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T CELL–DEPENDENT B CELL ACTIVATION

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Abstract

B cells obtain help from T cells in the antibody response by acting as antigen-specific antigen presenting cells. A direct signal through binding of antigen to membrane Ig can enhance B cell antigen presentation and T-dependent B cell activation, but is not required for a productive interaction between a small resting B cell and a differentiated helper T cell. As a result of helper T cell recognition of antigen on the B cell surface, the T cell becomes activated and in turn activates the B cell. T cell help has two components: lymphokines which act as growth and differentiation factors for B cells, and additional signals which require cell contact and enable B cells to respond to lymphokines. Contact help activity is regulated like lymphokine synthesis and secretion. Because contact help activity is retained by fixed, activated helper T cells and plasma membranes prepared from activated T cells, contact help is likely to be owing to new proteins expressed as membrane-bound lymphokines or activation antigens on helper T cells. Once induced, contact help can be delivered to B cells independently of recognition of antigen/class II MHC. A newly identified activation antigen of helper T cells, a ligand for the B cell differentiation antigen, CD40, is a key component of contact help. The roles of other T and B cell membrane molecules in contact help are reviewed.

INTRODUCTION

That the antibody response to protein antigens requires specific recognition of the same antigen particle by both B lymphocytes and T lymphocytes

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has been known for many years. B cells are the precursors of the antibody secreting cells, and T cells provide help. The mechanism of T cell help for B cells has been actively investigated, and much progress has been made (1, review), but a satisfactory description of T cell help remains elusive.

The pioneers of in vitro antibody responses discovered that mitogen or antigen-activated T cells secrete soluble molecules which can replace T cells in supporting antibody responses to red cells. It was proposed that local action of such factors could explain antigen-specific collaboration between T cells and B cells. This line of investigation led eventually to the identification of the lymphokines that act as growth and differentiation factors for B cells.

Other in vitro studies showed clearly that the requirement for T cells in the response to limiting concentrations of antigens cannot be replaced by lymphokines but requires antigen-specific, linked recognition of the same antigen particle by T cells and B cells, as does the antibody response in vivo. Also as previously shown in vivo, this interaction is restricted by allelic polymorphism at the major histocompatibility complex (MHC) (2, 3, 4—review). In spite of much effort, and a long detour through antigen-specific, MHC-restricted helper factors, the beginnings of an accurate scheme of T/B collaboration in the antibody response had to wait for a better understanding of how T cells recognize antigen and require appropriate MHC alleles on the cells with which they interact. We know now that T cells recognize antigen only on cell surfaces as peptide fragments bound to MHC molecules. B cells get help from T cells by acting as antigen-specific antigen presenting cells (APCs). Antigen recognition is sequential rather than simultaneous: First the B cell binds antigen with its antigen receptor membrane immunoglobulin (Ig) and internalizes and degrades the antigen; then it presents peptides from the antigen on the cell surface bound to class II MHC molecules; finally the T cell recognizes the processed antigen on the B cell surface, and mutual activation results. This scheme explains MHC restriction of T cell/B cell collaboration, as well as the ability of T cells recognizing internal peptide determinants to help B cells make antibodies that react with proteins only in their native conformation. This review begins with an introduction to B cells as APCs and then takes up the question: How does the T cell activate the B cell when it recognizes antigen on the B cell surface? Much of the evidence in this review is drawn from T cell–dependent polyclonal B cell responses in vitro.

B CELLS AS ANTIGEN PRESENTING CELLS

As soon as the outlines of how MHC molecules guide T cell recognition of antigen on cell surfaces became apparent, it was proposed that B cells

get help by acting as APCs (5, 6). Unfortunately, B cells failed to show APC function in vitro with primed T cells or T cell lines. In part, this was a technical problem, since APC populations were routinely irradiated to prevent them from contributing to the proliferative response of the T cells, and the APC function of resting B cells is radiation-sensitive (7). In fact, when the B cells were not irradiated, they responded polyclonally following polyclonal antigen presentation to antigen-specific helper T cell lines (8, 9). But resting B cells are also defective in the costimulatory signals that T cells require in order to proliferate following antigen recognition (10, review). Therefore, the first reports of B cells as stimulatory APC involved B cell tumor lines and normal B cells stimulated with anti-Ig (11).

Antigen Receptor–Mediated Antigen Presentation

The unique feature of B cells as APCs is their ability to use their clonally distributed antigen receptors to take up antigen into the processing pathway. Antigen processing and presentation mediated by membrane Ig was first demonstrated using normal B cells to present rabbit anti-mouse Ig to rabbit Ig-primed T cells, resulting in a T cell–proliferative response (11). Antigen-specific antigen presentation was then shown with purified, antigen-binding normal B cells (12) and also with antigen-specific, virus-transformed B cell lines (13). The polyclonal anti-Ig system was extended to show that small resting B cells respond with a vigorous polyclonal antibody response as a result of presenting antigen to T helper lines or hybridomas (14) and that they can process and present monovalent antigens bound to their antigen receptors (15) while in the resting state (16). In each case, receptor-mediated or antigen-specific antigen uptake and presentation was 100- to 10,000-fold more efficient than nonspecific antigen presentation (13, review). This remarkably efficient presentation of antigen by antigen-specific B cells accounts, at least in part, for the specificity of the antibody response.

Inducible Costimulatory Activities in B Cells

The in vitro experiments mentioned above showed that small B cells are efficient APCs for inducing helper signals from T cell lines and hybridomas, but helper signals can be induced from helper T cell lines or primed T cells without T cell proliferation. Other studies showed that small B cells are defective APCs for inducing primary antibody responses in vivo (17, 18) or T cell proliferative responses in vitro (19, review). Small B cells are also defective APC for the proliferative response of established Th1 lines because they fail to express a membrane-bound costimulatory activity (20, 21) and for Th2 lines, because they fail to make IL–1, which some Th2 lines require in order to respond to their own IL–4 in an autocrine fashion

(22, review). Costimulatory activity can be induced in B cells by activation, although different activation signals may induce different, as yet poorly defined, costimulatory activities. B cell blasts generally stimulate T cell proliferation well, and antigen-specific, proliferating B cells have been implicated in driving T cell expansion in vivo in antigen-primed lymph nodes (7, review). However, B cells are not required for T cell responses in vivo; T cell priming for lymph node proliferative responses is normal in mice with severe combined immunodeficiency that are reconstituted with T cells only (23).

If B cells are defective APC for primary T cell expansion and differentiation to helper function, how does the antibody response get started? In vitro studies discussed below support the concept that helper T cells must proliferate and differentiate from precursor cells to effector cells in order to gain helper function (24). There appears to be a requirement for another kind of APC to initiate the helper T cell arm of the antibody response (17, 18), perhaps a dendritic cell (19) or an adjuvant-activated macrophage (25).

B Cells as Tolerogenic Antigen-Presenting Cells

If resting B cells cannot induce proliferation and differentiation to helper function in resting T cells because of a lack of costimulatory activity, what are the consequences for the virgin T cell of recognizing antigen on a resting B cell? It has been proposed that primary T cells that recognize antigen in the absence of costimulation become functionally inactivated (26). In some Th1 clones, antigen recognition without costimulation results in a stable state of unresponsiveness called clonal anergy (27). Perhaps a virgin T cell becomes functionally inactivated when it encounters antigen on a resting B cell. In fact, transferred B cells have been shown to induce skin graft tolerance to an isolated class II alloantigen (28) and the H-Y minor histocompatibility antigen (Ephraim Fuchs, Polly Matzinger, *Science* 258: 1156–59). A foreign protein antigen targeted to B cells in vivo was a particularly effective tolerogen for antigen-reactive T cells in unprimed animals (29). These experiments support a role for B cells as antigen-specific APCs in acquired tolerance to protein antigens and in self tolerance to self antigens, especially those self antigens present in concentrations too low to induce tolerance through nonspecific antigen presentation by other types of APCs in the thymus, as argued elsewhere (10, review).

B LYMPHOCYTE ACTIVATION STAGES AND SUBSETS

Although the small, resting lymphocyte is poised to respond to external signals by proliferation and differentiation to effector function, the

response is not all-or-none. Lymphocyte activation is a multistep process regulated at each step. As implied above in the discussion of costimulation for T cells, multiple external signals result in an integrated cellular response. Uncovering causal connections among external signals, intracellular signaling pathways, and lymphocyte responses is difficult because the biochemical events associated with signaling pathways occur in minutes or seconds, while the lymphocyte response occurs over a period of days. It is helpful to consider B lymphocyte activation in stages: early events and priming, entry into cell cycle, progression through the cell cycle, and differentiation to Ig secretion and class switching, and to study signals which move the B cells through one stage to the next.

While in vitro B lymphocyte culture systems have been invaluable in defining this activation sequence and the various signals which can drive B cells along it, they have their limitations. B cells can be made to proliferate, secrete Ig, and switch Ig class in vitro, but these cultures are short-lived. With the exception of an anti-CD40-driven system (30), most of the B cells are dead or terminally differentiated to Ig-secreting cells within a week or at most two weeks. Culture systems that will generate long-lived memory B cells or propagatable nontransformed clonal B cell lines, or that undergo somatic mutation or affinity maturation, remain to be developed.

Also, normal B cells are heterogeneous in their ability to respond to activating signals in vitro. In particular, a fraction of freshly prepared B cells has less stringent activation requirements than the major population of small B cells (9), a fact which led some years ago to considerable confusion about whether T/B collaboration is in fact MHC-restricted in vitro (4, review). Subsequently, there has been an emphasis on the study of small, resting B lymphocytes, from which the partially activated cells have been removed on density gradients or by size fractionation using velocity sedimentation or centrifugal elutriation.

There are additional B lymphocyte subsets unrelated to changes accompanying acute activation. The major population in the mouse spleen are small, dense, resting, primary B lymphocytes some of which are recently differentiated from precursors in the bone marrow. Another population of small B cells is long-lived and includes the memory cells produced by clonal expansion during a previous exposure to antigen, although B cells can enter a long-lived compartment without deliberate antigen exposure (31). In addition to these differentiation states of the major population of B lymphocytes, there may be other, distinct B lymphocyte lineages with different life histories and functions. These include the marginal zone B cells, which account for the T cell–independent response to polysaccharide antigens (31) and the $Ly1^+$ ($CD5^+$) B cells in the mouse (32), which form a self renewing population in adults. Although there is little evidence that

CD5$^+$ B cells participate in normal thymus-dependent responses, they have high rates of spontaneous Ig secretion and may account for most of the circulating immunoglobulins and the natural antibody in unimmunized individuals.

Little is known about the extent to which these minor B cell subsets contribute to polyclonal T-dependent responses in vitro. The CD5$^+$ and marginal zone B cells are larger and less dense, and so may be removed when small, resting B cells are prepared. The frequency of resting B lymphocytes which respond to polyclonal T cell help is as high or higher than that responding to other activating signals (33–35). However, when not all the B cells respond, it raises the possibility of further heterogeneity in the major resting B cell population. This possibility becomes important in interpreting experiments in which different activating signals give rise to different outcomes: are the same B cells responding in different ways, or are different B cells responding? Unequivocal answers to questions about the timing of commitment to a particular outcome during B cell differentiation are often difficult to obtain. In the case of immunoglobulin class switching, the weight of evidence is strongly in favor of a pluripotent small B cell that can be driven in different directions by different signals (34–36).

THE ROLE OF SIGNAL 1 THROUGH MEMBRANE IMMUNOGLOBULIN

A key tenet of the clonal selection theory is the selection of clones committed to make particular antibodies by the interaction of antigen with a sample of the antibody displayed as an antigen receptor on the plasma membrane. The fact that B cells could be activated by antibodies specific for membrane Ig was taken as early direct evidence for the clonal selection theory. To accommodate other evidence on acquired tolerance and the requirement for linked recognition of hapten and carrier, Bretscher & Cohn (37) proposed that the B cell required two signals, the first through the antigen receptor, and the second from the carrier-specific helper T cell, which was targeted to the B cell by antigen. Later, based on experiments with haptenated lipopolysaccharide showing that immunogenicity could be a function of the intrinsic activating properties of the antigen, Coutinho & Möller proposed the "one non-specific signal" theory, in which signal 1 through membrane Ig was declared to be irrelevant in T-dependent responses to protein antigens as well (38).

Since then, abundant evidence has accumulated that B cells can be effectively activated through cross-linking of membrane Ig (39–41, reviews). This signal is likely to be essential for the T cell–independent

antibody response to polysaccharide antigens and other such highly multivalent, type 2 thymus-independent antigens, which lack peptide determinants for helper T cells. CBA/N mice bearing the *xid* mutation, which fail to respond to polysaccharide antigens, are also defective in response to membrane Ig cross-linking in vitro (42, 43). High concentrations of $F(ab')_2$ fragments of high affinity anti-IgM antibodies are mitogenic for purified small B cells, but responses can be enhanced and driven on to Ig secretion by provision of appropriate lymphokines. Submitogenic membrane Ig cross-linking can induce responsiveness to IL-4 and other lymphokines (44), and membrane Ig cross-linking upregulates expression of the IL-2 receptor (45), class II MHC (46), adhesion molecules (47), and costimulatory activity (20).

It would be surprising if these effects were irrelevant to T/B collaboration, and indeed, some early papers on polyclonal T/B collaboration established a requirement for signal 1 through membrane Ig in a response that was also dependent on MHC-restricted T cell help (48, 49). Other effects of Ig cross-linking on antigen presentation or T-dependent B cell responses have been reported (50, 51, review). On the other hand, a number of other investigators found excellent, T-dependent, MHC-restricted responses in the apparent absence of any involvement of membrane Ig, using alloreactive T cell lines or loading B cells with antigen nonspecifically at high antigen concentration (8, 9, 50, 52). When help was provided by an antigen-specific Th2 line, we were surprised to find that monovalent antigen was just as effective on a weight basis as divalent, activating antigen for proliferation and Ig secretion by small B cells (15). Moreover, in the same system, addition of a cross-linking, activating monoclonal anti-IgM antibody did not shift the dose response curve of monovalent antigen bound initially to membrane IgD (15).

Why is signal 1 required in some experiments and not others? We would argue that the experiments cited above demonstrate that signal 1 is in fact not necessary for T-dependent B cell activation, and that an appropriately activated T cell can provide all the necessary signals for B cell proliferation, Ig secretion, and class switching without a signal through membrane Ig. In the experiments in which a signal through membrane Ig is required, it is possible that the T cells employed could not deliver the full range of helper signals. For instance, as discussed further below, Th1 T cell lines are generally poor helpers in vitro but can be reconstituted with Th2 lymphokines. Signal 1 through the antigen receptor could induce high affinity IL-2 receptors or otherwise enable B cells to respond to insufficient helper signals. Alternatively, signal 1 may be necessary to allow the B cells to interact effectively with some T cells in order to induce the required helper signals, for instance, to induce costimulatory signals or to enhance

antigen presentation by increasing class II expression, adherence, or alloantigen expression.

It is not unlikely that signal 1, even when unnecessary for a vigorous antibody response, could be integrated along with contact help, cytokines, and other signals to affect the quality of the antibody response. For instance, anti-Ig strongly suppresses differentiation to high rate Ig secretion in the LPS response (53), and it may also influence switching to particular isotypes by effects on germline constant region transcription (54, 55).

The question of the role of signal 1 is harder to establish in vivo. Initially monovalent antigen can become multivalent by binding to cell surfaces directly or through antibody and Fc receptors or complement receptors. In addition to enhancing antigen presentation and costimulation as described above, signal 1 could play additional roles in vivo such as the preferential localization of antigen-specific B cells in appropriate microenvironments to meet antigen-specific T cells through enhanced adherence or chemotaxis, or selection of rare, high affinity somatic mutants from the progeny of germinal centers through direct signaling by antigen through antigen receptors. Anti-Ig cooperates with anti-CD40 to prevent apoptosis in germinal center centrocytes (56). A requirement for membrane IgD cross-linking was demonstrated in a model for a primary, T-dependent antibody response, the in vivo polyclonal IgG1 antibody response to anti-IgD antibodies (57).

CONTACT-DEPENDENT HELP

The Need for Contact-Dependent Help

No combination of characterized lymphokines induces clonal expansion in carefully prepared small resting B cells; there appear to be necessary short-range or contact-dependent signals delivered to the B cell when it presents antigen to the T cell (58, 59, reviews). This has been shown both indirectly by MHC restriction of T/B collaboration in specific antibody responses and directly in T-dependent polyclonal B cell responses. Soluble T cell–derived factors which have been reported to induce proliferation and differentiation of resting B cells have resisted isolation and molecular identification (60). Although B cells can be made responsive to lymphokines through strong cross-linking of membrane Ig by anti-Ig (40) or polysaccharide antigens (61), a signal through the antigen receptor is not required for a vigorous T cell–dependent B cell response as explained above, and the degree of cross-linking of membrane Ig which follows binding of soluble protein antigens to the B cell fails to induce responsiveness to lymphokines (62, 63).

Models of Contact-Dependent Help

One model for short-range interactions of T cells with antigen presenting cells is the killing of target cells by cytolytic T cells, which usually spare bystander cells. Although the mechanisms of killing are redundant and controversial, one mechanism involves directional secretion of pre-formed mediators by degranulation across the small intracellular space between the lymphocyte and the target cell (64). Helper factors have not been reported to be packaged in granules in helper T cells, but B cells might receive very high levels of stable lymphokines or postulated labile lymphokines by directional secretion of newly synthesized mediators. Helper T cells form tight conjugates with B cells (65), and there is functional (66) and microscopic (67) evidence for directional secretion of lymphokines toward the antigen-presenting B cell.

Other models for the delivery of contact help require direct interaction of membrane-bound molecules. That is, after all, how the T cell gets its activating signal through its antigen receptor and CD4 and the other accessory molecules involved in antigen recognition. It seems natural that the B cell should get simultaneous contact-dependent signals through its set of ligands for the molecules that activate the helper T cell. These include class II MHC (the ligand for the T cell antigen receptor, and CD4) in addition to the adhesion molecules like the LFA–1/ICAM–1 and CD2/LFA–3 ligand pairs, which also have signaling functions (68). In this model, B cell activation is a direct consequence of T cell recognition of antigen on the B cell surface.

Inducible Contact Help in Noncognate Systems

However, contact-dependent B cell activation can be divorced from antigen recognition in certain experimental systems in which the T cell is activated independently of antigen presentation by the responding B cell. This can be done by studying polyclonal "bystander" or "noncognate" responses of B cells that lack the appropriate MHC alleles or are not presenting antigen. In these systems, T cells are activated by mitomycin C–treated antigen-presenting B cells or plate-bound or cell-bound anti-CD3 or anti-T cell receptor antibodies. These bystander and noncognate responses are intense in the hands of many investigators, showing that antigen recognition can be separated functionally from the delivery of help (50, 69–76). For resting B cell responses, cell contact is required (72, 73, 75). These systems imply that some limiting component of the delivery of bystander or noncognate help requires activation of the T cell. In this model, activation is sequential rather than simultaneous: the B cell must first activate the T cell before it can receive activating signals from the T

cell. We have called this induced activity of helper T cells ITCH, for inducible T-dependent contact help (77).

More direct evidence for sequential rather than simultaneous activation of T and B cells during T/B conjugation was provided by Brian when she showed that plasma membranes from activated but not resting T cells could deliver contact-dependent help to B cells (78), a finding that has been reproduced and extended by others (79, 80). Similarly, Noelle and colleagues showed that activated T cells retain contact-dependent helper activity when they are metabolically inactivated by treatment with paraformaldehyde (81). This finding has also been reproduced and extended (77, 82). Fixed resting T cells and plasma membranes from resting T cells are not active. The acquisition of helper activity is transient and requires several hours of activation (59, review, 77). Acquisition of helper activity is sensitive to cyclosporin A (CsA) (79, 82, 83) and inhibitors of RNA and protein synthesis (59, 79, 82).

The contact helper activity of fixed cells and activated membranes also shows that directional secretion of pre-formed or newly synthesized helper factors is not an essential component of contact-dependent help, because the fixed cells and membranes are no longer capable of secretion, directional or otherwise, and retain activity during storage for several weeks at 4°. Induction of contact help requires a change in the helper T cell surface. The change could involve a modification of an existing membrane structure, for instance, the rapid increase in affinity of LFA–1 for ICAM–1 following T cell activation (84). However, the time course of induction of helper activity and the sensitivity to CsA and other drugs strongly suggests a requirement for synthesis of a new protein from a gene regulated like a lymphokine by T cell activation. Therefore, the limiting component of help in the noncognate system appears to be one or more membrane-bound lymphokines or transient T cell activation antigens that act by engaging receptors on B cells. The recently identified ligand for CD40 on activated helper T cells is an excellent candidate for such a molecule (85, 86) and is discussed below under its own heading.

Early Signals in Contact Help Depend on T Cell Activation

Although the non-cognate systems provide strong evidence for sequential activation of T cells and B cells during T/B collaboration, it can be argued that contact help is likely to be complex and redundant, and that other signaling pathways could be used in cognate interactions that result in simultaneous activation of the T cell and the B cell as a direct result of T cell recognition of antigen presented by the B cell. At limiting antigen concentrations and in some systems in which antigen concentration cannot be varied, bystander contact help is weak or absent (8, 52, 87). Even in

systems in which bystander help is easy to demonstrate, cognate help is more efficient than bystander help, especially when one measures Ig secretion rather than DNA synthesis in the responding B cells (14, 50, 70, 72). In a "cold target inhibition" experiment, mitomycin C-treated antigen-presenting B cells but not mitomycin C-treated bystander B cells compete with untreated B cells for limiting contact-dependent help signals (72). It has been argued that bystander help requires maximal, nonphysiological levels of helper T cell activation. To test whether cognate help involves growth signals delivered to the B cell as a direct consequence of Ag-specific conjugation, or whether the B cell must wait for some activation-induced change in the T cell in order to get its contact-dependent helper signal, one needs an early measure of the receipt of growth signals by the B cell.

To look as early as possible in the B cell activation sequence during T cell/B cell collaboration, we developed quantitative assays for the expression of the early activation genes c-*myc* and *egr*-1 during antigen specific T/B interaction (83). In our experiments, resting B cells are pulsed with monovalent antigen overnight and then mixed at high cell concentrations with helper T cells. After various periods of interaction at 37°, T and B cells are separated at 0° with antibody-coated magnetic beads, and RNA levels are analyzed with a probe protection assay.

The T cell–dependent, antigen-dependent c-*myc* signal in the B cells begins at 2 hr and peaks at 4 or 8 hr after T and B cell are mixed. We initially interpreted this rapid B cell response as evidence that growth signals can be delivered to the B cell without a requirement for lymphokine synthesis or induction of other helper machinery that needs to be made from new mRNA in the T cell. However, the same very early c-*myc* induction in B cells occurs when B cells receive help from T cells activated by plate-bound anti-CD3 without antigen. Also, the B cell c-*myc* response is sensitive to CsA acting on the T cells rather than the B cells. Therefore, this earliest growth response of the B cell in T/B conjugates also appears to require an inducible activity in the T cell (83). By this sensitive assay for growth signals, we found no indication of a B cell growth response triggered solely by the preformed membrane molecules involved in adhesion and T cell Ag recognition.

Early and Late Components of Contact Help

Although small B cells can receive growth signals as soon as 2 hr after conjugation with cloned helper T cells, it takes at least 6 to 8 hr of activation for T cells to gain the ability to induce DNA synthesis in B cells, when T cell activation is stopped by addition of paraformaldehyde or CsA, and excess IL-4 is provided (77, 88). The ligand for CD40, which is likely to be a key component of inducible contact help, is expressed very rapidly

on cloned T cell lines and may account for early contact help, but levels of CD40-ligand expression reach a peak at 3 or 4 hr of activation and begin to decline while helper activity of plasma membranes prepared from the same cells continues to increase (B. E. Castle, K. Kishimoto, M. L. Brown, M. R. Kehry, personal communication). Therefore, as mentioned above, there may well be multiple components of inducible contact help, some of which are expressed very early in the T cell activation sequence, and some of which are expressed later.

Requirements for Induction of Contact-Help Activity

Induction of contact help activity in continuous T cell lines follows the same time course as lymphokine synthesis and release, and it is likely to be regulated by the same intracellular signaling pathways originating from the T cell antigen receptor and the receptors for costimulatory signals on the T cell surface. Th1 and Th2 murine T cell lines show important differences in signaling pathways leading to lymphokine secretion (22, 89). The same differences, as revealed by sensitivity to various drugs that block intracellular signaling, apply to induction of contact help activity (77).

Contact help, like IL-4 production, is difficult to induce by polyclonal activation in resting normal T cells from healthy mice. Expression of these activities of differentiated helper T cells requires activation and culture for several days, followed by restimulation (24, 87, 90) (B. Whalen, D. C. Parker, unpublished). Therefore, it seems that precursors of helper T cells, like precursors of cytolytic T cells, must proliferate and differentiate to effector cells before they can express their differentiated functions efficiently (24). T memory helper cells from primed and rested animals, like in vivo primed cytolytic T cells, may also require a recent boost in vivo or in vitro to differentiate into effector cells (24), although in vivo T helper function in primed animals is generally radioresistant. Contact help can be induced directly in normal human peripheral blood T cells (82), but this population may include T cells mobilized by recent antigen exposure and primed for helper function.

THE ROLES OF CYTOKINES

The activity in supernatants of activated T cells originally called "T cell replacing factor" has turned out to be owing to a complex and variable mixture of cytokines. T cell replacing factor was resolved into various B cell growth and B cell differentiation factors by the early 1980s, but it was not until the availability of recombinant cytokines and neutralizing monoclonal antibodies that definitive experiments could be done. It is now

clear that the various cytokines involved in antibody responses in vitro, including IL–2, IL–4, IL–5, IL–6, IL–10, and γ-interferon, act in various combinations at various stages of the complex B cell activation sequence (91, 92). Different cytokines can produce similar effects, and the same cytokine can have different effects early and late in the activation sequence, depending also on the presence of other cytokines or signals. None is either necessary or sufficient for B cell growth or differentiation.

Th1 and Th2 Cells

The best insights into the roles of cytokines in T-dependent antibody responses have come from the study of helper activities of the Th1 and Th2 T cell lines in the mouse, which are distinguished by their stable patterns of lymphokine expression (36). Th1 cells secrete IL–2, γ-interferon, and lymphotoxin but not IL–4, IL–5, or IL–10, while Th2 lines secrete IL–4, IL–5, and IL–10 but not IL–2, γ-interferon, or lymphotoxin.

Both Th1 and Th2 cell lines are effective helper cells in vivo, including the generation of B cell memory and affinity maturation (93). Most Th2 cell lines are more effective helper cells than most Th1 lines for resting B cells in vitro (94), particularly for polyclonal responses (22, review), but Th1 cells help well if γ-interferon levels are low and cultures are supplemented with Th2 lymphokines (36), or if B cells belong to a subset expressing relatively high levels of class II antigen that is found in conventionally housed mice but not in sterilely housed mice (95). Therefore, both kinds of T cells deliver contact help signals, and antibody responses can be driven with either Th1 or Th2 lymphokines, although the requirements for induction of functional, high affinity IL–2 receptors on resting mouse B cells and their role in Th1-dependent responses have not been fully defined (J. Poudrier, T. Owens, personal communication).

A consistent and important difference between B cell antibody responses driven by Th1 and Th2 cell lines is in the classes of antibodies secreted by the responding B cells. Th1 helper cells preferentially induce IgG2a, while Th2 helpers induce IgG1 and IgE secretion (36, review). This difference appears to be owing entirely to the different cytokines produced, which direct switching by activating transcription of Ig constant region genes in their germline configurations (96). IL–4 enhances transcription of IgG1 and IgE germline constant region genes, and IL–4 is required for switching to IgE in vivo and in vitro (36). γ-interferon inhibits IgG1 and enhances IgG2a secretion. Contact helper signals delivered by membranes from activated Th1 and Th2 cells are equivalent (80, 97), although contact signals themselves may influence transcription of Ig heavy chain constant region germline genes and hence switching (98).

Lymphokines and Contact Help

Although some laboratories can detect a B cell proliferative response to contact help provided by activated and fixed T cells alone (77) or activated T cell membranes alone (79), these responses can be greatly enhanced by the addition of exogenous lymphokines (99, review). In the mouse, proliferation is enhanced by IL–4, and IL–4 accounts for all the proliferation enhancing activity in activated Th2 culture supernatant (80, and K. Kawakami, D. C. Parker, unpublished). Secretion of Ig and class switching depends on addition of IL–4 and IL–5 (80, 97). For human B cell responses to activated and fixed T cells, IL–2 plays a key role, although IL–4 and IL–6 can enhance Ig secretion (82). IL–4 is required for the human IgE response to contact with activated and fixed T cells (100). IL–4 also plays a unique role in the human B cell proliferative response and IgE response to anti-CD40 antibody (30, 101), and it is required for IgE secretion of mouse and human B cells in response to transfected cells expressing CD40-ligand, although the transfected cells induce B cell proliferation by themselves (85).

INTRACELLULAR SIGNALING PATHWAYS IN CONTACT HELP

Not much is known about intracellular signaling pathways in B cells in response to contact help. Strong induction of inositol phospholipid turnover and a rapid and transient increase in intracellular calcium ion concentration have been recently reported in a human B lymphoblastoid line presenting antigen to a human T cell line (102), but these signals had been sought unsuccessfully in normal human B cells receiving help from T cells (103). Changes in intracellular calcium ion concentration were also sought but not found in normal mouse B cells forming conjugates with anti-CD3 activated T cells (K. Kawakami, D. C. Parker, unpublished). A very rapid increase in cAMP concentration in B cells interacting with fixed, activated T cells has been reported (104). This increase could not be detected using activated plasma membranes (R. Noelle, personal communication). Early effects of contact help within 2 to 4 hr include induction of c-*myc* expression, as discussed above (83), and also appearance of a DNA binding protein in the NF-κB transcription factor family (A.-C. Lalmanach-Girard, T. C. Chiles, D. C. Parker, T. L. Rothstein, unpublished). Later effects include induction of ornithine decarboxylase activity (104) and casein kinase II activity (105), an increase in expression of CD23 (103, 106) and class II, and the increase in cellular size and RNA content that accompanies entry into G1 (81). Contact with T cells induces changes in

various other membrane proteins (107, and H. Wortis and D. C. Parker, unpublished). Activated T cell membranes induce transcripts from the IgG1 constant region (98).

A number of drugs block activation of B cells through membrane Ig cross-linking, including CsA, phorbol esters added at the time of activation, overnight incubation in phorbol ester to deplete PKC activity, and agents that raise cAMP. The same drugs do not block activation of B cells by contact with fixed, activated T cells, or block only at hundred-fold higher concentrations, as measured by DNA synthesis in the presence of added IL–4 (107a). This finding, and the lack of an obvious calcium and phosphatidyl inositol signal in small B cells, suggests that T cells use a signaling pathway that is distinct from that engaged through membrane Ig. Also, in the early activation gene experiments mentioned above (83), contact with T cells in either cognate or noncognate conjugates caused a large increase in c-*myc* mRNA levels without the corresponding increase in *egr*–1 levels that accompanies B cell activation by anti-Ig (108).

Contact help may involve a molecular interaction between a T cell membrane molecule and the membrane Ig complex. For instance, two laboratories have reported that CD4 associates directly with Ig (109, 110). Also, T cells bear an inducible Fc receptor for IgD (111). Alternatively, an inducible membrane protein on T cells might interact and cross-link membrane Ig or another molecule, such as CD21 (47, for references), associated with the membrane Ig complex. However, we think these possibilities are made unlikely by the experiments showing that the signaling pathways involved in contact help appear to be different from those engaged by cross-linking membrane Ig.

Progress on signaling pathways in contact help will be furthered by identification of the molecules involved in delivering help to the B cell across the B cell plasma membrane. With the appropriate reagents, signal transduction can then be studied following optimal cross-linking of individual molecules by monoclonal antibodies or soluble ligands or cell lines transfected with genes encoding membrane-bound ligands, as can now be done with CD40 and its ligand, a process described below.

MOLECULES INVOLVED IN DELIVERY OF CONTACT HELP

The number of ligand pairs involved in the T/B interaction is surprisingly large and growing. A partial list of ligand pairs and other surface molecules seeking ligands is shown in Table 1. Many of these molecules are known to be involved in T cell recognition of antigen on the B cell surface, either directly or as adhesion/signaling accessory molecules. Others act as

Table 1 Ligand pairs in T/B interaction

	T cell	B cell	Reference
Antigen recognition	TCR/CD3/CD4	Class II/peptide	158
	CD4	Class II	129
Adhesion/signaling	LFA-1	ICAM-1	68, 102, 137
	ICAM-1	LFA-1	138
	CD2	LFA-3	141
Costimulation	CD28	B7	144, 148
	CTLA-4	B7	150
	?	Heat stable antigen	159
Inducible contact help	CD40-ligand	CD40	85, 86
New ligand pairs	CD45Ro	CD22	146
	CD5	CD72 (Lyb-2)	145
	LAG-3	Class II	132
TNF receptor family	OX40	?	117
	CD27	?	122
	4-1BB	?	123
	Membrane TNF?	TNF receptor	118
Unknown	Fcδ receptor	IgD?	111
	CD4	Ig?	109, 110
	?	CD19	47
	?	CD20	47
	?	Bgp95	47

costimulatory molecules to modify the T cell response to antigen recognition. As argued above, we think it is unlikely that these molecules directly deliver essential, simultaneous, early growth signals to the B cell. On the other hand, to the extent that the B cell partners of these ligand pairs have been shown to act as signal transducing molecules, they are likely to modify either early signals or an ongoing interaction. From the point of view of a minimal model of contact help, the two most interesting classes of molecules are the activation antigens that appear rapidly on the T cell surface and the resident surface antigens on B cells that can activate B cells when cross-linked with monoclonal antibodies, including CD72 (Lyb2), CD40, CD19, CD20, and Bgp95 (47).

CD40-Ligand

At least five laboratories have independently identified what may be a single new surface protein, restricted in its expression to acutely activated $CD4^+$ T cells, that binds the B cell differentiation antigen, CD40, and activates B cells (85, 86; B. E. Castle, K. Kishimoto, M. L. Brown, M. R. Kehry, personal communication; S. Lederman, M. J. Yellin, G. Inghirami,

J. J. Lee, D. M. Knowles, L. Chess, *J. Immunol.* 149: 3817–26; P. Lane, A. Traunecker, S. Hubele, S. Inui, A. Lanzavecchia, D. Gray, *Eur. J. Immunol.* 22: 2573–78). Using a soluble CD40-Ig fusion protein, Armitage and colleagues (85) identified and cloned a CD40 ligand (CD40L) from a cDNA library made from a variant of the EL4 murine thymoma line, which they had selected for high expression of binding activity for soluble CD40-Ig. The cDNA encodes a predicted type II membrane protein of Mr about 33,000, with homology to tumor necrosis factor-α (TNF-α) and lymphotoxin (LT, also called TNF-β) (112). Expression of the message is strongly and rapidly induced in murine T cell lines by activation. A cell line transfected with the CD40-ligand clone induces proliferation and, in the presence of IL–4, IgE secretion from murine and human B cells. Noelle and colleagues (86) have isolated a hamster monoclonal antibody specific for an activation antigen on a murine T helper line that cross-competes with a soluble CD40-Ig fusion protein in binding to activated T helper cells, and that precipitates a protein of the same size as CD40-ligand. Both the antibody and the CD40-Ig molecule block the helper activity of membranes from activated T helper cells. Kehry and colleagues (B. E. Castle, K. Kishimoto, M. L. Brown, M. R. Kehry, personal communication) and Lane and colleagues (P. Lane, A. Traunecker, S. Hubele, S. Inui, A. Lanzavecchia, D. Gray, personal communication) each have data on the expression and helper function of CD40-ligand based on experiments with soluble CD40 molecules, in mouse and human systems, respectively. Lederman and colleagues have a monoclonal antibody specific for a variant subclone of the Jurkat human leukemic line which exhibits constitutive contact help activity (106). The help activity is blocked by a nonstimulatory form of anti-CD40. The monoclonal antibody detects a transient activation antigen of normal $CD4^+$ human T cells and inhibits the contact help activity of activated and fixed T cells. It stains rare $CD4^+$ T cells localized in the mantle and centrocytic zones of lymphoid follicles (S. Lederman, M. J. Yellin, G. Inghirami, J. J. Lee, D. M. Knowles, L. Chess, personal communication) and is likely to react with CD40-ligand or a very similar molecule.

The identification of CD40-ligand as a component of contact help will focus interest on CD40 as a signal transducing molecule. Anti-CD40 has recently been reported to induce c-*myc* expression (113). Antibodies to CD40 and several other B cell surface molecules upregulate cell adhesion through LFA-1 and other adhesion molecules (114). Anti-CD40 antibodies costimulate B cell proliferation together with IL–4, anti-Ig, or anti-CD20 antibodies (47, review). Cross-linking of CD40 induces rapid and extensive phosphorylation of CD20, and in the buoyant, activated tonsillar B cell fraction causes increases in phosphotyrosine and serine/threonine

kinase activity comparable to those produced by anti-Ig or anti-class II antibodies (115). Anti-CD40 antibody and IL–6, the latter by an indirect effect dependent on CD40, inhibit growth of CD40-transfected B cell lines (116).

Other Members of the CD40/TNF Receptor Family

CD40 is a member of a new class of membrane receptor proteins (117) which includes the low affinity nerve growth factor (NGF) receptor and the two TNF receptors for TNF-α and LT. TNF-α appears in Table 1 because it is a cytokine that has been reported to occur in an active, membrane-bound form (118), and it and LT, a Th1 lymphokine, act on the same receptors and are stimulatory for B cells (119). However, we were unable to inhibit contact help activity (C. Zarozinski, D. C. Parker, unpublished) with a soluble TNF receptor that blocks both TNF-α and LT activity (120). Another member of the receptor family is the Fas antigen, thought to be a trigger for apoptosis in thymocytes and other cell types (121). Three other members of this receptor family appear in Table 1 because they are T cell activation antigens: OX40 (117), CD27 (122), and the activation gene, 4–1BB (123). Of these, OX40 is of particular interest because its expression is restricted to $CD4^+$ T cells (117). However, since these molecules are homologous to receptors rather than their ligands, they probably costimulate or regulate helper T cell activation, rather than directly activate B cells.

Class II MHC

Because it is the B cell contribution to the ligand pair that is required for specific antigen recognition by the T cell, the class II MHC molecule is a favorite candidate for receipt of helper signals when the B cell presents antigen to the T cell. Class II MHC molecules are known to signal B cells by various pathways, and anti-class II antibodies have been frequently reported to have stimulatory or inhibitory effects on B lymphocyte responses (124–127). In combination with suboptimal stimulation with anti-Ig or anti-Ig and IL-4, anti-class II antibodies can induce B cell proliferation (127, 128).

Antibodies to class II molecules effectively block cognate help by blocking T cell antigen recognition. To study effects of anti-class II antibodies or other antibodies on the delivery rather than the induction of help, it is necessary to use bystander or noncognate systems. Of course, the noncognate systems by themselves argue against a role for an antigen-specific, MHC-restricted class II interaction in B cell activation, since the bystander and noncognate responses are not MHC-restricted. But it is known that class II can be engaged directly by CD4 (129), and CD4 redistributes to

the area of cell contact in T/B conjugates (130), and it has been argued that this interaction or a nonpolymorphic interaction with some portion of the T cell antigen receptor complex could participate directly in the delivery of noncognate help (81, 131). Alternatively, some component of inducible contact help on the T cell surface could engage class II; LAG–3 is a T cell activation gene which encodes a protein of unknown function that is closely related to CD4 and binds class II (132). Indeed, one could argue that effective signaling through class II in cognate systems should not be limited to the relatively rare interactions of a T cell antigen receptor with a particular class II peptide complex, of which only a few hundred may be adequate to trigger the T cell response (133).

The effects of anti-class II antibodies on noncognate responses have been mixed. Anti-CD4 or anti-class II antibodies reportedly can block delivery of help in noncognate systems by blocking a CD4/class II interaction (81, 134). We found that anti-class II antibodies against bystander alleles of class II do not block the bystander response (72). Others have also found that anti-CD4 or anti-class II antibodies fail to inhibit noncognate help from activated T cells, T cell membranes, or killed activated T cells (79, 86, 135). Looking very early in the activation sequence, we showed that anti-class II antibodies which block antigen recognition do not block the early c-*myc* signal in B cells induced by fixed, activated helper T cells (83). The possibility of negative signaling through class II could explain the apparent blocking effect of anti-class II antibodies in some experiments. Alternatively, the inability to block could be explained by proposing that the anti-class II antibodies replace the contact help signal from the T cell.

Recently, the availability of "knockout" mice lacking both I-A and I-E molecules produced by homologous recombination in embryonic stem cell lines (136) has allowed us to test unequivocally the requirement for class II molecules in the delivery of contact help in the noncognate model. We found that class II negative mice respond as well as class II positive heterozygous littermates by DNA synthesis and immunoglobulin secretion of various isotypes to Th1 or Th2 cells activated by plate-bound anti-CD3 (136a). Another laboratory has shown that MHC class II-negative variant B cells respond as well as class II-positive B cells in the rapid calcium and inositol phospholipid response of human B cell lines to contact with activated T cell clones (102). Therefore, class II molecules appear to play no essential role in the effector phase of help once the helper function is induced, at least in the noncognate models.

Adhesion/Signaling Molecules

In addition to engagement of the T cell antigen receptor and CD4 with the class II peptide complex, successful antigen recognition frequently

requires the participation of additional signaling/adhesion ligand pairs, of which LFA–1/ICAM–1 or ICAM–2 and CD–2/LFA–3 are the best studied (68). These interactions could also aid in the delivery of help to B cells, either as adhesion molecules to enhance delivery of signals by other molecules or as signaling molecules themselves. As discussed above for class II molecules, the role of these additional ligand pairs in the delivery of help is difficult to study in cognate systems because they are also required for the induction of help. For instance, in cognate or bystander interactions, anti-LFA–1 antibodies block B cell responses effectively by blocking antigen recognition and T cell activation (72). In various noncognate systems, the effects of antibodies to LFA–1 and ICAM–1 are again mixed, with several groups reporting no effect on B cell responses to activated T cell membranes (86, 99, 134). With anti-CD3-activated T cells or fixed, activated T cells, there is consistent evidence that the LFA–1/ICAM–1 interaction at least enhances the delivery of help (82, 135, 137, 138).

LFA–1 and ICAM–1 can be expressed on both T and B cells. Using T cell lines derived from a person whose T cells lack surface expression of LFA–1, one group (138) showed that LFA–1 on the T cell is not required for the delivery of help in a human noncognate system, and they suggested that ICAM–1 on the T cell engages LFA–1 on the B cell in the delivery of help. ICAM–1 is an activation antigen that is upregulated upon T cell activation (68). ICAM–1 has been shown to have a signaling role through engaging LFA–1 as well as an adhesion function in both T cells and B cells (102, 139, and references therein). A particular, atypical antibody to murine LFA–1 mimics the effects of IL–4 on B cells by binding to LFA–1 (140). Perhaps this antibody mimics the effects of ICAM–1 on B cells. Like IL–4, ICAM–1 on the T cell might deliver progression signals to CD40-ligand-activated B cells. However, another group concluded that a rapid, antigen-specific signal from a cloned T cell to an antigen-presenting B cell line required LFA–1 on the T cell and ICAM–1 on the B cell (102).

CD2 on the T cell has also been proposed to act in the delivery of help (141), although effects of CD2 on induction rather than delivery of help were not completely excluded. Several other groups have reported no effect of anti-CD2 on delivery of noncognate contact help (82, 134, 135). In the mouse, CD2 is expressed on B cells as well as T cells (142), and so it could be a target of an activating interaction. The murine homolog of the ligand for CD2, LFA–3, has not yet been identified. An additional ligand for CD2 in humans, CD59, has been identified recently (143).

Recently Identified Ligand Pairs

Using cDNA clones expressed as membrane proteins or as soluble fragments or fusion proteins, a number of new interactions have been found

among known T and B cell surface antigens. In addition to the CD40-ligand/CD40 interaction, newly identified interactions between T and B cell membrane molecules include CD28/B7 (144), CD5/CD72 (Lyb-2 in the mouse) (145), and CD45Ro/CD22 (146).

The CD28/B7 interaction is a key costimulatory signal for T cell IL-2 production and proliferation (144) and can determine whether antigen presentation results in T cell anergy or proliferation in Th1 lines (147). B7 is upregulated on activated B cells (148, 149) and so may determine in part whether B cells induce tolerance or drive T cell clonal expansion. CTLA-4 is T cell membrane protein which is also a ligand for B7. It is homologous and closely linked to CD28. It is expressed at lower levels but is of higher affinity for B7 than CD28 and was identified initially as a T cell activation gene (150, and references therein). At present, B7 has not been shown to deliver a signal to the B cell (149).

CD72 is a type II integral membrane protein on B cells whose external domain is homologous to that of asialoglycoprotein receptors and CD23. Antibodies to CD72 are mitogenic for murine and human B cells alone or in combination with cytokines. Its ligand, CD5, is expressed on all mature T cells and a subset of B cells, and CD5 can deliver activating signals to T cells when cross-linked with monoclonal antibodies (145, for references). Whether this interaction is costimulatory for T cells or B cells or both in T/B collaboration remains to be determined.

CD22, a marker of mature B cells, is a member of the Ig family and is homologous to myelin-associated glycoprotein. Antibodies to CD22 enhance responses to anti-Ig (47, review). CD45 is the major protein tyrosine phosphatase of lymphocytes and probably plays a key role in T and B lymphocyte activation through the antigen receptors. The CD45Ro isoform is a marker of $CD4^+$ memory T cells in humans, is the dominant CD45 isoform in murine T helper lines, and is upregulated further by acute activation (151, and references therein). A role for this interaction in T/B interactions remains to be established.

Other B Cell Surface Molecules Seeking Ligands

Several other B cell differentiation antigens of unknown function are candidates for receptors for contact help, because antibodies against them have various activating effects. Certain monoclonal antibodies to CD20, a membrane protein with four transmembrane domains used as a pan B cell marker, can activate resting B cells cooperatively with anti-CD40 (47, review, 113), and induce c-*myc* expression by a pathway distinct from that used by mIg (152). As mentioned earlier, anti-CD40 causes extensive phosphorylation of CD20 on serine and threonine. Antibodies to CD19, a member of the Ig family, and Bgp95, an uncloned B cell differentiation

antigen, each have activating effects similar in many respects to those of anti-Ig antibodies, but anti-CD19 antibodies inhibit rather than stimulate c-*myc* expression (47, review).

CONCLUDING REMARKS

The accumulating indirect evidence that MHC-restricted, cell contact–mediated T cell help depends on transient expression of new membrane molecules on helper T cells has been confirmed by the identification of CD40-ligand, which fits exactly the predicted properties of an effector molecule responsible for inducible contact help activity. Contact help has entered the molecular arena, and signal theory in T/B collaboration is about to give way to the study of signaling pathways. Other molecular interactions of known and yet to be discovered ligand pairs will play important roles in the induction and delivery of contact help. This review concentrates on the initial activating signals to resting B cells, but T and B cells continue to interact as proliferating lymphoblasts, and contact signals may be required for later rounds of B cell proliferation (153–155). Unlike CTL, which kill their targets by release of pre-formed mediators and recycle rapidly to kill again, helper T cells engage in a prolonged, perhaps monogamous (156) relationship with an antigen-specific B cell, and there is time for an extended, ongoing dialog in which each cell monitors and influences the activation state of the other. Membrane bound effector molecules are also likely to provide short-range signals to other cells that present antigen to $CD4^+$ T cells, such as macrophages, dendritic cells, and endothelial cells.

The idea that B cells get contact-dependent help by presenting antigen to T cells gave rise to the expectation that the class II molecules themselves would deliver critical activating signals in MHC-restricted T/B collaboration. So far, although anti-class II antibodies signal B cells, there is no good evidence for T cells acting through class II to deliver help. If only the induction but not the delivery of contact help is antigen-specific and MHC-restricted, how is the specificity of T/B collaboration maintained? The effector molecules for contact help could be among those that accumulate in the area of contact between the T cell and the B cell (1, review, 130), and so would not be available to bystanders unless produced in excess. In general, MHC-unrestricted contact help is easier to demonstrate in anti-CD3-driven systems than in bystander systems in which APCs are present. Also, lymphokine-mediated and contact-mediated bystander help have rarely been demonstrated in vivo, where effector mechanisms are tightly regulated and act against a background of nonspecific inhibition.

Membrane-bound effector molecules are more difficult to study than

soluble mediators, but new methods are being developed to identify them and study their functions separately and in combination. Also, the ability to produce mice with homozygous mutations in any cloned gene has created new opportunities to study the function of individual molecules in whole animals, as well as a source of normal cells lacking particular molecules for in vitro studies. In the next few years, knockout mice lacking a variety of cell surface antigens will become available for in vivo and in vitro studies of T/B collaboration.

As part of a larger program of identifying the costimulatory signals which enable T cells to distinguish self from not-self in the periphery (157), future work on T/B collaboration will also investigate how B cells acting as APCs can regulate T cells in immunity and tolerance. On the horizon are useful in vitro systems for reproducing the T cell–dependent germinal center environment, which gives rise to memory cells and in which somatic mutation and affinity maturation occur. More precise definition of cellular interactions, membrane molecules, and activation states in vitro will allow a more incisive approach to the old questions of functional subsets of primary and memory T and B lymphocytes in vivo, the lineage relationships among those subsets, how the rules for cell interactions change following exposure to antigen, and how the system breaks down in autoimmune disease.

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