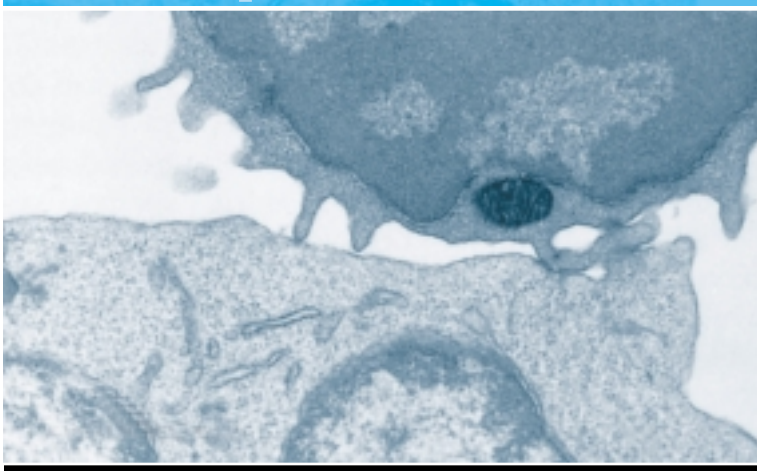
chapter 11

B-Cell Generation, Activation, and Differentiation



Initial Contact Between B and T Cells

The developmental process that results in production of plasma cells and memory B cells can be divided into three broad stages: generation of mature, immunocompetent B cells (maturation), activation of mature B cells when they interact with antigen, and differentiation of activated B cells into plasma cells and memory B cells. In many vertebrates, including humans and mice, the bone marrow generates B cells. This process is an orderly sequence of Ig-gene rearrangements, which progresses in the absence of antigen. This is the antigen-independent phase of B-cell development.

A mature B cell leaves the bone marrow expressing membrane-bound immunoglobulin (mIgM and mIgD) with a single antigenic specificity. These **naive B cells,** which have not encountered antigen, circulate in the blood and lymph and are carried to the secondary lymphoid organs, most notably the spleen and lymph nodes (see Chapter 2). If a B cell is activated by the antigen specific to its membrane-bound antibody, the cell proliferates (clonal expansion) and differentiates to generate a population of antibody-secreting plasma cells and memory B cells. In this activation stage, **affinity maturation** is the progressive increase in the average affinity of the antibodies produced and **class switching** is the change in the isotype of the antibody produced by the B cell from μ to γ, α, or ϵ. Since B cell activation and differentiation in the periphery require antigen, this stage comprises the antigen-dependent phase of B-cell development.

Many B cells are produced in the bone marrow throughout life, but very few of these cells mature. In mice, the size of the recirculating pool of B cells is about 2×10^8 cells. Most of these cells circulate as naive B cells, which have short life spans (half lives of less than 3 days to about 8 weeks) if they fail to encounter antigen or lose in the competition with other B cells for residence in a supportive lymphoid environment. Given that the immune system is able to generate a total antibody diversity that exceeds 10^9, clearly only a small fraction of this potential repertoire is displayed at any time by membrane immunoglobulin on recirculating B cells. Indeed, throughout the life span of an animal, only a small fraction of the possible antibody diversity is ever generated.

Some aspects of B-cell developmental processes have been described in previous chapters. The overall pathway, beginning with the earliest distinctive B-lineage cell, is described in sequence in this chapter. Figure 11-1 presents an overview of the major events in humans and mice. Most of this chapter applies to humans and mice, but important departures from these developmental pathways have been shown to occur in some other vertebrates. Finally, this chapter will consider the regulation of B-cell development at various stages.

B-Cell Maturation

The generation of mature B cells first occurs in the embryo and continues throughout life. Before birth, the yolk sac, fetal liver, and fetal bone marrow are the major sites of B-cell maturation; after birth, generation of mature B cells occurs in the bone marrow.

VISUALIZING CONCEPTS

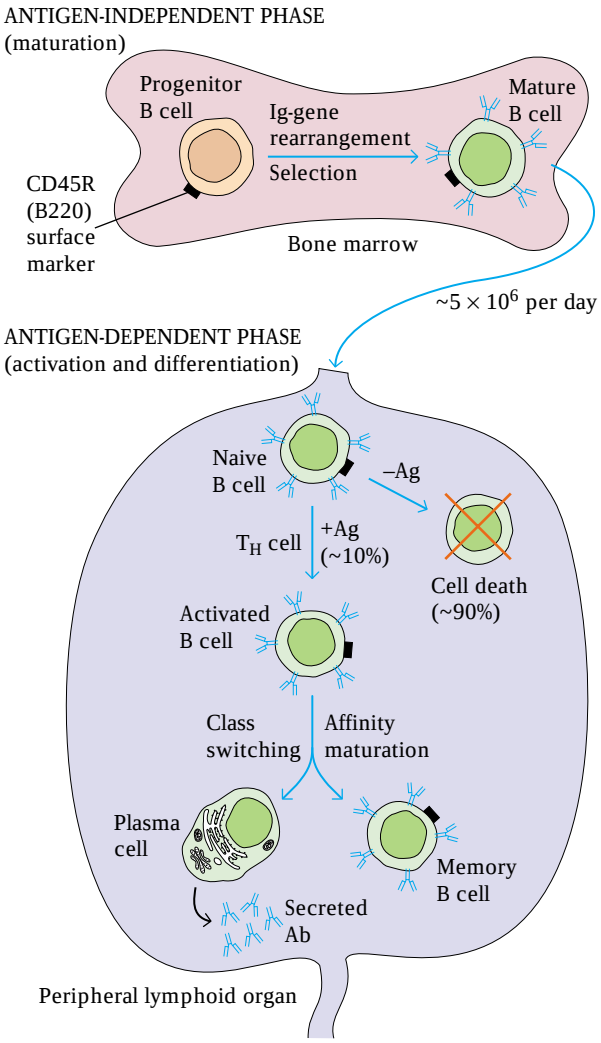


FIGURE 11-1 Overview of B-cell development. During the antigen-independent maturation phase, immunocompetent B cells expressing membrane IgM and IgD are generated in the bone marrow. Only about 10% of the potential B cells reach maturity and exit the bone marrow. Naive B cells in the periphery die within a few days unless they encounter soluble protein antigen and activated T_H cells. Once activated, B cells proliferate within secondary lymphoid organs. Those bearing high-affinity mIg differentiate into plasma cells and memory B cells, which may express different isotypes because of class switching. The numbers cited refer to B-cell development in the mouse, but the overall principles apply to humans as well.

Progenitor B Cells Proliferate in Bone Marrow

B-cell development begins as lymphoid stem cells differentiate into the earliest distinctive B-lineage cell—the **progenitor B cell (pro-B cell)**—which expresses a transmembrane tyrosine phosphatase called CD45R (sometimes called B220 in mice). Pro-B cells proliferate within the bone marrow, filling the extravascular spaces between large sinusoids in the shaft of a bone. Proliferation and differentiation of pro-B cells into **precursor B cells (pre-B cells)** requires the microenvironment provided by the bone-marrow stromal cells. If pro-B cells are removed from the bone marrow and cultured in vitro, they will not progress to more mature B-cell stages unless stromal cells are present. The stromal cells play two important roles: they interact directly with pro-B and pre-B cells, and they secrete various cytokines, notably IL-7, that support the developmental process.

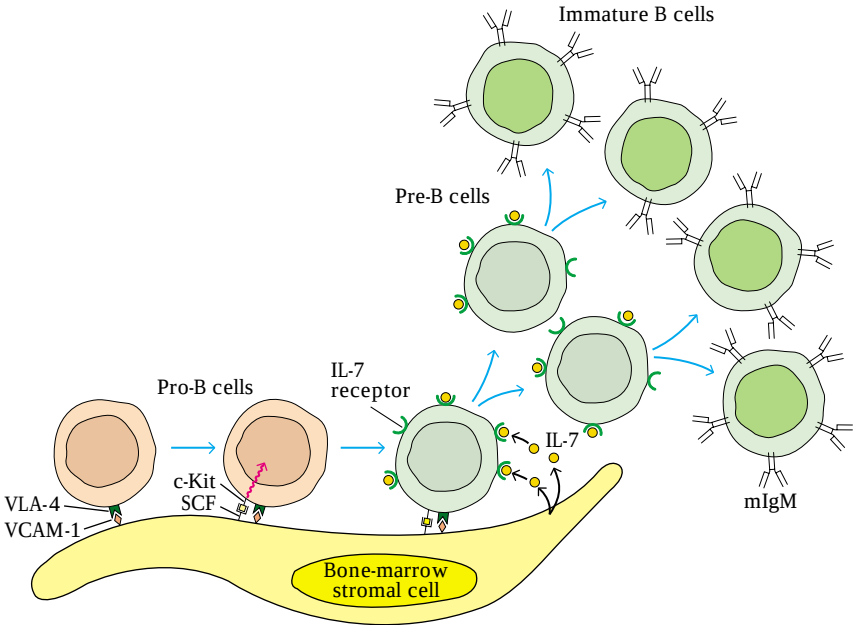


FIGURE 11-2 Bone-marrow stromal cells are required for maturation of progenitor B cells into precursor B cells. Pro-B cells bind to stromal cells by means of an interaction between VCAM-1 on the stromal cell and VLA-4 on the pro-B cell. This interaction promotes the binding of c-Kit on the pro-B cell to stem cell factor (SCF) on the stromal cell, which triggers a signal, mediated by the tyrosine kinase activity of c-Kit, that stimulates the pro-B cell to express receptors for IL-7. IL-7 released from the stromal cell then binds to the IL-7 receptors, inducing the pro-B cell to mature into a pre-B cell. Proliferation and differentiation evenutally produces immature B cells.

At the earliest developmental stage, pro-B cells require direct contact with stromal cells in the bone marrow. This interaction is mediated by several cell-adhesion molecules, including VLA-4 on the pro-B cell and its ligand, VCAM-1, on the stromal cell (Figure 11-2). After initial contact is made, a receptor on the pro-B cell called c-Kit interacts with a stromal-cell surface molecule known as stem-cell factor (SCF). This interaction activates c-Kit, which is a tyrosine kinase, and the pro-B cell begins to divide and differentiate into a pre-B cell and begins expressing a receptor for IL-7. The IL-7 secreted by the stromal cells drives the maturation process, eventually inducing down-regulation of the adhesion molecules on the pre-B cells, so that the proliferating cells can detach from the stromal cells. At this stage, pre-B cells no longer require direct contact with stromal cells but continue to require IL-7 for growth and maturation.

Ig-Gene Rearrangment Produces Immature B Cells

B-cell maturation depends on rearrangement of the immunoglobulin DNA in the lymphoid stem cells. The mechanisms of Ig-gene rearrangement were described in Chapter 5. First to occur in the pro-B cell stage is a heavy-chain D_H-to-J_H gene rearrangement; this is followed by a V_H-to-D_HJ_H rearrangement (Figure 11-3). If the first heavy-chain rearrangement is not productive, then V_H-D_H-J_H rearrangement continues on the other chromosome. Upon completion of heavy-chain rearrangement, the cell is classified as a pre-B cell. Continued development of a pre-B cell into an immature B cell requires a productive light-chain gene rearrangement. Because of allelic exclusion, only one light-chain isotype is expressed on the membrane of a B cell. Completion of a productive light-chain rearrangement commits the now immature B cell to a particular antigenic specificity determined by the cell's heavy-chain VDJ sequence and light-chain VJ sequence. Immature B cells express mIgM (membrane IgM) on the cell surface.

As would be expected, the recombinase enzymes RAG-1 and RAG-2, which are required for both heavy-chain and light-chain gene rearrangements, are expressed during the pro-B and pre-B cell stages (see Figure 11-3). The enzyme terminal deoxyribonucleotidyl transferase (TdT), which catalyzes insertion of N-nucleotides at the D_H-J_H and V_H-D_H-J_H coding joints, is active during the pro-B cell stage and ceases to be active early in the pre–B-cell stage. Because TdT expression is turned off during the part of the pre–B-cell stage when light-chain rearrangement occurs, N-nucleotides are not usually found in the V_L-J_L coding joints.

The bone-marrow phase of B-cell development culminates in the production of an IgM-bearing immature B cell. At this stage of development the B cell is not fully functional, and antigen induces death or unresponsiveness (anergy) rather than division and differentiation. Full maturation is signaled

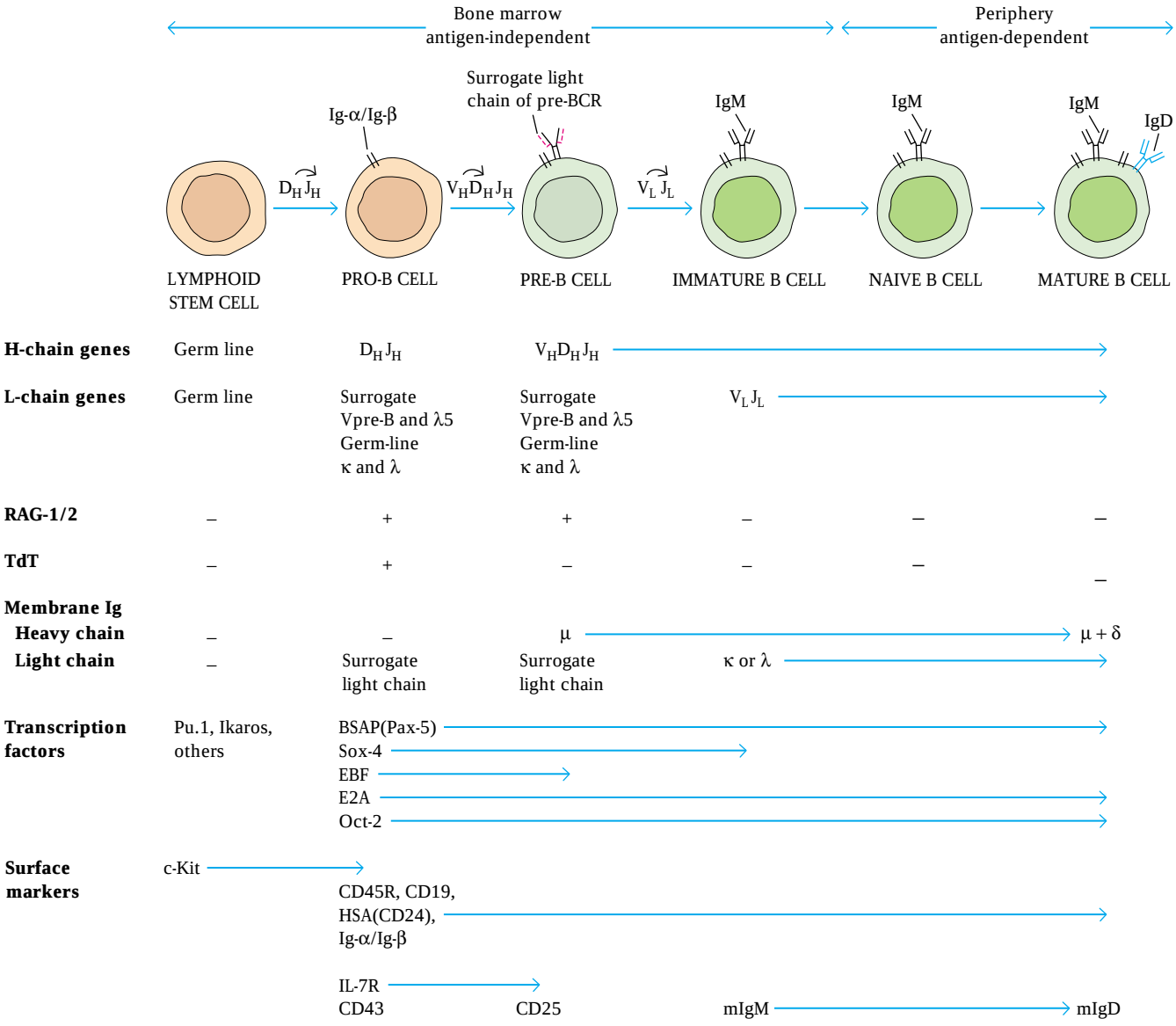


FIGURE 11-3 Sequence of events and characteristics of the stages in B-cell maturation in the bone marrow. The pre-B cell expresses a membrane immunoglobulin consisting of a heavy (H) chain and surrogate light chains, Vpre-B and λ5. Changes in the RNA processing of heavy-chain transcripts following the pre-B cell stage lead to synthesis of both membrane-bound IgM and IgD by mature B cells. RAG-1/2 = two enzymes encoded by recombination-activating genes; TdT = terminal deoxyribonucleotidyl transferase. A number of B-cell–associated transcription factors are important at various stages of B-cell development; some are indicated here.

by the co-expression of IgD and IgM on the membrane. This progression involves a change in RNA processing of the heavy-chain primary transcript to permit production of two mRNAs, one encoding the membrane form of the μ chain and the other encoding the membrane form of the δ chain (see Figure 5-19). Although IgD is a characteristic cell-surface marker of mature naive B cells, its function is not clear. However, since immunoglobulin δ knockout mice have essentially normal numbers of fully functional B cells, IgD is not essential to either B-cell development or antigen responsiveness.

The Pre–B-Cell Receptor Is Essential for B-Cell Development

As we saw in Chapter 10, during one stage in T-cell development, the β chain of the T-cell receptor associates with pre-Tα to form the pre–T-cell receptor (see Figure 10-1). A parallel situation occurs during B-cell development. In the pre-B cell, the membrane μ chain is associated with the **surrogate light chain,** a complex consisting of two proteins: a V-like sequence called **Vpre-B** and a C-like sequence called

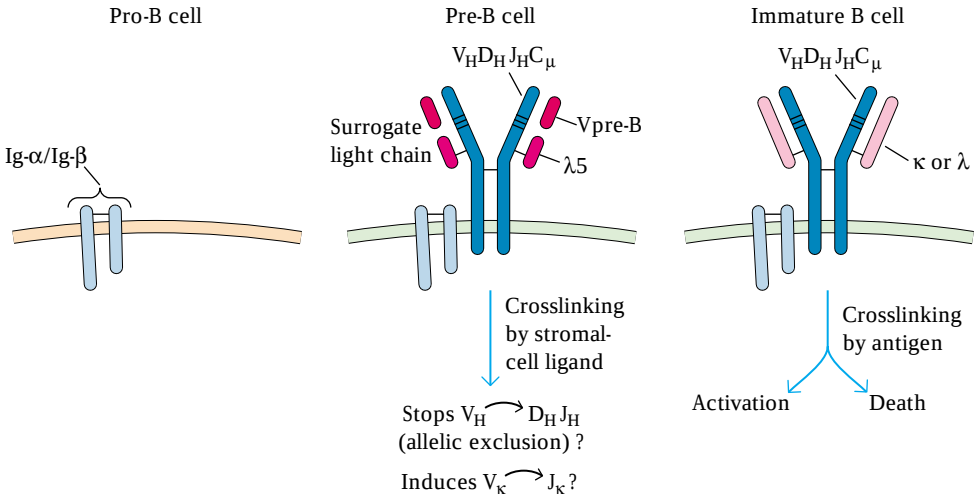


FIGURE 11-4 Schematic diagram of sequential expression of membrane immunoglobulin and surrogate light chain at different stages of B-cell differentiation in the bone marrow. The pre–B-cell receptor contains a surrogate light chain consisting of a Vpre-B polypeptide and a λ5 polypeptide, which are noncovalently associated. The immature B cell no longer expresses the surrogate light chain and instead expresses the κ or λ light chain together with the μ heavy chain.

λ5, which associate noncovalently to form a light-chain–like structure.

The membrane-bound complex of μ heavy chain and surrogate light chain appears on the pre-B cell associated with the Ig-α/Ig-β heterodimer to form the pre–B-cell receptor (Figure 11-4). Only pre-B cells that are able to express membrane-bound μ heavy chains in association with surrogate light chains are able to proceed along the maturation pathway.

There is speculation that the pre–B-cell receptor recognizes a not-yet-identified ligand on the stromal-cell membrane, thereby transmitting a signal to the pre-B cell that prevents V_H to D_HJ_H rearrangement of the other heavy-chain allele, thus leading to allelic exclusion. Following the establishment of an effective pre–B-cell receptor, each pre-B cell undergoes multiple cell divisions, producing 32 to 64 descendants. Each of these progeny pre-B cells may then rearrange different light-chain gene segments, thereby increasing the overall diversity of the antibody repertoire.

The critical role of the pre–B-cell receptor was demonstrated with knockout mice in which the gene encoding the λ5 protein of the receptor was disrupted. B-cell development in these mice was shown to be blocked at the pre-B stage, which suggests that a signal generated through the receptor is necessary for pre-B cells to proceed to the immature B-cell stage.

Knockout Experiments Identified Essential Transcription Factors

As described in Chapter 2, many different transcription factors act in the development of hematopoietic cells. Nearly a dozen of them have so far been shown to play roles in B-cell development. Experiments in which particular transcription factors are knocked out by gene disruption have shown that four such factors, **E2A, early B-cell factor (EBF), B-cell–specific activator protein (BSAP),** and **Sox-4** are particularly important for B-cell development (see Figure 11-3). Mice that lack E2A do not express RAG-1, are unable to make D_HJ_H rearrangements, and fail to express λ5, a critical component of the surrogate light chain. A similar pattern is seen in EBF-deficient mice. These findings point to important roles for both of these transcription factors early in B-cell development, and they may play essential roles in the early stages of commitment to the B-cell lineage. Knocking out the ***Pax-5*** gene, whose product is the transcription factor BSAP, also results in the arrest of B-cell development at an early stage. Binding sites for BSAP are found in the promoter regions of a number of B-cell–specific genes, including *Vpre-B* and λ5, in a number of Ig switch regions, and in the Ig heavy-chain enhancer. This indicates that BSAP plays a role beyond the early stages of B-cell development. This factor is also expressed in the central nervous system, and its absence results in severe defects in mid-brain development. Although the exact site of action of Sox-4 is not known, it affects early stages of B-cell activation. While Figure 11-3 shows that all of these transcription factors affect development at an early stage, some of them are active at later stages also.

Cell-Surface Markers Identify Development Stages

The developmental progression from progenitor to mature B cell is typified by a changing pattern of surface markers (see Figure 11-3). At the pro-B stage, the cells do not display the heavy or light chains of antibody but they do express CD45R,

which is a form of the protein tyrosine phophatase found on leukocytes, and the signal-transducing molecules Ig-α/Ig-β, which are found in association with the membrane forms of antibody in later stages of B-cell development. Pro-B cells also express CD19 (part of the B-cell coreceptor), CD43 (leukosialin), and CD24, a molecule also known as heat-stable antigen (HSA) on the surface. At this stage, c-Kit, a receptor for a growth-promoting ligand present on stromal cells, is also found on the surface of pro-B cells. As cells progress from the pro-B to the pre-B stage, they express many of the same markers that were present during the pro-B stage; however, they cease to express c-Kit and CD43 and begin to express CD25, the α chain of the IL-2 receptor. The display of the pre–B-cell receptor (pre-BCR) is a salient feature of the pre-B cell stage. After rearrangement of the light chain, surface immunoglobulin containing both heavy and light chains appears, and the cells, now classified as immature B cells, lose the pre-BCR and no longer express CD25. Monoclonal antibodies are available that can recognize all of these antigenic markers, making it possible to recognize and isolate the various stages of B-cell development by the techniques of immunohistology and flow cytometry described in Chapter 6.

B-1 B Cells Are a Self-Renewing B-Cell Subset

There is a subset of B cells, called B-1 B cells, that arise before B-2 B cells, the major group of B cells in humans and mice. In humans and mice, B-1 B cells compose about 5% of the total B-cell population. They appear during fetal life, express surface IgM but little or no IgD, and are marked by the display of CD5. However, CD5 is not an indispensable component of the B-1 lineage, it does not appear on the B-1 cells of rats, and mice that lack a functional CD5 gene still produce B-1 cells. In animals whose B-2 B cells are the major B-cell population, B-1 cells are minor populations in such secondary tissues as lymph nodes and spleen. Despite their scarcity in many lymphoid sites, they are the major B-cell type found in the peritoneum.

Although there is not a great deal of definitive information on the function of B-1 cells, several features set them apart from the B-2 B cells of humans and mice. During fetal life, B-1 cells arise from stem cells in bone marrow. However, in postnatal life this population renews itself by the proliferation of some B-1 cells in sites outside the bone marrow to form additional naive B-1 cells. The B-1 population responds poorly to protein antigens but much better to carbohydrate ones. Most of its members are IgM-bearing cells, and this population undergoes much less somatic hypermutation and class switching than the B-2 set of B cells does. Consequently, the antibodies produced by a high proportion of B-1 cells are of rather low affinity.

Self-Reactive B Cells Are Selected Against in Bone Marrow

It is estimated that in the mouse the bone marrow produces about 5×10^7 B cells/day but only 5×10^6 (or about 10%) are actually recruited into the recirculating B-cell pool. This means that 90% of the B cells produced each day die without ever leaving the bone marrow. Some of this loss is attributable to **negative selection** and subsequent elimination **(clonal deletion)** of immature B cells that express auto-antibodies against self-antigens in the bone marrow.

It has long been established that the crosslinkage of mIgM on immature B cells, demonstrated experimentally by treating immature B cells with antibody against the μ constant region, can cause the cells to die by apoptosis within the bone marrow. A similar process is thought to occur in vivo when immature B cells that express self-reactive mIgM bind to self-antigens in the bone marrow. For example, D. A. Nemazee and K. Burki introduced a transgene encoding the heavy and light chains of an IgM antibody specific for K^k, an H-2^k class I MHC molecule, into H-2^d and H-$2^{d/k}$ mice (Figure 11-5a,b). Since class I MHC molecules are expressed on the membrane of all nucleated cells, the endogenous H-2^k and H-2^d class I MHC molecules would be present on bone-marrow stromal cells in the transgenic mice. In the H-2^d mice, which do not express K^k, 25%–50% of the mature, peripheral B cells expressed the transgene-encoded anti-K^k both as a membrane antibody and as secreted antibody. In contrast, in the H-$2^{d/k}$ mice, which express K^k, no mature, peripheral B cells expressed the transgene-encoded antibody to H-2^k (Table 11-1). These results suggest that there is negative selection against any immature B cells expressing auto-antibodies on their membranes because these antibodies react with self-antigen

TABLE 11-1 Expression of transgene encoding IgM antibody to H-2^k class I MHC molecules

		EXPRESSION OF TRANSGENE	
Experimental animal	Number of animals tested	As membrane Ab	As secreted Ab (μg/ml)
Nontransgenics	13	(–)	<0.3
H-2^d transgenics	7	(+)	93.0
H-$2^{d/k}$ transgenics	6	(–)	<0.3

SOURCE: Adapted from D. A. Nemazee and K. Burki, 1989, *Nature* **337**:562.

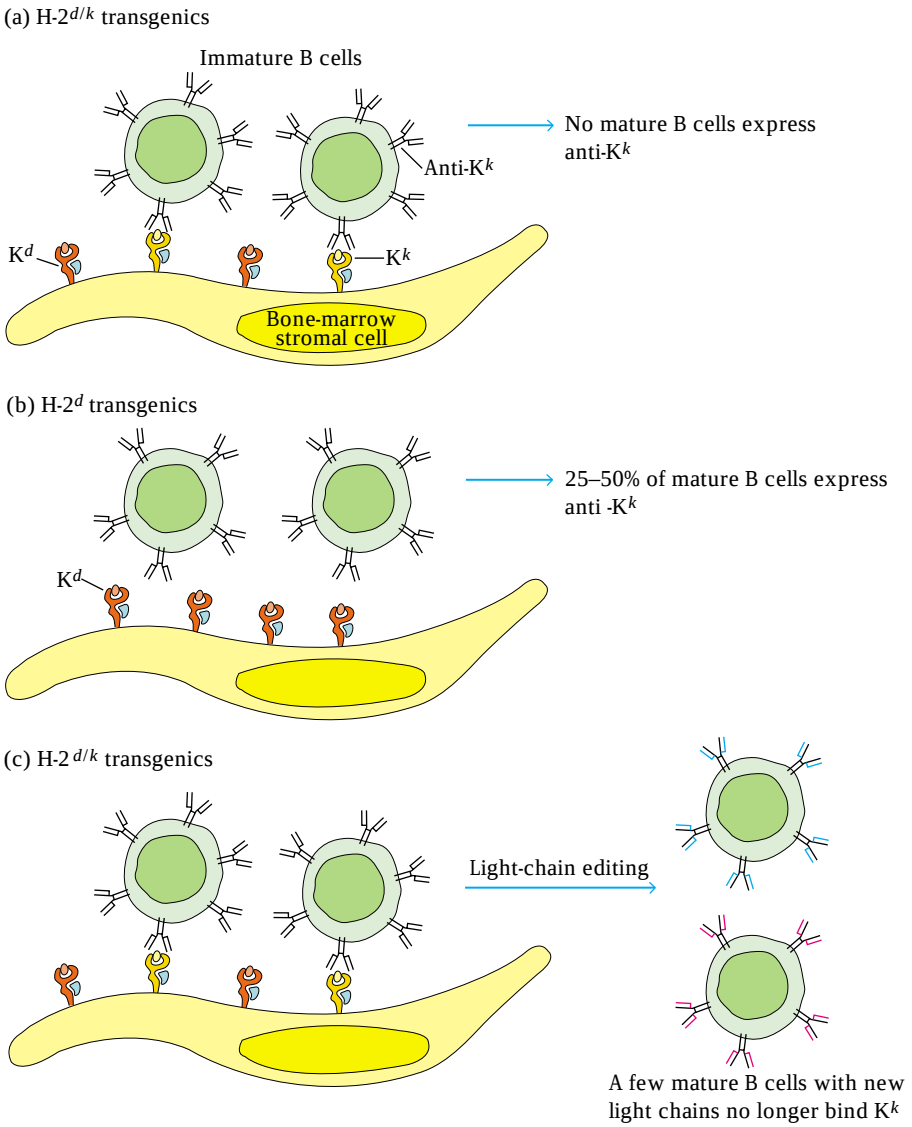


FIGURE 11-5 Experimental evidence for negative selection (clonal deletion) of self-reactive B cells during maturation in the bone marrow. The presence or absence of mature peripheral B cells expressing a transgene-encoded IgM against the H-2 class I molecule K^k was determined in H-2$^{d/k}$ mice (a) and H-2^d mice (b). In the H-2$^{d/k}$ transgenics, the immature B cells recognized the self-antigen K^k and were deleted by negative selection. In the H-2^d transgenics, the immature B cells did not bind to a self-antigen and consequently went on to mature, so that 25%–50% of the splenic B cells expressed the transgene-encoded anti-K^k as membrane Ig. More detailed analysis of the H-2$^{d/k}$ transgenics revealed a few peripheral B cells that expressed the transgene-encoded μ chain but a different light chain (c). Apparently, a few immature B cells underwent light-chain editing, so they no longer bound the K^k molecule and consequently escaped negative selection. *[Adapted from D. A. Nemazee and K. Burki, 1989,* Nature ***337:*** *562; S. L. Tiegs et al., 1993,* JEM ***177:****1009.]*

(e.g., the K^k molecule in H-2$^{d/k}$ transgenics) present on stromal cells, leading to crosslinking of the antibodies and subsequent death of the immature B cells.

Self-Reactive B Cells May Be Rescued by Editing of Light-Chain Genes

Later work using the transgenic system described by Nemazee and Burki showed that negative selection of immature B cells does not always result in their immediate deletion (Figure 11-5c). Instead, maturation of the self-reactive cell is arrested while the B cell "edits" the light-chain gene of its receptor. In this case, the H-2$^{d/k}$ transgenics produced a few mature B cells that expressed mIgM containing the μ chain encoded in the transgene, but different, endogenous light chains. One explanation for these results is that when some immature B cells bind a self-antigen, maturation is arrested; the cells up-regulate RAG-1 and RAG-2 expression and begin further

rearrangement of their endogenous light-chain DNA. Some of these cells succeed in replacing the κ light chain of the self-antigen reactive antibody with a λ chain encoded by endogenous λ-chain gene segments. As a result, these cells will begin to express an "edited" mIgM with a different light chain and a specificity that is not self-reactive. These cells escape negative selection and leave the bone marrow.

B-Cell Activation and Proliferation

After export of B cells from the bone marrow, activation, proliferation, and differentiation occur in the periphery and require antigen. Antigen-driven activation and clonal selection of naive B cells leads to generation of plasma cells and memory B cells. In the absence of antigen-induced activation, naive B cells in the periphery have a short life span, dying within a few weeks by apoptosis (see Figure 11-1).

Thymus-Dependent and Thymus-Independent Antigen Have Different Requirements for Response

Depending on the nature of the antigen, B-cell activation proceeds by two different routes, one dependent upon T_H cells, the other not. The B-cell response to **thymus-dependent (TD) antigens** requires direct contact with T_H cells, not simply exposure to T_H-derived cytokines. Antigens that can activate B cells in the absence of this kind of direct participation by T_H cells are known as **thymus-independent (TI) antigens.** TI antigens are divided into types 1 and 2, and they activate B cells by different mechanisms. Some bacterial cell-wall components, including lipopolysaccharide (LPS), function as *type 1 thymus-independent (TI-1) antigens. Type 2 thymus-independent (TI-2) antigens* are highly repetitious molecules such as polymeric proteins (e.g., bacterial flagellin) or bacterial cell-wall polysaccharides with repeating polysaccharide units.

Most TI-1 antigens are polyclonal B-cell activators **(mitogens);** that is, they are able to activate B cells regardless of their antigenic specificity. At high concentrations, some TI-1 antigens will stimulate proliferation and antibody secretion by as many as one third of all B cells. The mechanism by which TI-1 antigens activate B cells is not well understood. When B cells are exposed to lower concentrations of TI-1 antigens, only those B cells specific for epitopes of the antigen will be activated. These antigens can stimulate antibody production in nude mice (which lack a thymus and thus are greatly deficient in T cells), and the response is not greatly augmented by transferring T cells into these athymic mice, indicating that TI-1 antigens are truly T-cell independent. The prototypic TI-1 antigen is **lipopolysaccharide (LPS),** a major component of the cell walls of gram-negative bacteria. At low concentrations, LPS stimulates the production of antibodies specific for LPS. At high concentrations, it is a polyclonal B-cell activator.

TI-2 antigens activate B cells by extensively crosslinking the mIg receptor. However, TI-2 antigens differ from TI-1 antigens in three important respects. First, they are not B-cell mitogens and so do not act as polyclonal activators. Second, TI-1 antigens will activate both mature and immature B cells, but TI-2 antigens activate mature B cells and inactivate immature B cells. Third, although the B-cell response to TI-2 antigens does not require direct involvement of T_H cells, cytokines derived from T_H cells are required for efficient B-cell proliferation and for class switching to isotypes other than IgM. This can be shown by comparing the effect of TI-2 antigens in mice made T-cell–deficient in various ways. In nude mice, which lack thymus-derived T cells but do contain a few T cells that arise from other sites that probably lie in the intestine, TI-2 antigens do elicit B-cell responses. TI-2 antigens do not induce antibody production in mice that cannot express either αβ or γδ TCRs because the genes encoding the TCR β and δ chains have been knocked out. Administration of a few T cells to these TCR-knockout mice restores their ability to elicit B-cell responses to TI-2 antigens.

The humoral response to thymus-independent antigens is different from the response to thymus-dependent antigens (Table 11-2). The response to TI antigens is generally weaker, no memory cells are formed, and IgM is the predominant antibody secreted, reflecting a low level of class switching. These differences highlight the important role played by T_H cells in generating memory B cells, affinity maturation, and class switching to other isotypes.

Two Types of Signals Drive B Cells into and Through the Cell Cycle

Naive, or resting, B cells are nondividing cells in the G_0 stage of the cell cycle. Activation drives the resting cell into the cell cycle, progressing through G_1 into the S phase, in which DNA is replicated. The transition from G_1 to S is a critical restriction point in the cell cycle. Once a cell has reached S, it completes the cell cycle, moving through G_2 and into mitosis (M).

Analysis of the progression of lymphocytes from G_0 to the S phase revealed similarities with the parallel sequence in fibroblast cells. These events could be grouped into two categories, competence signals and progression signals. Competence signals drive the B cell from G_0 into early G_1, rendering the cell competent to receive the next level of signals. Progression signals then drive the cell from G_1 into S and ultimately to cell division and differentiation. Competence is achieved by not one but two distinct signaling events, which are designated *signal 1* and *signal 2*. These signaling events are generated by different pathways with thymus-independent and thymus-dependent antigens, but both pathways include signals generated when multivalent antigen binds and cross-links mIg (Figure 11-6). Once the B cell has acquired an effective competence signal in early activation, the interaction of cytokines and possibly other ligands with the B-cell membrane receptors provides progression signals.

TABLE 11-2 Properties of thymus-dependent and thymus-independent antigens

		TI ANTIGENS	
Property	TD antigens	Type 1	Type 2
Chemical nature	Soluble protein	Bacterial cell-wall components (e.g., LPS)	Polymeric protein antigens; capsular polysaccharides
Humoral response			
Isotype switching	Yes	No	Limited
Affinity maturation	Yes	No	No
Immunologic memory	Yes	No	No
Polyclonal activation	No	Yes (high doses)	No

Transduction of Activating Signals Involves Ig-α/Ig-β Heterodimers

For many years, immunologists questioned how engagement of the Ig receptor by antigen could activate intracellular signaling pathways. All isotypes of mIg have very short cytoplasmic tails. Both mIgM and mIgD on B cells extend into the cytoplasm by only three amino acids; the mIgA tail consists of 14 amino acids; and the mIgG and mIgE tails contains 28 amino acids. In each case, the cytoplasmic tail is too short to be able to generate a signal by associating with intracellular signaling molecules, such as tyrosine kinases and G proteins. The discovery that membrane Ig is associated with the disulfide-linked heterodimer Ig-α/Ig-β, forming the **B-cell receptor(BCR),** solved this longstanding puzzle. Though it was originally thought that two Ig-α/Ig-β heterodimers associated with one mIg to form the B-cell receptor, careful biochemical analysis has shown that only one Ig-α/Ig-β heterodimer associates with a single mIg molecule to form the receptor complex. (Figure 11-7). Thus the BCR is functionally divided into the ligand-binding immunoglobulin molecule and the signal-transducing Ig-α/Ig-β heterodimer. A similar functional division marks the pre-BCR, which transduces signals via a complex consisting of an Ig-α/Ig-β heterodimer and μ heavy chains combined with the surrogate light chain (see Figure 11-4). The Ig-α chain has a long cytoplasmic tail containing 61 amino acids; the tail of the Ig-β chain contains 48 amino acids. The cytoplasmic tails of both Ig-α and Ig-β contain the 18-residue motif termed the **immunoreceptor tyrosine-based activation motif (ITAM;** see Figure 11-7) which has already been described in several molecules of the T-cell–receptor complex (see Figure 10-11). Interactions with the cytoplasmic tails of Ig-α/Ig-β transduce the stimulus produced by crosslinking of mIg molecules into an effective intracellular signal.

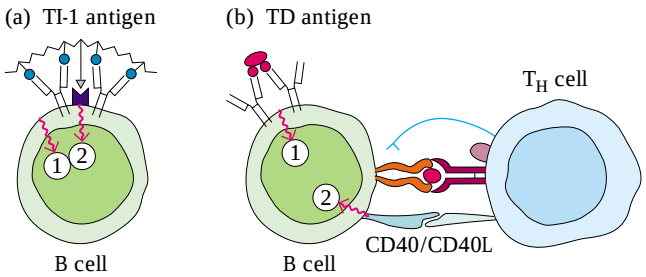


FIGURE 11-6 An effective signal for B-cell activation involves two distinct signals induced by membrane events. Binding of a type 1 thymus-independent (TI-1) antigen to a B cell provides both signals. A thymus-dependent (TD) antigen provides signal 1 by crosslinking mIg, but a separate interaction between CD40 on the B cell and CD40L on an activated T_H cell is required to generate signal 2.

In the BCR and the TCR, as well as in a number of receptors for the Fc regions of particular Ig classes (FcεRI for IgE; FcγRIIA, FcγRIIC, FcγRIIIA for IgG), ligand binding and signal transduction are mediated by a multimeric complex of proteins that is functionally compartmentalized. The ligand-binding portions of these complexes (mIg in the case of the BCR) is on the surface of the cell, and the signal-transducing portion is mostly or wholly within the cell. As is true of the TCR, signaling from the BCR is mediated by protein tyrosine kinases (PTKs). Furthermore, like the TCR, the BCR itself has no PTK activity; this activity is acquired by recruitment of a number of different kinases, from nearby locations within the cell, to the cytoplasmic tails of the signal. Phosphorylation of tyrosines within the ITAMs of the BCR by receptor associated PTKs is among the earliest events in B-cell activation and plays a key role in bringing other critical PTKs to the BCR and in their activation. The antigen-mediated crosslinking of BCRs initiates a number of signaling cascades that ultimately result in the cell's responses to the crosslinking of its surface immunoglobulin by antigen. The crosslinking of BCRs results in the induction of many signal-transduction pathways

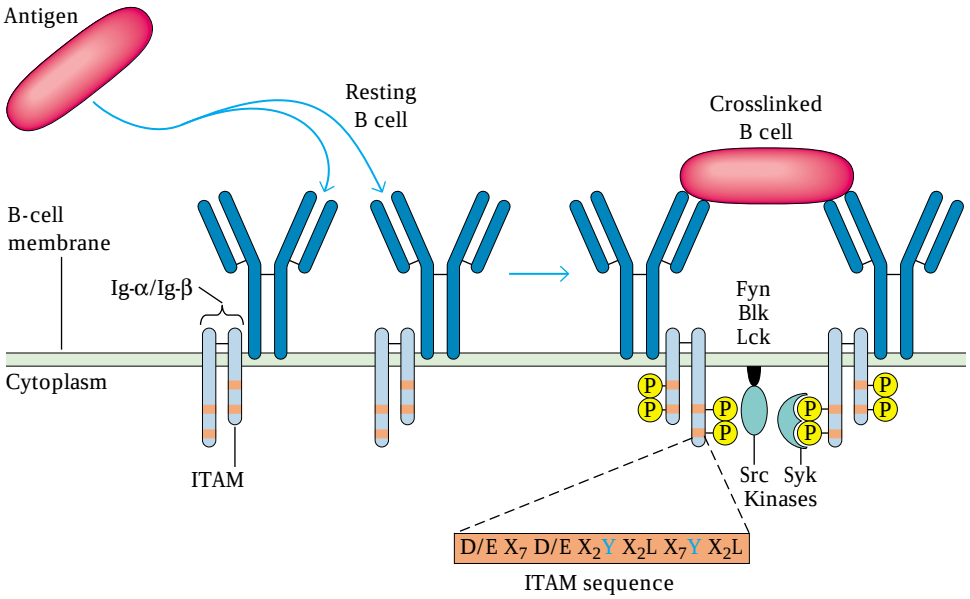


FIGURE 11-7 The initial stages of signal transduction by an activated B-cell receptor (BCR). The BCR comprises an antigen-binding mIg and one signal-transducing Ig-α/Ig-β heterodimer. Following antigen crosslinkage of the BCR, the immunoreceptor tyrosine-based activation motifs (ITAMs) interact with several members of the Src family of tyrosine kinases (Fyn, Blk, and Lck), activating the kinases. The activated enzymes phosphorylate tyrosine residues on the cytoplasmic tails of the Ig-α/Ig-β heterodimer, creating docking sites for Syk kinase, which is then also activated. The highly conserved sequence motif of ITAMs is shown with the tyrosines (Y) in blue. D/E indicates that an aspartate or a glutamate can appear at the indicated position, and X indicates that the position can be occupied by any amino acid.

and the activation of the B cell. Figure 11-8 shows many parallels between B-cell and T-cell activation. These include:

- *Compartmentalization of function within receptor subunits:* Both the B-cell and T-cell pathways begin with antigen receptors that are composed of an antigen-binding and a signaling unit. The antigen-binding unit confers specificity, but has cytoplasmic tails too short to transduce signals to the cytoplasm of the cell. The signaling unit has long cytoplasmic tails that are the signal transducers of the receptor complex.
- *Activation by membrane-associated Src protein tyrosine kinases:* The receptor-associated PTKs (Lck in T cells and Lyn, Blk, and Fyn in B cells) catalyze phosphorylations during the early stages of signal transduction that are essential to the formation of a functional receptor signaling complex.
- *Assembly of a large signaling complex with protein-tyrosine-kinase activity:* The phosphorylated tyrosines in the ITAMs of the BCR and TCR provide docking sites for the molecules that endow these receptors with PTK activity; ZAP-70 in T cells and Syk in B cells.
- *Recruitment of other signal-transduction pathways:* Signals from the BCR and TCR result in the production of the second messengers IP_3 and DAG. IP_3 causes the release of Ca^{2+} from intracellular stores, and DAG activates PKC. A third important set of signaling pathways are those governed by the small G proteins Ras and Rac that are also activated by signals received through the TCR or BCR.
- *Changes in gene expression:* One of the important outcomes of signal-transduction processes set in motion with engagement of the BCR or the TCR is the generation or translocation to the nucleus of active transcription factors that stimulate or inhibit the transcription of specific genes.

Failures in signal transduction can have severe consequences for the immune system. The Clinical Focus on X-linked agammaglobulinemia describes the effect of defective signal transduction on the development of B cells.

The B-Cell–Coreceptor Complex Can Enhance B-Cell Responses

Stimulation through antigen receptors can be modified significantly by signals through coreceptors. Recall that co-stimulation through CD28 is an essential feature of effective positive stimulation of T lymphocytes, while signaling through CTLA-4 inhibits the response of the T cell. In B cells

VISUALIZING CONCEPTS

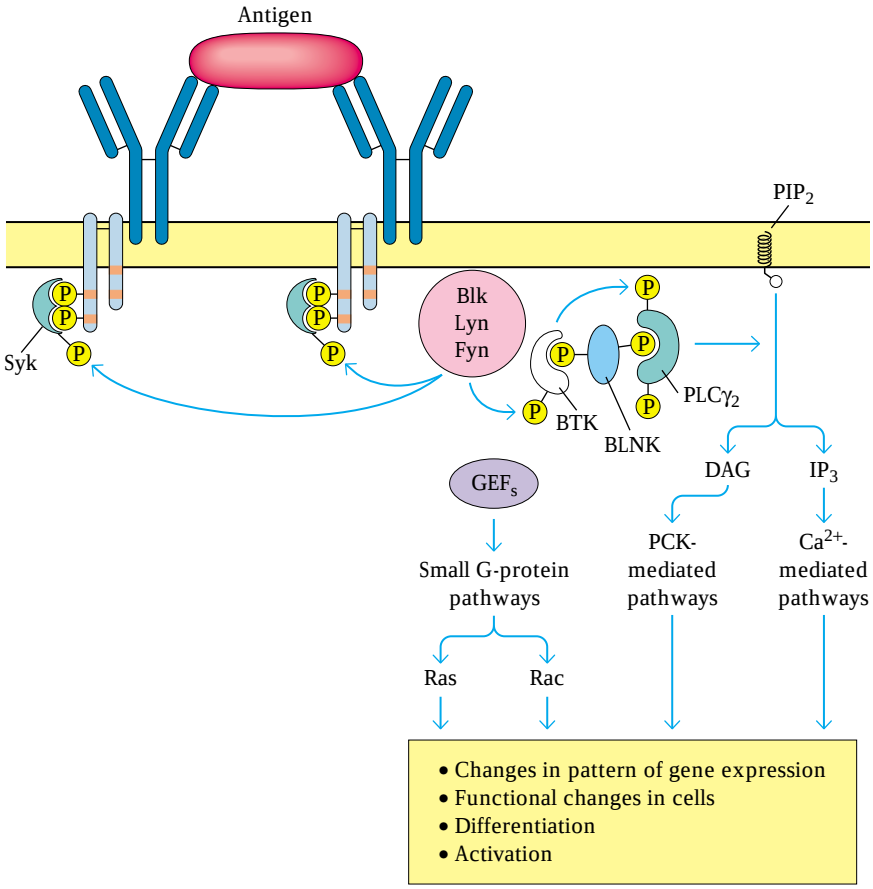


FIGURE 11-8 Some of the many signal-transduction pathways activated by the BCR. In one pathway, Syk activates PLCγ_2 by tyrosine phosphorylation. PLCγ_2 then hydrolyzes PIP_2, a membrane phospholipid, to produce the second messengers DAG and IP_3. DAG and Ca^{2+} released by the action of IP_3 collaboratively activate the PKC, which induces additional signal-transduction pathways. The activated receptor complex also generates signals that activate the Ras pathway. Activated Ras initiates a cascade of phosphorylations that culminates in the activation of transcription factors that up-regulate the expression of many genes.

a component of the B-cell membrane, called the **B-cell coreceptor,** provides stimulatory modifying signals.

The B-cell coreceptor is a complex of three proteins: CD19, CR2 (CD21), and TAPA-1 (CD81) (Figure 11-9). CD19, a member of the immunoglobulin superfamily, has a long cytoplasmic tail and three extracellular domains. The CR2 component is a receptor of C3d, a breakdown product of the complement system, which is an important effector mechanism for destroying invaders (Chapter 13); note that the involvement of C3d in the pathway for coreceptor activity reveals different arms of the immune system interacting with each other. CR2 also functions as a receptor for a membrane molecule and the transmembrane protein TAPA-1. In addition to the stimulatory coreceptor, another molecule, CD22, which is constitutively associated with the B-cell receptor in resting B cells, delivers a negative signal that makes B-cells more difficult to activate. As shown in Figure 11-9, the CR2 component of the coreceptor complex binds to complement-coated antigen that has been captured by the mIg on the B cell. This crosslinks the coreceptor to the BCR and allows the CD19 component of the coreceptor to interact with the Ig-α/Ig-β component of the BCR. CD19 contains six tyrosine residues in its long cytoplasmic tail and is a major substrate of the protein tyrosine kinase activity that is mediated by crosslinkage of the BCR. Phosphorylation of CD19 permits it to bind a number of signaling molecules, including the protein tyrosine kinase Lyn.

The delivery of these signaling molecules to the BCR complex contributes to the activation process, and the coreceptor

CLINICAL FOCUS

X-Linked Agammaglobulinemia: A Failure in Signal Transduction and B-Cell Development

X-linked agammaglobulinemia is a genetically determined immunodeficiency disease characterized by the inability to synthesize all classes of antibody. It was discovered in 1952 by O. C. Bruton in what is still regarded as an outstanding example of research in clinical immunology. Bruton's investigation involved a young boy who had mumps 3 times and experienced 19 different episodes of serious bacterial infections during a period of just over 4 years. Because pneumococcus bacteria were isolated from the child's blood during 10 of the episodes of bacterial infection, attempts were made to induce immunity to pneumococcus by immunization with pneumococcus vaccine. The failure of these efforts to induce antibody responses prompted Bruton to determine whether the patient could mount antibody responses when challenged with other antigens. Surprisingly, immunization with diphtheria and typhoid vaccine preparations did not raise humoral responses in this patient. Electrophoretic analysis of the patient's serum revealed that although normal amounts of albumin and other typical serum proteins were present, gamma globulin, the major antibody fraction of serum, was absent. Having traced the immunodeficiency to a lack of antibody, Bruton tried a bold new treatment. He administered monthly doses of human immune serum globulin. The patient's experience of a fourteen-month period free of bacterial sepsis established the usefulness of the immunoglobulin replacement for the treatment of immunodeficiency.

Though initially called Bruton's agammaglobulinemia, this hereditary immunodeficiency disease was renamed X-linked agammaglobulinemia, or X-LA, after the discovery that the responsible gene lies on the X chromosome. The disease has the following clinical features:

- Because this defect is X-linked, almost all afflicted individuals are male.
- Signs of immunodeficiency may appear as early as 9 months after birth, when the supply of

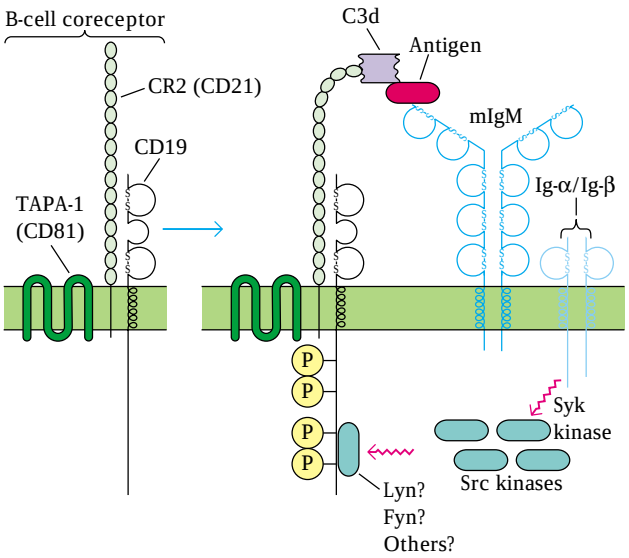


FIGURE 11-9 The B-cell coreceptor is a complex of three cell membrane molecules: TAPA-1 (CD81), CR2 (CD21), and CD19. Binding of the CR2 component to complement-derived C3d that has coated antigen captured by mIg results in the phosphorylation of CD19. The Src-family tyrosine kinase Lyn binds to phosphorylated CD19. The resulting activated Lyn and Fyn can trigger the signal-transduction pathways shown in Figure 11-8 that begin with phospholipase C.

complex serves to amplify the activating signal transmitted through the BCR. In one experimental in vitro system, for example, 10^4 molecules of mIgM had to be engaged by antigen for B-cell activation to occur when the coreceptor was not involved. But when CD19/CD2/TAPA-1 coreceptor was crosslinked to the BCR, only 10^2 molecules of mIgM had to be engaged for B-cell activation. Another striking experiment highlights the role played by the B-cell coreceptor. Mice were immunized with either unmodified lysozyme or a hybrid protein in which genetic engineering was used to join hen's egg lysozyme to C3d. The fusion protein bearing 2 or 3 copies of C3d produced anti-lysozyme responses that were 1000 to 10,000 times greater than those to lysozyme alone. Perhaps coreceptor phenomena such as these explain how naive B cells that often express mIg with low affinity for antigen are able to respond to low concentrations of antigen in a primary response. Such responses, even though initially of low affinity, can play a significant role in the ultimate generation of high-affinity antibody. As described later in this chapter, response to an antigen can lead to affinity maturation, resulting in higher average affinity of the B-cell population. Finally, two experimental observations indicate that the CD19 component of the B-cell coreceptor can play a role independent of CR2, the complement receptor. In normal mice, artifically crosslinking the BCR with anti-BCR antibodies results in the

maternal antibody acquired in utero has decreased below protective levels.

- There is a high frequency of infection by *Streptococcus pneumoniae* and *Haemophilus influenzae;* bacterial pneumonia, sinusitis, meningitis, or septicemia are often seen in these patients.
- Although infection by many viruses is no more severe in these patients than in normal individuals, long-term antiviral immunity is usually not induced.
- Analysis by fluorescence microscopy or flow cytometry shows few or no mature B cells in the blood.

Studies of this disease at the cellular and molecular level provide insights into the workings of the immune system. A scarcity of B cells in the periphery explained the inability of X-LA patients to make antibody. Studies of the cell populations in bone marrow traced the lack of B cells to failures in B-cell development. The samples displayed a ratio of pro-B cells to pre-B cells 10 times normal, suggesting inhibition of the transition from the pro– to the pre–B-cell stage. The presence of very few mature B cells in the marrow indicated a more profound blockade in the development of B cells from pre-B cells.

In the early 1990s, the gene responsible for X-LA was cloned. The normal counterpart of this gene encodes a protein tyrosine kinase that has been named Bruton's tyrosine kinase (Btk) in honor of the resourceful and insightful physician who discovered X-LA and devised a treatment for it. Parallel studies in mice have shown that the absence of Btk causes a syndrome known as xid, an immunodeficiency disease that is essentially identical to its human counterpart, X-LA. Btk has turned out to play important roles in B-cell signaling. For example, crosslinking of the B-cell receptor results in the phosphorylation of a tyrosine residue in the catalytic domain of Btk. This activates the protein-tyrosine-kinase activity of Btk, which then phosphorylates phospholipase C-γ_2 (PLC-γ_2); in vitro studies of cell cultures in which Btk has been knocked out show compromised PLC-γ_2 activation. Once activated, PLC-γ_2 hydrolyzes membrane phospholipids, liberating the potent second messengers IP_3 and DAG. As mentioned earlier, IP_3 causes a rise in intracellular Ca^{2+}, and DAG is an activator of protein kinase C (PKC). Thus, Btk plays a pivotal role in activating a network of intracellular signals vital to the function of mature B cells and earlier members of the B-cell lineage. Research has shown that it belongs to a family of PTKs known as Tec kinases; its counterpart in T cells is Itk. The insights gained from studies of X-LA, xid, and Btk are impressive examples of how the study of pathological states can clarify the workings of normal cells.

stimulation of some of the signal-transduction pathways characteristic of B-cell activation. On the other hand, treatment of B cells from mice in which CD19 has been knocked out with anti-BCR antibody fails to induce these pathways. Furthermore, CD19 knockout mice make greatly diminished antibody response to most antigens.

T_H Cells Play Essential Roles in Most B-Cell Responses

As noted already, activation of B cells by soluble protein antigens requires the involvement of T_H cells. Binding of antigen to B-cell mIg does not itself induce an effective competence signal without additional interaction with membrane molecules on the T_H cell. In addition, a cytokine-mediated progression is required for B-cell proliferation. Figure 11-10 outlines the probable sequence of events in B-cell activation by a thymus-dependent (TD) antigen. This process is considerably more complex than activation induced by thymus-independent (TI) antigens.

FORMATION OF T-B CONJUGATE

After binding of antigen by mIg on B cells, the antigen is internalized by receptor-mediated endocytosis and processed within the endocytic pathway into peptides. Antigen binding also initiates signaling through the BCR that induces the B cell to up-regulate a number of cell-membrane molecules, including class II MHC molecules and the co-stimulatory ligand B7 (see Figure 11-10a). Increased expression of both of these membrane proteins enhances the ability of the B cell to function as an antigen-presenting cell in T_H-cell activation. B-cells could be regarded as helping their helpers because the antigenic peptides produced within the endocytic processing pathway associate with class II MHC molecules and are presented on the B-cell membrane to the T_H cell, inducing its activation. It generally takes 30–60 min after internalization of antigen for processed antigenic peptides to be displayed on the B-cell membrane in association with class II MHC molecules.

Because a B cell recognizes and internalizes antigen specifically, by way of its membrane-bound Ig, a B cell is able to present antigen to T_H cells at antigen concentrations that are 100 to 10,000 times lower than what is required for presentation by macrophages or dendritic cells. When antigen concentrations are high, macrophages and dendritic cells are effective antigen-presenting cells, but, as antigen levels drop, B cells take over as the major presenter of antigen to T_H cells.

Once a T_H cell recognizes a processed antigenic peptide displayed by a class II MHC molecule on the membrane of a

(a) Antigen crosslinks mIg, generating signal (1), which leads to increased expression of class II MHC and co-stimulatory B7. Antigen–antibody complexes are internalized by receptor-mediated endocytosis and degraded to peptides, some of which are bound by class II MHC and presented on the membrane as peptide–MHC complexes.

(b) T_H cell recognizes antigen–class II MHC on B-cell membrane. This plus co-stimulatory signal activates T_H cell.

(c) 1. T_H cell begins to express CD40L.
2. Interaction of CD40 and CD40L provides signal (2).
3. B7-CD28 interactions provide co-stimulation to the T_H cell.

(d) 1. B cell begins to express receptors for various cytokines.
2. Binding of cytokines released from T_H cell in a directed fashion sends signals that support the progression of the B cell to DNA synthesis and to differentiation.

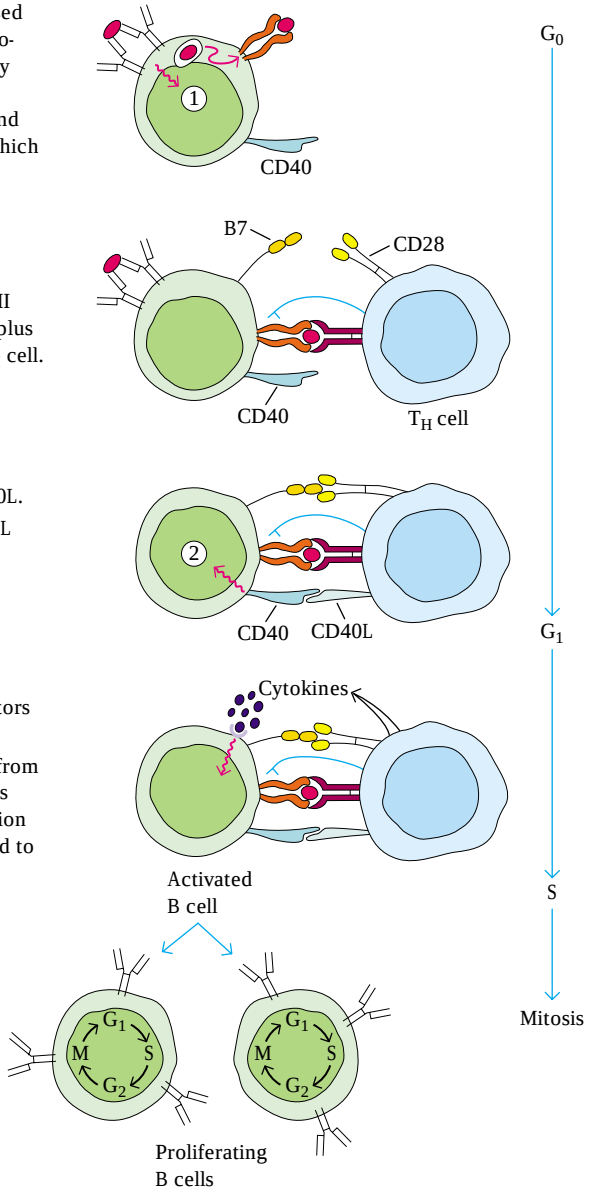


FIGURE 11-10 Sequence of events in B-cell activation by a thymus-dependent antigen. The cell-cycle phase of the interacting B cell is indicated on the right.

B cell, the two cells interact to form a T-B conjugate (Figure 11-11). Micrographs of T-B conjugates reveal that the T_H cells in antigen-specific conjugates have reorganized the Golgi apparatus and the microtubular-organizing center toward the junction with the B cell. This structural adjustment facilitates the release of cytokines toward the antigen-specific B cell.

CONTACT-DEPENDENT HELP MEDIATED BY CD40/CD40L INTERACTION

Formation of a T-B conjugate not only leads to the directional release of T_H-cell cytokines, but also to the up-regulation of CD40L (CD154), a T_H-cell membrane protein that then interacts with CD40 on B cells to provide an essential signal for T-cell–dependent B-cell activation. CD40 belongs to the tumor necrosis factor (TNF) family of cell-surface proteins and soluble cytokines that regulate cell proliferation and programmed cell death by apoptosis. CD40L belongs to the TNF receptor (TNFR) family. Interaction of CD40L with CD40 on the B cell delivers a signal (signal 2) to the B cell that, in concert with the signal generated by mIg crosslinkage (signal 1), drives the B cell into G_1 (see Figure 11-10c). The signals from CD40 are transduced by a number of intracellular signaling pathways, ultimately resulting in changes in gene expression. Stud-

ies have shown that although CD40 does not have kinase activity, its crosslinking is followed by the activation of protein tyrosine kinases such as Lyn and Syk. Crosslinking of CD40 also results in the activation of phospholipase C and the subsequent generation of the second messengers IP_3 and DAG, and the activation of a number of transcription factors. *Ligation* of CD40 also results in its association with members of the TNFR-associated factor (TRAF) family. A consequence of this interaction is the activation of the transcription factor NF-6B.

Several lines of evidence have identified the CD40/CD40L interaction as the mediator of contact-dependent help. The role of an inducible T_H-cell membrane protein in B-cell activation was first revealed by experiments in which naive B cells were incubated with antigen and plasma membranes prepared from either activated or resting T_H-cell clones. Only the membranes from the activated T_H cells induced B-cell proliferation, suggesting that one or more molecules expressed on the membrane of an activated T_H cell engage receptors on the B cell to provide contact-dependent help. Furthermore, when antigen-stimulated B cells are treated with anti-CD40 monoclonal antibodies in the absence of T_H cells, they become activated and proliferate. Thus, engagement of CD40, whether by antibodies to CD40 or by CD40L, is critical in providing signal 2 to the B cell. If appropriate cytokines are also added to this experimental system, then the proliferating B cells will differentiate into plasma cells. Conversely, antibodies to CD40L have been shown to block B-cell activation by blocking the CD40/CD40L interaction.

SIGNALS PROVIDED BY T_H-CELL CYTOKINES

Although B cells stimulated with membrane proteins from activated T_H cells are able to proliferate, they fail to differentiate unless cytokines are also present; this finding suggests that both a membrane-contact signal and cytokine signals are necessary to induce B-cell proliferation and differentiation. As noted already, electron micrographs of T-B conjugates reveal that the antigen-specific interaction between a T_H and a B cell induces a redistribution of T_H-cell membrane proteins and cytoskeletal elements that results in the polarized release of cytokines toward the interacting B cell.

Once activated, the B cell begins to express membrane receptors for various cytokines, such as IL-2, IL-4, IL-5, and others. These receptors then bind the cytokines produced by the interacting T_H cell. The signals produced by these cytokine-receptor interactions support B-cell proliferation and can induce differentiation into plasma cells and memory B cells, class switching, and affinity maturation. Each of these events is described in a later section.

Mature Self-Reactive B Cells Can Be Negatively Selected in the Periphery

Because some self-antigens do not have access to the bone marrow, B cells expressing mIgM specific for such antigens cannot be eliminated by the negative-selection process in the bone marrow described earlier. To avoid autoimmune responses from such mature self-reactive B cells, some process for deleting them or rendering them inactive must occur in peripheral lymphoid tissue.

A transgenic system developed by C. Goodnow and his coworkers has helped to clarify the process of negative selection of mature B cells in the periphery. Goodnow's experimental system included two groups of transgenic mice

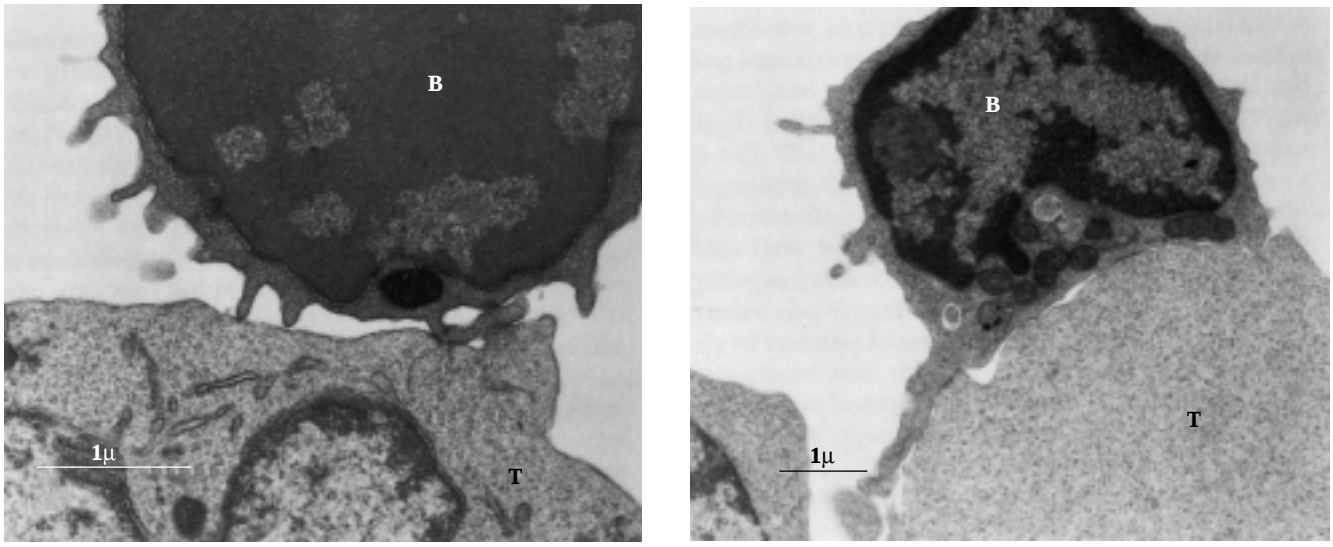


FIGURE 11-11 Transmission electron micrographs of initial contact between a T cell and B cell (left) and of a T-B conjugate (right). Note the broad area of membrane contact between the cells after formation of the conjugate. *[From V. M. Sanders et al., 1986,* J. Immunol. ***137:****2395.]*

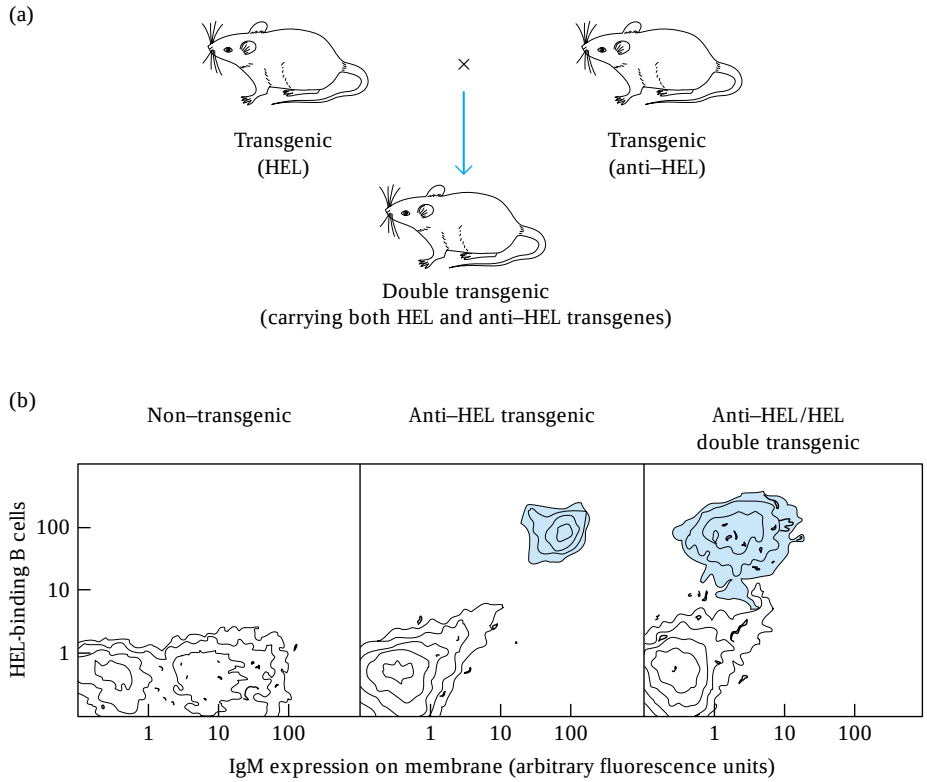


FIGURE 11-12 Goodnow's experimental system for demonstrating clonal anergy in mature peripheral B cells. (a) Production of double-transgenic mice carrying transgenes encoding HEL (hen egg-white lysozyme) and anti-HEL antibody. (b) Flow cytometric analysis of peripheral B cells that bind HEL compared with membrane IgM levels. The number of B cells binding HEL was measured by determining how many cells bound fluorescently labeled HEL. Levels of membrane IgM were determined by incubating the cells with anti-mouse IgM antibody labeled with a fluorescent label different from that used to label HEL. Measurement of the fluorescence emitted from this label indicated the level of membrane IgM expressed by the B cells. The nontransgenics (*left*) had many B cells that expressed high levels of surface IgM but almost no B cells that bound HEL above the background level of 1. Both anti-HEL transgenics (*middle*) and anti-HEL/HEL double transgenics (*right*) had large numbers of B cells that bound HEL (blue), although the level of membrane IgM was about twentyfold lower in the double transgenics. The data in Table 11-3 indicate that the B cells expressing anti-HEL in the double transgenics cannot mount a humoral response to HEL.

(Figure 11-12a). One group carried a hen's egg-white lysozyme (HEL) transgene linked to a metallothionine promoter, which placed transcription of the HEL gene under the control of zinc levels. The other group of transgenic mice carried rearranged immunoglobulin heavy- and light-chain transgenes encoding anti-HEL antibody; in normal mice, the frequency of HEL-reactive B cells is on the order of 1 in 10^3, but in these transgenic mice the rearranged anti-HEL transgene is expressed by 60%–90% of the mature peripheral B cells. Goodnow mated the two groups of transgenics to produce "double-transgenic" offspring carrying both the HEL and anti-HEL transgenes. Goodnow then asked what effect HEL, which is expressed in the periphery but not in the bone marrow, would have upon the development of B cells expressing the anti-HEL transgene.

The Goodnow double-transgenic system has yielded several interesting findings concerning negative selection of B cells (Table 11-3). He found that double-transgenic mice expressing high levels of HEL (10^{-9} M) continued to have mature, peripheral B cells bearing anti-HEL membrane antibody, but these B cells were functionally nonresponsive; that is, they were **anergic.** The flow-cytometric analysis of B cells from double-transgenic mice showed that, while large numbers of anergic anti-HEL cells were present, they expressed IgM at levels about 20-fold lower than anti-HEL single transgenics (Figure 11-12b). Further study demonstrated that the double transgenics had both surface IgM and IgD, indicating that the anergy was induced in mature rather than immature B cells. When these mice were given an immunizing dose of HEL, few anti-HEL plasma cells were induced and the serum anti-HEL titer was low.

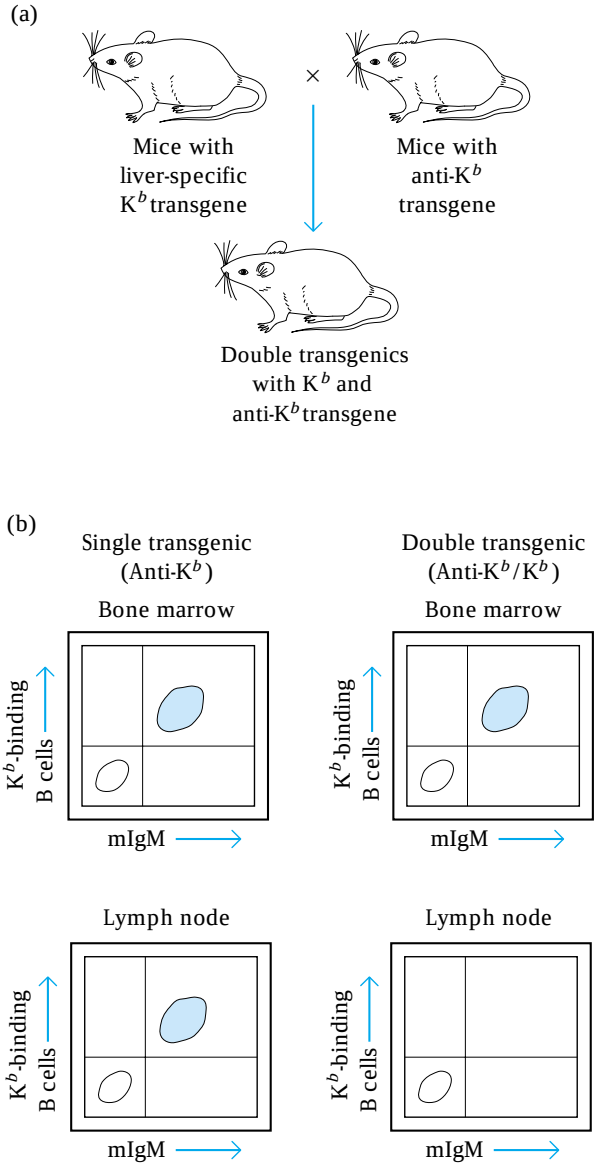
To study what would happen if a class I MHC self-antigen were expressed only in the periphery, Nemazee and Burki modified the transgenic system used in the experiments on

TABLE 11-3 Expression of anti-HEL transgene by mature peripheral B cells in single and double-transgenic mice

Experimental group	HEL level	Membrane anti-HEL	Anti-HEL PFC/spleen*	Anti-HEL serum titer*
Anti-HEL single transgenics	None	+	High	High
Anti-HEL/HEL single transgenics (Group 1)	10^{-9} M	+	Low	Low

* Experimental animals were immunized with hen egg-white lysozyme (HEL). Several days later, hemolytic plaque assays for the number of plasma cells secreting anti-HEL antibody were performed and the serum anti-HEL titers were determined. PFC = plaque-forming cells; see Figure 23-1 for a description of the plaque assay.

SOURCE: Adapted from C. C. Goodnow, 1992, *Annu. Rev. Immunol.* **10:**489.



negative selection in the bone marrow described previously (Figure 11-5a). They first produced a transgene consisting of the class I K^b gene linked to a liver-specific promoter, so that the class I K^b molecule could be expressed only in the liver. Transgenic mice expressing an anti-K^b antibody on their B cells also were produced, and the two groups of transgenic mice were then mated (Figure 11-13a). In the resulting double-transgenic mice, the immature B cells expressing anti-K^b mIgM would not encounter class I K^b molecules in the bone marrow. Flow-cytometric analysis of the B cells in the double transgenics showed that immature B cells expressing the transgene-encoded anti-K^b cells were present in the bone marrow but not in the peripheral lymphoid organs (Figure 11-13b). In the previous experiments of Nemazee and Burki, the class I MHC self-antigen (H-2^k) was expressed on all nucleated cells, and immature B cells expressing the transgene-encoded antibody to this class I molecule were selected against and deleted in the bone marrow (see Figure 11-5a). In their second system, however, the class I self-antigen (K^b) was expressed only in the liver, so that negative selection and deletion occurred at the mature B-cell stage in the periphery.

FIGURE 11-13 Experimental demonstration of clonal deletion of self-reactive mature peripheral B cells by Nemazee and Burki. (a) Production of double-transgenic mice expressing the class I K^b molecule and anti-K^b antibody. Because the K^b transgene contained a liver-specific promoter, K^b was not expressed in the bone marrow of the transgenics. (b) Flow-cytometric analysis of bone marrow and peripheral (lymph node) B cells for K^b binding versus membrane IgM (mIgM). In the double transgenics, B cells expressing anti-K^b (blue) were present in the bone marrow but were absent in the lymph nodes, indicating that mature self-reactive B cells were deleted in the periphery.

The Humoral Response

This section considers the differences between the primary and secondary humoral response and the use of hapten-carrier conjugates in studying the humoral response.

Primary and Secondary Responses Differ Significantly

The kinetics and other characteristics of the humoral response differ considerably depending on whether the humoral response results from activation of naive lymphocytes (primary response) or memory lymphocytes (secondary response). In both cases, activation leads to production of secreted antibodies of various isotypes, which differ in their ability to mediate specific effector functions (see Table 4-2).

The first contact of an exogenous antigen with an individual generates a primary humoral response, characterized by the production of antibody-secreting plasma cells and memory B cells. As Chapter 3 showed, the kinetics of the primary response, as measured by serum antibody level, depend on the nature of the antigen, the route of antigen administration, the presence or absence of adjuvants, and the species or strain being immunized.

In all cases, however, a primary response to antigen is characterized by a lag phase, during which naive B cells undergo clonal selection, subsequent clonal expansion, and differentiation into memory cells or plasma cells (Figure 11-14). The lag phase is followed by a logarithmic increase in serum antibody level, which reaches a peak, plateaus for a variable time, and then declines. The duration of the lag phase varies with the nature of the antigen. Immunization of mice with an antigen such as sheep red blood cells (SRBCs) typically results in a lag phase of 3–4 days. Eight or nine successive cell divisions of activated B cells during days 4 and 5 then generate plasma and memory cells. Peak plasma-cell levels are attained at day 4–5; peak serum antibody levels are attained by around day 7–10. For soluble protein antigens, the lag phase is a little longer, often lasting about a week, peak plasma-cell levels are attained by 9–10 days, and peak serum titers are present by around 14 days. During a primary humoral response, IgM is secreted initially, often followed by a switch to an increasing proportion of IgG. Depending on the persistence of the antigen, a primary response can last for various periods, from only a few days to several weeks.

The memory B cells formed during a primary response stop dividing and enter the G_0 phase of the cell cycle. These cells have variable life spans, with some persisting for the life of the individual. The capacity to develop a secondary humoral response (see Figure 11-14) depends on the existence of this population of memory B cells as well as memory T cells. Activation of memory cells by antigen results in a secondary antibody response that can be distinguished from the primary response in several ways (Table 11-4). The secondary response has a shorter lag period, reaches a greater mag-

VISUALIZING CONCEPTS

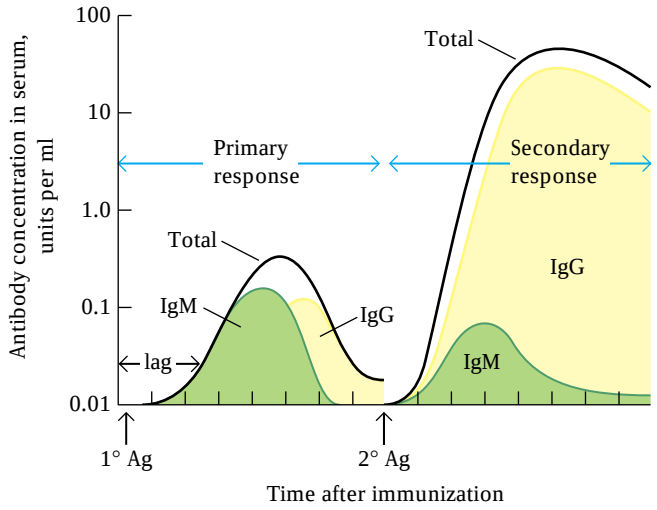


FIGURE 11-14 Concentration and isotype of serum antibody following primary (1°) and secondary (2°) immunization with antigen. The antibody concentrations are plotted on a logarithmic scale. The time units are not specified because the kinetics differ somewhat with type of antigen, administration route, presence or absence of adjuvant, and the species or strain of animal.

TABLE 11-4 Comparison of primary and secondary antibody responses

Property	Primary response	Secondary response
Responding B cell	Naive (virgin) B cell	Memory B cell
Lag period following antigen administration	Generally 4–7 days	Generally 1–3 days
Time of peak response	7–10 days	3–5 days
Magnitude of peak antibody response	Varies depending on antigen	Generally 100–1000 times higher than primary response
Isotype produced	IgM predominates early in the response	IgG predominates
Antigens	Thymus-dependent and thymus-independent	Thymus-dependent
Antibody affinity	Lower	Higher

nitude, and lasts longer. The secondary response is also characterized by secretion of antibody with a higher affinity for the antigen, and isotypes other than IgM predominate.

A major factor in the more rapid onset and greater magnitude of secondary responses is the fact that the population of memory B cells specific for a given antigen is considerably larger than the population of corresponding naive B cells. Furthermore, memory cells are more easily activated than naive B cells. The processes of affinity maturation and class switching are responsible for the higher affinity and different isotypes exhibited in a secondary response. The higher levels of antibody coupled with the overall higher affinity provide an effective host defense against reinfection. The change in isotype provides antibodies whose effector functions are particularly suited to a given pathogen.

The existence of long-lived memory B cells accounts for a phenomenon called "original antigenic sin," which was first observed when the antibody response to influenza vaccines was monitored in adults. Monitoring revealed that immunization with an influenza vaccine of one strain elicited an antibody response to that strain but, paradoxically, also elicited an antibody response of greater magnitude to another influenza strain that the individual had been exposed to during childhood. It was as if the memory of the first antigen exposure had left a life-long imprint on the immune system. This phenomenon can be explained by the presence of a memory-cell population, elicited by the influenza strain encountered in childhood, that is activated by cross-reacting epitopes on the vaccine strain encountered later. This process then generates a secondary response, characterized by antibodies with higher affinity for the earlier viral strain.

T Helper Cells Play a Critical Role in the Humoral Response to Hapten-Carrier Conjugates

As Chapter 3 described, when animals are immunized with small organic compounds (haptens) conjugated with large proteins (carriers), the conjugate induces a humoral immune response consisting of antibodies both to hapten epitopes and to unaltered epitopes on the carrier protein. Hapten-carrier conjugates provided immunologists with an ideal system for studying cellular interactions of the humoral response, and such studies demonstrated that the generation of a humoral antibody response requires recognition of the antigen by both T_H cells and B cells, each recognizing different epitopes on the same antigen. A variety of different hapten-carrier conjugates have been used in immunologic research (Table 11-5).

One of the earliest findings with hapten-carrier conjugates was that a hapten had to be chemically coupled to a larger carrier molecule to induce a humoral response to the hapten. If an animal was immunized with both hapten and carrier separately, very little or no hapten-specific antibody was generated. A second important observation was that, in order to generate a secondary antibody response to a hapten,

TABLE 11-5 Common hapten-carrier conjugates used in immunologic research

Hapten-carrier acronym	Hapten	Carrier protein
DNP-BGG	Dinitrophenol	Bovine gamma globulin
TNP-BSA	Trinitrophenyl	Bovine serum albumin
NIP-KLH	5-Nitrophenyl acetic acid	Keyhole limpet hemocyanin
ARS-OVA	Azophenylarsonate	Ovalbumin
LAC-HGG	Phenyllactoside	Human gamma globulin

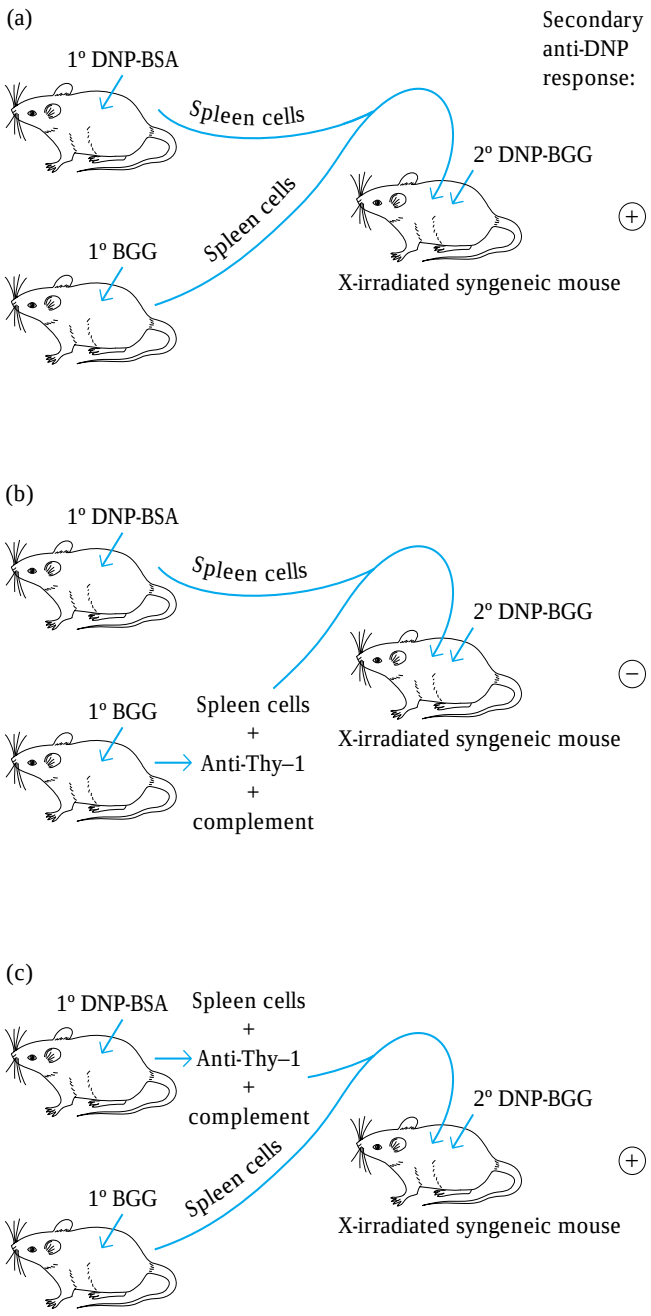


FIGURE 11-15 Cell-transfer experiments demonstrating that hapten-primed and carrier-primed cells are separate populations. (a) X-irradiated syngeneic mice reconstituted with spleen cells from both DNP-BSA–primed mice and BGG-primed mice and challenged with DNP-BGG generated a secondary anti-DNP response. (b) Removal of T cells from the BGG-primed spleen cells, by treatment with anti-Thy-1 antiserum, abolished the secondary anti-DNP response. (c) Removal of T cells from the DNP-BSA–primed spleen cells had no effect on the secondary response to DNP. These experiments show that carrier-primed cells are T cells and hapten-primed cells are B cells.

the animal had to be again immunized with the same hapten-carrier conjugate used for the primary immunization. If the secondary immunization was with the same hapten but conjugated to a different, unrelated carrier, no secondary anti-hapten response occurred. This phenomenon, called the **carrier effect,** could be circumvented by priming the animal separately with the unrelated carrier.

Similar experiments conducted with a cell-transfer system showed that cells immunized against the hapten and cells immunized against the carrier were distinct populations. In these studies, one mouse was primed with the DNP-BSA conjugate and another was primed with the unrelated carrier BGG, which was not conjugated to the hapten. In one experiment, spleen cells from both mice were mixed and injected into a lethally irradiated syngeneic recipient. When this mouse was challenged with DNP conjugated to the unrelated carrier BGG, there was a secondary anti-hapten response to DNP (Figure 11-15a). In a second experiment, spleen cells from the BGG-immunized mice were treated with anti-T-cell antiserum (anti-Thy-1) and complement to lyse the T cells. When this T-cell–depleted sample was mixed with the DNP-BSA–primed spleen cells and injected into an irradiated mouse, no secondary anti-hapten response was observed upon immunizing with DNP-BGG (Figure 11-15b). However, similar treatment of the DNP-BSA–primed spleen cells with anti-Thy-1 and complement did not abolish the secondary anti-hapten response to DNP-BGG (Figure 11-15c). Later experiments, in which antisera were used to specifically deplete $CD4^+$ or $CD8^+$ T cells, showed that the $CD4^+$ T-cell subpopulation was responsible for the carrier effect. These experiments demonstrate that the response of hapten-primed B cells to the hapten-carrier conjugate requires the presence of carrier-primed $CD4^+$ T_H cells specific for carrier epitopes. (It is important to keep in mind that the B-cell response is not limited to the hapten determinant; in fact some B cells do react to epitopes on the carrier; however, the assay can be conducted in such a manner as to detect only anti-hapten responses.)

The experiments with hapten-carrier conjugates revealed that both T_H cells and B cells must recognize antigenic determinants on the same molecule for B-cell activation to occur. This feature of the T- and B-cell interaction in the humoral response is called associative, or linked, recognition. The conclusions drawn from hapten-carrier experiments apply to the humoral response to antigens in general and support the requirement for T-cell help in B-cell activation described earlier in this chapter.

In Vivo Sites for Induction of Humoral Responses

In vivo activation and differentiation of B cells occurs in defined anatomic sites whose structure places certain restrictions on the kinds of cellular interactions that can take place.

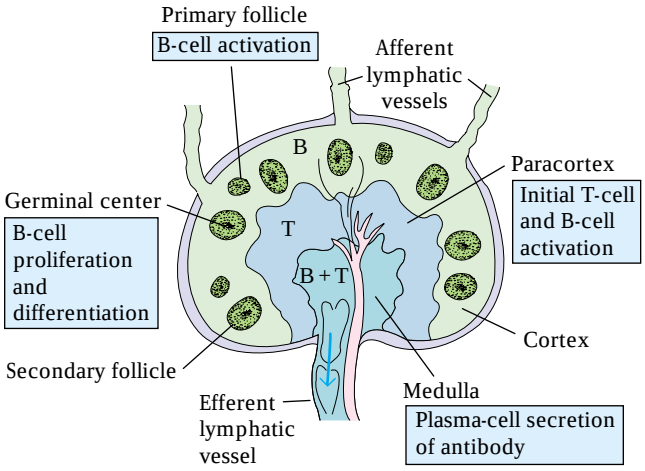


FIGURE 11-16 Schematic diagram of a peripheral lymph node showing anatomic sites at which various steps in B-cell activation, proliferation, and differentiation occur. The cortex is rich in B cells and the paracortex in T cells; both B and T cells are present in large numbers in the medulla. A secondary follicle contains the follicular mantle and a germinal center.

When an antigen is introduced into the body, it becomes concentrated in various peripheral lymphoid organs. Blood-borne antigen is filtered by the spleen, whereas antigen from tissue spaces drained by the lymphatic system is filtered by regional lymph nodes or lymph nodules. The following description focuses on the generation of the humoral response in lymph nodes.

A lymph node is an extremely efficient filter capable of trapping more than 90% of any antigen carried into it by the afferent lymphatics. Antigen or antigen-antibody complexes enter the lymph nodes either alone or associated with antigen-transporting cells (e.g., Langerhans cells or dendritic cells) and macrophages. As antigen percolates through the cellular architecture of a node, it will encounter one of three types of antigen-presenting cells: interdigitating dendritic cells in the paracortex, macrophages scattered throughout the node, or specialized follicular dendritic cells in the follicles and germinal centers. Antigenic challenge leading to a humoral immune response involves a complex series of events, which take place in distinct microenvironments within a lymph node (Figure 11-16). Slightly different pathways may operate during a primary and secondary response because much of the tissue antigen is complexed with circulating antibody in a secondary response.

Once antigen-mediated B-cell activation takes place, small foci of proliferating B cells form at the edges of the T-cell–rich zone. These B cells differentiate into plasma cells secreting IgM and IgG isotypes. Most of the antibody produced during a primary response comes from plasma cells in these foci. (A similar sequence of events takes place in the spleen, where initial B-cell activation takes place in the T-cell–rich periarterial lymphatic sheath, PALS; see Figure 2-19).

A few days after the formation of foci within lymph nodes, a few activated B cells, together with a few T_H cells, are thought to migrate from the foci to primary follicles. These follicles then develop into secondary follicles, which provide a specialized microenvironment favorable for interactions between B cells, activated T_H cells, and follicular dendritic cells. Note that although they share the highly branched morphology of dendritic cells derived from bone marrow, follicular dendritic cells do not arise in bone marrow, do not express class II MHC molecules, and do not present antigen to $CD4^+$ T cells. Follicular dendritic cells have long extensions, along which are arrayed Fc receptors and complement receptors. These receptors allow follicular dendritic cells to retain and present antigen-antibody complexes for long periods of time, even months, on the surface of the cell. Activated B cells (together with some activated T_H cells) may migrate towards the center of the secondary follicle, forming a germinal center.

Germinal Centers and Antigen-Induced B-Cell Differentiation

Germinal centers arise within 7–10 days after initial exposure to a thymus-dependent antigen. During the first stage of germinal-center formation, activated B cells undergo intense proliferation. These proliferating B cells, known as centroblasts, appear in human germinal centers as a well-defined **dark zone** (Figure 11-17). Centroblasts are distinguished by their large size, expanded cytoplasm, diffuse chromatin, and absence or near absence of surface Ig. Centroblasts eventually give rise to centrocytes, which are small, nondividing B cells that now express membrane Ig. The centrocytes move from the dark zone into a region containing follicular dendritic cells called the **light zone,** where some centrocytes make contact with antigen displayed as antigen-antibody complexes on the surface of follicular dendritic cells. Three important B-cell differentiation events take place in germinal centers: affinity maturation, class switching, and formation of plasma cells and memory B cells. In general, affinity maturation and memory-cell formation require germinal centers. However some class switching and significant plasma-cell formation occur outside germinal centers.

Affinity Maturation Is the Result of Repeated Mutation and Selection

The average affinity of the antibodies produced during the course of the humoral response increases remarkably during the process of affinity maturation, an effect first noticed by H. N. Eisen and G. W. Siskind when they immunized rabbits

VISUALIZING CONCEPTS

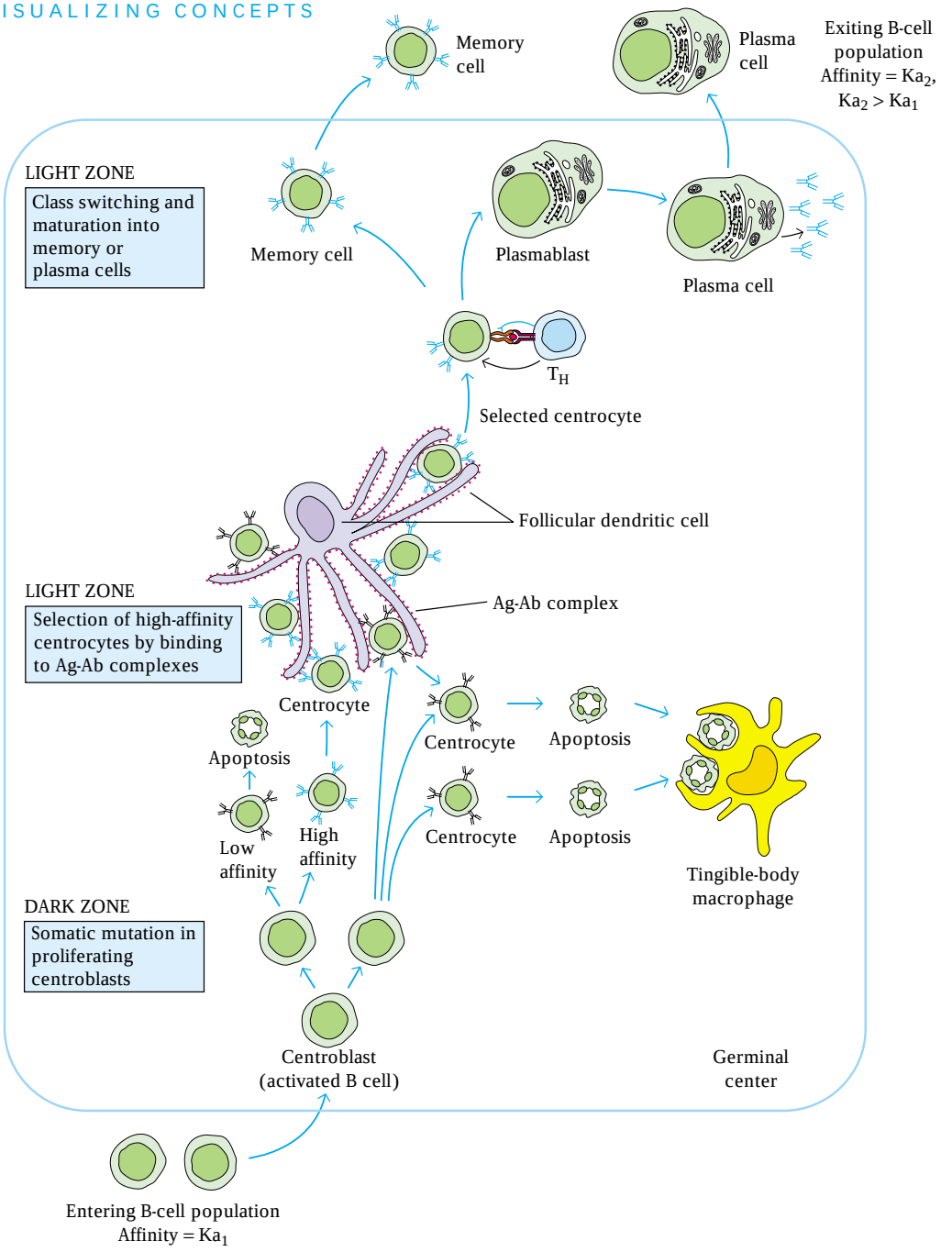


FIGURE 11-17 Overview of cellular events within germinal centers. Antigen-stimulated B cells migrate into germinal centers, where they reduce expression of surface Ig and undergo rapid cell division and mutation of rearranged immunoglobulin V region genes within the dark zone. Subsequently, division stops and the B cells migrate to the light zone and increase their expression of surface Ig. At this stage they are called centrocytes. Within the light zone centrocytes must interact with follicular dendritic cells and T helper cells to survive. Follicular dendritic cells bind antigen-antibody complexes along their long extensions and the centrocytes must compete with each other to bind antigen. B cells bearing high-affinity membrane immunoglobulin (antibodies shown in blue) are most likely to compete successfully. Those that fail this antigen-mediated selection (antibodies shown in black) die by apoptosis. B cells that pass antigen selection and receive a second survival signal from T_H cells differentiate into either memory B cells or antibody-secreting plasma cells. The encounter with T_H cells may also induce class switching. A major outcome of the germinal center is to generate higher affinity B cells (Ka_2) from B cells of lower affinity (Ka_1).

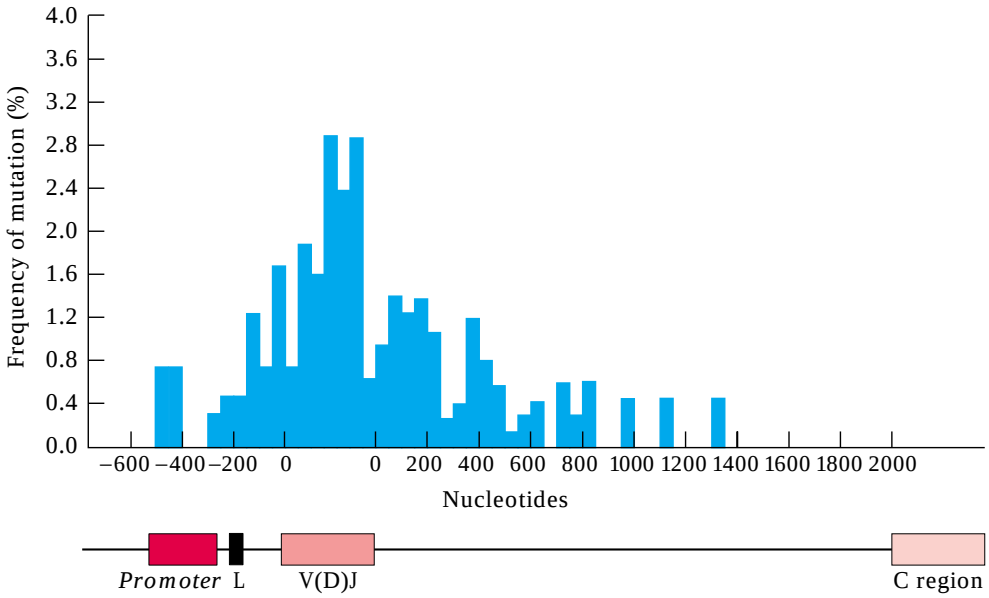


FIGURE 11-18 The frequency of somatic hypermutation decreases with the distance from the rearranged V(D)J gene. Experimental measurement of the mutation frequency shows that few if any mutations are seen upstream of the promoter of the rearranged gene. Mutations do not extend into the portion of the gene encoding the constant region becaue there are no mutations at positions more than about 1.5 kb 3′ of the rearranged gene. *[Adapted from P. Gearhart, in* Fundamental Immunology, *3rd ed., 1993, p. 877.]*

with the hapten-carrier complex DNP-BGG. The affinity of the serum anti-DNP antibodies produced in response to the antigen was then measured at 2, 5, and 8 weeks after immunization. The average affinity of the anti-DNP antibodies increased about 140-fold from 2 weeks to 8 weeks. Subsequent work has shown that affinity maturation is mainly the result of somatic hypermutation.

THE ROLE OF SOMATIC HYPERMUTATION

Monitoring of antibody genes during an immune response shows that extensive mutation of the Ig genes that respond to the infection takes place in B cells within germinal centers. A direct demonstration that germinal centers are the sites of somatic hypermutation comes from the work of G. Kelsoe and his colleagues. These workers compared the mutation frequencies in B cells isolated from germinal centers with those from areas of intense B-cell activation outside the germinal centers. To do so, they prepared thin sections of spleen tissue from animals immunized with the hapten 4-hydroxy-3-nitrophenylacetyl (NP) conjugated with chicken gamma globulin as a carrier. This system is convenient because the initial response to this hapten is dominated by a particular heavy-chain gene rearrangement and the use of a γ light chain (in mice, >95% of antibodies bear κ light chains). Consequently, antibodies against the idiotype of this antibody can be used to readily distinguish responding B cells. Using antibodies to the idiotype and immunohistological staining techniques, these workers identified B cells bearing anti-NP antibody in germinal centers and nongerminal-center foci of B-cell activation present in thin sections cut from the spleens of recently immunized mice. They isolated these B cells by microdissection, used PCR to amplify the immunoglobulin genes of each individual cell, and then cloned and sequenced the immunoglobulin genes. Many mutations were found in the immunoglobulin genes obtained from B cells in germinal centers, few in the genes obtained from activated B cells in nongerminal-center foci. When the mutated sequences of the collection of B cells from germinal centers was examined, it was apparent that many of the cells had sequences that were sufficiently similar that they were likely to be related by common descent from the same precursor cell. Detailed analysis of the sequences allowed these workers to build genealogic trees in which one could clearly see the descent of progeny from progenitors by progressive somatic hypermutation.

The introduction of point mutations, deletions, and insertions into the rearranged immunoglobulin genes is strikingly focused. Figure 11-18 shows that the overwhelming majority of these mutations occur in a region that extends from about 0.5 kb 5′ to about 1.5 kb 3′ of the V(D)J segments of rearranged immunoglobulin genes. Although the hypermutation process delivers mutations throughout the V region, antigen-driven selection results in the eventual emergence of immunoglobulin genes in which the majority of the mutations lie within the three complementarity-determining regions (CDRs). It has been estimated that the mutation rate

during somatic mutation is approximately 10^{-3}/base pair/division, which is a millionfold greater than the normal mutation rate for other genes of humans or mice cells. Since the heavy- and light-chain V(D)J segments total about 700 base pairs, this rate of mutation means that, for every two cell divisions it undergoes, a centroblast will acquire a mutation in either the heavy- or light-chain variable regions. The extremely high rates and precise targeting of somatic hypermutation are remarkable features that are unique to the immune system. Determining the molecular basis of this extraordinary process remains a challenge in immunology.

Because somatic mutation occurs randomly, it will generate a few cells with receptors of higher affinity and many cells with receptors of unchanged or lower affinity for a particular antigen. Therefore, selection is needed to derive a population of cells that has increased affinity. The germinal center is the site of selection. B cells that have high-affinity receptors for the antigen are likely to be positively selected and leave the germinal center; those with low affinity are likely to undergo negative selection and die in the germinal center.

THE ROLE OF SELECTION

Somatic hypermutation of heavy- and light-chain variable-region genes occurs when centroblasts proliferate in the dark zone of the germinal center. Selection takes place in the light zone, among the nondividing centrocyte population. The most important factor influencing selection is the ability of the membrane Ig molecules on the centrocyte to recognize and bind antigen displayed by follicular dendritic cells (FDCs). Because the surfaces of FDCs are richly endowed with both Fc receptors and complement receptors, antigen complexed with antibody or antigen that has been bound by C3 fragments generated during complement activation (see Chapter 13) can bind to FDCs by antibody or C3 bridges. A centrocyte whose membrane Ig binds and undergoes cross-linking by FDC-bound antigen receives a signal that is essential for its survival. Those that fail to receive such signals die. However, centrocytes must compete for the small amounts of antigen present on FDCs;. Because the amount of antigen is limited, centrocytes with receptors of high affinity are more likely to be successful in binding antigen than those of lower affinity (see Figure 11-17).

While antigen binding is necessary for centrocyte survival, it is not sufficient. A centrocyte must also receive signals generated by interaction with a $CD4^+$ T_H cell to survive. An indispensable feature of this interaction is the engagement of CD40 on the B cell (centrocyte) by CD40L on the helper T cell. It is also necessary that processed antigen on class II MHC molecules of the B cell interact with the TCR of the collaborating T_H-cell. Centrocytes that fail to receive either the T_H-cell or the antigen-membrane Ig signal undergo apoptosis in the germinal center. Indeed, one of the striking characteristics of the germinal center is the extensive cell death by apoptosis that takes place there. This is clearly evident in the presence of condensed chromatin fragments, indicative of apoptosis, in tingible-body macrophages, an unusual type of macrophage that removes cells by phagocytosis from lymphoid tissues.

CLASS SWITCHING

Antibodies perform two important activities: the specific binding to an antigen, which is determined by the V_H and V_L domains; and participation in various biological effector functions, which is determined by the isotype of the heavy-chain constant domain. As described in Chapter 5, class switching allows any given V_H domain to associate with the constant region of any isotype. This enables antibody specificity to remain constant while the biological effector activities of the molecule vary. A number of cytokines affect the decision of what Ig class is chosen when an IgM-bearing cell undergoes the class switch (Figure 11-19). The role of cytokines in class switching is explored further in Chapter 12.

As noted earlier, the humoral response to thymus-dependent antigens is marked by extensive class switching to isotypes other than IgM, whereas the antibody response to thymus-independent antigens is dominated by IgM. In the case of thymus-dependent antigens, membrane interaction between CD40 on the B cell and CD40L on the T_H cell is essential for the induction of class switching. The importance of the CD40/CD40L interaction is illustrated by the **X-linked hyper-IgM syndrome,** an immunodeficiency disorder in which T_H cells fail to express CD40L. Patients with this disorder produce IgM but not other isotypes. Such patients fail to generate memory-cell populations, fail to form germinal centers, and their antibodies fail to undergo somatic hypermutation.

Memory B Cells and Plasma Cells are Generated in Germinal Centers

After B cells are selected in the germinal center for those bearing high-affinity mIg for antigen displayed on follicular dendritic cells, some B cells differentiate into plasma cells and others become memory B cells (see Figure 11-17). While germinal centers are important sites of plasma-cell generation, these Ig-secreting cells are fomed in other sites as well. Plasma cells generally lack detectable membrane-bound immunoglobulin and instead synthesize high levels of secreted antibody (at rates as high as 1000 molecules of Ig per cell per second). Differentiation of mature B cells into plasma cells requires a change in RNA processing so that the secreted form of the heavy chain rather than the membrane form is synthesized. In addition, the rate of transcription of heavy- and light-chain genes is significantly greater in plasma cells than in less-differentiated B cells. Several authors have suggested that the increased transcription by plasma cells might be explained by the synthesis of higher levels of transcription factors that bind to immunoglobulin enhancers. Some mechanism also must coordinate the increase in transcription of heavy-chain and light-chain genes, even though these genes are on different chromosomes.

As indicated above, B cells that survive selection in the light zone of germinal centers also differentiate into memory

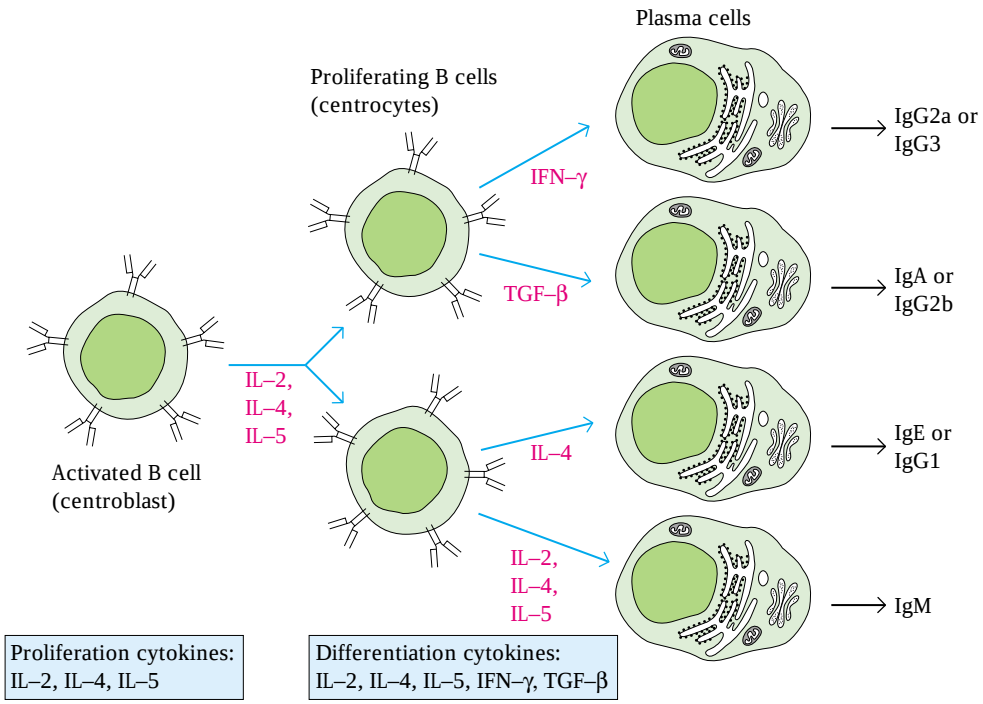


FIGURE 11-19 The interactions of numerous cytokines with B cells generate signals required for proliferation and class switching during the differentiation of B cells into plasma cells. Binding of the proliferation cytokines, which are released by activated T_H cells, provides the progression signal needed for proliferation of activated B cells. Similar or identical effects may be mediated by cytokines beyond the ones shown. Class switching in the response to thymus-dependent antigens also requires the CD40/CD40L interaction, which is not shown here.

cells. Some properties of naive and memory B cells are summarized in Table 11-6. Except for membrane-bound immunoglobulins, few membrane molecules have been identified that distinguish naive B cells from memory B cells. Naive B cells express only IgM and IgD; as a consequence of class switching, however, memory B cells express additional isotypes, including IgG, IgA, and IgE.

Regulation of B-Cell Development

A number of transcription factors that regulate expression of various gene products at different stages of B-cell development have been identified. Among these are NF-6B, BSAP, Ets-1, c-Jun, Ikaros, Oct-2, Pu.1, EBF, BCF, and E2A. Like all

TABLE 11-6 Comparison of naive and memory B cells

Property	Naive B cell	Memory B cell
Membrane markers		
Immunoglobulin	IgM, IgD	IgM, IgD(?), IgG, IgA, IgE
Complement receptor	Low	High
Anatomic location	Spleen	Bone marrow, lymph node, spleen
Life span	Short-lived	May be long-lived
Recirculation	Yes	Yes
Receptor affinity	Lower average affinity	Higher average affinity due to affinity maturation*
Adhesion molecules	Low ICAM-1	High ICAM-1

* Affinity maturation results from somatic mutation during proliferation of centroblasts and subsequent antigen selection of centrocytes bearing high-affinity mIg.

transcription factors, these DNA-binding proteins interact with promoter or enhancer sequences, thereby either stimulating or inhibiting transcription of the associated gene. Analyses of the effects of knocking out the gene that encodes a particular transcription factor have provided clues about the role of some factors in B-cell development. For example, in knockout mice that carry a disrupted *Ikaros* gene, there is a general failure of lymphocyte development, and pro-B cells fail to develop in the bone marrow.

One of the most critical B-cell transcription factors, *B-cell–specific activator protein (BSAP)*, which has been previously mentioned (see Figure 11-3), appears to function as a master B-cell regulator. It is expressed only by B-lineage cells and influences all the cell stages during B-cell maturation. Moreover, recent evidence indicates that BSAP also influences the final differentiation events leading to the formation of memory B cells and plasma cells. The latter are the only B-lineage cells that do not express BSAP. BSAP binds to promoter or enhancer sequences of various B-cell–specific genes, including the λ5 and Vpre-B genes of the surrogate light chain, the J-chain gene of polymeric IgM, and the 3′α heavy-chain enhancer region, one of the two enhancers that lie 3′ of the α gene in heavy-chain germ-line DNA. In addition, BSAP binds to various immunoglobulin heavy-chain switch sites and to several genes involved in B-cell activation.

The heavy-chain 3′α enhancer ($E_{3'\alpha}$) contains binding sites for several transcription factors in addition to BSAP. Binding of BSAP to $E_{3'\alpha}$ appears to influence B-cell development by preventing the binding of other transcription factors. For example, when BSAP levels are high, this factor appears to block binding of NF-αP to the 3′α enhancer, thereby blocking transcription of the heavy-chain gene and promoting formation of memory B cells. When BSAP levels are low, NF-αP can bind to $E_{3'\alpha}$. As a result, transcription of the immunoglobulin heavy-chain gene is increased, leading to formation of plasma cells.

Regulation of the Immune Effector Response

Upon encountering an antigen, the immune system can either develop an immune response or enter a state of unresponsiveness called **tolerance.** The development of immunity or tolerance, both of which involve specific recognition of antigen by antigen-reactive T or B cells, must be carefully regulated since an inappropriate response—whether it be immunity to self-antigens or tolerance to a potential pathogen—can have serious and possibly life-threatening consequences.

Regulation of the immune response takes place in both the humoral and the cell-mediated branch. Every time an antigen is introduced, important regulatory decisions determine the branch of the immune system to be activated, the intensity of the response, and its duration. Chapter 12 describes the importance of the cytokines to the orchestration of appropriate immune responses. In addition to cytokines, other regulatory mechanisms may also play important immunoregulatory roles. Greater knowledge about these regulatory events, which are still not well understood, may allow the deliberate manipulation of immune responses, selectively up-regulating desirable responses and down-regulating undesirable ones.

Different Antigens Can Compete with Each Other

The immunologic history of an animal influences the quality and quantity of its immune response. A naive animal responds to antigen challenges very differently from a previously primed animal. Previous encounter with an antigen may have rendered the animal tolerant to the antigen or may have resulted in the formation of memory cells. In some cases, the presence of a competing antigen can regulate the immune response to an unrelated antigen. This **antigenic competition** is illustrated by injecting mice with a competing antigen a day or two before immunization with a test antigen. For example, the response to horse red blood cells (HRBCs) is severely reduced by prior immunization with sheep red blood cells (SRBCs) and vice versa (Table 11-7). Although antigenic competition is a well-established phenomenon, its molecular and cellular basis is not understood.

The Presence of Antibody Can Suppress the Response to Antigen

Like many biochemical reactants, antibody exerts feedback inhibition on its own production. Because of antibody-mediated suppression, certain vaccines (e.g., those for measles and mumps) are not administered to infants before the age of 1 year. The level of naturally acquired maternal IgG, which the fetus acquires by transplacental transfer, remains high for about 6 months after birth. If an infant is immunized with

TABLE 11-7 Antigenic competition between SRBCs and HRBCs

IMMUNIZING ANTIGEN		HEMOLYTIC PLAQUE ASSAY (DAY 8)*	
Ag1 (day 0)	Ag2 (day 3)	Test Ag	PPC/10^6 spleen cells
None	HRBC	HRBC	205
SRBC	HRBC	HRBC	13
None	SRBC	SRBC	626
HRBC	SRBC	SRBC	78

* See Figure 23-1 for a description of the plaque assay.

measles or mumps vaccine while this maternal antibody is still present, the humoral response is low and the production of memory cells is inadequate to confer long-lasting immunity. If an animal is immunized with a specific antigen and is injected with preformed antibody to that same antigen just before or within a few days after antigen priming, the immune response to the antigen is reduced as much as 100-fold.

There are two explanations for antibody-mediated suppression. One is that the circulating antibody competes with antigen-reactive B cells for antigen inhibiting the clonal expansion of the B cells. The second explanation is that binding of antigen-antibody complexes by Fc receptors on B cells reduces signalling by the B-cell-receptor complex.

As the antibody response proceeds, antibody feedback produces inhibition of the response. As more secreted IgG molecules become involved in antigen-antibody complexes, the Ig portions of these complexes become bound to Fcγ receptors present on the B cell membane and the antigen of the complex binds the Ig of B-cell receptors. This crosslinking brings Fcγ receptors into close association with activated B-cell-receptor complexes, allowing phosphatases bound to the cytoplasmic tails of the Fc receptor to dephosphorylate sites in the BCR complex that are necessary to maintain B-cell activation. As a consequence, the activity of the B cell is progressively down-regulated as the amount of IgG bound to antigen increases. Evidence for such competition between passively administered antibody and antigen-reactive B cells comes from studies in which it took over 10 times more low-affinity anti-DNP antibody than high-affinity anti-DNP antibody to induce comparable suppression. Furthermore, the competition for antigen between passively administered antibody and antigen-reactive B cells drives the B-cell response toward higher-affinity antibody. Only the high-affinity antigen-reactive cells can compete successfully with the passively administered antibody for the available antigen.

SUMMARY

- B cells develop in bone marrow and undergo antigen-induced activation and differentiation in the periphery. Activated B cells can give rise to antibody-secreting plasma cells or memory B cells.
- During B-cell development, sequential Ig-gene rearrangements transform a pro-B cell into an immature B cell expressing mIgM with a single antigenic specificity. Further development yields mature naive B cells expressing both mIgM and mIgD.
- When a self-reactive BCR is expressed in the bone marrow, negative selection of the self-reactive immature B cells occurs. The selected cells are deleted by apoptosis or undergo receptor editing to produce non-self-reactive mIg. B cells reactive with self-antigens encountered in the periphery are rendered anergic.
- In the periphery, the antigen-induced activation and differentiation of mature B cells generates an antibody response. The antibody response to proteins and most other antigens requires T_H cells. These are thymus-dependent or simply T-dependent (TD) responses. Responses to some antigens, such as certain bacterial cell-wall products (e.g., LPS) and polymeric molecules with repeating epitopes, do not require T_H cells and are independent (TI) antigens. The vast majority of antigens are dependent.
- B-cell activation is the consequence of signal-transduction process triggered by engagement of the B-cell receptor that ultimately leads to many changes in the cell, including changes in the expression of specific genes.
- B- and T-cell activation share many parallels, including: compartmentalization of function within receptor subunits; activation by membrane-associated protein tyrosine kinases; assembly of large signaling complexes with protein–tyrosine-kinase activity; and recruitment of several signal-transduction pathways.
- The B-cell coreceptor can intensify the activating signal resulting from crosslinkage of mIg. This may be particularly important during the primary response to low concentrations of antigen.
- Activation induced by TD antigens requires contact-dependent help delivered by the interaction between CD40 on B cells and CD40L on activated T_H cells. The CD40/CD40L interaction is essential for B-cell survival, the formation of germinal centers, the generation of memory-cell populations, and somatic hypermutation.
- The properties of the primary and secondary antibody responses differ. The primary response has a long lag period, a logarithmic rise in antibody formation, a short plateau, and then a decline. IgM is the first antibody class produced, followed by a gradual switch to other classes, such as IgG. The secondary response has a shorter lag time, a more rapid logarithmic phase, a longer plateau phase, and a slower decline than the primary response. Mostly IgG and other isotypes are produced in the secondary response rather than IgM, and the average affinity of antibody produced is higher.
- Within a week or so of exposure to a TD antigen, germinal centers form. Germinal centers are sites of somatic hypermutation of rearranged immunoglobulin genes. Germinal centers are the sites of affinity maturation, formation of memory B cells, class switching, and plasma-cell formation.

References

Benschop, R. J., and J. C. Cambier. 1999. B-cell development: signal transduction by antigen receptors and their surrogates. *Curr. Opin. Immunol.* **11**:143.

Berek, C. 1999. Affinity Maturation. In *Fundamental Immunology*, 4th ed., edited by W. E. Paul. Lippincott-Raven, Philadelphia and New York.

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Berland, R. and H. H. Wortis. 2002. Origins and functions of B-1 cells with notes on the role of cd5. *Annu. Rev. Immunol.* **20:**253.

Bruton, O. C. 1952. Agammaglobulinemia. *Pediatrics* **9:**722.

Hardy, R. R., and K. Hayakawa. 2001. B-cell development pathways. *Annu. Rev. Immunol.* **19:**595.

Jacob, J., G. Kelsoe, K. Rajewsky, and U. Weiss. 1991. Intraclonal generation of antibody mutants in germinal centres. *Nature* **354:**389.

Manis, J. P., M. Tian, and F. W. Alt. 2002. Mechanism and control of class-switch recombination. *Trends Immunol.* **23:**31.

Matsuuchi, L., and M. R. Gold. 2001. New views of BCR structure and organization. *Curr. Opin. Immunol.* **13:**270.

Melchers, F., and A. Rolink. 1999. B-lymphocyte development and biology. In *Fundamental Immunology,* 4th ed., edited by W. E. Paul. Lippincott-Raven, Philadelphia and New York.

Meffre, E., R. Casellas, and M. C. Nussenzweig. 2000. Antibody regulation of B-cell development. *Nature Immunology* **1:**379.

Papavasiliou, F. N., and D. G. Schatz. 2002. Somatic hypermutation of immunoglobulin genes. Merging mechanisms for genetic diversity. *Cell* **109:**S35.

USEFUL WEB SITES

http://www.ncbi.nlm.nih.gov/Omim/>

http://www.ncbi.nlm.nih.gov/htbinpost/Omim/getmim

The Online Mendelian Inheritance in Man Web site contains a subsite that lists more than a dozen different inherited diseases associated with B-cell defects.

http://www.bioscience.org/knockout/knochome.htm>

The Frontiers in Bioscience Database of Gene Knockouts features information on the effects of knockouts of many genes important to the development and function of B cells.

Study Questions

CLINICAL FOCUS QUESTION Patients with X-linked agammaglobulinemia are subject to infection by a broad variety of pathogens. Suppose you have three sources of highly purified human immunoglobulin (HuIg) for the treatment of patients with X-linked agammaglobulinemia. The human Ig from all three sources is equally free of disease-causing agents and is equally well tolerated by recipients, but the number of donors whose blood was pooled for the preparation of each source differs widely: 100 individuals for source A, 1000 for source B, and 60,000 for source C. Which would you choose and what is the basis of your choice?

1. Indicate whether each of the following statements concerning B-cell maturation is true or false. If you think a statement is false, explain why.
   a. Heavy chain V_H-D_H-J_H rearrangement begins in the pre–B-cell stage.
   b. Immature B cells express membrane IgM and IgD.
   c. The enzyme terminal deoxyribonucleotidyl transferase (TdT) is active in the pre–B-cell stage.
   d. The surrogate light chain is expressed by pre-B cells.
   e. Self-reactive B cells can be rescued from negative selection by the expression of a different light chain.
   f. In order to develop into immature B cells, pre-B cells must interact directly with bone-marrow stromal cells.
   g. Most of the B cells generated every day never leave the bone marrow as mature B cells.

2. You have fluorescein (Fl)-labeled antibody to the μ heavy chain and a rhodamine (Rh)-labeled antibody to the δ heavy chain. Describe the fluorescent-antibody staining pattern of the following B-cell maturational stages assuming that you can visualize both membrane and cytoplasmic staining: (a) progenitor B cell (pro-B cell); (b) precursor B cell (pre-B cell); (c) immature B cell; (d) mature B cell; and (e) plasma cell before any class switching has occurred.

3. Describe the general structure and probable function of the B-cell–coreceptor complex.

4. In the Goodnow experiment demonstrating clonal anergy of B cells, two types of transgenic mice were compared: single transgenics carrying a transgene-encoded antibody against hen egg-white lysozyme (HEL) and double transgenics carrying the anti-HEL transgene and a HEL transgene linked to the zinc-activated metallothionine promoter.
   a. In both the single and double transgenics, 60%–90% of the B cells expressed anti-HEL membrane-bound antibody. Explain why.
   b. How could you show that the membrane antibody on these B cells is specific for HEL and how could you determine its isotype?
   c. Why was the metallothionine promoter used in constructing the HEL transgene?
   d. Design an experiment to prove that the B cells, not the T_H cells, from the double transgenics were anergic.

5. Discuss the origin of the competence and progression signals required for activation and proliferation of B cells induced by (a) soluble protein antigens and (b) bacterial lipopolysaccharide (LPS).

6. Fill in the blank(s) in each statement below (a–i) with the most appropriate term(s) from the following list. Terms may be used more than once or not at all.

dark zone	centroblasts	memory B cells
light zone	centrocytes	plasmablasts
paracortex	follicular dendritic cells	T_H cells
cortex	medulla	

   a. Most centrocytes die by apoptosis in the _____.
   b. Initial activation of naive B cells induced by thymus-dependent antigens occurs within the _____ of lymph nodes.
   c. _____ are rapidly dividing B cells located in the _____ of germinal centers.
   d. _____ expressing high-affinity mIg interact with antigen captured by _____ in the light zone.
   e. Class switching occurs in the _____ and requires direct contact between B cells and _____.
   f. Centrocytes expressing mIg specific for a self-antigen present in the bone marrow are subjected to negative selection in the _____.

g. Within lymph nodes, plasma cells are found primarily in the _____ of secondary follicles.
h. Generation of _____ in the _____ of germinal centers is induced by interaction of centrocytes with IL-1 and CD3.
i. Somatic hypermutation, which occurs in proliferating _____, is critical to affinity maturation.

7. Activation and differentiation of B cells in response to thymus-dependent (TD) antigens requires T_H cells, whereas the B-cell response to thymus-independent (TI) antigens does not.

a. Discuss the differences in the structure of TD, TI-1, and TI-2 antigens and the characteristics of the humoral responses induced by them.
b. Binding of which classes of antigen to mIg provides an effective competence signal for B-cell activation?

8. B-cell–activating signals must be transduced to the cell interior to influence developmental processes. Yet the cytoplasmic tails of all isotypes of mIg on B cells are too short to function in signal transduction.

a. How do naive B cells transduce the signal induced by crosslinkage of mIg by antigen?
b. Describe the general result of signal transduction in B cells during antigen-induced activation and differentiation.

9. In some of their experiments, Nemazee and Burki mated mice carrying a transgene encoding K^b, a class I MHC molecule, linked to a liver-specific promoter with mice carrying a transgene encoding antibody against K^b. In the resulting double transgenics, K^b-binding B cells were found in the bone marrow but not in lymph nodes. In contrast, the anti-K^b single transgenics had K^b-binding B cells in both the bone marrow and lymph nodes.

a. Was the haplotype of the mice that received the transgenes H-2^b or some other haplotype?
b. Why was the K^b transgene linked to a liver-specific promoter in these experiments?
c. What do these results suggest about the induction of B-cell tolerance to self-antigens?

10. Indicate whether each of the following statements is true or false. If you believe a statement is false, explain why.

a. Cytokines can regulate which branch of the immune system is activated.
b. Immunization with a hapten-carrier conjugate results in production of antibodies to both hapten and carrier epitopes.
c. All the antibodies secreted by a single plasma cell have the same idiotype and isotype.
d. If mice are immunized with HRBCs and then are immunized a day later with SRBCs, the antibody response to the SRBCs will be much higher than that achieved in control mice immunized only with SRBCs.

11. Four mice are immunized with antigen under the conditions listed below (a–d). In each case, indicate whether the induced serum antibodies will have high affinity or low affinity and whether they will be largely IgM or IgG.

a. A primary response to a low antigen dose
b. A secondary response to a low antigen dose
c. A primary response to a high antigen dose
d. A secondary response to a high antigen dose

12. DNA was isolated from three sources: liver cells, pre-B lymphoma cells, and IgM-secreting myeloma cells. Each DNA sample was digested separately with the restriction enzymes *Bam*HI and *Eco*RI, which cleave germ-line heavy-chain and κ light-chain DNA as indicated in part (a) of the figure below. The digested samples were analyzed by Southern blotting using a radiolabeled $C_\mu 1$ probe with the *Bam*HI digests (blot #1) and a radiolabeled C_κ probe with the *Eco*RI digests (blot #2). The blot patterns are illustrated in part (b) of the figure. Based on this information, which DNA sample (designated A, B, or C) was isolated from the (a) liver cells, (b) pre-B lymphoma cells, and (c) IgM-secreting plasma cells? Explain your assignments.

For use with Question 12