components are involved in the effective operation of the T-cell receptor for antigen and therefore in determining whether the cell will respond in a given situation [6]. It is hoped therefore that future studies of the role of the major histocompatibility complex will help elucidate the identity of the T-cell receptor and define the requirements for T-cell triggering.

The influence of Ir genes is likely to be relevant, not so much in the overall response to complex antigens (e.g., bacterial or viral) in which multiple antigenic determinants are involved, but rather in the response to low concentrations of poorly immunogenic molecules, for example, tumor-specific antigens. Evidently, Ir genes must have been selected for survival value during evolution, and the many instances of an association between histocompatibility type and susceptibility to disease (chapter 9) support this notion. Unfortunately, we do not understand the pathogenetic mechanisms that underlie such relationships, and it is to be hoped that new techniques will be developed to elucidate these mechanisms. Only then might we be able to predict susceptibility to disease with a greater degree of certainty.

In the last seven years, it has become evident that T cells may become involved in controlling a wide variety of cellular and humoral immune responses by producing quantitative and qualitative modifications [7]. Evidence has been obtained to show that cooperative interactions occur between T cells and macrophages, between T and B cells, and possibly among T cells themselves. Antigen-activated T cells are known to release several factors that may be specific or nonspecific with respect to the antigenic determinants concerned. "Lymphokines" are essentially nonspecific effectors of cellular immunity responsible for various effects on monocytes, macrophages, and polymorphonuclear leukocytes. They may recruit leukocytes that produce the inflammatory lesions of delayed-type hypersensitivity, and they may enhance the microbicidal activity of macrophages important for cellular resistance to infection. It was largely as a result of the work of Mackaness [8] that evidence has accumulated to show that sensitized T lymphocytes release factors that "activate" macrophages to kill some intracellular bacteria. A similar mechanism plays a role in resistance to some viruses. The situation with other infectious agents is not clear, but T-cell deprivation does augment susceptibility to some protozoan and metazoan parasites and to some fungi. In view of this point, it is essential to investigate, in these systems, the possible involvement of T cells and macrophages and their role in protective immunity. Although the feeling has been expressed that immunology was born from the demand for protection against infectious disease and that essentially what was needed has been provided, it may be worthwhile pointing out that a recent World Health Organization survey [9] estimates that approximately 200 million people are infected with schistosomes, 300 million with filariae and other helminths, 250 million with malaria, and 1,000 million with a variety of intestinal parasites. Furthermore, of the immunologic methods available, none can satisfactorily halt the spread of gonococcal and syphilitic infections.

In many humoral immune responses, T and B lymphocytes work together, not separately, as part of a network in which collaborative and feedback loops determine the outcome [7]. Both facilitation and suppression of

antibody production have been observed, but the exact mechanism involved is not known. A vast amount of in vitro work has been done in an attempt to determine this mechanism (chapter 8). As a result, certain antigen-specific T-cell factors have been implicated as mediators. One such factor is claimed to be IgT, a T cell-derived immunoglobulin with a capacity for binding to macrophages. Another is a nonimmunoglobulin, antigen-specific product coded by genes that, in mice, lie close to the K end of the histocompatibility complex (chapter 9). It is sometimes difficult to relate in vitro events to the in vivo situation. Thus, IgG responses are much more T-cell dependent than are IgM responses, and most investigators who have used in vitro systems to generate such T-cell factors have confined their observations to IgM responses. Furthermore, germinal center formation, which is a characteristic marker of effective T- and B-cell cooperation in vivo, has not been reproduced in any in vitro model. Other theories of T- and B-cell cooperation have invoked the operation of nonspecific T-cell factors. Since the fundamental function of T cells is cellular immunity, and since nonspecific factors are instrumental in many T cell-mediated responses and often act through macrophages, it is not unreasonable to assume that similar nonspecific factors indirectly influence B-cell responsiveness [7]. T cells and macrophages may thus exert a crucial role by removing excess antigen from the immediate microenvironment of the B cell and therefore prevent B-cell paralysis (chapter 6). This belief is in accordance with the observed correlations among antigen persistence, susceptibility of potential IgG or high-affinity antibody-forming cell precursors to paralysis, and absence of a T-cell influence. Whatever may be the mechanism of T- and B-cell interaction, it is clear that the existence of a dual system of interacting cell types allows more precise multilevel regulation of antibody production of crucial importance in the development of immunologic memory and immunologic tolerance.

The evidence for interaction among subsets of T cells is not as convincing as that for T and B cells. T cell-dependent suppression may be an example of one form of T-T interaction. It is not, however, clear whether suppressor T cells exist as distinct cellular entities or whether T cells from tolerant (or suppressed) animals are not responding because they are coated with an excess of antigen-antibody complexes, some of which could "leak" onto other T cells, thus preventing the latter from responding. It is probable that the mechanism of T cell-dependent suppression will become elucidated in the next few years. Another, but more controversial, form of T-T interaction may operate via "transfer factor" (TF). This phenomenon has been observed in the human but has not been reproduced unequivocally in any other animal species. It appears that human TF is a dialyzable component of molecular weight less than 10,000 daltons that acts essentially as an *initiator* of cellular immunity, converting naive lymphocytes (presumably

T cells) to an antigen-responsive state [10]. These lymphocytes can now behave as the original sensitized lymphocytes from which TF was extracted. It is now 20 years since the phenomenon was first described in the human species, and there have been reports of spectacular success in controlling infections with TF. In view of this success, it is imperative to attempt to reproduce the TF phenomenon in an animal model, not only to determine critically whether such a factor that regulates T cell-mediated immunity is universal, but also to explore precisely its mechanism of action.

It is evident that a deeper understanding of the mechanisms by which T and B cells interact will pave the way for practical methods of manipulating selectively different sets of lymphocytes and their responses. This procedure may eventually be applicable in clinical medicine for use in various infectious diseases, vaccinating procedures, anergic states, immune deficiency diseases, allergic conditions, autoimmune diseases, cancer, and transplantation of tissues or organs.

Progress in immunophylogenetic studies is described in chapter 4. The capacity to develop specific transplantation immunity, concomitantly with some degree of immunologic memory, appears to have been demonstrated in two phyla of advanced invertebrates: annelid worms and echinoderms [11]. In the annelids, adoptive transfer of such immunity has been achieved with coelomocytes (cells akin to leukocytes), not with serum. In lower invertebrates, there is evidence for an ability to recognize alien cells but not for the diversification of effector functions, nor for memory. Thus, for example, a hyperplastic proliferative contact reaction is seen in coelenterates and tunicates. It has been suggested that two separate sets of recognition patterns might have evolved. One would be akin to immunoglobulin (though not necessarily identical) and would be responsible for enabling the T cells of higher vertebrates to be activated by a multiplicity of antigens to perform the functions of cellular immunity. The other would be a more primitive recognition molecule that would enable T cells to recognize, on other cells of the same species, the major histocompatibility antigens of that species and to react by blast transformation and proliferation in the "mixed lymphocyte reaction." It remains a challenge to the immunophylogeneticist to clarify the evolutionary relationships among such recognition molecules, immunoglobulins, and cell surface (histocompatibility) antigens.

Burnet has often stressed that one of the most important functions of lymphocytes is to maintain the genetic integrity of the body [12]. The discovery that neonatal thymectomy (which impairs T cell-mediated immunity) renders animals more susceptible to the oncogenic activity of certain viruses [13] certainly supports the notion that T cells constantly scan other cells and eliminate those with altered antigenicity. More recently, identical observations have been made in "athymic nude" mice [14]. T cells may thus constitute