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Internal Activity of the Immune System and its Physiologic Significance

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A. Introduction

After the introduction of Jerne's network theory 10 years ago, modern views of the immune system have been strongly influenced by the ideas of *autonomy* previously developed by those working on complex systems such as the brain. This "new immunology" attempts to describe the normal immune system as it is, the fundamental characteristics of its organization and, in the words of Francisco Varela, "the landscape of its eigen behaviours." Within this perspective, the study of the internal activity of the immune system, of the generation and decay of its cellular and molecular components, is likely to provide more relevant information as to the physiology of immune activity than the study of immune responses to the injection of large doses of antigen, as in classical approaches. Obviously, we aim to describe and understand the basis for this inner life of the immune system, embedded as it is in a multitude of molecular components that constitute the "self" of the individual, and exposed to the surrounding "noise" of the environment. We do not use artificial priming with antigen, neither antibodies nor antiidiotypes as surrogates of antigens in the induction of immune responses. On the other hand, the interpretation of such internal activity, as well as any hypothesis on the internal mechanisms inducing effector cells and their specificity, must necessarily rely on detailed knowledge of lymphocyte physiology: the mechanisms by which lymphocytes are turned on and turned off, and how their proliferation and maturation to effector functions are regulated as well as the nature of the functionally relevant molecules expressed at the surface of these cells.

In a meeting on human leukemia, what we have to say risks being completely out of place. We neither work with human cells, nor are we concerned with leukemia. Moreover, our perspectives and approaches may well lead us nowhere. The profound motivation to follow them is our dissatisfaction with current approaches to biologic systems, particularly within immunology, and their failure to solve problems (such as that of cancer and autoimmune pathology) within classical frameworks of thought and experimentation. We have provided detailed references in a previous publication [1].

B. Organizational Closure in the Normal Immune System

I. Internal Activity

It has now become clear that the normal immune system does not need environmental stimulation to be directed into relatively high levels of activity. This activity can be measured not only at the level of production and decay of lymphocytes, but also in the generation of effector cells.

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Thus, germ-free mice maintained on low molecular weight, chemically defined diets, possess in their spleens numbers of immunoglobulin-secreting cells that are quite similar, if not superior, to those found in the spleens of conventionally bred and infected mice. Interestingly, such mice have no plasma cells in the lymph nodes, an observation which actually provides a control for the "antigen-free" state of these mice. The ontogenic development of "natural plasma cells" in the spleen of these mice is perfectly comparable to that of mice born from normal mothers and bred in an infected environment with a normal diet [2]. It would appear, therefore, that at least within the spleen compartment, the immune system shows an activity of its own, which leads to the generation of large numbers of high rate immunoglobulin-secreting cells. Such an environment-independent activity is also observed in the other lymphocyte compartments. As we have recently shown, normal mice contain in their spleens activated effector cells of both the helper and the suppressor type, in roughly the same numbers as background effector cells of the B lymphocyte lineage. It is yet to be determined whether or not such "natural" generation of effector T cells is truly internal, by studying "antigen-free" mice, but we think it very likely that this is indeed the case. It is obvious, however, that even those carefully maintained animals are not antigen free, as the immune system is in contact with its own antigens and with those constituting the soma.

II. A Formal Network of Idiotypes

We have therefore turned experimentally to the study of the mutual interactions among the molecular and cellular components of the immune system, as well as between the immune system and other molecules found in the internal environment. To this end, we have isolated large collections of hybridomas, both of B and T lymphocytes, from newborn untreated animals, aiming at obtaining representative samples of the lymphocytes that have been activated in the internal environment. The analysis of these collections has already provided the first experimental evidence for the existence of a formal idiotypic network in the developing immune system. Thus, natural antibodies isolated from a single newborn mouse show an astonishingly high frequency of mutual reactions, demonstrating a degree of *connectivity* and *redundancy* in the developing immune system which might be expected on theoretical grounds as an attribute of stable networks. We are thus far unable to decide on the functional relevance of such idiotypic interactions. Experiments are at present being carried out, trying to analyze these aspects.

III. Mutual Influence of the T and B Cell Repertoires

The detailed study of these collections of naturally activated cells in normal mice has led us to an interesting conclusion: the specificity repertoire of background-activated cells is unique to each individual, even if newborn mice from the same litter of an inbred cross are compared. On the other hand, we can of course expect that the diversity repertoire of individual mice is submitted to genetic constraints, that is, limited to the possibilities allowed by gene families such as immunoglobulins, MHC, and T cell receptors. Within these potentialities, however, the individuality of immune systems defined by available repertoires appears to be established somatically by the connectivity between its cellular elements. Bearing in mind such organizational closure and internal activity, we have paid particular attention to the influences that antibody repertoires might have on T cell repertoires and the reverse, that is, the influences that T cell repertoires of both the helper and cytolytic type (as well as MHC genes) might have on natural antibody repertoires. Three clear-cut examples already exist, supporting the existence of these mutual influences which determine the specificity of the internal activity of the immune system.

One such example is already a few years old. It described the H-2 *and* immunoglobulin allotype control of a function of the normal immune system which

consists in reproducing increased circulating levels of a given idiotype upon injection of nanogram amounts of same idiotype. Since idiotypes that had this property of "autoreproduction" are consistently found as natural antibodies in the serum of normal mice, we have concluded that natural antibody repertoires may well be under the influence of H-2 and, consequently, are at least in part selected on the basis of T cell specificities and activities. We have further suggested that among natural antibodies (i.e., products of cells that were internally activated), a relatively high frequency of idiotypic profiles resembling MHC products could be expected on the basis of the predominant anti-H-2 specificities in the T cell compartment. This hypothesis has been recently confirmed by isolating natural idiotypes which are internal images of self-MHC antigens.

As a mirror image of this type of influence, we have also found that helper cell repertoires, particularly the expression of idiotypes on clonally distributed receptors, is controlled not only by MHC-linked genes, but also by immunoglobulin heavy-chain genes. We have further shown that the influence of immunoglobulin genes on such repertoires is indirect and results from internal complementarities established between the two repertoires, because helper cell idiotypic repertoires are profoundly altered in mice deprived from birth of the antibody/B cell system.

We conclude from all these observations that a normal immune system is characterized by a high degree of internal activity which results from mutual specific complementarities between T and B cell repertoires. Effector cells are induced in the internal environment and themselves regulate the levels of activity in the normal system and determine, within the "noise" that surrounds the immune system, what makes sense to it and can therefore perturb its equilibrium and modify its activity.

C. The Normality of Autoreactivity

A large body of evidence has accumulated over the last few years indicating the existence of immune reactivities directed to other components of the immune system itself. Thus, antibodies, helper cells, and cytolytic T cells have been shown to recognize idiotypic determinants on other antibodies or on T lymphocytes. Furthermore, normal autoreactivity of T lymphocytes that appears to be stimulated by self-I-A under some conditions, has led to a large number of descriptions of what is called autologous MLR. It appears, therefore, that autoreactivity is a normal component within the immune system itself, as one would expect from a complex autonomous system that is self-organized. For a number of years, quite independently from these observations, a considerable number of reports have dealt with the existence in normal individuals of lymphocyte precursors in the B cell lineage with specificities for determinants expressed on other proteins of the "self" internal environment. The prevalent concept, however, is that in the absence of effective helper activity which is thought to be eliminated (T cell tolerance to self-determinants is a widely accepted concept), such B cell precursors will not be induced to antibody formation in the normal immune system. Autoimmunity has invariably been considered as pathologic and the approaches to its pathogenesis have been the search for either the abnormal expression (qualitative or quantitative) of a self-antigen, or the abnormal occurrence of one or more lymphocyte clones that should have been "forbidden."

More recently, however, considerable evidence has accumulated for the existence of autoreactive antibodies in the pool of natural circulating immunoglobulin. In the analysis of natural antibodies in newborn mice, we have observed that a very large fraction of these internally induced antibodies show extensive reactions with self-antigens. Other observations in adult individuals, both mice and humans, have led Avrameas and his collaborators to infer the invariable presence of autoreactive antibodies in the normal serum of these species. It follows that the presence of autoantibodies is not correlated with autoimmune pathology, a conclusion that had already been suggested by some workers in the field of autoimmunity. It becomes important, therefore, to separate the

physiology from the *pathology* of autoreactivity, and to evaluate its physiologic relevance. It also appears to us that the study of the internal activity of the normal immune system, which is formally more similar to pathologic situations due to autoreactivity, may be more likely to lead us to the solution of these problems than the study of immune responses, developed within systemic strategies and clonal patterns of lymphocyte behavior which are definitely very different from those that can be observed in the normal physiology of the immune system.

Reference

1. Coutinho et al. (1984) Immunol Rev 79:151–168
2. Benner R, personal communication