

The Membrane Receptor Complex Regulating Induction and Clonal Expansion of Normal Murine B Lymphocytes

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Induction of resting B lymphocytes results from the interaction of competent ligands or helper cells with "triggering receptors." Subsequent clonal expansion and performance are thought to be regulated by the interaction of selective growth or maturation factors with specific receptors on induced B cells. A set of membrane molecules of B lymphocytes, including IgM, IgD, IA, IE, lipopolysaccharide receptors, receptors for Fc and C3b, and other non-immunoglobulin structures recognized by some antiidiotypic antibodies, display ligand-induced relationships. Functional studies also indicate that this group of molecules functions as a complex that regulates delivery of inductive signals, the expression of growth and maturation receptors, and/or the outcome of the interactions of these receptors with the corresponding factors.

Network concepts [1] have radically altered classic perspectives of the immune system. Defense against infection is no longer the only or even the major aspect in immune physiology: the immune system is turned inward and includes images of all molecular patterns. No distinction can be made between the epitopes of "self" and "nonself." In spite of this profound conceptual revolution, some properties of bacterial products in their interaction with lymphocytes make it unescapable to conclude that the immune system started, at least in part, by being a protective, antiinfectious organ. The remnants of this evolutionary past are numerous and quite spectacular [2-4]. Perhaps the most widely analyzed is the ability of lipid A to stimulate a large fraction (10%-50%) of all B lymphocytes in the mouse.

The mere consideration of this phenomenon is sufficient for us to conclude that "internal images" of lipid A do not exist in vertebrates and to segregate properties of lipid A from the universe of the epitope and the molecular basis of those reactions from that of antibody recognition. In fact it has been established that B lymphocyte reactivity to

lipid A is controlled by a locus on chromosome 4 [5, 6] that appears to determine the expression of membrane molecules that specifically bind lipid A and are competent to generate triggering signals [7]. The study of the biochemistry of those putative lipid A receptors has been hampered by the difficulty in preparing monospecific reagents. On the other hand, recent progress in the analysis of B cell activation, growth, and maturation provide new perspectives for the role of "triggering" or "mitogen" receptors in B cell physiology. We shall briefly discuss this progress here.

Physiology of B Cell Activation and Clonal Performances

The analysis of B cell responses stimulated by helper T lymphocytes has revealed three distinct phases: (1) induction of resting B lymphocytes to a state of reactivity to nonspecific factors [8], which requires direct cell-to-cell interactions and participation of IA and IE molecules recognized on the responding B cell membrane by allospecific [9] or major histocompatibility complex (MHC)-restricted helper cells [10]; (2) proliferation of "induced" B cells upon interaction of cell type-specific growth factors (BCGF) with appropriate receptors, which are not expressed by resting cells [11]; and (3) maturation of proliferating B cells, regulated by nonspecific factors (BCMF) competent for stimulating increased rates of synthesis and secretion of immunoglobulins [12, 13]. At

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least one type of BCGF is produced by T lymphocytes, and although definitive evidence is still lacking in this respect, helper cells appear to be competent in the production of maturation factors as well [12].

The fact that there are stringent requirements for nonspecific factors in B cell responses, although not surprising in light of some current concepts of lymphocyte activation [2–4], raised similar questions for mitogen-activated systems. B cell responses to lipopolysaccharide (LPS), for example, had previously been described as independent of T cells and macrophages [14, 15], a finding that implies lipid A could function directly as an inducer, a growth factor, and a maturation signal for normal B lymphocytes. Improved conditions for accessory-cell depletion, however, have suggested to a number of investigators [16–19] that both LPS-dependent growth and maturation of B lymphocytes is macrophage dependent. In turn, this conclusion would imply that LPS-activated macrophages are competent to produce growth and maturation factors, perhaps distinct from those with a T cell origin. It is also clear, however, that LPS-activated B cell blasts are reactive to both BCGF and BCMF derived from T cells [11], a fact demonstrating that B lymphocyte growth can be brought about by independent pathways.

On the other hand, an absolute macrophage dependence of LPS-induced response must be considered with reservations. First of all, in those experiments, there are no indications on selective losses during purification of B cells that presumably could respond directly to LPS by proliferation. The available results only indicate that some B lymphocyte subsets require macrophages to proliferate in LPS-stimulated cultures. Secondly, it is impossible to distinguish, in the activity of macrophages, simple pleiotropic or “filler” effects from a specific function, such as the production of a growth factor. Finally, several B cell lymphomas, cloned and maintained *in vitro* in the absence of any other cell type, have been shown to respond to LPS with an increase in proliferation and maturation.

These recent experiments, however, have contributed one general conclusion, namely that induction of resting B cells is accessory-cell independent, even if the subsequent phases in the response are not. Before these latter details are settled, therefore, we shall consider here only the role of

lipid A receptors on the induction step in B lymphocyte responses and its regulation by other membrane molecules.

A Molecular Complex on the B Lymphocyte Membrane

We and others have observed ligand-induced interactions between several independent B cell-membrane molecules [20–25]. These interactions are demonstrated either in “cocapping” experiments or by the consequences of specific ligand binding to a given molecule, in the absence of redistribution, on the availability of other receptors that can be either inhibited or enhanced. Such interactions have delineated a complex that includes antibody receptors of both IgD and IgM classes, class II MHC products (both IA and IE molecules), LPS receptors, receptors for Fc and C3b, and other less-defined structures, such as putative products of the *Mls* locus and those recognized by some antiidiotypic antibodies. Most interestingly, there is a definite position of the various molecules in the complex, and there appears to exist a hierarchy or directionality of interactions, as shown by the analysis of the different combinations. Finally, the general properties of this complex and the type of specific interactions observed change with the stage of activation of the cell, in particular early after exposure to LPS.

These observations acquire particular importance because the functional counterpart of those interactions can be demonstrated readily. Thus, antibodies against several components of this complex interfere with or modulate the ability of LPS to induce B lymphocytes. Furthermore, the kinetics of LPS-dependent induction of reactivities to growth factors is closely paralleled by the shift from resting- to activated-type interactions as well as by the loss of the ability of the same antibodies to modulate responses. In this respect, the recent finding of Herrlich et al. [26]—that synthesis of a protein currently designated “invariant I-chain” is drastically reduced in activated B lymphocytes—may be of value for a complete understanding of this process. More recent experiments would actually suggest a role for Ii in maintaining the topography in the complex and the regulation of Ii synthetic rates by LPS (P. Herrlich, L. Forni, and A. Coutinho, unpublished observations). To be confirmed, these indications would ascribe a pri-

mary role for those multimolecular interactions at the B lymphocyte membrane in the triggering process. It follows that the mere binding of lipid A to its specific receptor might not be sufficient for inducing B cells in the absence of a chain of reactions involving the other components in the complex, as suggested by the inhibition observed with anti-IgM [27] and anti-Ia antibodies [28] directed to various domains of either IA or IE molecules [29]. In this respect, it should be noted that, depending on the relative position of each of these molecules in the complex, the effects of ligand binding vary in accordance with the type of inductive signals used. For example, anti-IgD is not inhibitory of LPS-induced responses [30] in contrast to IgM, which, however, fails to inhibit helper cell-dependent activation [31]. These observations also demonstrate that some of the components in the complex are inductive or triggering receptors, such as the lipid A receptor [7] or IA and IE [9], while others, such as IgD or IgM—at least in our own experience—are not [32].

The model for B cell activation presented here offers an explanation for apparently “abnormal” induction via ligand binding to nontriggering receptors as well as for many observations on the synergy between ligands of different specificities. Thus, under selected experimental conditions that modulate interactions in the complex, the availability of competent triggering receptors might increase above threshold levels and reveal or enhance the activity of inducing ligands present at subliminal levels. It is likely, on the other hand, that initial inductive reactions simply lead to the functional expression of receptors for nonspecific growth and maturation factors. The selective expression of receptors for one or the other type of factors is also likely to be regulated via that complex which consequently, controls the balance between proliferation and terminal maturation in activated B lymphocyte clones.

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