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ADVANCES IN IMMUNOLOGY, VOL. 45

Cellular Interactions in the Humoral Immune Response

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I. Introduction

The immune system has evolved primarily to combat infection by pathogenic organisms. It is characterized by its virtually infinite repertoire of specificities, its highly specialized effector components, its complex regulatory mechanisms, and its mobility. In contrast to most other organ systems, the immune system is not confined to a single site in the body; immunocytes and their secreted molecules traffic within and among lymphoid organs and various body compartments. Hence, a highly complex system of communication has developed among the various cell types in the immune sytem. One important mechanism of communication is the requirement for interactions among cells for the activation and differentiation of resting B lymphocytes into antibody-secreting cells. These cellular interactions involve both cell/cell contact and the release of mediators (cytokines) that can act in either an autocrine or paracrine fashion on cells both within and outside the immune system.

In the present review we will discuss the interactions between T and B cells and the role of accessory cells and cytokines in the generation of specific antibody responses. The first portion of the review is a historic perspective (Section I), followed by a summary of present-day concepts (Sections II-V); in the final section we speculate on how the different components of the immune system might function *in vivo* (Section VI).

A. Models of T Cell/B Cell Interactions

In 1966, Claman and his co-workers provided the first direct evidence that T and B cells interact in the generation of an antibody response to sheep red blood cells (SRBCs)* (1). Lethally irradiated mice were

*Abbreviations used: ABC, antigen-binding cell; AIDS, acquired immune deficiency syndrome; APC, antigen-presenting cell; BCGF, B cell growth factor; BCDFγ, B cell differentiation factor for IgG_1; BSF-1, B cell stimulatory factor-1; BSF-2, B cell stimulatory factor-2; C, constant region; CML, cell-mediated lysis; CSF, colony-stimulating factor; DNP, dinitrophenyl; DTH, delayed-type hypersensitivity; EAF,

injected with thymocytes or bone marrow cells from normal or immune syngeneic donors. The cell transfer was followed by challenge with antigen (SRBC) and the spleen cells from the recipient mice were assayed at various times for the secretion of hemolytic antibodies. Such experiments showed that neither thymus-derived nor bone marrow-derived cells could, on their own, elicit an anti-SRBC response. However, when the two types of cells were present in the same recipient, an antibody response was elicited. Studies by Davies *et al.* (2) and Mitchell and Miller (3–7) confirmed and extended these conclusions and established that the antibody-forming cell precursors were derived from the bone marrow population and that thymus cells could recognize and react specifically with antigen, but did not, themselves, produce antibody. It was suggested that the thymocytes or their mature progeny played a role in helping the B cells to differentiate into antibody-producing cells.

With the advent of *in vitro* tissue culture techniques developed by Mishell and Dutton (8, 9) and Marbrook (10, 11), it was possible to further elucidate the roles of different cell types under more controlled experimental conditions. Using *in vitro* culture, Mosier and his colleagues (12–14) first demonstrated that when spleen cells were separated by virtue of their ability to adhere to plastic, neither the adherent nor nonadherent population of cells could, on their own, make an antibody response. The cells in the adherent population required for an antibody response were macrophages, while the cells in the nonadherent population were primarily T and B cells. Experiments carried out by many investigators confirmed that T and B cells were required for both primary and

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eosinophil activation factor; EBV, Epstein-Barr virus; EDF, eosinophil differentiation factor; FCS, fetal calf serum; FDC, follicular dendritic cell; FITC, fluorescein isothiocyanate; HGF, hybridoma growth factor; HIV, human immunodeficiency virus; HSA, human serum albumin; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; Ig, immunoglobulin; IL, interleukin; Ir, immune response (gene); K_d, dissociation constant; KHF, killer helper factor; KLH, keyhole limpet hemocyanin; LAF, lymphocyte-activating factor; LAK, lymphokine-activated killer cell; LFA, lymphocyte function associated; LPS, lipopolysaccharide; LT, lymphotoxin; MABC, memory ABC; MAF, macrophage-activating factor; MHC, major histocompatibility complex; mAbs, monoclonal antibodies; MTOC, microtubule organizing center; NK, natural killer cell; OVA, ovalbumin; PC, phosphorylcholine; PCT-GF, plasmacytoma growth factor; PFC, plaque-forming cell; PGE_2, prostaglandin E_2; PKC, protein kinase C; PMA, phorbol myristate acetate; PNA, peanut agglutinin; PPD, purified protein derivative; r, recombinant; R, receptor; RAMIg, rabbit antimouse Ig; SDS, sodium dodecyl sulfate; sIg, surface immunoglobulin; SN, supernatant; SRBC, sheep red blood cell; Tc, cytotoxic T cell; TCGF, T cell growth factor; TcR, T cell receptor; TD, thymus dependent; Th, T helper cell; TI, thymus independent; TNF, tumor necrosis factor; TNP-ABCs, trinitrophenyl-antigen-binding cells; TNP-MABCs, memory TNP-ABCs; TRF, T cell replacing factor; Ts, T suppressor cell; TT, tetanus toxoid; V, variable region.

secondary antibody responses and that macrophages served as accessory cells (7, 12, 15).

B. Interactions between T and B Cells Specific for Different Determinants on the Same Antigen (Hapten-Carrier Effect)

An important but initially perplexing observation was the demonstration of the "hapten-carrier" effect. It had been known for many years that immunization with a small nonimmunogenic molecule or hapten was effective only when the hapten was coupled to a "carrier" molecule which was immunogenic. It was demonstrated that cooperative interactions between distinct lymphocytes specific for carrier and haptenic determinants were essential for the development of an antihapten antibody response. The first direct evidence for cooperative participation of two cells with distinct determinant specificities was obtained by Mitchison (16). Spleen cells obtained from syngeneic donor mice that had been immunized with a hapten-carrier conjugate secreted antihapten antibodies following challenge with a homologous hapten-carrier conjugate, but not with a heterologous conjugate containing the correct hapten but another carrier. In contrast, when spleen cells from donors immunized with a hapten-carrier conjugate were mixed with spleen cells from donors immunized with another carrier, a good secondary response could be obtained using the hapten conjugated to the second carrier. Thus, cells specific for the second carrier helped the hapten-specific B cells to make an antihapten response. It was later shown by Raff and colleagues (17, 18) that the carrier-specific cooperating cells, or helper cells, were thymus derived, whereas the antihapten antibody-forming cells were bone marrow derived. These experiments were made possible by the identification of a marker on thymus-derived cells called θ (now Thy-1), and the development of an antibody against θ, which, in the presence of complement, could lyse the thymus-derived cells. By eliminating θ^+ cells from cell mixtures, it was demonstrated that they were responsible for carrier specificity and for cooperating with the B cells in the elaboration of an antibody response to the hapten. The phenomenon of cooperation between carrier-specific T cells and hapten-specific B cells was also demonstrated independently by Rajewsky *et al.* (19), who immunized rabbits with a hapten-carrier conjugate and observed that the animals made a significant antihapten antibody response if they received a supplemental intervening immunization with free, unconjugated carrier. It was further demonstrated that the intervening immunization with the carrier primed a second helper T cell population, which could then cooperate with the hapten-specific B cell

population in responding to the hapten–carrier conjugate. While subsequent *in vitro* studies went a long way in defining the cell types involved in the linked response to hapten and carrier, they also pointed to the vast array of artifacts involved in obtaining antibody responses *in vitro* as opposed to *in vivo*. Numerous papers focused on the roles played by the constituents of the tissue culture media, fetal calf serum (FCS), plastic ware, and the addition of reagents (such as 2-mercaptoethanol) in obtaining optimal antibody responses *in vitro* (9, 20–24). During this time, immunologists were faced with the frustrating problem that it was often difficult to repeat experiments among different laboratories because of technical differences in the culture systems. Nevertheless, with time, it became evident that the basic tenet of T cell/B cell collaboration involving hapten-specific B cells and carrier-specific T cells was correct, albeit with some qualifications. For example, experiments carried out by Mäkelä and his associates (25, 26) and others (27–31) established that an increasing density of repeating antigenic epitopes correlated with decreasing dependency on T cells, and that epitope density affected not only the magnitude of the response, but the isotype of antibody secreted as well. Additional variables that influenced the *in vitro* antibody response included the physical nature of the carrier (particulate or soluble), virgin versus memory cells, and differences among mouse strains (32, 33).

C. The Role of T Cells on the Quality of the Antibody Response

As discussed above, experiments carried out in the 1960s demonstrated that T cells are required for B cells to respond to most antigens. It was further demonstrated that T cells were also required to induce the progeny of the B cells to switch from the secretion of IgM to IgG antibodies (34–42). Furthermore, other experiments showed that T lymphocytes were involved in affinity maturation of the antibody response and that the more "T independent" the response, the poorer the affinity maturation (39, 43–46). It was concluded that T cells play an essential role not only in the activation of resting B cells, but also in isotype switching and affinity maturation of this response. Thus, T cells can regulate the levels and affinity of serum antibodies of different isotypes. Different Ig isotypes are essential for providing the most effective means of eliminating a given type of pathogen, e.g., viral, parasitic, or bacterial. Finally, T cells play a major role in generating memory B cells which can respond more effectively to subsequent challenge with antigen (47).

D. Suppression versus Enhancement of the Immune Response

Gershon and Kondo (48, 49) were the first to develop the concept of suppressor T cells. In general, suppression appeared to be nonspecific (50, 51); however, there were claims of antigen-specific suppressor T (Ts) cells (52-55). Subsequently, additional mechanisms for down regulating immune responses were suggested, including antibody-mediated suppression generated by immune complexes (2, 56-68) or antiidiotypic antibodies (69-71). It is now apparent that at least some types of suppressor cells can be distinguished from helper T (Th) cells by virtue of the expression of different surface markers. More recent evidence suggests that suppression may be mediated, at least in certain cases, by soluble factors elaborated by the suppressor cells that can act on helper cells and, possibly, on other immunocytes as well (72). The mechanisms by which Ts cells manifested their effect were controversial and have still not been well defined. These observations led to experiments in which it was demonstrated that supernatants (SNs) from activated T cells could, in some instances, substitute for T cells in enhancing or suppressing the immune response (73). In retrospect, much of the confusion concerning suppression can be accounted for by two findings: (1) different subsets of T cells secrete a large array of different lymphokines which exert both helper and suppressor activities (74, 75); (2) soluble factors act primarily on cells which had been activated by a T cell/B cell cooperative interaction and which can induce the partially activated B cells to differentiate and, in some cases, can suppress this differentiation (50, 51).

E. The Role of the Major Histocompatibility Complex in T Cell/B Cell Interaction

A large number of experiments in the 1970s established the relationship between immune response (Ir) genes, encoded by the major histocompatibility complex (MHC), and the ability of T and B cells to interact in the elaboration of an immune response (76-79). Molecules encoded by the MHC, in particular the I region of the MHC, influenced the ability of T cells to interact with macrophages and B cells (76, 78, 80-84). This implied that the products of Ir genes (now class II MHC genes) were involved in cellular interactions between T cells and B cells and indicated that in order for a T cell to recognize a B cell or an antigen-presenting cell (APC), recognition of class II molecules was necessary. T cell receptors (TcRs) involved in recognizing class II molecules were either the same or different from those recognizing antigen. In the last

few years, the basis for genetic restriction has been elucidated and the concept has emerged that a single TcR can recognize *both* the antigenic moiety and the MHC molecule on the surface of an APC (85, 86). It has also been shown that accessory molecules on both T and B cells influence the interaction of the two cells.

F. Antigen-Specific Receptors on T and B Cells

Perhaps no issue in immunology has had a more colorful and controversial history than the elucidation of the antigen-specific receptors on T and B cells. Studies in the early 1960s demonstrated that B cells express antibody molecules that served as antigen-specific receptors (87–97). It was initially thought that the antigen-specific receptors were the same as the major class of immunoglobulin (Ig) in the serum, e.g., IgG (97). However, studies in the early and mid-1970s established that the major antigen-specific receptors on the B cells are monomeric IgM (98) and IgD molecules (99), both of which share the same antigen specificity (90, 100, 101) and idiotype (102–104), and each of which contains a C-terminal transmembrane domain responsible for anchoring the receptor in the membrane of the B cell (105–109). It was later reported that B cells can express other classes of Ig on their surface depending upon their state of differentiation (110–112) and that these receptors can also express transmembrane segments (112). Furthermore, B cells often express more than one isotype of surface Ig (sIg) (110–112). Based on previous studies, it was assumed that the Ig receptors on B cells are responsible for recognizing the haptenic portion of a hapten–carrier conjugate, whereas, the T cells express a receptor of a different specificity that recognizes portions of the carrier. For a long time, there was heated controversy concerning the nature of the TcR which divided the immunologic community into two camps, i.e., those who were convinced that the TcR was Ig (113) and those who were not (114). In 1980, pioneering experiments by Hedrick *et al.* (115) and Yanagi *et al.* (116) demonstrated that the receptor expressed on the T cell was indeed different from that expressed on the B cell. The major TcR consists of disulfide-bonded α and β chains, each having a M_r of 45,000–55,000. The α and β chains express transmembrane and cytoplasmic domains. It was further shown that the two chains of the receptor can be divided into variable (V) and constant (C) region segments (117–119) which show structural similarities to V and C domains of Ig (116, 120–122). Indeed, the TcR is encoded by V, D, and J segments of DNA, in a manner analogous to the Ig receptor on the B cell (123). The $\alpha\beta$ dimer of the TcR recognizes both antigen and portions of the MHC molecule (123–125). Finally, it was shown that other surface molecules on the T cell

[such as cluster-designation (CD)3, CD4, CD8, lymphocyte function-associated antigen (LFA)-1] interact with the $\alpha\beta$ complex or with molecules on the B cell (125, 126). More recently, δ and γ chains of the TcR have been identified but their role in antigen recognition has not been fully defined, even though T cells expressing $\delta\gamma$ receptors have been identified (127-130). Most importantly, T cells, in contrast to B cells, do not recognize soluble native antigen, but recognize "processed" antigens (131-136) or endogenously synthesized antigens (137) that are bound to the surface of APCs, e.g., macrophages, dendritic cells, Langerhans cells, B cells, or tumor target cells. In the case of exogenous antigen, the presented form of the antigen arises as a consequence of a series of intracellular processing events after the native antigen binds to and is internalized by the APC (138). It is thought that these processing events involve degradation and/or denaturation of the antigen, recycling of fragments of denatured antigen to the surface of the presenting cell, and association of the fragments with class II MHC-encoded molecules. Recent studies supporting this concept will be presented later in the chapter.

G. The Antigen-Bridging Model of T Cell/B Cell Interaction

The original model postulated by Mitchison (16) to describe associative recognition of antigen by T and B cells suggested that receptors on B and T cells recognize the hapten and carrier portion of the native antigen, respectively, and, hence, antigen bridges the two cells. Following bridging by antigen, the T cell delivers activating signals to the B cell. This model has now been revised to include the current-day notion that the B cells bind the haptenic portion of the antigen, internalize the hapten-carrier complex, degrade the carrier, and present peptide fragments of the carrier in association with a class II molecule to specific Th cells. Th cells also recognize peptides of the processed carrier (taken up nonspecifically) on the surface of a macrophage or other accessory cells (138). In fact, both Th and B cells may interact on the surface of a macrophage where the B cells recognize the native hapten and the T cells, the processed carrier. This becomes important in the physiological setting of a lymphoid organ where T cells and B cells are concentrated in different portions of the organ (139). In this system, it is attractive to postulate trafficking of cells through the lymph node to a common site where antigen is present in sufficient concentration to bind to the relevant cells and where these cells can interact.

In the following sections of this review, we discuss the mechanisms underlying the hapten-carrier effect, T cell/B cell interaction, the role

of Th cells in inducing affinity maturation and Ig class switching, genetic restriction, T cell suppression, and the role of accessory cells and soluble mediators in T cell/B cell interaction and activation. Furthermore, the role of the MHC will be discussed both in the context of processing and presentation of antigen to T cells by APCs and in the context of T cell/B cell interaction. The focus of this review will be on the mechanisms involved in the interaction between T and B cells, and the functional outcome of these interactions.

II. Antigen Processing and Presentation

A. Requirement for Processing

Between 1970 and 1980, experimental findings related to antigen processing and presentation established that (1) T cells are required for the generation of antibody by the differentiated progeny of activated B cells responding to TD antigens; (2) B and T cells do not recognize the same epitope on a TD antigen; (3) the antigenic epitopes recognized by T and B cells must be physically linked on the antigen in order for the antigen to elicit an antibody response; and (4) T cells cannot bind native, soluble antigen, but have receptors for "processed" antigen associated with class II molecules on APCs. These findings suggested that APCs must first bind native antigen and, at a later time, present some other form of this antigen to a T cell in an antigen-specific, MHC-restricted manner. As a result of the interaction of the T cell with an APC, the T cell becomes activated. The term "presentation" can be used to define the capacity of APCs to express altered forms of antigen to T cells. Antigen "processing" describes the steps by which an APC converts native antigen to a form which can be recognized by a T cell. Processing may involve proteolytic degradation, denaturation, or modification of the antigen. Processing also involves the association of antigenic fragments with class II molecules and the expression of these complexes of processed antigen and class II molecules on the surface of APCs.

The earliest studies to correlate antigen catabolism with presentation were carried out by Ziegler and Unanue (140), who exposed macrophages to the bacterium *Listeria monocytogenes*. Following extensive washing of these treated macrophages, *Listeria*-primed T cells were added to the cultures and were allowed to bind. Unbound T cells were decanted from the cultures and the depletion of antigen-specific cells was assessed. These experiments showed that (1) a processing period of approximately 1 h is required before the T cells can bind to the antigen-pulsed macrophages;

(2) antigen presentation is decreased after treatment of the APCs with lysosomotropic agents; (3) presentation is inhibited when the macrophages are fixed prior to exposure to antigens; and (4) after the APC has been exposed to antigen for at least 1 h, subsequent fixation or treatment with lysosomotropic agents no longer affects their ability to present antigen. These experiments also demonstrated that the binding of T cells to antigen-presenting macrophages is "restricted" by class II molecules and that the native antigen is ingested and catabolized by the macrophages.

B. Different Types of APCs

The macrophage was the first APC to be identified. Monocytes/macrophages exist both in the circulation and in the tissues, and together with polymorphonuclear leukocytes (PMNs) form the first line of defense against foreign pathogens. Macrophages have the ability to engulf particulate and soluble antigens, to degrade them, and to present antigen fragments to T cells (138).

More recent studies have demonstrated that in addition to macrophages, other cells can also act as effective APCs. In general, all cells capable of presenting antigen to T cells constitutively express class II antigens on their surface or can be induced to do so. These cells include dendritic cells (141–144), Kupffer cells (145), Langerhans cells (146–148), vascular endothelial cells (146), Schwann cells (149), astrocytes (150), thymic stromal cells (151), human dermal fibroblasts (152), B lymphocytes (153), and human class II^+ T cells (154). Although the role played by these APC cells *in vivo* is not completely understood, evidence from a number of laboratories suggests that macrophages, B lymphocytes, and dendritic cells are the major APCs present in lymphoid tissues.

Steinman and co-workers (141–144) have demonstrated that dendritic cells are very efficient APCs. In fact, their studies suggest that the dendritic cell may be a more important cell than the macrophage for the initial activation of resting T cells *in vivo*. Another special type of APC is the follicular dendritic cell (FDC) (155, 156). This cell is unique in that it carries immune complexes of native antigen on its surface in a nonprocessed form. *In vivo*, the FDC may serve as a reservoir for native antigen which can be subsequently bound and processed by other APCs and, in particular, B cells.

Since 1980, a number of groups have demonstrated that B cells can process and present antigen to T cells both *in vitro* and *in vivo* (153). This demonstration supports the earlier hypotheses of antigen bridging in T cell/B cell interactions. Once it became clear that B cells could bind native antigen, process it, and present processed fragments of

antigen to T cells in an MHC-restricted manner, the mechanism of cognate interaction between B and T cells became clearer. In the remainder of this section we focus on antigen processing and presentation by B cells, a comparison of the ability of B cells versus other APCs to present antigen, and proposals concerning the roles of the different APCs *in vivo*.

C. B Cells as APCs

Benacerraf first hypothesized that B cells were major APCs (157). This was deduced from two findings: (1) B cells express high densities of class II molecules, which were thought to be involved in T cell/B cell communication, and (2) since the initiation of an antibody response requires the "bridging" of T and B cells, it would be logical to presume that B cells should function as APCs. The first report to clearly demonstrate that this was the case was that of Chesnut and Grey (158). Using rabbit antimouse Ig (RAMIg) as an antigen and rabbit Ig-specific T cells, they circumvented the problem of the low frequency of B cells which could specifically bind to a purified protein antigen, because RAMIg can bind to *all* sIg^+ B cells while antigen binds to $<0.01\%$ of B cells. Their experimental model system differed from those used in the earlier studies of Bergholtz and Thorsby (159), Hiramine and Hojo (160), and Kammer and Unanue (161), in which soluble protein antigens were bound nonspecifically (i.e., not via clonally distributed sIg molecules) to B cells. In such studies, the presentation of antigens by B cells versus macrophages was reported to be much less efficient. In contrast, using the "antigen-specific" system (RAMIg), Chesnut and Grey (158) demonstrated that B cells and macrophages could present RAMIg to T cells with similar efficiency. In contrast, normal rabbit IgG was presented by macrophages as effectively as RAMIg, but, at the same low concentrations, it was not presented by the B cells (since it had no anti-Ig activity and thus would bind poorly to B cells). Using this and similar controls, it was convincingly demonstrated that B cells, rather than contaminating macrophages or dendritic cells, are involved in antigen presentation and that antigen presentation is MHC restricted (158).

Although Chesnut and Grey established that B cells could present antigen, it was unclear whether uptake of antigen by resting B cells required binding of antigen to sIg receptors, or whether B cells, like macrophages, could bind antigen nonspecifically. In this regard, resting B cells were compared with both cycling neoplastic B cells (BAL) and macrophages for their capacity to present the antigen keyhole limpet hemocyanin (KLH) to specific T cells (162). Using secretion of IL-2 rather than proliferation as an assay for T cell activation obviated the need

of irradiating the presenting B cells (which also proliferate as a consequence of T cell-mediated activation). Under these conditions, normal splenic B cells depleted of T cells and macrophages were unable to present KLH to T cells except at very high antigen or cell concentrations. In contrast, both the BAL and the macrophages were very efficient APCs and induced significant levels of IL-2 secretion from the T cells at 100-fold lower cell numbers. When normal primed T cells (instead of T hybridoma cells) were used as responding cells, the normal B cells were, again, very poor APCs for KLH. Although the BAL were better APCs than the B cells, they were 50 times less efficient than splenic adherent cells.

Two issues were raised by the aforementioned experiments. (1) There were marked differences in the capacity of different B cells to present antigen to T cells and this appeared to be dependent upon their state of activation; i.e., resting B cells were less effective than activated or neoplastic B cells. (2) There was a dichotomy between the ability of B cells to present antigens which they could bind specifically via sIg, e.g., RAMIg, versus nonspecifically, e.g., KLH and ovalbumin (OVA). This latter point raised two further questions: (1) whether presentation of antigen by resting B cells was physiologically relevant under conditions where the B cells did not express sIg receptors specific for the antigen in question, and (2) whether resting B cells could process irrelevant antigen (as opposed to RAMIg) as effectively as macrophages.

Between 1983 and 1985, a number of papers were published with conflicting views as to whether resting B cells could act as APCs. Cowing and co-workers (163, 164) and Grey and co-workers (131, 165-167) showed that resting B cells could not act as APCs, while Parker and associates (168, 169) and Pierce and colleagues (170) demonstrated that they could. This controversy was partially resolved by Ashwell *et al.* (171) who showed that resting B cells could present antigen, but that this event was radiosensitive. This was subsequently confirmed in several reports (163, 172, 173). In earlier experiments by some groups, the resting B cells had been irradiated prior to using them as APCs.

Although studies have now confirmed the exquisite radiosensitivity of the presentation process in resting B cells, this may not be the entire explanation for the lack of antigen-presenting activity reported previously. In 1985, Krieger and co-workers (167) irradiated B cells with 500 rads and subsequently examined them for their ability to act as APCs following Percoll density gradient fractionation into cells of low and high density. Only the low-density cells were capable of eliciting a significant T cell response. These results conflicted with those of Parker and co-workers (169, 174), who separated B cells on the basis of density using a