10 The Humoral Immune Response

Many pathogens multiply in the body's extracellular spaces, and even intracellular pathogens can spread by moving through the extracellular fluids. The extracellular spaces are protected by the **humoral immune response**, in which antibodies produced by B cells act to destroy extracellular microorganisms and their products, and prevent the spread of intracellular infections. As we introduced in Section 1-20, antibodies contribute to immunity in three main ways: **neutralization**, **opsonization**, and **complement activation** (Fig. 10.1). Antibodies can bind to pathogens and prevent their ability to enter and infect cells, and therefore are thus said to neutralize the pathogen; antibodies may also bind bacterial toxins, preventing their action or ability to enter cells. Antibodies also facilitate opsonization, the uptake of the pathogens by phagocytes, by binding to Fc receptors through their constant regions (C regions). Finally, antibodies bound to pathogens can activate proteins of the classical pathway of the complement system, as we described in Chapter 2. This can increase opsonization by placing other complement proteins onto the pathogen's surface, help recruit phagocytic cells to the site of infection, and activate the membrane-attack complex, which can directly lyse certain microorganisms by forming pores in their membranes. The choice of which effector mechanisms are used is influenced by the heavy-chain isotype of the antibodies produced, which determines their class (see Section 5-12).

In the first part of this chapter, we describe the interactions of naive B cells with antigen and with helper T cells that lead to the activation of B cells and antibody production. Some microbial antigens can provoke antibody production without T-cell help, but activation of naive B cells by antigens usually involves help from **T follicular helper** (T_{FH}) cells (see Section 9-20). Activated B cells then differentiate into antibody-secreting **plasma cells** and memory B cells. Most antibody responses undergo a process called affinity maturation, in which antibodies of greater affinity for their target antigen are produced by the somatic hypermutation of antibody variable-region (V-region) genes. We examine the molecular mechanism of somatic hypermutation and its immunological consequences, as well as class switching—a process that generates the different classes of antibodies that confer functional diversity on the antibody response. Both affinity maturation and class switching occur only in B cells and require T-cell help. In the second part of the chapter, we introduce the distributions and functions of various classes of antibody, in particular those secreted into mucosal sites. In the third part of the chapter, we discuss in detail how the Fc region of the antibody engages various effector mechanisms to contain and eliminate infections. Like the T-cell response, the humoral immune response produces immunological memory, and this is discussed in Chapter 11.

IN THIS CHAPTER

B-cell activation by antigen and helper T cells.

The distributions and functions of immunoglobulin classes.

The destruction of antibody-coated pathogens via Fc receptors.

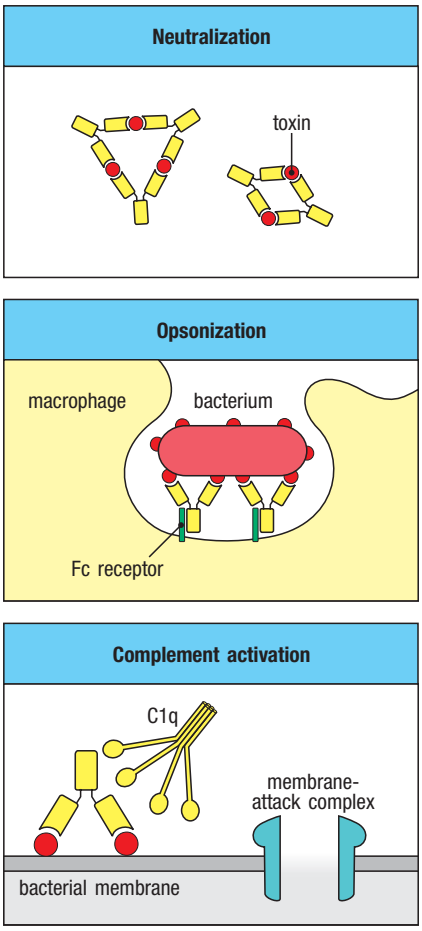


Fig. 10.1 Antibodies mediate the humoral immune response through neutralization, opsonization, and complement activation. After being secreted by plasma cells, antibodies protect the host from infection in three main ways. They can inhibit the toxic effects or infectivity of pathogens or their products by binding to them, a process called neutralization (top panel). When bound to pathogens, the antibody's Fc region can bind to Fc receptors on accessory cells, such as macrophages and neutrophils, helping these cells to ingest and kill the pathogen. This process is called opsonization (middle panel). Antibodies can trigger complement by activating C1, the first step in the classical complement pathway. Deposition of complement proteins enhances opsonization and can also directly kill certain bacterial cells by activating the membrane-attack complex (bottom panel).

B-cell activation by antigen and helper T cells.

The surface immunoglobulin that serves as the **B-cell receptor** (**BCR**) plays two roles in B-cell activation in response to pathogens. Like the antigen receptor on T cells, the BCR initiates a signaling cascade upon binding antigens derived from the microbe. In addition, the BCR can deliver the antigen to intracellular sites for antigen processing, so that antigenic peptides bound to MHC class II molecules can be returned to the B-cell surface. These peptide:MHC class II complexes are recognized by antigen-specific helper T cells that have already differentiated in response to the same pathogen. The effector T cells express surface molecules and cytokines that help the B cell to proliferate and to differentiate into antibody-secreting cells and into memory B cells, and a structure called the germinal center (see Section 10-6) is formed during an intermediate phase of the antibody response, before the emergence of long-term plasma cells that generate antibody or of memory B cells. Some microbial antigens can activate B cells directly in the absence of T-cell help, and the ability of B cells to respond directly to these antigens provides a rapid response to many important pathogens. However, the fine tuning of antibody responses to increase the affinity of the antibody for the antigen and the switching to most immunoglobulin classes other than IgM depend on the interaction of antigen-stimulated B cells with helper T cells and other cells in the peripheral lymphoid organs. Thus, antibodies induced by microbial antigens alone tend to have lower affinity and to be less functionally versatile than those induced with T-cell help.

10-1 Activation of B cells by antigen involves signals from the B-cell receptor and either T_{FH} cells or microbial antigens.

As we learned in Chapter 8, activation of naive T cells requires signals derived from the T-cell receptor as well as co-stimulatory signals provided by professional antigen-presenting cells. Similarly, in addition to signals derived from the B-cell receptor, naive B cells also require accessory signals that can arise either from a helper T cell or, in some cases, directly from microbial constituents (Fig. 10.2).

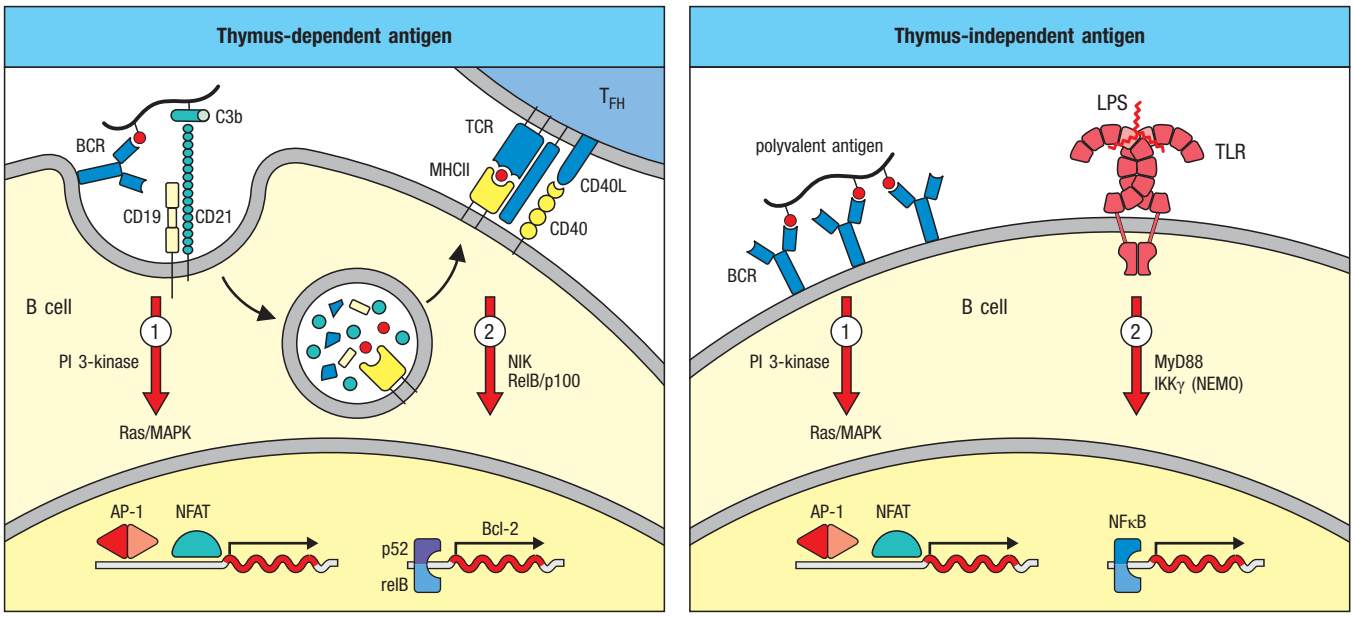


Fig. 10.2 A second signal is required for B-cell activation by either thymus-dependent or thymus-independent antigens. The first signal (indicated as 1) required for B-cell activation is delivered through its antigen receptor (BCR) and activates several pathways as described in Chapter 7. Signaling by the BCR is enhanced by the co-receptors CD21 and CD19, which interact with C3b on opsonized microbial surfaces. For thymus-dependent antigens (first panel), a second signal (indicated as 2) is delivered by a helper T cell (T_{FH}) that recognizes degraded fragments of the antigen as peptides bound to MHC class II molecules on the B-cell surface. CD40L on the T_{FH} cell binds to CD40 on the B cell, activating the non-canonical NFκB signaling pathway via NFκB-inducing kinase (NIK). This induces expression of pro-survival genes such as Bcl-2 (see Section 7-17). For thymus-independent antigens (second panel), a second signal can be delivered through Toll-like receptors that recognize antigen-associated TLR ligands, such as bacterial lipopolysaccharide (LPS) or bacterial DNA, as described in Chapter 3.

Protein antigens alone are unable to induce antibody responses in animals or humans who lack T cells, and they are therefore known as **thymus-dependent** or **TD antigens**, and typically involve antigen-specific T-cell help. The T cells involved are T_{FH} cells that reside in the lymphoid tissues and are not fully differentiated T_H1, T_H2, or T_H17 effector cells. To receive T-cell help, the B cell must display antigen on its surface in a form that a T cell can recognize. This occurs when antigen bound by surface immunoglobulin on the B cell is internalized and degraded within the B cell and peptides derived from it are returned to the cell surface in a complex with MHC class II molecules (see Fig. 10.2, first panel). When the T_{FH} cell recognizes these peptide:MHC complexes, it provides the B cell with signals that favor survival and induce proliferation. These signals include the activation of **CD40** on B cells by T_{FH} expression of its ligand, **CD40L** (CD154), and production of various cytokines by T_{FH} cells, including **IL-21** (Fig. 10.3). CD40 signaling activates the **non-canonical NFκB pathway** (see Section 7-23) and enhances B-cell survival by inducing the expression of anti-apoptotic molecules such as Bcl-2. IL-21 signaling activates STAT3 and enhances cellular proliferation and differentiation into plasma cells and memory B cells. Other cytokines produced by T_{FH} cells include IL-6, TGF-β, IFN-γ, and IL-4, which provide signals that can regulate the type of antibody produced, as we will see in Section 10-12. These cytokines are also made by other differentiated effector subsets (described in Chapter 9), but T_{FH} cells are distinct from these. For example, T_{FH} cells transcribe the IL-4 gene using regulatory elements that are independent of the transcription factors GATA-3 and STAT6, which are responsible for IL-4 production by T_H2 cells.

While B-cell responses to protein antigens rely on help from T cells, some microbial constituents can induce antibody production in the absence of helper T cells. These microbial antigens are known as **thymus-independent** or **TI antigens** because they can induce antibody responses in individuals who have no T lymphocytes. Such antigens are typically highly repetitive molecules, such as the polysaccharides of bacterial cell walls, and can cross-link the BCR on B cells. In such cases, a second signal can be derived from direct recognition of a common microbial constituent such as LPS that can activate TLR signaling in the B cell (see Fig. 10.2, second panel), activating the **NFκB pathway**, as described in Chapter 3. Thymus-independent antibody responses provide some protection against extracellular bacteria, and we will return to them later.

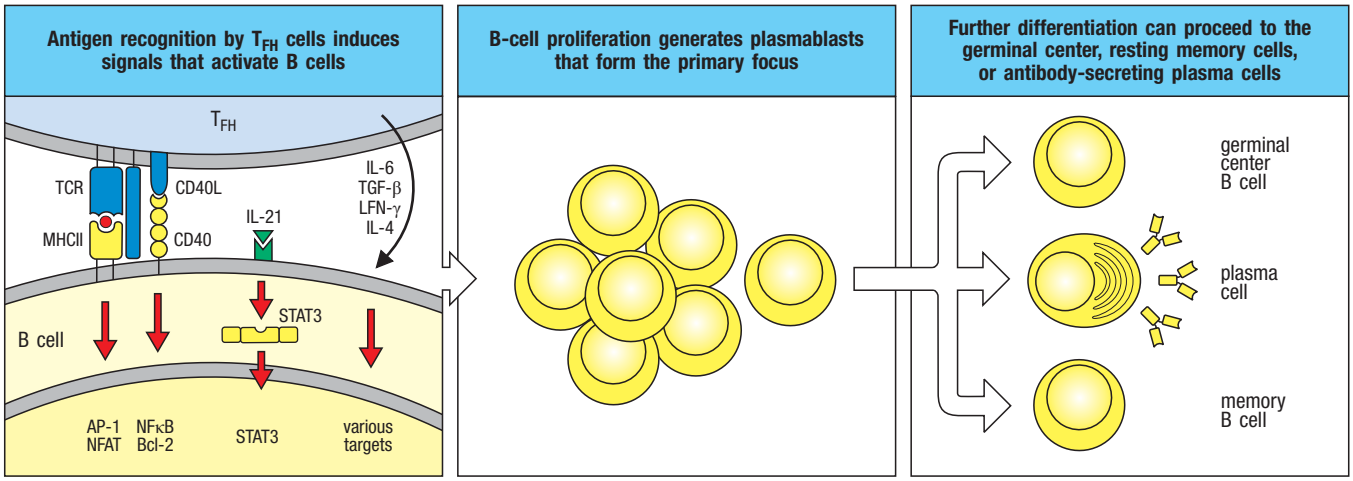


Fig. 10.3 T_{FH} cells provide several signals that activate B cells and control their subsequent differentiation. After antigen binding to the B-cell receptor delivers the first signal for B-cell activation (not shown), the T_{FH} cell delivers additional signals when it recognizes a peptide:MHC class II complex on the B-cell surface (first panel). Besides expression of CD40 ligand, the T_{FH} cell secretes several important cytokines. Included among them is IL-21, which activates the transcription factor STAT3 to enhance B-cell proliferation and survival. T_{FH} cells can also produce cytokines that will regulate isotype switching (see Section 10-12). After receiving these signals, activated B cells begin to proliferate (second panel), enter the germinal center, and eventually become plasma cells or memory B cells (third panel).

10-2 Linked recognition of antigen by T cells and B cells promotes robust antibody responses.

B-cell activation by antigens on microbial surfaces can be greatly stimulated by the concurrent deposition of complement on these pathogens. The **B-cell co-receptor complex** contains the cell-surface proteins CD19, CD21, and CD81 (see Fig. 7.27). When **CD21**, or complement receptor 2 (CR2), binds to the complement fragments C3d and C3dg that are deposited on microbial surfaces (see Section 2-13), it is brought near to the activated B-cell receptor bound to the same surface. CD21 and CD19 are associated with each other, and CD19 becomes phosphorylated by the activated B-cell receptor. This recruits PI 3-kinase, which then stimulates several downstream pathways, enhancing proliferation, differentiation, and antibody production (see Fig. 10.2, arrow 1). This effect is shown dramatically when mice are immunized with the experimental antigen hen egg-white lysozyme that is coupled to three linked molecules of C3dg. In this case the dose of modified lysozyme needed to induce antibody in the absence of added adjuvant is as little as 1/10,000 of that needed with the unmodified lysozyme.

For T-dependent antibody responses, the T cells involved are activated by the same antigen as is recognized by the B cells; this is called **linked recognition**. However, the peptide recognized by the T_{FH} cell is likely to differ from the protein epitope recognized by the B cell's antigen receptor. Natural antigens, such as viruses and bacteria, contain multiple proteins and carry both protein and carbohydrate epitopes. For linked recognition to occur, the peptide recognized by the T cell must be physically associated with the antigen recognized by the B cell's receptor, so that the B cell can take up and present the appropriate peptide to the T cell. For example, a B cell that recognizes an epitope on a viral coat protein will internalize the complete virus particle. The B cell can degrade multiple viral proteins into peptides for display on MHC class II molecules on the B-cell surface. CD4 T cells specific for such viral peptides may have been activated by dendritic cells earlier in the infection, and some will have differentiated into T_{FH} cells. When these T_{FH} cells are activated by B cells presenting their peptide, they are stimulated to provide specific signals that help B cells to generate antibodies against the viral coat protein (**Fig. 10.4**).

Linked recognition relies on the concentration of the appropriate peptide for presentation by MHC class II molecules on the B-cell surface. B cells whose B-cell receptor binds a particular antigen are 10,000 times more efficient at displaying peptide fragments of that antigen on their MHC class II molecules than are B cells that process the antigen through macropinocytosis alone. Linked recognition was originally discovered through studies of the production of antibodies against haptens, which are small chemical groups that cannot elicit antibody responses on their own (see Appendix I, Section A-1). But haptens that are coupled to a carrier protein become immunogenic—known as the **hapten carrier effect**—for two reasons. The protein can carry multiple hapten groups, allowing it to cross-link B-cell receptors. Also, T cells that are activated against peptides of the carrier protein can become T_{FH} cells and strengthen the antibody response to the hapten. Accidental coupling of a hapten to a protein is responsible for the allergic responses shown by many people to the antibiotic penicillin, which reacts with host proteins to form a coupled hapten that can stimulate an antibody response, as we will learn in Chapter 14.

Linked recognition works to preserve self-tolerance, since autoreactive antibodies will arise only if self-reactive T_{FH} and self-reactive B cells are present at the same time. This is discussed further in Chapter 15. Vaccine design can take advantage of linked recognition, as in the vaccine used to immunize infants against *Haemophilus influenzae* type b (see Section 16-26).

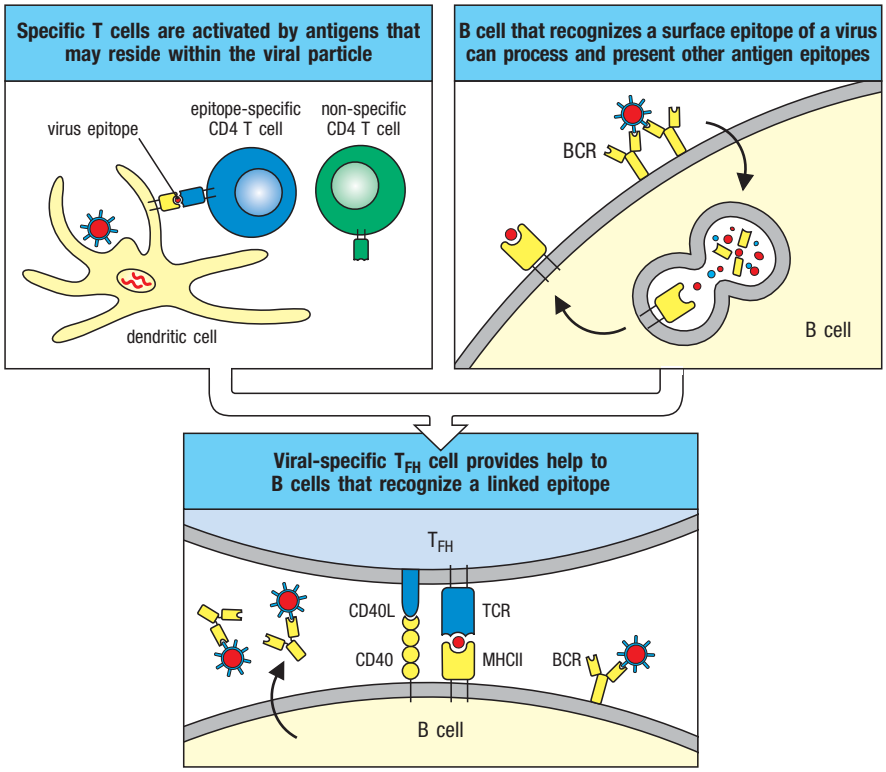


Fig. 10.4 T cells and B cells must recognize antigens contained within the same molecular complex in order to interact. In this example, an internal viral protein harbors a peptide epitope (shown as red) that is presented by MHC class II molecules and is recognized by a CD4 T cell. The virus also harbors a native epitope on an external viral coat protein (shown as blue) that is recognized by the surface immunoglobulin on a B cell. If the virus is captured and presented by a dendritic cell, a peptide-specific CD4 T cell (blue) becomes activated (top left panel), whereas nonspecific T cells (green) remain inactive. If the virus is recognized by a specific B cell (top right panel), peptides derived from internal viral proteins are processed and presented by MHC class II molecules. When the activated T cell recognizes its peptide on this B cell (bottom panel), the T cell will deliver various accessory signals to the B cell that promote antibody production against the coat protein. This process is known as linked recognition.

10-3 B cells that encounter their antigens migrate toward the boundaries between B-cell and T-cell areas in secondary lymphoid tissues.

The frequency of naive lymphocytes specific for a particular antigen is extremely low (less than 1 in 10,000). Thus, the chance of a random encounter between a T and a B cell with the same antigen specificity should be less than 1 in 10^8, making it remarkable that B cells ever interact with T_{FH} cells with similar antigen specificity. For these reasons, linked recognition requires a precise regulation of the migration of activated B and T cells—orchestrated by several sets of ligands and receptors—into specific locations within the lymphoid tissues, which serves to increase the chances of a productive interaction (Fig. 10.5).

Naive T cells and B cells express the **sphingosine 1-phosphate receptor, S1PR1**, which they use to egress from the peripheral lymphoid tissues (see Section 9-7). However, before they exit, they are retained and initially occupy two distinct zones, the **T-cell areas** and the **primary lymphoid follicles** (or B-cell areas or B-cell zones), respectively (see Figs 1.18–1.20). These zones are established by different patterns of chemokine receptor expression and chemokine production. Naive T cells express the chemokine receptor **CCR7**, and localize to zones where its ligands, **CCL19** and **CCL21**, are highly expressed by stromal cells and dendritic cells (see Section 9-3). Circulating naive B cells express **CXCR5**, and when they migrate into lymphoid tissues, they enter the primary lymphoid follicles, where the chemokine **CXCL13** is abundant. Within the follicle, stromal cells and a specialized cell type, the **follicular dendritic cell** (**FDC**), secrete CXCL13. The FDC is a nonphagocytic cell of nonhematopoietic origin that bears numerous long processes; it functions by trapping antigen using complement receptors on its cell surface for access by B cells in the follicle.

Once in the follicle, naive B cells encounter the soluble TNF-family cytokine **BAFF** (see Section 8-8), which is secreted by FDCs, stromal cells, and dendritic cells and which acts as a survival factor for B cells. BAFF can act through three

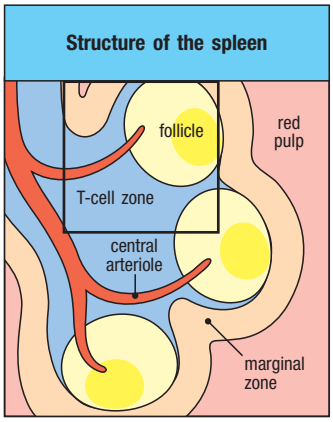
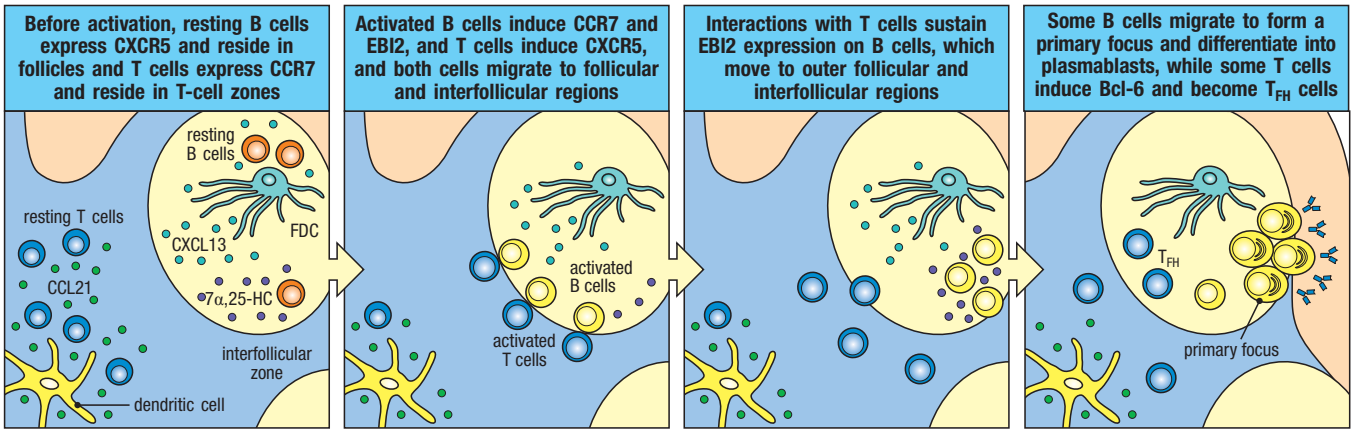


Fig. 10.5 Antigen-binding B cells meet T cells at the border between the T-cell area and a B-cell follicle in secondary lymphoid tissues. Antigens enter the spleen from the blood and collect in T-cell zones and follicles (first panel). Naive CCR7-positive T cells and CXCR5-positive B cells migrate to distinct regions where the chemokines CCL19 and CCL21, or CXCL13 and 7α, 25-hydroxycholesterol (7α, 25-HC), respectively, are being produced (second panel). If a B cell encounters its antigen, either on a follicular dendritic cell (FDC) or a macrophage, it increases expression of CCR7 and migrates toward the border with the T-cell zone (third panel). T cells activated by antigen-presenting dendritic cells induce expression of CXCR5 and migrate to this same border, where linked recognition induces further B-cell proliferation. After 2 to 3 days, B cells reduce expression of CCR7, but retain EBI2 and migrate in response to 7α, 25-HC to the outer follicle and interfollicular regions (fourth panel). After another day or so, some B cells cluster in the interfollicular regions near the red pulp, proliferate, and differentiate into plasmablasts, forming a primary focus with terminal differentiation into antibody-secreting plasma cells. T cells that retain EBI2 expression may remain in the follicle and induce Bcl-6 expression to become T_{FH} cells that participate with B cells there to form a germinal center reaction.



receptors, but its major actions in promoting survival are through **BAFF-R** (Fig. 10.6). BAFF-R signals through **TRAF3** (see Section 3-7) to activate the non-canonical NFκB pathway, as described for CD40 (see Fig. 7.31), and, like CD40 signaling, induces expression of Bcl-2. Two other receptors for BAFF are **TACI** and **BCMA**, although BAFF has a relatively low affinity for BCMA. TACI and BCMA also bind the related cytokine **APRIL**, and they signal through TRAF2, 5, and 6 to induce signaling pathways involved in B-cell activation.

Antigens derived from microorganisms and viruses are transported into lymph nodes via the afferent lymph, and into the spleen via the blood. Opsonized antigens bearing C3b or C3dg accumulate in the B-cell follicles because they are trapped by complement receptors CR1 and CR2 expressed on the surface of FDCs. Opsonized particulate antigens can also be taken up by specialized macrophages residing in the **subcapsular sinus** (SCS) of lymph nodes and the **marginal sinus** of the spleen, regions that are both adjacent to the B-cell

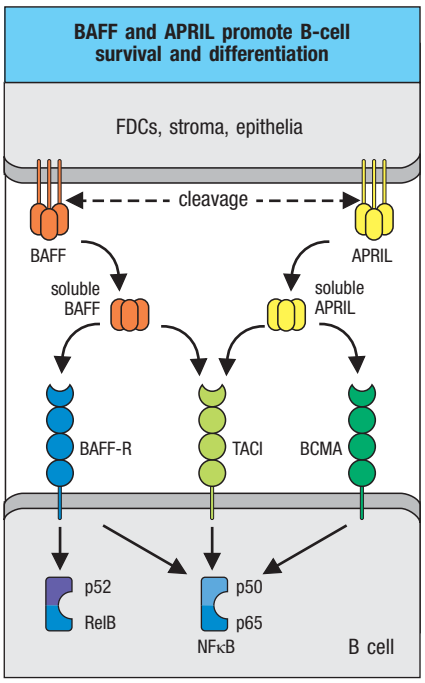


Fig. 10.6 BAFF and APRIL promote B-cell survival and regulate differentiation. BAFF (B-cell activating factor, also called B-lymphocyte stimulator, or BLyS) and APRIL (a proliferation-inducing ligand) are both members of the TNF superfamily of cytokines. They are initially produced as membrane-bound trimers by several cell types. BAFF is produced by FDCs and other cells in the B-cell follicle, where it supports B-cell survival. Its main receptor, BAFF-R, signals in a manner similar to CD40 (see Fig. 7.31) through TRAF3 and NIK to activate both the non-canonical NFκB pathway, leading to the RelB:p52 transcription factor, and the canonical p50:p65 NFκB pathway. BAFF also binds to the receptors TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor) and BCMA (B-cell maturation antigen), although its affinity for the latter is relatively weak. These receptors activate the canonical NFκB pathway.

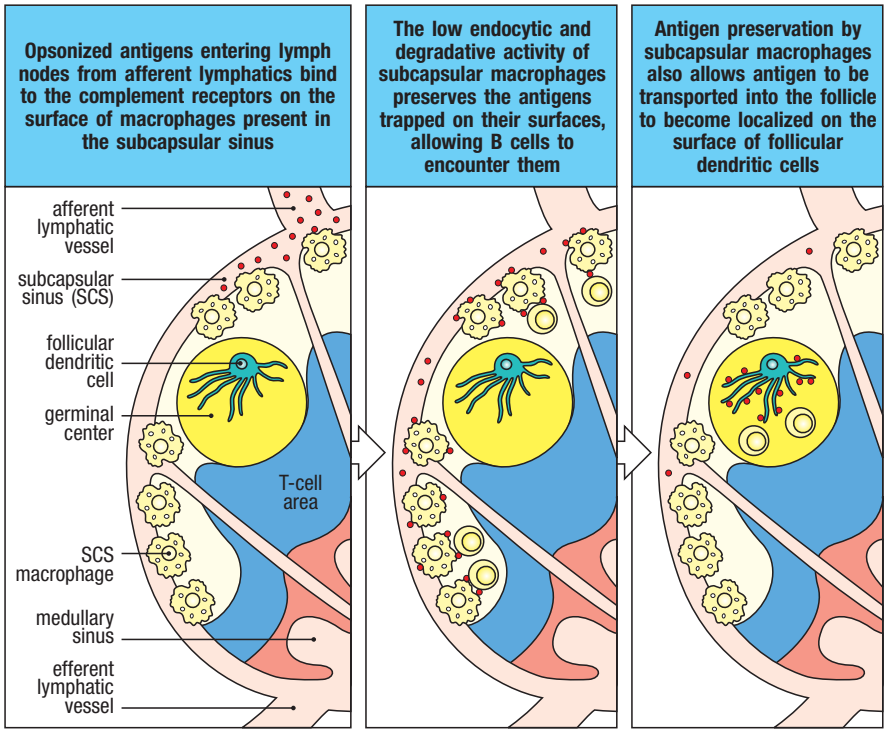


Fig. 10.7 Opsonized antigens are captured and preserved by subcapsular sinus macrophages. Macrophages residing in the lymph node subcapsular sinus (SCS) express complement receptors 1 and 2 (CR1 and CR2, respectively), are poorly endocytic, and have reduced levels of lysosomal enzymes compared with macrophages in the medulla. Opsonized antigen arriving from the afferent lymphatics binds to CR1 and CR2 on the surface of SCS macrophages. Instead of being completely degraded by these macrophages, some antigen is retained on the cell surface, where it can be presented and transferred to the surface of follicular B cells. B cells are then able to transport the antigen into the follicle, where it can be trapped on the surfaces of follicular dendritic cells.

follicles (Fig. 10.7). These macrophages seem to retain the antigen on their surface rather than ingesting and degrading it. These antigens can then be sampled and carried by antigen-specific follicular B cells. B cells of any antigen specificity could also acquire antigen from these macrophages via their complement receptors and transport it within the follicle. In the spleen, marginal zone B cells shuttle between that site and the follicle, carrying antigen trapped in the marginal zone for deposition on FDCs. SCS macrophages can also actively function to restrict the dissemination of infection. In mice, infection of these macrophages in lymph nodes by vesicular stomatitis virus (VSV), a relative of rabies virus, triggers the cells to produce interferon and to recruit plasmacytoid dendritic cells (pDCs). Type I interferon produced by pDCs restricts further viral spread, which would otherwise eventually pass on to the central nervous system.

After a naive follicular B cell first encounters specific antigen displayed by FDCs or macrophages, within a few hours it will become positioned in the outer follicles of lymphoid tissue close to the sites where antigen enters the lymph node or spleen. This positioning is orchestrated by the B cell's expression of a chemokine receptor, **EBI2** (GPR183), whose ligands are oxysterols such as 7α, 25-dihydroxycholesterol. The precise source of these ligands is still unclear, but they are abundant in the outer follicular and interfollicular regions. After sampling antigens there for 6 hours to 1 day, the B cell induces expression of CCR7, which functions together with EBI2 to distribute activated B cells along the interface between the B-cell follicle and the T-cell zone, where CCL21 is expressed.

During an immune response, T cells are activated within the T-cell zones by dendritic cells. When naive T cells are activated, some will proliferate, differentiate into effector cells, downregulate expression of S1P1, and exit the lymphoid tissue. However, others will induce expression of CXCR5 and migrate to the border with the B-cell follicle. There, T cells can encounter B cells activated during the same response, increasing the chance that they might recognize linked antigens presented by activated B cells that have recently moved to this location (see Fig. 10.5).

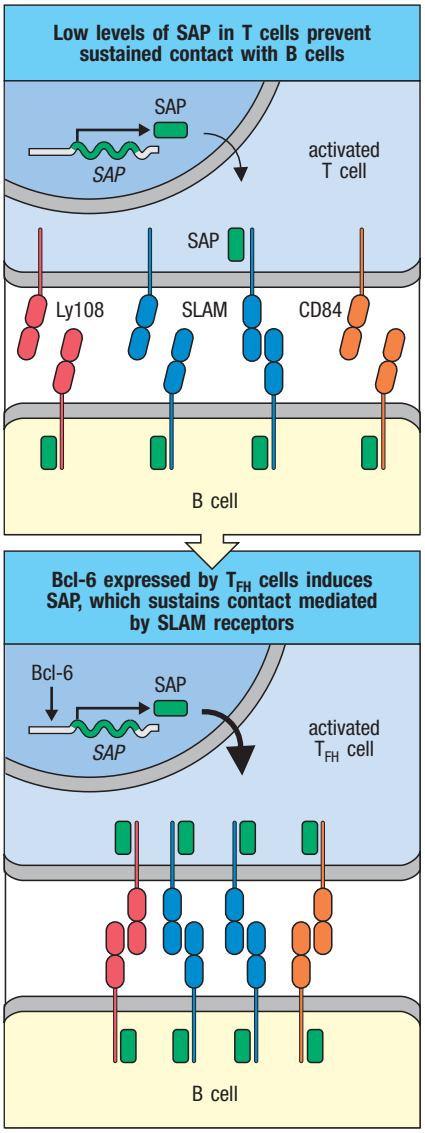


Fig. 10.8 Induction of SAP in T_{FH} cells allows SLAM family receptors to mediate sustained contact with B cells. The SLAM receptor family members SLAM, Ly108, and CD84 are expressed on T cells and B cells and mediate homotypic interactions that lead to adhesion between cells. SLAM can also enhance signaling by the T-cell receptor to augment production of cytokines such as IL-21 that help B cells. The SLAM-associated molecule SAP is a signaling adapter that is required for one SLAM receptor to sustain binding with another. T cells initially express SAP at low levels that are insufficient for sustained adhesion between T and B cells. Fully differentiated T_{FH} cells express high levels of the transcription factor Bcl-6, which induces higher levels of SAP expression. This level is sufficient to sustain cell–cell interactions and allow for the delivery of CD40L and cytokine signals to B cells.

10-4 T cells express surface molecules and cytokines that activate B cells, which in turn promote T_{FH}-cell development.

When T_{FH} cells encounter an activating peptide presented by B cells, the T_{FH} cells respond by expressing receptors and cytokines that in turn activate B cells. As mentioned above, the induced expression of CD40L on T_{FH} cells activates CD40 on B cells to increase B-cell survival, and also induces B-cell expression of co-stimulatory molecules, especially those of the B7 family. Activated T cells also express **CD30 ligand** (**CD30L**), which binds to **CD30** expressed on B cells and promotes B-cell activation. Mice lacking CD30 show reduced proliferation of activated B cells in lymphoid follicles and weaker secondary humoral responses than normal. T_{FH} cells also secrete several cytokines that regulate B-cell proliferation and antibody production. Primary among these is **IL-21**, which is produced early in immune responses by T_{FH} cells and which activates the transcription factor **STAT3** in B cells to support proliferation and differentiation. IL-21 exerts similar autocrine effects on T_{FH} cells. Later in the antibody response, T_{FH} cells will produce other cytokines, such as IL-4 and IFN-γ, that are characteristic of the other T helper subsets (described in Chapter 9). These will impact B-cell differentiation, particularly class switching, as we discuss later.

The ability of T_{FH} cells to successfully deliver these signals to B cells depends on intimate contact between these cells. Specific adhesion molecules, including several Ig superfamily receptors of the **SLAM** (**signaling lymphocyte activation molecule**) family, are involved that prolong and stabilize cell–cell contact. T_{FH} cells and B cells both express SLAM (CD150), **CD84**, and **Ly108**, which promote cell adhesion through homotypic binding interactions (Fig. 10.8). The cytoplasmic regions of these SLAM family receptors all interact with an adaptor protein, **SAP** (SLAM-associated protein), which is expressed highly by T_{FH} cells and which is necessary for prolonging cell–cell contact mediated by these receptors. The SAP gene is inactivated in **X-linked lymphoproliferative syndrome**, which is associated with a T-cell and NK-cell lymphoproliferative disorder and with a defect in antibody production due to failed interactions between T_{FH} cells and B cells in the germinal center, discussed below. The regulated migration of activated B cells and T_{FH} cells to the same location in the peripheral lymphoid organ increases the chance that linked recognition will occur and deliver appropriate help for B-cell differentiation. Antigen-stimulated B cells that fail to interact with T cells that recognize the same antigen die within 24 hours.

This first interaction between T and B cells not only provides important help to B cells, but also influences T-cell differentiation by signals provided by the B cell. Activated B cells express **ICOSL**, a member of the B7 family of co-stimulatory molecules and a ligand for **ICOS** (inducible co-stimulatory protein), which is expressed by T cells. This T- and B-cell interaction, provided by linked recognition, activates ICOS signaling in T cells and is important for the completion of T_{FH} differentiation (see Section 7-21), leading to induction of the transcription factors **Bcl-6** and **c-Maf**. These transcription factors are required for SAP production and the consequent sustained contact between B and T_{FH} cells.

10-5 Activated B cells differentiate into antibody-secreting plasmablasts and plasma cells.

After their initial encounter, B cells that have received T-cell help migrate from the follicle border to continue to proliferate and differentiate. Two to three days after activation, B cells begin to decrease expression of CCR7 and to increase expression of EBI2 (see Fig. 10.5). Decreased expression of CCR7 causes B cells to move away from the boundary with the T-cell zone: EBI2

directs their migration back to the interfollicular regions and the subcapsular sinus in the lymph nodes, or, in the spleen, to the splenic bridging channels, a region between the T-cell area and the red pulp. Here, some B cells will form an emerging aggregate of differentiating B cells called the **primary focus**, which in lymph nodes is located in the medullary cords, where lymph drains out of the node, and in the spleen can be seen as extrafollicular foci in the splenic red pulp. Primary foci are apparent by about 5 days after an infection or immunization with an antigen not previously encountered.

B cells proliferate in the primary focus for several days, and this constitutes the first phase of the primary humoral immune response. Some of these proliferating B cells differentiate into antibody-synthesizing **plasmablasts** in the primary focus. Not all B cells activated by the initial interaction with T_{FH} cells will move into the primary focus. Some will migrate into the lymphoid follicle, where they may eventually differentiate into plasma cells, as described below. Plasmablasts are cells that have begun to secrete antibody, yet are still dividing and express many of the characteristics of activated B cells that allow their interaction with T cells. After a few more days, the plasmablasts in the primary focus stop dividing and may eventually die. Subsequently, long-lived plasma cells will develop and migrate to the bone marrow, where they will continue antibody production. Since many long-lived plasma cells are generated long after the primary focus has dissipated, it is likely that they do not arise directly from plasmablasts in the primary focus, but rather from B cells that entered the germinal center reaction.

The properties of resting B cells, plasmablasts, and plasma cells are compared in Fig. 10.9. The differentiation of a B cell into a plasma cell is accompanied by many morphological changes that reflect a commitment to the production of large amounts of secreted antibody, which can constitute up to 20% of all the protein synthesized by a plasma cell. Plasmablasts and plasma cells have a prominent perinuclear Golgi apparatus and an extensive rough endoplasmic reticulum that is rich in immunoglobulin molecules that are being synthesized and exported into the lumen of the endoplasmic reticulum for secretion. Plasmablasts have relatively large numbers of B-cell receptors on the cell surface, whereas plasma cells have many fewer. This low level of surface immunoglobulin on plasma cells may still be physiologically important, since their survival seems to be determined in part by their ability to continue to bind antigen. Plasmablasts still express B7 co-stimulatory molecules and MHC class II molecules; by contrast, plasma cells turn down the expression of MHC class II molecules. Nevertheless, T cells still provide important signals for plasma-cell differentiation and survival, such as IL-6 and CD40 ligand.

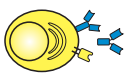
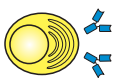
	Intrinsic properties			Inducible by antigen stimulation		
B-lineage cell	Surface Ig	Surface MHC class II	High-rate Ig secretion	Growth	Somatic hyper-mutation	Class switch
 Resting B cell	High	Yes	No	Yes	Yes	Yes
 Plasmablast	High	Yes	Yes	Yes	Unknown	Yes
 Plasma cell	Low	Yes	Yes	No	No	No

Fig. 10.9 Plasma cells secrete antibody at a high rate but can no longer respond to antigen. Resting naive B cells have membrane-bound immunoglobulin (usually IgM and IgD) and MHC class II molecules on their surface. Although their V genes do not carry somatic mutations, B cells can take up antigen and present it to helper T cells. The T cells in return induce the B cells to proliferate and to undergo isotype switching and somatic hypermutation, but B cells do not secrete significant amounts of antibody during this period. Plasmablasts have an intermediate phenotype. They secrete antibody but retain substantial surface immunoglobulin and MHC class II molecules and so can continue to take up and present antigen to T cells. Plasmablasts early in the immune response and those activated by T-independent antigens have usually not undergone somatic hypermutation and class switching, and therefore secrete IgM. Plasma cells are terminally differentiated cells that secrete antibodies. Plasma cells have very low levels of surface immunoglobulin but can express MHC class II molecules and may suppress T_{FH} activity in a negative feedback pathway while differentiating. Early in the immune response they differentiate from unswitched activated B cells and secrete IgM; later in the response they derive from activated B cells that entered the germinal center reaction and underwent class switching and somatic hypermutation. Plasma cells have lost the ability to change the class of their antibody or undergo further somatic hypermutation.

X-linked Lymphoproliferative Syndrome

Recent evidence indicates that even the low level of MHC class II molecules expressed on plasma cells functions to present cognate antigen to T_{FH} cells, and acts to suppress IL-21 production and Bcl-6 expression, thus acting as a feedback pathway to regulate ongoing B-cell responses. While some plasma cells survive for only days to a few weeks after their final differentiation, others are very long lived and account for the persistence of antibody responses.

10-6 The second phase of a primary B-cell immune response occurs when activated B cells migrate into follicles and proliferate to form germinal centers.

Not all B cells activated by T_{FH} cells will migrate to the outer follicle and eventually establish a primary focus. Instead, some move into a primary lymphoid follicle together with their associated T_{FH} cells (Fig. 10.10), where they continue to proliferate and ultimately form a **germinal center**; follicles with germinal centers are also called **secondary lymphoid follicles**. Downregulating EBI2 by B cells appears to favor germinal center formation. In mice lacking EBI2 expression in B cells, antigen-activated B cells remain near the border with the T-cell zone and are able to form germinal centers, but generate fewer plasmablasts.

Germinal centers are composed mainly of proliferating B cells, but antigen-specific T cells make up about 10% of germinal center lymphocytes and provide indispensable help to the B cells. The germinal center is an area of active cell division that forms within a surrounding region of resting B cells in the primary follicle. Proliferating germinal center B cells displace the resting B cells toward the periphery of the follicle, forming a **mantle zone** of resting cells around the two distinguishable areas of activated B cells, called the **light zone** and the **dark zone** (Fig. 10.11, left panel). The germinal center grows in size as the immune response proceeds, and then shrinks and finally disappears when the infection is cleared. Germinal centers are present for about 3–4 weeks after initial antigen exposure.

The primary focus and the germinal center reaction differ in the quality of antibody that they produce. Plasmablasts, germinal center B cells, and early memory B cells begin to emerge during the first 4–5 days of an immune response. Plasmablasts in primary foci primarily secrete antibodies of the IgM isotype that offer some immediate protection. In contrast, B cells in the germinal center reaction undergo several processes that produce antibodies that are more effective in eliminating infections. These processes include **somatic hypermutation**, which alters the V regions of immunoglobulin genes (see below), and which enables a process called **affinity maturation**, which selects for the survival of mutated B cells that have a high affinity for the antigen.

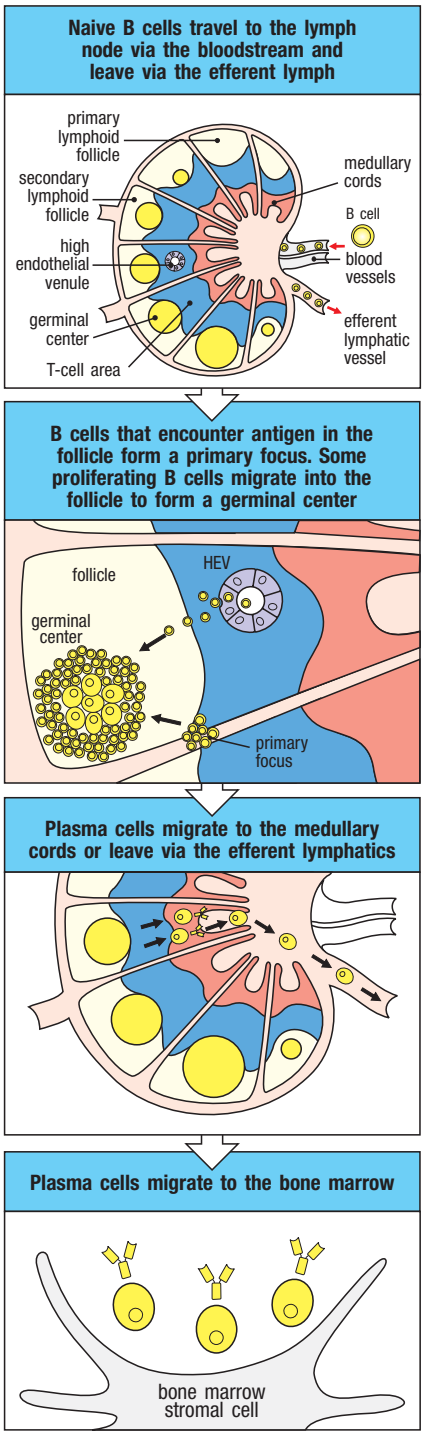


Fig. 10.10 Activated B cells form germinal centers in lymphoid follicles. Activation of B cells in a lymph node is shown here. Top panel: naive circulating B cells enter lymph nodes from the blood via high endothelial venules (HEV) and are attracted by chemokines into the primary lymphoid follicle; if these B cells do not encounter antigen in the follicle, they leave via the efferent lymphatic vessel. Second panel: B cells that have bound antigen move to the border with the T-cell area, where they may encounter activated helper T cells specific for the same antigen; these T cells interact with the B cells and activate them to start proliferation and differentiation into plasmablasts. Some B cells activated at the T-cell–B-cell border migrate to form a primary focus of antibody-secreting plasmablasts in the interfollicular regions (spleen) or medullary cords (lymph nodes), whereas others move back into the follicle, where they continue to proliferate and form a germinal center. Germinal centers are sites of sustained B-cell proliferation and differentiation. Follicles in which germinal centers have formed are known as secondary follicles. Within the germinal center, B cells begin their differentiation into either antibody-secreting plasma cells or memory B cells. Third and fourth panels: plasma cells leave the germinal center and migrate to the medullary cords, or leave the lymph node altogether via the efferent lymphatics and migrate to the bone marrow.

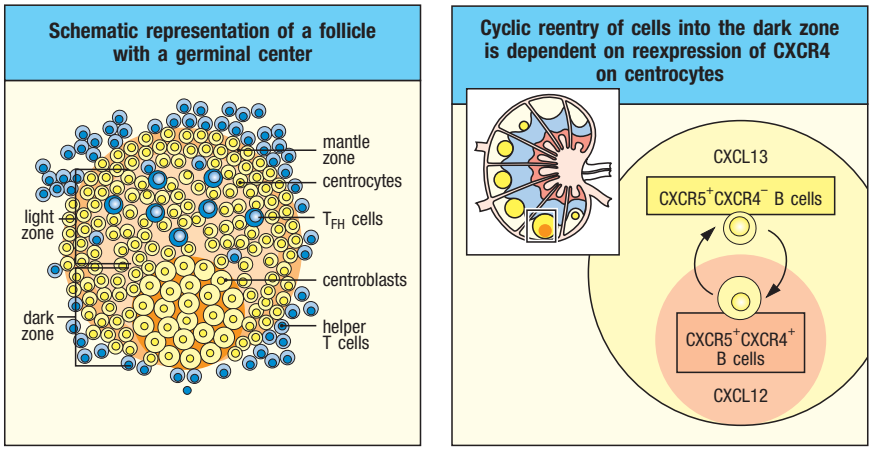
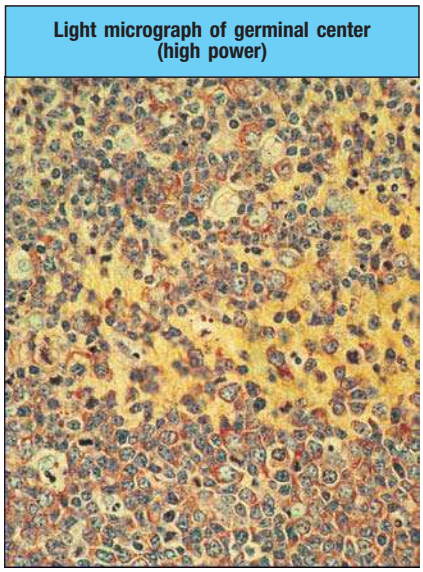


Fig. 10.11 The structure of a germinal center. The germinal center is a specialized microenvironment in which B-cell proliferation, somatic hypermutation, and selection for strength of antigen binding all occur. Closely packed centroblasts, which express CXCR4 and CXCR5, form the 'dark zone' of the germinal center; the less densely packed 'light zone' contains centrocytes, which express only CXCR5. Stromal cells in the dark zone produce CXCL12, which attracts the CXCR4-expressing centroblasts. Cyclic reentry describes the process by which B cells can lose and gain expression of CXCR4 and thus move from the light zone to the dark zone and back again.

In addition, **class switching** allows the selected B cells to produce antibodies with a variety of effector functions. These B cells will differentiate either into plasma cells that secrete higher-affinity and class-switched antibody in the latter part of the primary immune response, or into memory B cells as described in Chapter 11.

B cells in the germinal center divide rapidly, every 6–8 hours. Initially, these rapidly proliferating B cells, called **centroblasts**, express the chemokine receptors CXCR4 and CXCR5 but markedly reduce their expression of surface immunoglobulin, particularly of IgD. Centroblasts proliferate in the dark zone of the germinal center, named for its densely packed appearance (Fig. 10.12). Stromal cells in the dark zone produce **CXCL12** (SDF-1), a ligand for CXCR4 that acts to retain centroblasts in this region. As time goes on, some centroblasts reduce their rate of cell division, enter the growth phase, pausing at the G_2/M phase of the cell cycle, reduce CXCR4 expression, and begin to produce higher levels of surface immunoglobulin. These B cells are termed **centrocytes**. The loss of CXCR4 allows centrocytes to move into the light zone, a less densely packed area containing abundant FDCs that produce the chemokine CXCL13 (BLC), a ligand for CXCR5 (see Fig. 10.11, right panel). The B cells proliferate in the light zone, but to a lesser extent than in the dark zone.



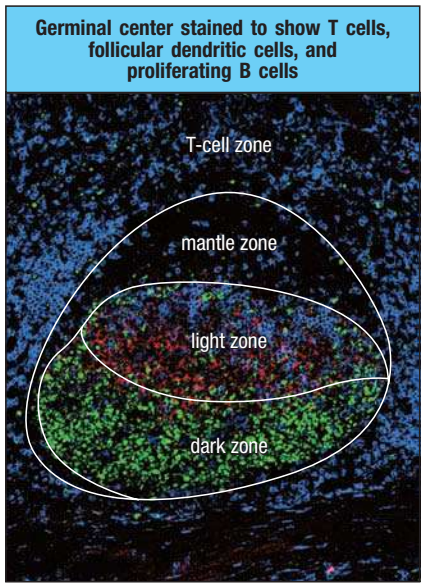


Fig. 10.12 Germinal centers are sites of intense cell proliferation and cell death. The photomicrograph (first panel) shows a section through a human tonsillar germinal center. Closely packed centroblasts, seen in the lower part of this photomicrograph, form the so-called dark zone of the germinal center. Above this region is the less densely packed light zone. The second panel shows immunofluorescent staining of a germinal center. B cells are found in the dark zone, light zone, and mantle zone. Proliferating cells are stained green for Ki67, an antigen expressed in nuclei of dividing cells, revealing the rapidly proliferating centroblasts in the dark zone. The dense network of follicular dendritic cells, stained red, mainly occupies the light zone. Centrocytes in the light zone proliferate to a lesser degree than centroblasts. Small recirculating B cells occupy the mantle zone at the edge of the B-cell follicle. Large masses of CD4 T cells, stained blue, can be seen in the T-cell zones, which separate the follicles. There are also significant numbers of T cells in the light zone of the germinal center; CD4 staining in the dark zone is associated mainly with CD4-positive phagocytes, that digest B cells that die there. Photographs courtesy of I. MacLennan.

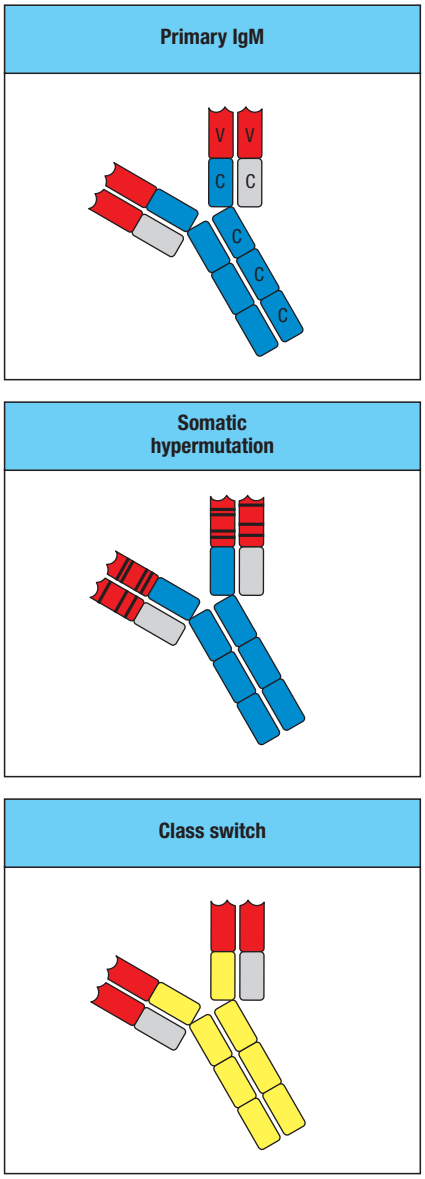


Fig. 10.13 The primary antibody repertoire is diversified by three processes that modify the rearranged immunoglobulin gene. First panel: the primary antibody repertoire is initially composed of IgM-containing variable regions (red) produced by V(D)J recombination and constant regions (blue) from the μ gene segment. The range of reactivity of this primary repertoire can be further modified by somatic hypermutation, by class switch recombination at the immunoglobulin loci, and in some species by gene conversion (not shown). Second panel: somatic hypermutation results in mutations (shown as black lines) being introduced into the heavy-chain and light-chain V regions (red), altering the affinity of the antibody for its antigen. Third panel: in class switch recombination, the initial μ heavy-chain C regions (blue) are replaced by heavy-chain regions of another isotype (shown as yellow), modifying the effector activity of the antibody but not its antigen specificity.

10-7 Germinal center B cells undergo V-region somatic hypermutation, and cells with mutations that improve affinity for antigen are selected.

Somatic hypermutation introduces mutations that change anywhere from one to a few amino acids in the immunoglobulin, producing closely related B-cell clones that differ subtly in specificity and antigen affinity (Fig. 10.13). These mutations in the V genes are initiated by an enzyme called **activation-induced cytidine deaminase**, or **AID**, which is expressed only by germinal center B cells. Before describing the enzymatic mechanisms initiated by AID, we first present a general overview of this process in which random mutations can improve antibody affinity.

The immunoglobulin V-region genes accumulate mutations at a rate of about one base pair change per 10^3 base pairs per cell division, while the rate of mutations in the rest of the cell's DNA is much lower: around one base pair change per 10^{10} base pairs per cell division. Somatic hypermutation also affects some DNA flanking the rearranged V gene, but does not generally extend into the C-region exons. Since each V region is encoded by about 360 base pairs and about three out of every four base changes will alter the amino acid encoded, there is about a 50% chance during each B-cell division that a mutation will occur to the receptor.

The point mutations accumulate in a stepwise manner as the descendants of each B cell proliferate in the germinal center to form B-cell clones (Fig. 10.14). An altered receptor can affect the ability of a B cell to bind antigen and thus will affect the fate of the B cell in the germinal center. Most mutations have a negative impact on the ability of the B-cell receptor to bind the original antigen, by preventing the correct folding of the immunoglobulin molecule or by blocking the complementarity-determining regions from binding antigen. Detrimental mutations may alter conserved framework regions (see Fig. 4.7) and disrupt basic immunoglobulin structure. Cells that harbor such detrimental mutations are eliminated by apoptosis in a process of negative selection, either because they can no longer make a functional B-cell receptor or because they cannot take up antigen as well as sibling B cells (Fig. 10.15). Germinal centers are filled with apoptotic B cells that are quickly engulfed by macrophages, giving rise to the characteristic **tingible body macrophages**. These contain dark-staining nuclear debris in their cytoplasm. Negative selection is implied by the relative scarcity of amino acid replacements in the framework regions, reflecting the loss of cells that had mutated any one of the many residues that are critical for immunoglobulin V-region folding. This process prevents rapidly dividing B cells from expanding to numbers that would overwhelm the lymphoid tissues. Less frequently, mutations may improve the affinity of a B-cell receptor for antigen, and these mutations will be selectively expanded (see Fig. 10.15) because the cells expressing receptors with such mutations will have an increased survival rate compared with low-affinity cells. Positive selection is evident in the accumulation of numerous amino acid replacements in the complementarity-determining regions, which determine antibody specificity

MOVIE 10.1

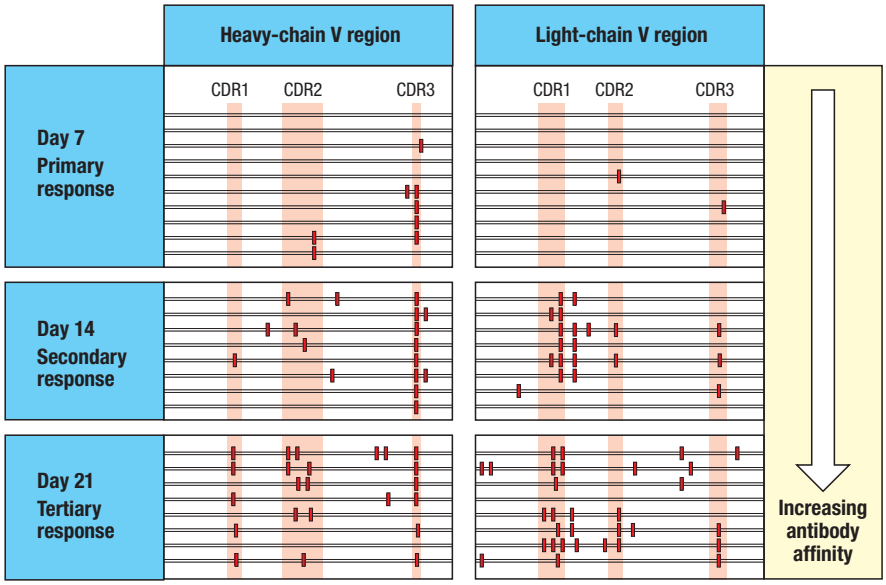


Fig. 10.14 Somatic hypermutation introduces mutations into the rearranged immunoglobulin variable (V) regions that improve antigen binding. The process of somatic hypermutation can be tracked by sequencing immunoglobulin V regions from hybridomas (clones of antibody-producing cells; see Appendix I, Section A-7) established at different time points after the experimental immunization of mice. The result of one experiment is depicted here. Each V region sequenced is represented by a horizontal line. The complementarity-determining regions CDR1, CDR2, and CDR3 are shown by pink shading. Mutations that change the amino acid sequence are represented by red bars. Within a few days of immunization, the V regions within a particular clone of responding B cells begin to acquire mutations, and over the course of the next week more mutations accumulate (top panels). B cells whose V regions have accumulated deleterious mutations and can no longer bind antigen die. B cells whose V regions have acquired mutations that improve the affinity of the B-cell receptor for antigen are able to compete more effectively for antigen, and receive signals that drive their proliferation and expansion. The antibodies they produce also have this improved affinity. This process of mutation and selection can continue in the lymph node germinal center through multiple cycles in response to secondary and tertiary immune responses elicited by further immunization with the same antigen (center and bottom panels). In this way, the antigen-binding efficiency of the antibody response is improved over time.

and affinity (see Fig. 10.14), a process we discuss in the next section. The result of selection for enhanced binding to antigen is that the nucleotide changes that alter amino acid sequences, and thus protein structure, tend to be clustered in the CDRs of the immunoglobulin V-region genes, whereas silent, or neutral, mutations that preserve amino acid sequence and do not alter protein structure are scattered throughout the V region.

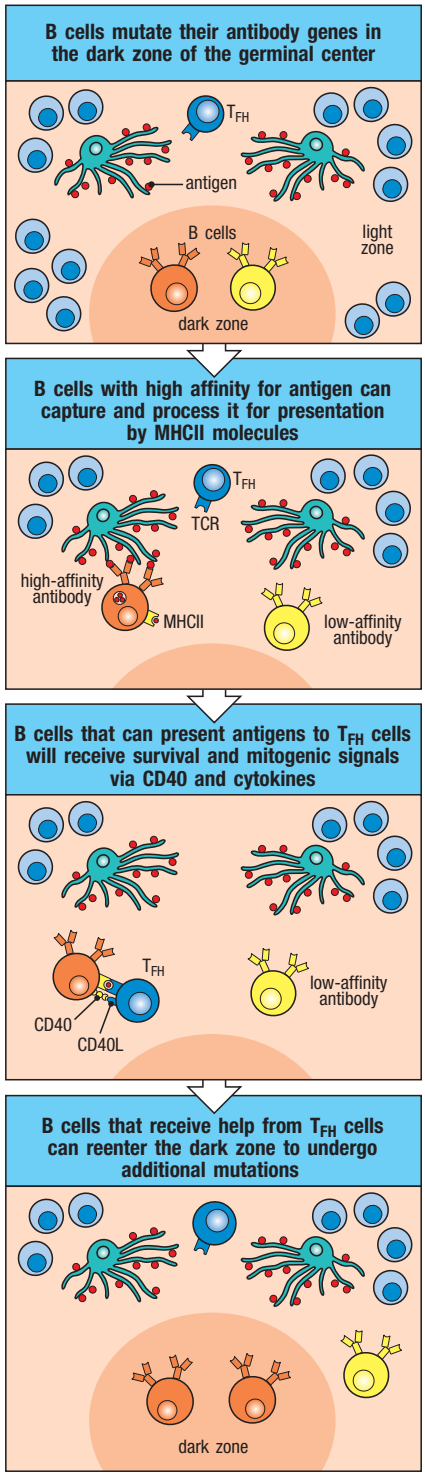


Fig. 10.15 Selection for high-affinity mutants in the germinal center relies on help provided by T_{FH} cells. After activated B cells interact with T_{FH} cells at the follicle border, they migrate to germinal centers (GCs), where the following events depicted here occur. In the dark zone of the GC, somatic hypermutation alters the immunoglobulin V regions (first panel). In some B cells (yellow), the mutated B-cell receptor (BCR) will have low or no affinity for the antigen, while in other B cells (orange) the mutated BCR affinity may be higher. After exiting the dark zone, the B cells with higher-affinity BCRs will capture antigen (red) trapped on follicular dendritic cells (FDCs) and then process and present it on MHC class II molecules (second panel). B cells with low-affinity BCRs will fail to capture and present antigen. B cells that present linked antigen epitopes to T_{FH} cells will receive help through CD40L and IL-21, which promote survival and proliferation. B cells that lack antigen on MHC class II molecules receive no help and will eventually die (third panel). Some of the proliferating B cells undergo repeated cycles of entry to the dark zone, mutation, and selection (fourth panel), and other progeny B cells undergo differentiation to either memory B cells or plasma cells (not shown).

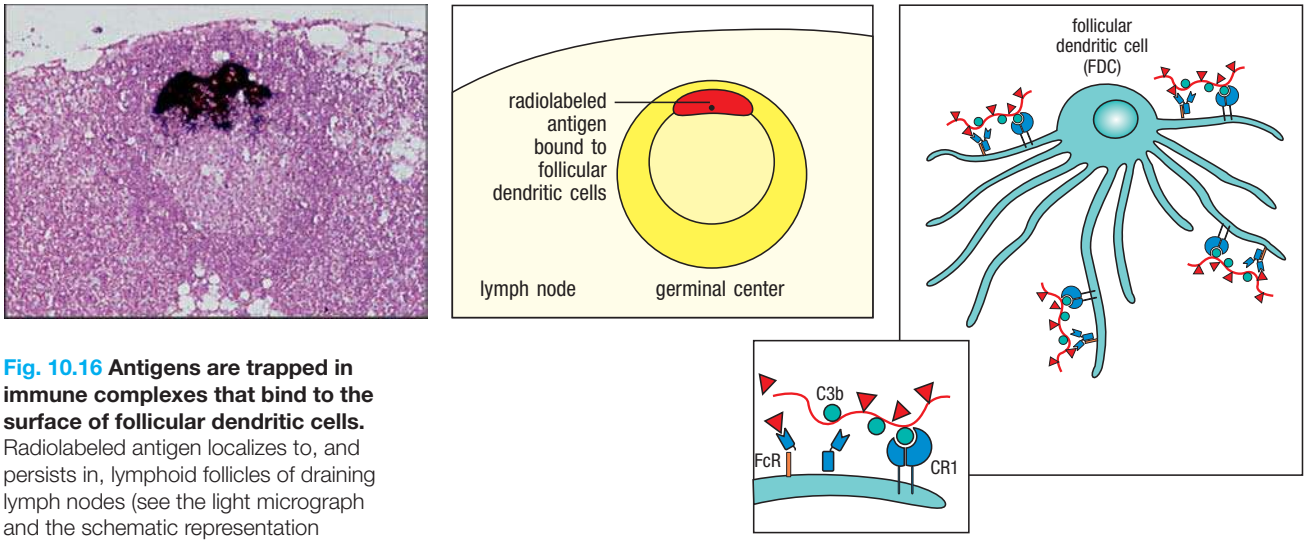


Fig. 10.16 Antigens are trapped in immune complexes that bind to the surface of follicular dendritic cells. Radiolabeled antigen localizes to, and persists in, lymphoid follicles of draining lymph nodes (see the light micrograph and the schematic representation (middle panel), showing a germinal center in a lymph node). The intense dark staining shows the localization in the germinal center of radiolabeled antigen that had been injected 3 days previously. The antigen is in the form of antigen:antibody:complement complexes bound to Fc receptors and to complement receptors CR1 or CR2 on the surface of the follicular dendritic cell (FDC), as depicted in the right-hand panel and inset. These complexes are not internalized, as such antigen can persist in this form for long periods. Photograph courtesy of J. Tew.

10-8 Positive selection of germinal center B cells involves contact with T_{FH} cells and CD40 signaling.

Selection of B cells with improved affinity for antigen occurs in increments. It was originally discovered *in vitro* that resting B cells could be kept alive by simultaneously cross-linking their B-cell receptors and ligating their cell-surface CD40. *In vivo* these signals are delivered by antigen and by T_{FH} cells, respectively. The details of selection in the germinal center have become more clear recently from *in vivo* two-photon microscopic studies (see Appendix I, A-10) that show that positive selection of a B cell depends on the B cell's ability to take up antigen, and to receive signals delivered by T_{FH} cells. It is thought that somatic hypermutation occurs in the centroblasts in the dark zone; when a centroblast reduces its rate of proliferation and becomes a centrocyte, it increases the number of B-cell receptors on its surface and moves to the light zone, where there are abundant FDCs. Antigen can be trapped and stored for long periods in the form of immune complexes on FDCs (Fig. 10.16 and Fig. 10.17). The centrocyte's ability to bind antigen determines its relative ability to acquire

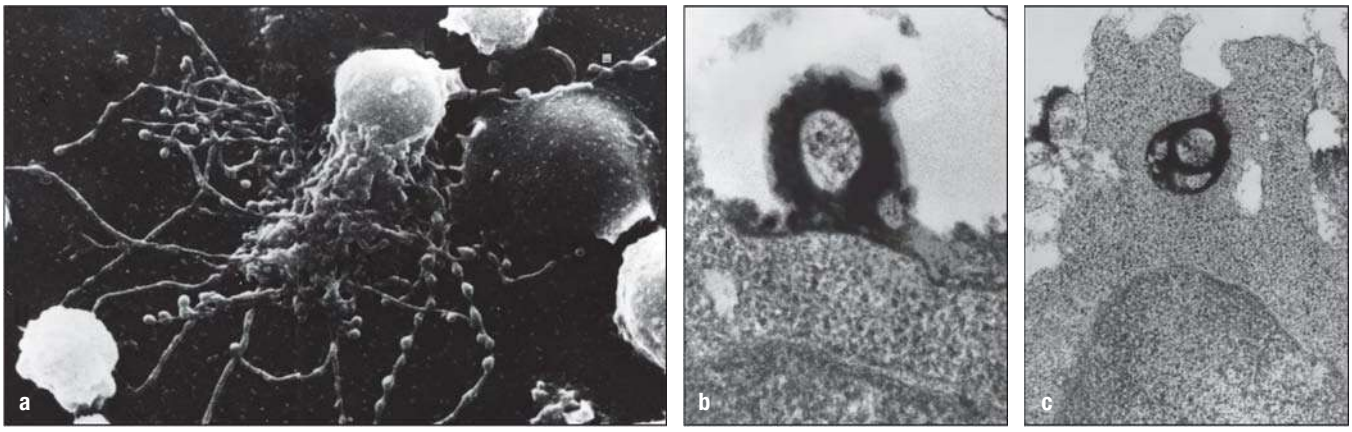


Fig. 10.17 Immune complexes bound to FDCs form iccosomes, which are released and can be taken up by B cells in the germinal center. FDCs have a prominent cell body and many dendritic processes. Immune complexes, bound to complement receptors and Fc receptors on the FDC surface, become clustered, forming prominent 'beads' along the dendrites (a). An intermediate form of FDC is shown, which has both straight filiform dendrites and others that are becoming beaded. These beads are shed from the cell as iccosomes (immune complex-coated bodies), which can bind to a B cell in the germinal center (b) and be taken up by it (c). In panels b and c, the iccosome has been formed with immune complexes containing horseradish peroxidase, which is electron-dense and therefore appears dark in the transmission electron micrographs. Photographs courtesy of A.K. Szakal.

antigen, in competition with the other clonally related centrocytes harboring different mutations. Centrocytes whose receptors bind antigen better will capture and present more peptides on their surface MHC class II molecules. T_{FH} cells in the germinal center recognize these peptides and, as before, are activated to deliver signals to the B cell that promote survival. Centrocytes whose mutations reduce antigen-binding affinity will take up less antigen, and so will receive weaker survival signals from T_{FH} cells. Successful B cells will reexpress CXCR4 and return to the dark zone, where they will undergo additional rounds of division, in effect becoming centroblasts again. Germinal center B cells that fail to acquire sufficient antigen from FDCs to engage T_{FH} cells will become apoptotic and be lost. This process of B-cell migration within the germinal center is known as the **cyclic reentry model** (see Fig. 10.11, right panel). In this way, the affinity and specificity of B cells are continually refined during the germinal center response, through affinity maturation (see Section 10-6). The selection process can be quite stringent: although 50–100 B cells may seed the germinal center, most of them leave no progeny, and by the time the germinal center reaches maximum size, it is typically composed of the descendants of only one or a few B cells.

In the germinal center, T_{FH} cells and B cells interact to deliver signals that are important for both cells (see Section 10-4). Mice that lack ICOS are deficient in the germinal center reaction and have severely reduced class-switched antibody responses due to defective T_{FH}-cell function. CD40 signaling in B cells is activated by CD40L on T_{FH} cells and increases expression of the survival molecule Bcl-X_L, a relative of Bcl-2. These interactions also include signaling by SLAM family receptors through the adapter protein SAP, as discussed above. Two-photon intravital microscopy has revealed that mice lacking the SLAM receptor CD84 have reduced numbers of conjugates between antigen-specific T cells and B cells in germinal centers, and these mice also have a reduced humoral response to antigen.

10-9 Activation-induced cytidine deaminase (AID) introduces mutations into genes transcribed in B cells.

Now that we have discussed the cellular processes involved in somatic hypermutation and affinity maturation, we will delve into the details of the mutation process itself. The enzyme AID is important for both somatic hypermutation and class switch recombination, as mice lacking AID have defects in both processes. People with mutations in the *AID* gene that inactivate the enzyme—that is, have **activation-induced cytidine deaminase deficiency**, or **AID deficiency**—also lack both somatic hypermutation and class switching. This condition leads to the production of predominantly IgM antibodies and the absence of affinity maturation, a syndrome known as **hyper IgM type 2 immunodeficiency** (discussed in Chapter 13).

AID is related to enzymes that deaminate cytosine to uracil in making nucleotide precursors for RNA and DNA synthesis. Its closest homolog, **APOBEC1** (apolipoprotein B mRNA editing catalytic polypeptide 1), is an RNA-editing enzyme that deaminates cytosine in the context of RNA. However, AID fulfills its activity in antibody gene diversification by acting on cytosine in the DNA of the immunoglobulin locus. When AID deaminates cytidine residues in the immunoglobulin V regions, somatic hypermutation is initiated; when cytidine residues in switch regions are deaminated, class switch recombination is initiated.

AID can deaminate cytidine residues in single-stranded DNA but not double-stranded DNA (Fig. 10.18). For AID to act, AID target genes are typically being transcribed, so that the DNA double helix is temporarily unwound. Since AID is expressed only in germinal center B cells, targeting of the immunoglobulin genes takes place only in these cells and in the actively transcribed rearranged

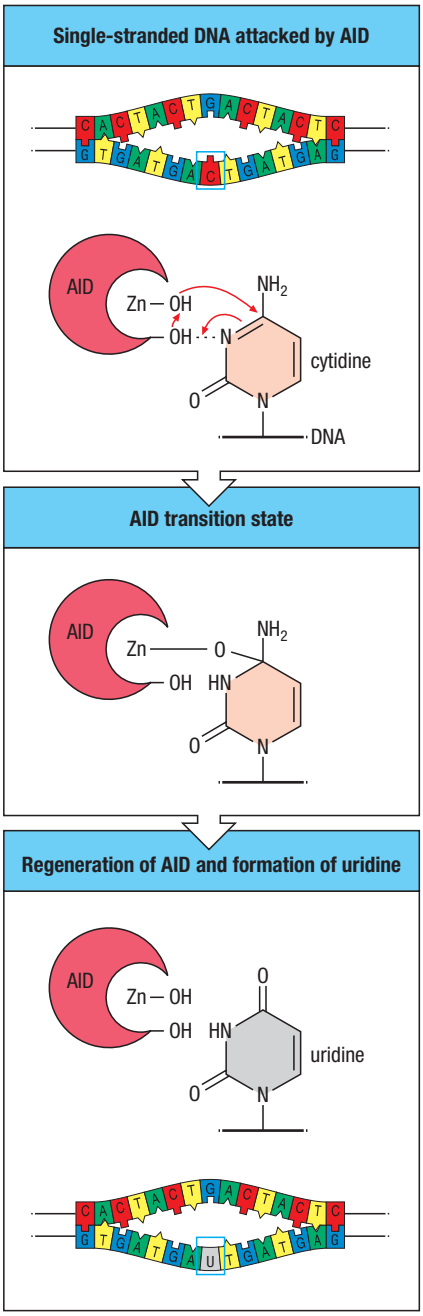


Fig. 10.18 Activation-induced cytidine deaminase (AID) is the initiator of mutations in somatic hypermutation, gene conversion, and class switching. The activity of AID, which is expressed only in B cells, requires access to the cytidine side chain of a single-stranded DNA molecule (first panel), which is normally prevented by the hydrogen bonding in double-stranded DNA. AID initiates a nucleophilic attack on the exposed cytosine ring (second panel), which is resolved by the deamination of the cytidine to form uridine (third panel).

Activation-Induced Cytidine Deaminase Deficiency

V regions where RNA polymerase generates transient single-stranded regions. Somatic hypermutation does not occur in loci that are not being actively transcribed. Rearranged V_H and V_L genes are mutated even if they are 'nonproductive' rearrangements and are not being expressed as protein, as long as they are being transcribed. Some actively transcribed genes in B cells besides those for immunoglobulins can also be affected by the somatic mutation process, but at a much lower rate.

10-10 Mismatch and base-excision repair pathways contribute to somatic hypermutation following initiation by AID.

The uridine produced by AID represents a dual lesion in DNA; not only is uridine foreign to normal DNA, but it is now a mismatch with the guanosine nucleoside on the opposite DNA strand. The presence of uridine in DNA can trigger several types of DNA repair—including the **mismatch repair** and the **base-excision repair** pathways—which further alter the DNA sequence. The various repair processes lead to different mutational outcomes (Fig. 10.19). In the mismatch repair pathway, the presence of uridine is detected by the mismatch repair proteins **MSH2** and **MSH6** (MSH2/6). They recruit nucleases that remove the complete uridine nucleotide along with several adjacent nucleotides from the damaged DNA strand. This is followed by a fill-in 'patch repair' by a DNA polymerase; unlike the process in all other cells, in B cells this DNA synthesis is error-prone and tends to introduce mutations at nearby A:T base pairs.

The initial steps in the base-excision repair pathway are shown in Fig. 10.20. In this pathway, the enzyme **uracil-DNA glycosylase** (**UNG**) removes the uracil base from the uridine to create an abasic site in the DNA. If no further modification is made, this will result at the next round of DNA replication in the random insertion of a nucleotide opposite the abasic site by DNA polymerase, leading to mutation. The action of UNG may, however, be followed by the action of another enzyme, **apurinic/apyrimidinic endonuclease 1** (**APE1**), which excises the abasic residue to create a single-strand discontinuity (known as a single-strand nick) in the DNA at the site of the original cytidine. Repair of the single-strand nick proceeding through double-strand breaks may result in gene conversion. Gene conversion is not used in the diversification of immunoglobulin genes in humans and mice, but is of importance in some other mammals and in birds.

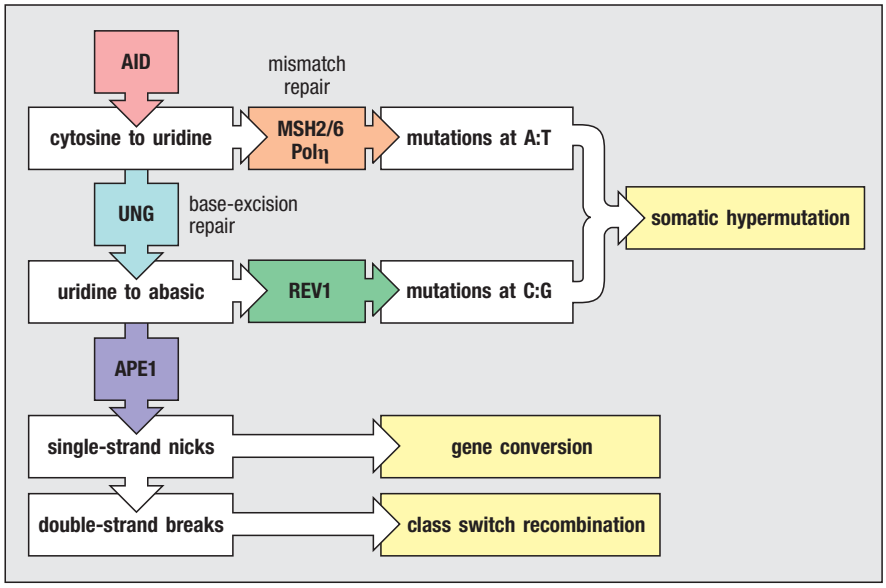


Fig. 10.19 AID initiates DNA lesions whose repair leads to somatic hypermutation, class switch recombination, or gene conversion. When AID converts a cytidine (C) to uridine (U) in the DNA of an immunoglobulin gene, the final mutation produced depends on which repair pathways are used. Somatic hypermutation can result from either the mismatch repair (MSH2/6) pathway combined with error-prone polymerase activity of Polη, or the base-excision repair (UNG) pathway. Acting together, these can generate point mutations at and around the site of the original C:G pair. REV1 is a DNA repair enzyme that can synthesize DNA, or recruit other enzymes that can synthesize DNA, over the abasic sites in damaged DNA. REV1 itself will insert only C opposite the abasic site, but it can help recruit other polymerases that can also insert A, G, and T. The end result is insertion of a random nucleotide at the C:G residues where AID initially acted. Both class switch recombination and gene conversion require the formation of a single-strand break in the DNA. A single-strand break is formed when apurinic/apyrimidinic endonuclease 1 (APE1) removes a damaged residue from the DNA as part of the repair process (see Fig. 10.20, bottom two panels). In class switch recombination, single-strand breaks made in two of the so-called switch regions upstream of the C-region genes are converted to double-strand breaks. The cell's machinery for repairing double-strand breaks, which is very similar to the later stages of V(D)J recombination, then rejoins the DNA ends in a way that leads to a recombination event in which a different C-region gene is brought adjacent to the rearranged V region. Gene conversion results from the broken DNA strand using homologous sequences flanking the immunoglobulin gene as a template for repair DNA synthesis, thus replacing part of the gene with new sequences.

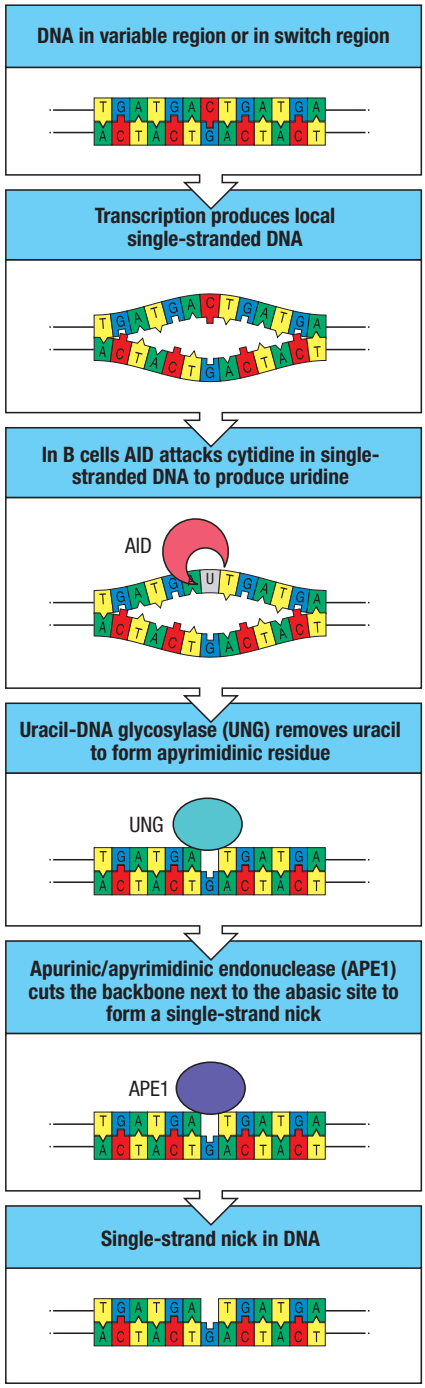


Fig. 10.20 The base-excision repair pathway produces single-strand nicks in DNA by the sequential actions of AID, uracil-DNA glycosylase (UNG), and apurinic/apyrimidinic endonuclease 1 (APE1). Double-stranded DNA (first panel) can be made accessible to AID by transcription that unwinds the DNA helix locally (second panel). AID, which is specifically expressed in activated B cells, converts cytidine residues to uridines (third panel). The ubiquitous base-excision repair enzyme UNG can then remove the uracil ring from uridine, creating an abasic site (fourth panel). The repair endonuclease APE1 then cuts the sugar–phosphate DNA backbone next to the abasic residue (fifth panel), thereby forming a single-strand nick in the DNA (sixth panel). APE1 does not excise ribose to form a single-strand nick in DNA, but rather cuts the DNA backbone to yield a 5′-deoxyribosephosphate terminus that is then removed by, for example, DNA polymerase b.

Somatic hypermutation involves both mutation at the original cytidines targeted by AID and mutation at nearby non-cytidine nucleotides. If the original U:G mismatch is recognized by UNG, then an abasic site will be generated in the DNA (see Fig. 10.19). If no further modification is made to this site, it can be replicated without instructive base pairing from the template strand by a class of **error-prone 'translesion' DNA polymerases** that normally repair gross damage to DNA, such as that caused by ultraviolet (UV) radiation. These polymerases can incorporate any nucleotide into the new DNA strand opposite the abasic site, and after a further round of DNA replication this can result in a stable mutation at the site of the original C:G base pair.

In the mismatch repair pathway in B cells, but not in other cell types, the DNA lesion is repaired by error-prone DNA polymerases rather than by more accurate polymerases that faithfully copy the undamaged template strand. Individuals with a defect in the translesion polymerase **Polη** have relatively fewer mutations than usual at A:T, but not at C:G, in their hypermutated immunoglobulin V regions. This fact suggests that Polη is the repair polymerase involved in this pathway of somatic hypermutation. These individuals also have a form of **xeroderma pigmentosum**, a condition resulting from the inability of their cells to repair DNA damage caused by UV radiation.

10-11 AID initiates class switching to allow the same assembled V_H exon to be associated with different C_H genes in the course of an immune response.

All the progeny of a particular B cell activated in an immune response will express the same V_H gene that was generated during its development in the bone marrow, although the gene may be modified by somatic hypermutation. In contrast, that B cell's progeny may express several different C-region isotypes as the cells mature and proliferate during the immune response. The first antigen receptors expressed by B cells are IgM and IgD, and the first antibody produced in an immune response is always IgM. Later in the immune response, the same assembled V region may be expressed in IgG, IgA, or IgE antibodies. This change is known as class switching (or **isotype switching**), and, unlike the expression of IgD, it involves irreversible DNA recombination. It is stimulated in the course of an immune response by external signals such as cytokines released by T_{FH} cells.

MOVIE 5.2

Switching from IgM to the other immunoglobulin classes occurs only after B cells have been stimulated by antigen. It is achieved through **class switch recombination**, which is a type of nonhomologous DNA recombination that is guided by stretches of repetitive DNA known as **switch regions**. Switch regions lie in the intron between the J_H gene segments and the C_μ gene, and at equivalent sites upstream of the genes for each of the other heavy-chain isotypes, with the exception of the δ gene, which does not require DNA rearrangement for its expression (Fig. 10.21, first panel). When a B cell switches from the coexpression of IgM and IgD to the expression of another subtype, DNA recombination occurs between S_μ and the S region immediately upstream of

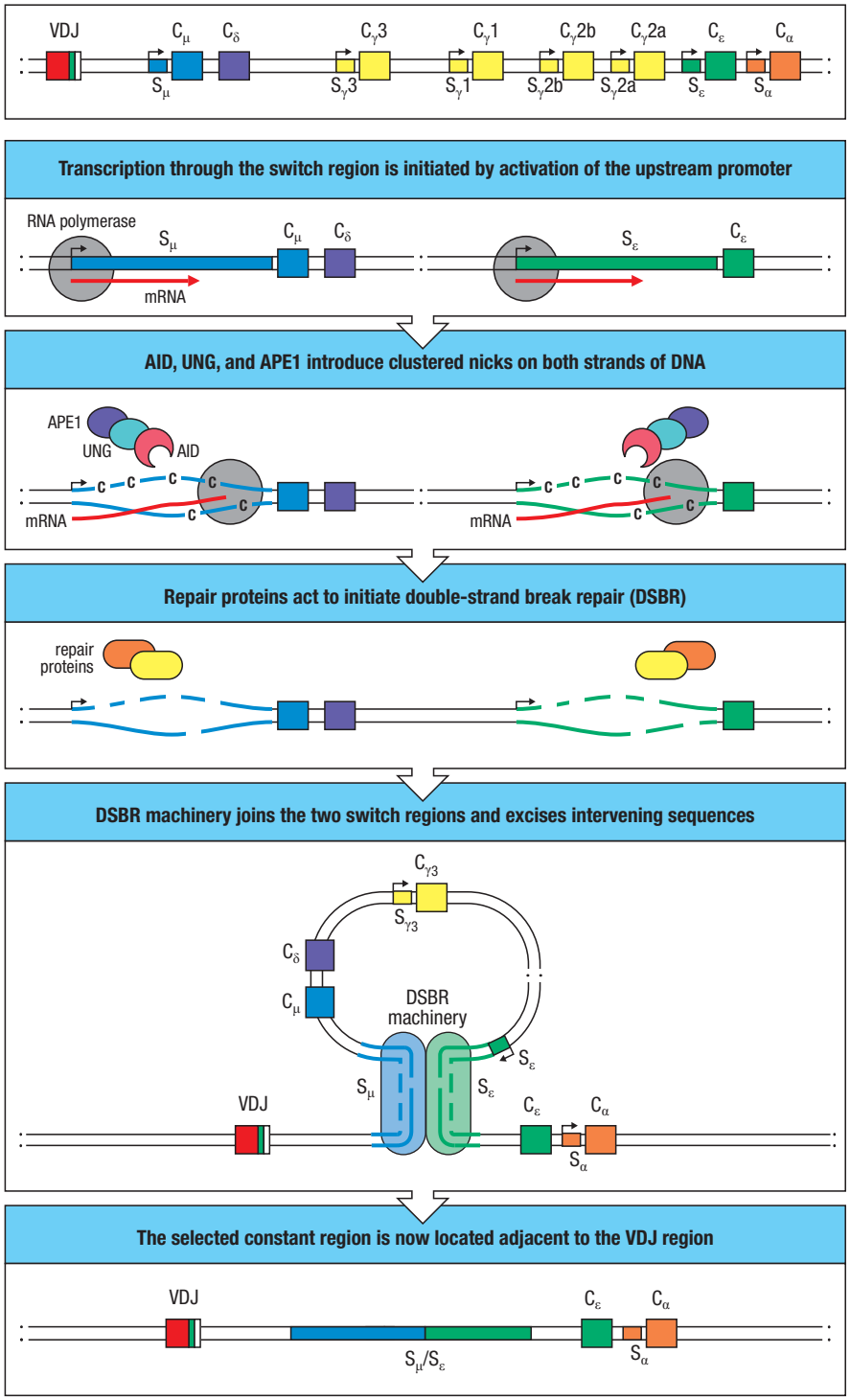


Fig. 10.21 Class switching involves recombination between specific switch signals. The top panel shows the organization of a rearranged immunoglobulin heavy-chain locus before class switching. Second panel: this figure illustrates switching between the μ and ε isotypes in the mouse heavy-chain locus. Switch regions (S) are repetitive DNA sequences that guide class switching and are found upstream of each of the immunoglobulin C-region genes, with the exception of the δ gene. Switching is guided by the initiation of transcription by RNA polymerase (shaded circle) through these regions from promoters (shown as arrows) located upstream of each S. Due to the repetitive sequences, RNA polymerase can stall within the S regions, allowing these regions to serve as substrates for AID, and subsequently for UNG and APE1. Third panel: these enzymes introduce a high density of single-strand nicks into the non-template DNA strand and the template strand. Staggered nicks are converted to double-strand breaks by a mechanism that is not yet understood. Fourth panel: these breaks are then recognized by the cell's double-strand break repair machinery, which involves DNA-PKcs, Ku proteins, and other repair proteins. Bottom two panels: the two switch regions, in this case S_μ and S_ε, are brought together by the repair proteins, and class switching is completed by excision of the intervening region of DNA (including C_μ and C_δ) and ligation of the S_μ and S_ε regions.

the new constant-region gene. In such a recombination event, the C_μ coding regions and the entire intervening DNA between C_μ and the S region undergoing rearrangement are deleted. Figure 10.21 illustrates switching from C_μ to C_ε in the mouse. All switch recombination events produce genes that can encode a functional protein, because the switch sequences lie in introns and therefore cannot cause frameshift mutations.

The enzyme AID initiates class switch recombination, and acts only on regions of DNA being transcribed. Certain properties of the switch region sequences

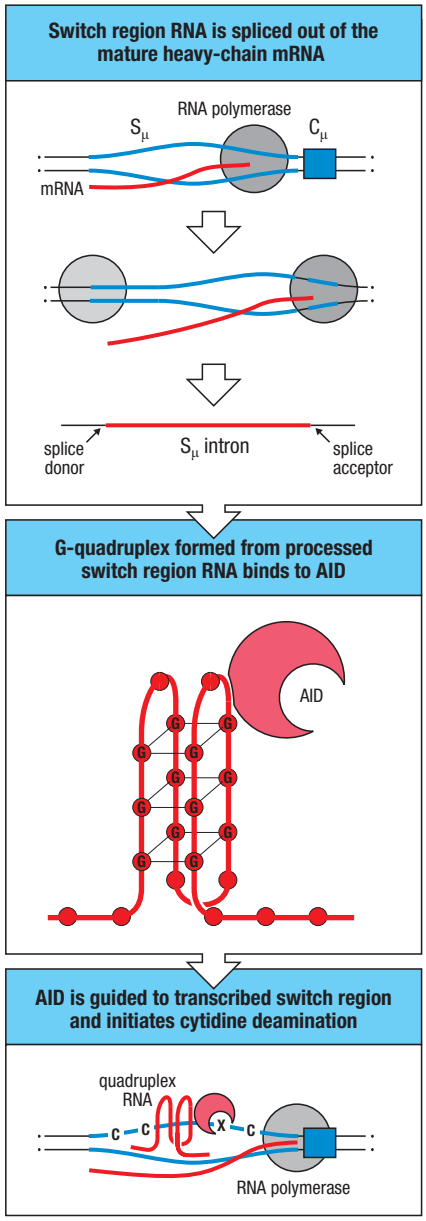


Fig. 10.22 RNA processed from switch region introns interacts with AID and guides its activity. Top panel: promoters upstream of each switch region initiate transcription by RNA polymerase upstream of a rearranged V_H gene, as in the case of C_μ, shown here, or a noncoding exon for all other constant regions. In all cases, the switch region itself lies within an intron upstream of the exons encoding the constant regions. This intronic switch region RNA is removed from the primary RNA transcript by splicing at specific splice acceptor and donor sites. Middle panel: after splicing, the switch region RNA is further processed and its repetitive elements allow the formation of putative G-quadruplex structures. Evidence indicates that these RNAs are able to bind AID, as implied in the cartoon. Bottom panel: the RNA acts as a guide to bring AID to the switch region by the ability of the G-quadruplex to hybridize with the original DNA template strand from which it was transcribed.

promote the accessibility to AID when they are being transcribed. Each switch region consists of many repeats of a G-rich sequence element on the non-template strand. For example, S_μ consists of about 150 repeats of the sequence (GAGCT)*n*(GGGGGT), where *n* is usually 3 but can be as many as 7. The sequences of the other switch regions (S_γ, S_α, and S_ε) differ in exact sequence but all contain repeats of the GAGCT and GGGGGT sequences. It appears that movement of RNA polymerase through this highly repetitive region is occasionally halted—called **polymerase stalling**. This may be caused by bubble-like structures, called **R-loops**, that form when the transcribed RNA displaces the non-template strand of the DNA double helix (see Fig. 10.21, third panel) due to having many G residues in tandem on one strand.

Polymerase stalling seems closely connected with the recruitment of AID to specific switch regions being transcribed. A multisubunit RNA processing/degradation complex, the **RNA exosome**, associates with AID and accumulates on transcribed switch regions, and the protein **Spt5** associates with the stalled polymerase; both are necessary for AID to generate double-stranded breaks. Recent evidence indicates that AID is selectively guided to the transcribed switch by an additional mechanism. After an RNA polymerase has completed transcription of one RNA template, the intron RNA harboring the switch region is spliced out. This RNA is processed to generate an RNA structure, called a **G-quadruplex**, that is based on the G-rich repetitive element of the switch region (Fig. 10.22). This G-quadruplex serves a dual purpose, both binding to AID and also associating with the switch region from which it was transcribed, based on its sequence complementarity. Thus the G-quadruplex guides AID to the appropriate switch region, where particular palindromic sequences, such as AGCT, act as good substrates to allow its cytidine deaminase activity to act on both strands concurrently. In this way, the G-quadruplex functions in a manner similar to the synthetic guide RNAs that deliver the Cas9 endonuclease to specific genomic regions, as described in Appendix I, Section A-35).

Following the generation of double-stranded breaks in switch regions, general cellular mechanisms for repairing these breaks lead to the nonhomologous recombination between switch regions that results in class switching (see Fig. 10.21, fourth and fifth panels). The ends to be joined are brought together by the alignment of repetitive sequences common to the different switch regions, and rejoining of the DNA ends then leads to excision of all DNA between the two switch regions and the formation of a chimeric region at the junction. Loss of AID completely blocks class switching, but deficiency of UNG in both mice and humans severely impairs class switching, suggesting sequential actions of AID and UNG in generating DNA breaks. Joining of DNA ends is probably mediated by classic nonhomologous end joining (as in V(D)J recombination) as well as by a poorly understood alternative end-joining pathway. Class switching is sometimes impaired in the disease **ataxia telangiectasia**, which is caused by mutations in the DNA-PKcs-family kinase **ATM**, a known DNA repair protein. The role of ATM in class switching is not yet entirely clear, however.

Ataxia Telangiectasia

10-12 Cytokines made by T_{FH} cells direct the choice of isotype for class switching in T-dependent antibody responses.

Now that we understand the general mechanisms that control DNA rearrangements of class switching, we are ready to explain how a particular heavy-chain is selected during an immune response. It is the choice of antibody isotype that ultimately determines the effector function of antibodies, and we will see that this choice is largely controlled by the cytokines that are produced by T_{FH} cells in the germinal center reaction.

As discussed above, interactions between germinal center B cells and T_{FH} cells are essential for class switching to occur. The required interactions occur through the interplay of CD40 on B cells with CD40 ligand on activated helper T cells. Genetic deficiency of CD40 ligand greatly reduces class switching and causes abnormally high levels of plasma IgM, a condition known as **hyper IgM syndrome**. People with this defect lack antibodies of classes other than IgM and exhibit severe humoral immunodeficiency, manifested as repeated infections with common bacterial pathogens. Much of the IgM in hyper IgM syndromes may be induced by thymus-independent antigens on the pathogens that chronically infect these patients. Nevertheless, people with CD40 ligand deficiency can make IgM antibodies in response to thymus-dependent antigens, which indicates that in the B-cell response, CD40L–CD40 interactions are most important in enabling a sustained response that includes class switching and affinity maturation, rather than in the initial activation of B cells.

CD40 Ligand Deficiency

The selection of the particular C region for class switch recombination is not random but is regulated by the cytokines produced by T_{FH} cells and other cells during the immune response. Different cytokines preferentially induce switching to different isotypes (Fig. 10.23). Cytokines induce class switching in part by inducing the production of RNA transcripts through the switch regions that lie 5′ to each heavy-chain C gene segment. When activated B cells are exposed to IL-4, for example, transcription from promoters that lie upstream of the switch regions of $C_\gamma 1$ and C_ε can be detected a day or two before switching occurs. This will make it possible for switch to occur to either of these two heavy-chain C genes, but in any particular germinal center B cell, recombination will occur in only one. In the example of class switching shown in Fig. 10.21, transcription through the S_ε regions caused the rearrangement between the S_μ and S_ε regions, making the IgE isotype antibody. This results because IL-4 signaling activates the transcription factor **STAT6**, which initiates transcription of the Iε promoter upstream of the S_ε region. Other cytokines activate other promoters upstream of other switch regions to produce other antibody classes. T_{FH} cells also produce IL-21, which promotes switching to IgG1 and IgG3. Transforming growth factor (TGF)-β induces switching to IgG2b ($C_\gamma 2b$) and IgA (C_α). IL-5 promotes switching to IgA, and interferon (IFN)-γ induces switching to IgG2a and IgG3.

	Role of cytokines in regulating expression of antibody classes						
Cytokines	**IgM**	**IgG3**	**IgG1**	**IgG2b**	**IgG2a**	**IgE**	**IgA**
IL-4	Inhibits	Inhibits	Induces		Inhibits	Induces	
IL-5							Augments production
IFN-γ	Inhibits	Induces	Inhibits		Induces	Inhibits	
TGF-β	Inhibits	Inhibits		Induces			Induces
IL-21		Induces	Induces				Induces

Fig. 10.23 Different cytokines induce switching to different antibody classes. The individual cytokines induce (violet) or inhibit (red) the production of certain antibody classes. Much of the inhibitory effect is probably the result of directed switching to a different class. The actions of IL-21 on class switching are regulated by IL-4. These data are drawn from experiments with mouse cells.

10-13 B cells that survive the germinal center reaction eventually differentiate into either plasma cells or memory cells.

When B cells have undergone affinity maturation and class switching, some eventually exit from the light zone and start to differentiate into plasma cells that produce large amounts of antibody. In B cells, the transcription factors Pax5 and **Bcl-6** inhibit the expression of transcription factors required for plasma-cell differentiation, and both Pax5 and Bcl-6 are downregulated when the B cell starts differentiating. The transcription factor IRF4 then induces the expression of **BLIMP-1**, a transcriptional repressor that switches off genes required for B-cell proliferation, class switching, and affinity maturation. B cells in which BLIMP-1 is induced become plasma cells; they cease proliferating, increase the synthesis and secretion of immunoglobulins, and change their cell-surface properties. Plasma cells downregulate CXCR5 and upregulate CXCR4 and α_4:β_1 integrins so that they can leave the germinal centers and home to peripheral tissues.

Some plasma cells deriving from germinal centers in lymph nodes or spleen migrate to the bone marrow, where a subset live for a long period, whereas others migrate to the medullary cords in lymph nodes or splenic red pulp. B cells that have been activated in germinal centers in mucosal tissues, and which are predominantly switched to IgA production, stay within the mucosal system. A splice variant of **XBP1** (X-box binding protein 1) is expressed in plasma cells and helps to regulate their secretory capacity. Plasma cells in bone marrow receive signals from stromal cells that are essential for their survival, and they can be very long lived, whereas plasma cells in the medullary cords or red pulp are not long lived. XBP1 is also required for plasma cells to colonize bone marrow successfully. Plasma cells in the bone marrow are the source of long-lasting high-affinity class-switched antibody.

Other germinal center B cells differentiate into **memory B cells**. Memory B cells are long-lived descendants of cells that were once stimulated by antigen and had proliferated in the germinal center. They divide very slowly if at all; they express surface immunoglobulin but secrete no antibody, or do so only at a low rate. Because the precursors of some memory B cells arise from the germinal center reaction, memory B cells can inherit the genetic changes that occur there, including somatic hypermutation and the gene rearrangements that result in a class switch. The signals that control which path of differentiation a B cell takes are still being investigated. We will briefly return to memory B cells in Chapter 11.

10-14 Some antigens do not require T-cell help to induce B-cell responses.

Humans and mice with T-cell deficiencies are able to produce antibodies against thymus-independent (TI) antigens, which we introduced in Section 10-1. These antigens include certain bacterial polysaccharides, polymeric proteins, and lipopolysaccharides, which are able to stimulate naive B cells in the absence of T-cell help. These nonprotein bacterial products cannot elicit classical T-cell responses, yet they induce antibody responses in normal individuals. In addition, there are TI antigens that are not derived from bacteria; these include plant-derived mitogens and lectins, viral antigens, and superantigens, and some parasite-derived antigens.

Thymus-independent antigens fall into two classes, TI-1 and TI-2, which activate B cells by two different mechanisms. **TI-1 antigens** rely on activity that can directly induce B-cell division without T-cell help. We now understand that TI-1 antigens contain molecules that cause the proliferation and differentiation of most B cells regardless of their antigen specificity; this is known as **polyclonal activation** (Fig. 10.24, top panels). TI-1 antigens are therefore

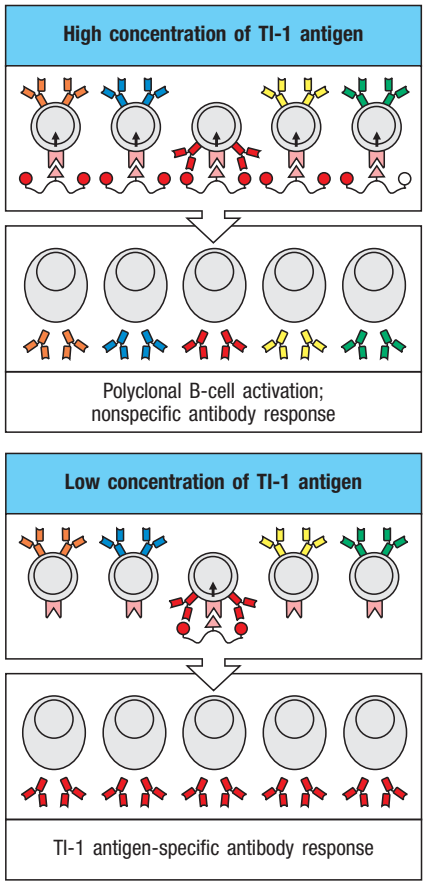


Fig. 10.24 TI-1 antigens induce polyclonal B-cell responses at high concentrations, and antigen-specific antibody responses at low concentrations. At high antigen concentration, the signal delivered by the B-cell-activating moiety of TI-1 antigens is sufficient to induce B-cell proliferation and antibody secretion in the absence of specific antigen binding to surface immunoglobulin. Thus, all B cells respond (top panels). At low concentration, only B cells specific for the TI-1 antigen bind enough of it to focus its B-cell activating properties onto the B cell; this gives a specific antibody response to epitopes on the TI-1 antigen (lower panels).

often called **B-cell mitogens**, a mitogen being a substance that induces cells to undergo mitosis. For example, LPS and bacterial DNA are both TI-1 antigens because they activate TLRs expressed by B cells (see Section 3-5) and can act as a mitogen. Naive murine B cells express most TLRs constitutively, but naive human B cells do not express high levels of most TLRs until they receive stimulation through the B-cell receptor. So by the time a B cell has been stimulated by antigen through its B-cell receptor, it is likely to express several TLRs and be responsive to stimulation by TLR ligands that accompany the antigens. Thus, when B cells are exposed to concentrations of TI-1 antigens that are 10^3–10^5 times lower than those used for polyclonal activation, only those B cells whose B-cell receptors specifically bind the TI-1 antigen become activated. At these low concentrations, amounts of TI-1 antigen sufficient for B-cell activation can only be concentrated on the B-cell surface with the aid of this specific binding (see Fig. 10.24, bottom panels). B-cell responses to TI-1 antigens in the early stages of an infection may be important in defense against several extracellular pathogens, but they do not lead to affinity maturation or memory B cells, both of which require antigen-specific T-cell help.

The second class of thymus-independent antigens—**TI-2 antigens**—consists of molecules that have highly repetitive structures, such as bacterial capsular polysaccharides. These contain no intrinsic B-cell-stimulating activity. Whereas TI-1 antigens can activate both immature and mature B cells, TI-2 antigens can activate only mature B cells; immature B cells, as we saw in Section 8-6, are inactivated by encounter with repetitive epitopes. Infants and young children up to about 5 years of age do not make fully effective antibody responses against polysaccharide antigens, and this might be because most of their B cells are immature.

Responses to several TI-2 antigens are prominently made by **marginal zone B cells**, a subset of nonrecirculating B cells that line the border of the splenic white pulp, and by **B-1 cells** (see Section 8-9). Marginal zone B cells are rare at birth and accumulate with age; they might therefore be responsible for most physiological TI-2 responses, which increase in efficiency with age. TI-2 antigens probably act by simultaneously cross-linking a critical number of B-cell receptors on the surface of antigen-specific mature B cells (Fig. 10.25, left

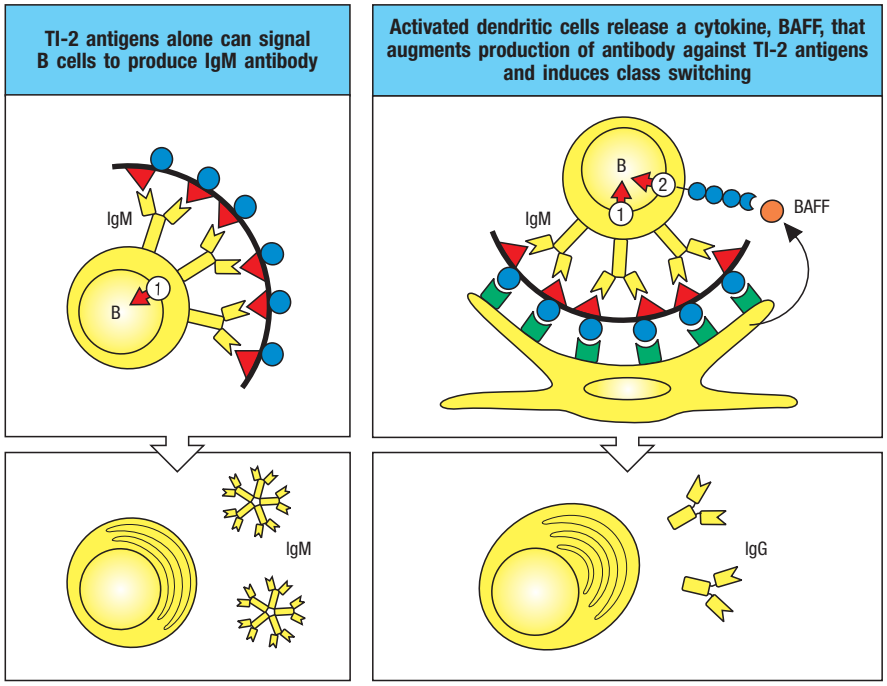


Fig. 10.25 B-cell activation by TI-2 antigens requires, or is greatly enhanced by, cytokines. Multiple cross-linking of the B-cell receptor by TI-2 antigens can lead to IgM antibody production (left panels), but there is evidence that in addition cytokines greatly augment these responses, and also lead to isotype switching (right panels). It is not clear where such cytokines originate, but one possibility is that dendritic cells, which may be able to bind the antigen through innate immune-system receptors on their surface and so present it to the B cells, secrete a soluble TNF-family cytokine called BAFF, which can activate class switching by the B cell.

panels). Dendritic cells and macrophages can provide co-stimulatory signals for activation of B cells by TI-2 antigens. One of these co-stimulatory signals is BAFF, which can be secreted by dendritic cells and interacts with the receptor TACI on the B cell (see Fig. 10.25, right panels). The density of TI-2 antigen epitopes is critical; excessive cross-linking of B-cell receptors renders mature B cells unresponsive or anergic, as in immature B cells, while too low a density may be insufficient for activation.

An important class of TI-2 antigens arises during infection by **capsulated bacteria**. Many common extracellular bacterial pathogens are surrounded by a polysaccharide capsule that enables them to resist ingestion by phagocytes. The bacteria not only escape direct destruction by phagocytes but also avoid stimulating T-cell responses against bacterial peptides presented by macrophages. IgM antibodies rapidly produced against the capsular polysaccharide independent of peptide-specific T-cell help will coat the bacteria, promoting their ingestion and destruction by phagocytes early in the infection.

Not all antibodies against bacterial polysaccharides are produced strictly through this TI-2 mechanism. We mentioned earlier the importance of antibodies against the capsular polysaccharide of *Haemophilus influenzae* type b in protective immunity to this bacterium. The immunodeficiency disease **Wiskott–Aldrich syndrome** is caused by defects in T cells that impair their interaction with B cells (see Chapter 13). Patients with Wiskott–Aldrich syndrome respond poorly to protein antigens, but, unexpectedly, also fail to make IgM and IgG antibody against polysaccharide antigens and are highly susceptible to infection with encapsulated bacteria such as *H. influenzae*. The failure to make IgM seems to be due in part to greatly reduced development of the marginal zone of the spleen, which contains B cells responsible for making much of the 'natural' IgM antibody against ubiquitous carbohydrate antigens. Thus, IgM and IgG antibodies induced by TI-2 antigens are likely to be an important part of the humoral immune response in many bacterial infections, and in humans at least, the production of class-switched antibodies to TI-2 antigens might normally rely on some degree of T-cell help.

Wiskott–Aldrich Syndrome

As well as producing IgM, TI responses can include switching to certain other antibody classes, such as IgG3 in the mouse. This is probably the result of help from dendritic cells (see Fig. 10.25, right panels), which provide secreted cytokines such as BAFF and membrane-bound signals to proliferating plasmablasts as they respond to TI antigens. The distinguishing features of thymus-dependent, TI-1, and TI-2 antibody responses are summarized in **Fig. 10.26**.

Summary.

B-cell activation by many antigens requires both binding of the antigen by the B-cell surface immunoglobulin—the B-cell receptor—and interaction of the B cell with antigen-specific helper T cells. Helper T cells recognize peptide fragments derived from the antigen internalized by the B cells and displayed by the B cells as peptide:MHC class II complexes. Follicular helper T cells stimulate B cells by conjugation in germinal centers, with binding of CD40 ligand on the T cell to CD40 on the B cell, and by their release of cytokines, such as IL-21. Activated B cells also express molecules, such as ICOSL, that can stimulate T cells. The initial interaction between B and T cells occurs at the border of the T-cell and B-cell areas of secondary lymphoid tissue, to which antigen-activated helper T cells and B cells migrate in response to chemokines. Further interactions between T cells and B cells continue after migration into the follicle and the formation of a germinal center.

Fig. 10.26 Properties of different classes of antigen that elicit antibody responses. Some data indicate a minor role for T cells in antibody responses to TI-2 antigens; robust responses to TI-2 antigens can be observed in T-cell-deficient mice.

	TD antigen	TI-1 antigen	TI-2 antigen
Antibody response in infants	Yes	Yes	No
Antibody production in congenitally athymic individual	No	Yes	Yes
Antibody response in absence of all T cells	No	Yes	Yes
Primes T cells	Yes	No	No
Polyclonal B-cell activation	No	Yes	No
Requires repeating epitopes	No	No	Yes
Examples of antigen	Diphtheria toxin Viral hemagglutinin Purified protein derivative (PPD) of *Mycobacterium tuberculosis*	Bacterial lipopolysaccharide *Brucella abortus*	Pneumococcal polysaccharide *Salmonella* polymerized flagellin Dextran Hapten-conjugated Ficoll (polysucrose)

T cells induce a phase of vigorous B-cell proliferation in the germinal center reaction and direct the differentiation of clonally expanded B cells into either antibody-secreting plasma cells or memory B cells. Immunoglobulin genes expressed in B cells are diversified in the germinal center reaction by somatic hypermutation and class switching, initiated by activation-induced cytidine deaminase (AID). Unlike V(D)J recombination, these processes occur only in B cells. Somatic hypermutation diversifies the V region through the introduction of point mutations that are selected for providing greater affinity for the antigen as the immune response proceeds. Class switching does not affect the V region but increases the functional diversity of immunoglobulins by replacing the C_μ region in the immunoglobulin gene, which is first expressed with another heavy-chain C region to produce IgG, IgA, or IgE antibodies. Class switching provides antibodies with the same antigen specificity but distinct effector capacities. The switching to different antibody isotypes is regulated by cytokines released from helper T cells. Some nonprotein antigens stimulate B cells in the absence of linked recognition by peptide-specific helper T cells. Responses to these thymus-independent antigens are accompanied by only limited class switching and do not induce memory B cells. However, such responses have a crucial role in host defense against pathogens whose surface antigens cannot elicit peptide-specific T-cell responses.

The distributions and functions of immunoglobulin classes.

Extracellular pathogens can invade most sites within the body, and so antibodies must be equally widely distributed to combat them. Most classes of antibodies are distributed by diffusion from their site of synthesis, but specialized transport mechanisms are required to deliver antibodies across the epithelial surfaces lining the mucosa of organs such as the lungs and intestine. The particular heavy-chain isotype of the antibody can either limit antibody diffusion

or engage specific transporters that deliver the antibody across epithelia. This part of the chapter describes these mechanisms and the antibody classes that use them to enter compartments of the body where their particular effector functions are appropriate. Here we restrict our discussion to the protective functions of antibodies that result solely from their binding to pathogens, and in the next part of the chapter, we discuss the effector cells and molecules that are specifically engaged by different antibody classes.

10-15 Antibodies of different classes operate in distinct places and have distinct effector functions.

Pathogens most commonly enter the body across the epithelial barriers of the mucosa lining the respiratory, digestive, and urogenital tracts, or through damaged skin. Less often, insects, wounds, or hypodermic needles introduce microorganisms directly into the blood. Antibodies protect all the body's mucosal surfaces, tissues, and blood from such infections; these antibodies serve to neutralize the pathogen or promote its elimination before it can establish a significant infection.

The different classes of antibodies (see Fig. 5.19) are adapted to function in different compartments of the body. Their functional activities and distributions are listed in Fig. 10.27. Because a given V region can become associated with any C region through class switching, the progeny of a single B cell can produce antibodies that share the same specificity yet provide all of the protective functions appropriate for each body compartment. All naive B cells express cell-surface IgM and IgD. IgM is the first antibody secreted by activated B cells but is less than 10% of the immunoglobulin found in plasma. Little IgD antibody is produced at any time, while IgE contributes a small but biologically important part of the immune response. IgG and IgA are the predominant antibody classes. IgE contributes a small but biologically important part of the response. The overall predominance of IgG is also due in part to its longer lifetime in the plasma (see Fig. 5.20).

Functional activity	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	++	++	++	++	++	–
Opsonization	+	–	++	*	++	+	+	–
Sensitization for killing by NK cells	–	–	++	–	++	–	–	–
Sensitization of mast cells	–	–	+	–	+	–	–	+++
Activates complement system	+++	–	++	+	+++	–	+	–

Distribution	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	–	–	–	–	–	+++ (dimer)	–
Transport across placenta	–	–	+++	+	++	+/–	–	–
Diffusion into extravascular sites	+/–	–	+++	+++	+++	+++	++ (monomer)	+
Mean serum level ($mg \cdot ml^{-1}$)	1.5	0.04	9	3	1	0.5	2.1	3×10^{-5}

Fig. 10.27 Each human immunoglobulin class has specialized functions and a unique distribution. The major effector functions of each class (+++) are shaded in dark red, whereas lesser functions (++) are shown in dark pink, and very minor functions (+) in pale pink. The distributions are marked similarly, with actual average levels in serum being shown in the bottom row. IgA has two subclasses, IgA1 and IgA2. The IgA column refers to both. *IgG2 can act as an opsonin in the presence of an Fc receptor of the appropriate allotype, found in about 50% of people of Caucasian descent.

IgM antibodies are produced first in a humoral immune response and tend to be of low affinity. However, IgM molecules form pentamers that are stabilized by a single J-chain molecule (see Fig. 5.23) and have 10 antigen-binding sites, conferring higher overall avidity when binding to multivalent antigens such as bacterial capsular polysaccharides. This higher avidity of the pentamer compensates for the low affinity of the individual antigen-binding site within the IgM monomers. Because of the large size of the pentamers, IgM is found mainly in the bloodstream and, to a lesser extent, in the lymph, rather than in intercellular spaces within tissues. The pentameric structure of IgM makes it especially effective in activating the complement system, as we will see in the last part of this chapter. IgM hexamers can also form, and these fix complement much more efficiently than pentamers, possibly because C1q is also a hexamer. However, the *in vivo* role of IgM hexamers in protecting against infections has not been fully established.

Infection of the bloodstream has serious consequences unless it is controlled quickly, and the rapid production of IgM and its efficient activation of the complement system are important in controlling such infections. Some IgM is produced by conventional B cells that have not undergone class switching, but most is produced by B-1 cells residing in the peritoneal cavity and pleural spaces and by marginal zone B cells of the spleen. These cells secrete antibodies against commonly encountered carbohydrate antigens, including those of bacteria, and do not require T-cell help; they therefore provide a preformed repertoire of IgM antibodies in blood and body cavities that can recognize invading pathogens (see Section 8-9).

Antibodies of the other classes—IgG, IgA, and IgE—are smaller, and diffuse easily out of the blood into the tissues. IgA can form dimers (see Fig. 5.23), but IgG and IgE are always monomeric. The affinity of the individual antigen-binding sites for their antigen is therefore critical for the effectiveness of these antibodies, and most of the B cells expressing these classes have been selected in the germinal centers for their increased affinity for antigen after somatic hypermutation. IgG4 is the least abundant of the IgG subclasses, but has the unusual ability to form hybrid antibodies. One IgG4 heavy chain and attached light chain can split from the original heavy-chain dimer and reassociate with a different IgG4 heavy chain–light chain pair, forming a bivalent IgG4 antibody with two distinct antigen specificities.

IgG is the principal class of antibody in blood and extracellular fluid, whereas IgA is the principal class in secretions, the most important being those from the epithelia lining the intestinal and respiratory tracts. IgG efficiently opsonizes pathogens for engulfment by phagocytes and activates the complement system, but IgA is a less potent opsonin and a weak activator of complement. IgG operates mainly in the tissues, where accessory cells and molecules are available, whereas dimeric IgA operates mainly on epithelial surfaces, where complement and phagocytes are not normally present; therefore IgA functions chiefly as a neutralizing antibody. Monomeric IgA can be produced by plasma cells that differentiate from class-switched B cells in lymph nodes and spleen, and it acts as a neutralizing antibody in extracellular spaces and in the blood. This monomeric IgA is predominantly of the subclass IgA1; the ratio of IgA1 to IgA2 in the blood is 10:1. The IgA antibodies produced by plasma cells in the gut are dimeric and predominantly of subclass IgA2; the ratio of IgA2 to IgA1 in the gut is 3:2.

Finally, IgE antibody is present only at very low levels in blood or extracellular fluid, but is bound avidly by receptors on **mast cells** that are found just beneath the skin and mucosa and along blood vessels in connective tissue. Antigen binding to this cell-associated IgE triggers mast cells to release powerful chemical mediators that induce reactions such as coughing, sneezing, and vomiting, which in turn can expel infectious agents, as discussed later in this chapter.

10-16 Polymeric immunoglobulin receptor binds to the Fc regions of IgA and IgM and transports them across epithelial barriers.

In the mucosal immune system, IgA-secreting plasma cells are found predominantly in the lamina propria, which lies immediately below the basement membrane of many surface epithelia. From there the IgA antibodies can be transported across the epithelium to its external surface, for example to the lumen of the gut or of the bronchi (Fig. 10.28). IgA antibody synthesized in the lamina propria is secreted as a dimeric IgA molecule associated with a single J chain. This polymeric form of IgA binds specifically to a receptor called the **polymeric immunoglobulin receptor** (**pIgR**), which is present on the basolateral surfaces of the overlying epithelial cells. When the pIgR has bound a molecule of dimeric IgA, the complex is internalized and carried in a transport vesicle through the cytoplasm of the epithelial cell to its luminal surface. This process is called transcytosis. IgM also binds to the pIgR and can be secreted into the gut by the same mechanism. Upon reaching the luminal surface of the enterocyte, the antibody is released into the mucous layer covering the gut lining by proteolytic cleavage of the extracellular domain of the pIgR. The cleaved extracellular domain of the pIgR is known as **secretory component** (frequently abbreviated to **SC**) and remains associated with the antibody. Secretory component is bound to the part of the Fc region of IgA that contains the binding site for the Fcα receptor I, which is why secretory IgA does not bind to this receptor. Secretory component serves several physiological roles. It binds to mucins in mucus, acting as 'glue' to bind secreted IgA to the mucous layer on the luminal surface of the gut epithelium, where the antibody binds and neutralizes gut pathogens and their toxins (see Fig. 10.28). Secretory component also protects the antibodies against cleavage by gut enzymes.

The principal sites of IgA synthesis and secretion are the gut, the respiratory epithelium, the lactating breast, and various other exocrine glands such as the salivary and tear glands. It is believed that the primary functional role of IgA antibodies is to protect epithelial surfaces from infectious agents, just as IgG antibodies protect the extracellular spaces inside tissues. By binding bacteria, virus particles, and toxins, IgA antibodies prevent the attachment of bacteria and viruses to epithelial cells and the uptake of toxins, and provide the first line of defense against a wide variety of pathogens. IgA is also thought to have an additional role in the gut, that of regulating the gut microbiota (see Chapter 12). The alveolar spaces in the lower respiratory tract lack the thicker mucosal layer characteristic of the upper respiratory tract, because efficient gas diffusion would be impeded by a mucous layer covering the alveolar epithelium. IgG can rapidly transudate into these spaces and is the major isotype responsible for protection there.

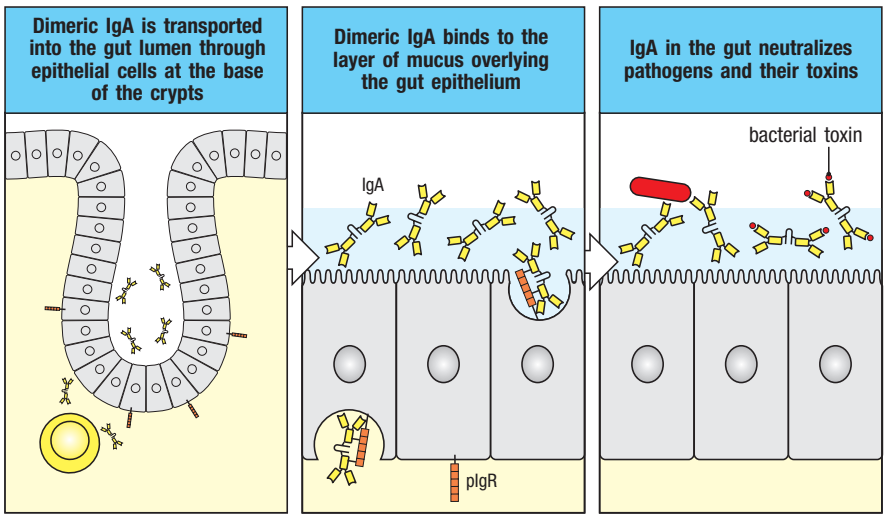


Fig. 10.28 Dimeric IgA is the major class of antibody present in the lumen of the gut. IgA is synthesized by plasma cells in the lamina propria and transported into the lumen of the gut through epithelial cells at the base of the crypts. Dimeric IgA binds to the layer of mucus overlying the gut epithelium and acts as an antigen-specific barrier to pathogens and toxins in the gut lumen

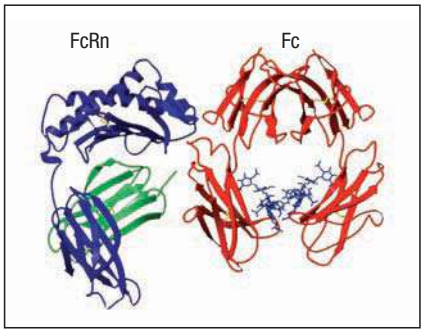


Fig. 10.29 The neonatal Fc receptor (FcRn) binds to the Fc portion of IgG. The structure of a molecule of FcRn (blue) is shown bound to one chain of the Fc portion of IgG (red), at the interface of the $C_\gamma2$ and $C_\gamma3$ domains, with the $C_\gamma2$ region at the top. The β_2-microglobulin component of the FcRn is green. The dark-blue structure attached to the Fc portion of IgG is a carbohydrate chain, reflecting glycosylation. FcRn transports IgG molecules across the placenta in humans and also across the gut in rats and mice. It also has a role in maintaining the levels of IgG in adults. Although only one molecule of FcRn is shown binding to the Fc portion, it is thought that it takes two molecules of FcRn to capture one molecule of IgG. Courtesy of P. Björkman.

10-17 The neonatal Fc receptor carries IgG across the placenta and prevents IgG excretion from the body.

Newborn infants are especially vulnerable to infection, having had no previous exposure to the microbes in the environment they enter at birth. IgA antibodies are secreted in breast milk and are transferred to the gut of the newborn infant, where they provide protection from newly encountered bacteria until the infant can synthesize its own protective antibody. IgA is not the only protective antibody that a mother passes on to her baby. Maternal IgG is transported across the placenta directly into the bloodstream of the fetus during intrauterine life; human babies at birth have as high levels of plasma IgG as their mothers, and with the same range of antigen specificities. The selective transport of IgG from mother to fetus is due to an IgG transport protein in the placenta, **FcRn** (**neonatal Fc receptor**), which is closely related in structure to MHC class I molecules. Despite this similarity, FcRn binds IgG quite differently from the binding of peptide to MHC class I molecules, because its peptide-binding groove is occluded. It binds to the Fc portion of IgG molecules (Fig. 10.29). Two molecules of FcRn bind one molecule of IgG, bearing it across the placenta. Maternal IgG is ingested by the newborn animal from its mother's milk and colostrum, the protein-rich fluid secreted by the early postnatal mammary gland. In this case, FcRn transports the IgG from the lumen of the neonatal gut into the blood and tissues. Interestingly, FcRn is also found in adults in the gut and liver and on endothelial cells. Its function in adults is to maintain the levels of IgG in plasma, which it does by binding antibody, endocytosing it, and recycling it to the blood, thus preventing its excretion from the body.

By means of these specialized transport systems, mammals are supplied from birth with antibodies against pathogens common in their environments. As they mature and make their own antibodies of all isotypes, these are distributed selectively to different sites in the body (Fig. 10.30). Thus, throughout life, class switching and the distribution of antibody classes throughout the body provide effective protection against infection in extracellular spaces.

10-18 High-affinity IgG and IgA antibodies can neutralize toxins and block the infectivity of viruses and bacteria.

Pathogens can cause damage to a host by producing toxins or by infecting cells directly, and antibodies can protect by blocking both of these actions. Many bacteria cause disease by secreting toxins that damage or disrupt the function of the host's cells (Fig. 10.31). To affect cells, many toxins consist of separate domains for exerting toxicity and for binding to specific cell-surface receptors by which they enter cells. Antibodies that bind a toxin's receptor-binding site can prevent cell entry and protect cells from attack (Fig. 10.32). Antibodies that act in this way to neutralize toxins are referred to as **neutralizing antibodies**. Most toxins are active at nanomolar concentrations: a single molecule of diphtheria toxin can kill a cell. To neutralize toxins, therefore, antibodies must be able to diffuse into the tissues and bind the toxin rapidly and with high affinity. The ability of IgG antibodies to diffuse easily throughout the

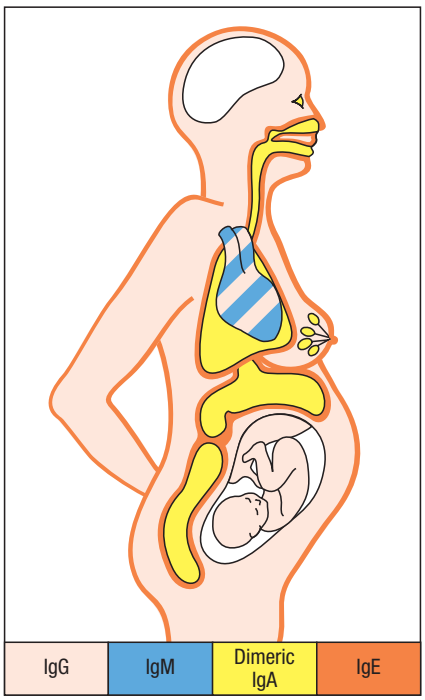


Fig. 10.30 Immunoglobulin classes are selectively distributed in the body. IgG and IgM predominate in blood (shown here for simplicity by IgM and IgG in the heart), whereas IgG and monomeric IgA are the major antibodies in extracellular fluid within the body. Dimeric IgA predominates in secretions across epithelia, including breast milk. The fetus receives IgG from the mother by transplacental transport. IgE is found mainly associated with mast cells just beneath epithelial surfaces (especially of the respiratory tract, gastrointestinal tract, and skin). The brain is normally devoid of immunoglobulin.

Disease	Organism	Toxin	Effects *in vivo*
Tetanus	*Clostridium tetani*	Tetanus toxin	Blocks inhibitory neuron action, leading to chronic muscle contraction
Diphtheria	*Corynebacterium diphtheriae*	Diphtheria toxin	Inhibits protein synthesis, leading to epithelial cell damage and myocarditis
Gas gangrene	*Clostridium perfringens*	Clostridial toxin	Phospholipase activation, leading to cell death
Cholera	*Vibrio cholerae*	Cholera toxin	Activates adenylate cyclase, elevates cAMP in cells, leading to changes in intestinal epithelial cells that result in loss of water and electrolytes
Anthrax	*Bacillus anthracis*	Anthrax toxic complex	Increases vascular permeability, leading to edema, hemorrhage, and circulatory collapse
Botulism	*Clostridium botulinum*	Botulinum toxin	Blocks release of acetylcholine, leading to paralysis
Whooping cough	*Bordetella pertussis*	Pertussis toxin Tracheal cytotoxin	ADP-ribosylation of G proteins, leading to lymphoproliferation Inhibits cilia and causes epithelial cell loss
Scarlet fever	*Streptococcus pyogenes*	Erythrogenic toxin Leukocidin Streptolysins	Vasodilation, leading to scarlet fever rash Kill phagocytes, allowing bacterial survival
Food poisoning	*Staphylococcus aureus*	Staphylococcal enterotoxin	Acts on intestinal neurons to induce vomiting. Also a potent T-cell mitogen (SE superantigen)
Toxic-shock syndrome	*Staphylococcus aureus*	Toxic-shock syndrome toxin	Causes hypotension and skin loss. Also a potent T-cell mitogen (TSST-1 superantigen)

Fig. 10.31 Many common diseases are caused by bacterial toxins. The toxins shown here are all exotoxins—proteins secreted by the bacteria. High-affinity IgG and IgA antibodies protect against these toxins. Bacteria also have nonsecreted endotoxins, such as lipopolysaccharide, which are released when the bacterium dies and may also mediate pathogenesis of disease. Host responses to exotoxins are more complex because the innate immune system has receptors for some endotoxins, such as TLR-4 (see Chapter 3).

extracellular fluid, and their high affinity for antigen once affinity maturation has taken place, make them the principal antibodies that neutralize toxins in tissues. High-affinity IgA antibodies similarly neutralize toxins at the mucosal surfaces of the body.

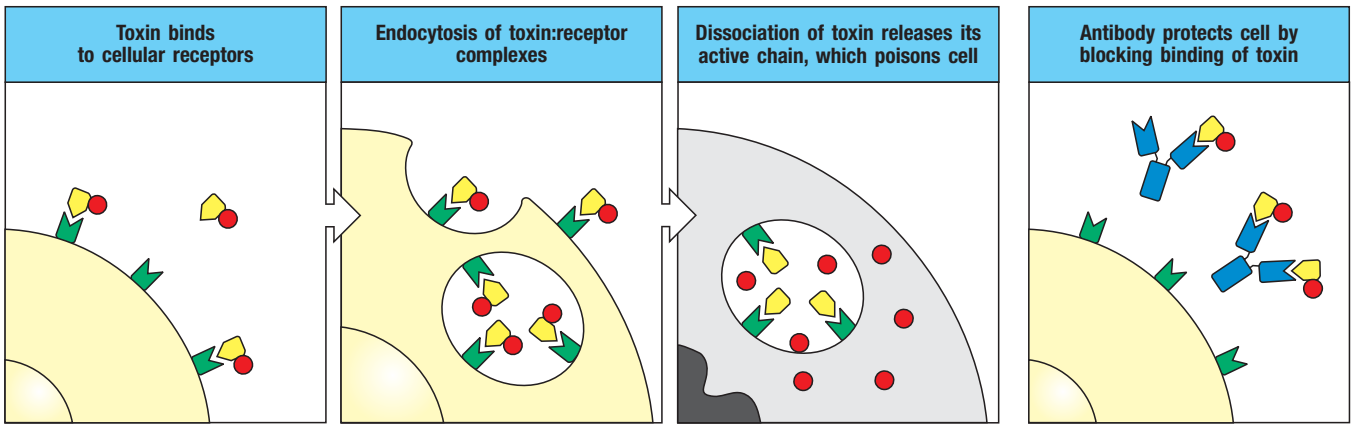


Fig. 10.32 Neutralization of toxins by IgG antibodies protects cells from damage. The damaging effects of many bacteria are due to the toxins they produce (see Fig. 10.31). These toxins are usually composed of several distinct moieties. One part of the toxin molecule binds a cell-surface receptor, which enables the molecule to be internalized. Another part of the toxin molecule then enters the cytoplasm and poisons the cell. Antibodies that inhibit toxin binding can prevent, or neutralize, these effects.

Diphtheria and tetanus toxins are two bacterial toxins in which the toxic and receptor-binding functions are on separate protein chains. It is therefore possible to immunize individuals, usually as infants, with modified toxin molecules in which the toxic chain has been denatured. These modified toxins, called **toxoids**, lack toxic activity but retain the receptor-binding site. Thus, immunization with the toxoid induces neutralizing antibodies that protect against the native toxin.

Some insect or animal venoms are so toxic that a single exposure can cause severe tissue damage or death. For these the adaptive immune response is too slow to be protective. Exposure to these venoms is a rare event, and protective vaccines have not been developed for use in humans. Instead, neutralizing antibodies are generated by immunizing other species, such as horses, with insect and snake venoms to produce anti-venom antibodies, or **antivenins**. The antivenins are injected into exposed individuals to protect them against the toxic effects of the venom. Transfer of antibodies in this way is known as **passive immunization** (see Appendix I, Section A-30).

Animal viruses infect cells by binding to a particular cell-surface receptor. These are often cell-type-specific proteins that determine which cells a virus can infect, or its **tropism**. Many antibodies that neutralize viruses do so by directly blocking the binding of virus to surface receptors (Fig. 10.33). The **hemagglutinin** of influenza virus, for example, binds to terminal sialic acid residues on the carbohydrates of glycoproteins present on epithelial cells of the respiratory tract. It is known as hemagglutinin because it recognizes and binds to similar sialic acid residues on chicken red blood cells and agglutinates these red blood cells. Antibodies against the hemagglutinin can prevent infection by the influenza virus. Such antibodies are called **virus-neutralizing antibodies**, and, as with the neutralization of toxins, high-affinity IgA and IgG antibodies are particularly important. However, antibodies can also neutralize viruses by interfering with the fusion mechanisms used to enter the cell's cytoplasm after binding to surface receptors.

Many bacteria have cell-surface molecules called **adhesins** that enable them to bind to the surface of host cells. This adherence is crucial to the ability of these bacteria to cause disease, whether they subsequently enter the cell, as do *Salmonella* species, or remain attached to the cell surface as extracellular pathogens (Fig. 10.34). *Neisseria gonorrhoeae*, the causative agent of the sexually transmitted disease gonorrhea, has a cell-surface protein known as **pilin** that enables the bacterium to adhere to the epithelial cells of the urinary and reproductive tracts and is essential to its infectivity. Antibodies against pilin can inhibit this adhesive reaction and prevent infection.

IgA antibodies secreted onto the mucosal surfaces of the intestinal, respiratory, and reproductive tracts are particularly important in inhibiting the colonization of these surfaces by pathogens and in preventing infection of the epithelial cells. Adhesion of bacteria to cells within tissues can also contribute to pathogenesis, and IgG antibodies against adhesins protect tissues from damage in much the same way as IgA antibodies protect mucosal surfaces.

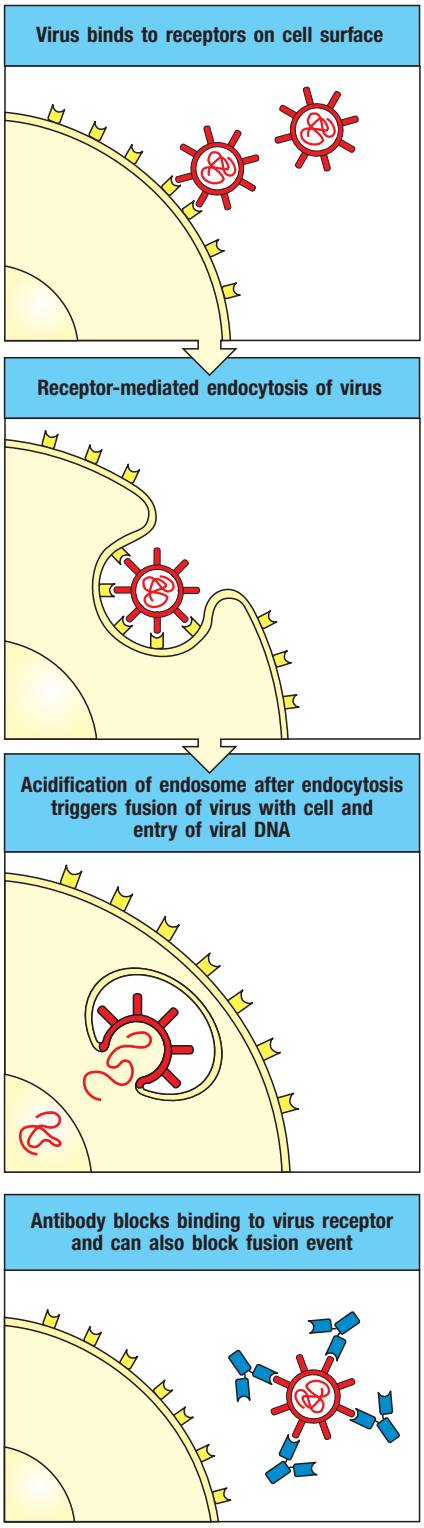


Fig. 10.33 Viral infection of cells can be blocked by neutralizing antibodies. For a virus to multiply within a cell, it must introduce its genes into the cell. The first step in entry is usually the binding of the virus to a receptor on the cell surface. For enveloped viruses, as shown in the figure, entry into the cytoplasm requires fusion of the viral envelope and the cell membrane. For some viruses this fusion event takes place on the cell surface (not shown); for others it can occur only within the more acidic environment of endosomes, as shown here. Non-enveloped viruses must also bind to receptors on cell surfaces, but they enter the cytoplasm by disrupting endosomes. Antibodies bound to viral surface proteins neutralize the virus, inhibiting either its initial binding to the cell or its subsequent entry.

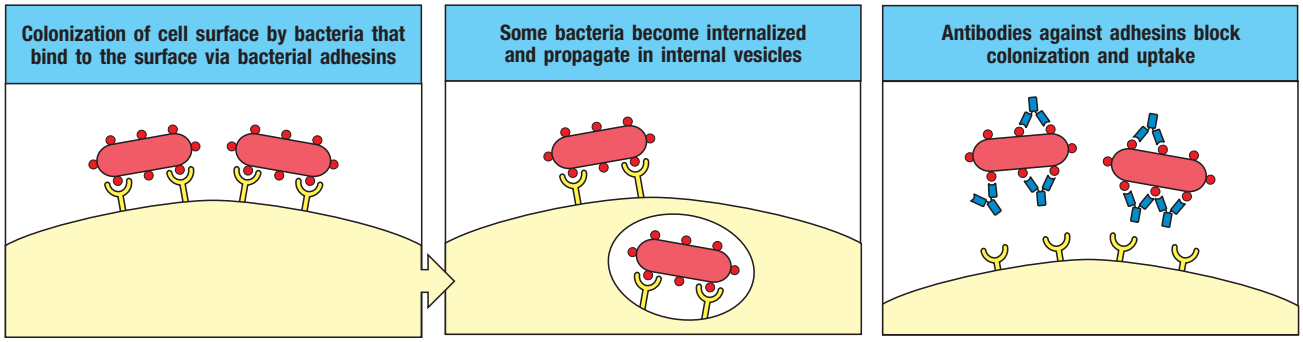


Fig. 10.34 Antibodies can prevent the attachment of bacteria to cell surfaces. Many bacterial infections require an interaction between the bacterium and a cell-surface receptor. This is particularly true for infections of mucosal surfaces. The attachment process involves very specific molecular interactions between bacterial adhesins and their receptors on host cells; antibodies against bacterial adhesins can block such infections.

10-19 Antibody:antigen complexes activate the classical pathway of complement by binding to C1q.

Chapter 2 introduced the complement system as an essential component of innate immunity. Complement activation can proceed in the absence of antibody via the **lectin pathway** through the actions of mannose-binding lectin (MBL) and ficolins. But complement is also an important effector of antibody responses via the **classical pathway**. The different pathways of complement activation converge to coat pathogen surfaces or antigen:antibody complexes with covalently attached complement fragment C3b, which acts as an opsonin to promote uptake and removal by phagocytes. In addition, the terminal complement components can form a membrane-attack complex that damages some bacteria.

In the classical pathway, complement activation is triggered by C1, a complex of C1q and the serine proteases C1r and C1s (see Section 2-7). Complement activation is initiated when antibodies that are attached to the surface of a pathogen then bind to C1 via C1q (Fig. 10.35). C1q can be bound by either

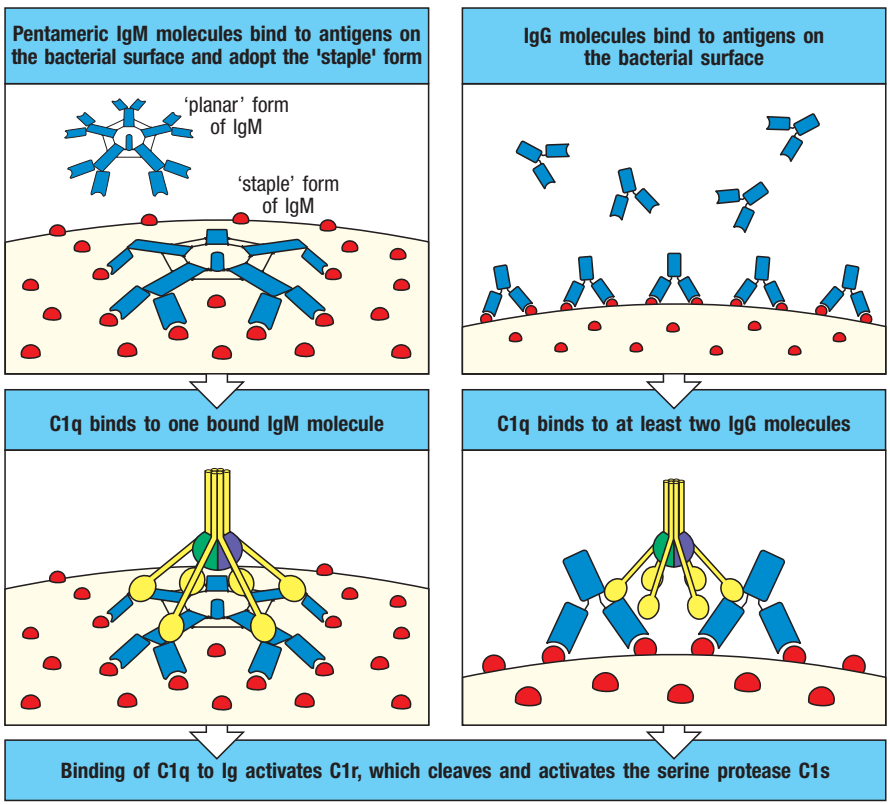


Fig. 10.35 The classical pathway of complement activation is initiated by the binding of C1q to antibody on a pathogen surface. When a molecule of IgM binds several identical epitopes on a pathogen surface, it is bent into the 'staple' conformation, which allows the globular heads of C1q to bind to the Fc regions of IgM (left panels). Multiple molecules of IgG bound on the surface of a pathogen allow the binding of a single molecule of C1q to two or more Fc regions (right panels). In both cases, the binding of C1q to the Fc regions induces a conformational change that activates the associated C1r, which becomes an active enzyme that cleaves the pro-enzyme C1s, generating a serine protease that initiates the classical complement cascade (see Chapter 2).

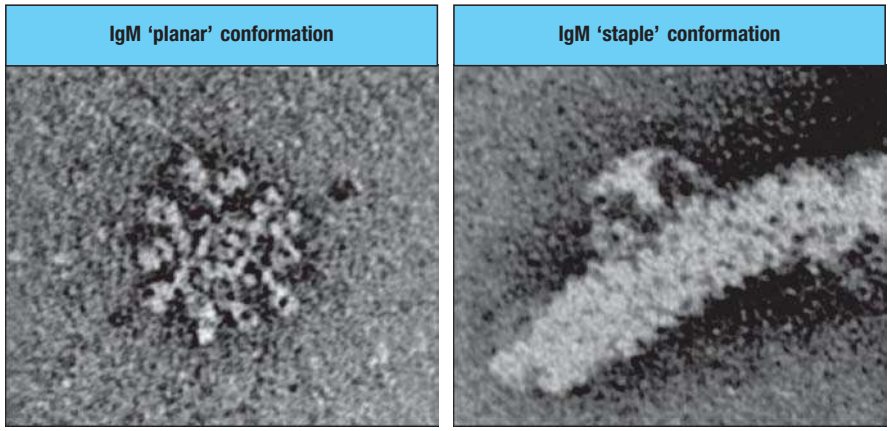


Fig. 10.36 The two conformations of IgM. The left panel shows the planar conformation of soluble IgM; the right panel shows the 'staple' conformation of IgM bound to a bacterial flagellum. Photographs (×760,000) courtesy of K.H. Roux.

IgM or IgG antibodies, but, because of the structural requirements of binding to C1q, neither of these antibody classes can activate complement in solution; the complement reactions are initiated only when the antibodies are already bound to multiple sites on a cell surface, normally that of a pathogen.

Each globular head of a C1q molecule can bind to one Fc region, and binding of two or more heads activates the C1 complex. In plasma, the **pentameric IgM** molecule has a planar conformation that does not bind C1q (Fig. 10.36, left panel); however, binding to the surface of a pathogen deforms the IgM pentamer so that it looks like a staple (see Fig. 10.36, right panel), and this distortion exposes binding sites for the C1q heads. As mentioned in Section 10-15, IgM hexamers can also form but comprise less than 5% of total serum IgM. Hexameric IgM activates complement about 20 times more efficiently than its pentameric form, possibly because C1q is also a hexamer. The *in vivo* role of IgM hexamers in protecting against infections has not been fully established, and it has even been suggested that IgM hexamers are too reactive and may be harmful.

Although C1q binds with low affinity to some subclasses of IgG in solution, the binding energy required for C1q activation is achieved only when a single molecule of C1q can bind two or more IgG molecules that are held within 30–40 nm of each other as a result of binding antigen. This requires that multiple molecules of IgG be bound to a single pathogen or to an antigen in solution. For this reason, IgM is much more efficient than IgG in activating complement. The binding of C1q to a single bound IgM molecule, or to two or more bound IgG molecules (see Fig. 10.35), leads to activation of the protease activity of C1r, triggering the complement cascade.

10-20 Complement receptors and Fc receptors both contribute to removal of immune complexes from the circulation.

Fc receptors confer the distinct effector functions to the various antibody isotypes by interacting with their Fc regions. One such function is the clearance from the circulation of antigen:antibody complexes (immune complexes), which can include toxins, or debris from dead host cells and microorganisms, bound by neutralizing antibodies. Immune complexes can be cleared by the binding of the antibody's Fc region to Fc receptors expressed on various phagocytic cells in tissues. This clearance is also helped by complement activation (described in the last section), which occurs when the Fc region activates C1q. The deposition of C4b and C3b onto the immune complex aids clearance by binding to complement receptor 1 (CR1) on the surface of erythrocytes (see Section 2-13 for a description of the different types of complement receptors). The erythrocytes transport the bound complexes of antigen, antibody, and complement to the liver and spleen. Here, macrophages bearing CR1 and Fc

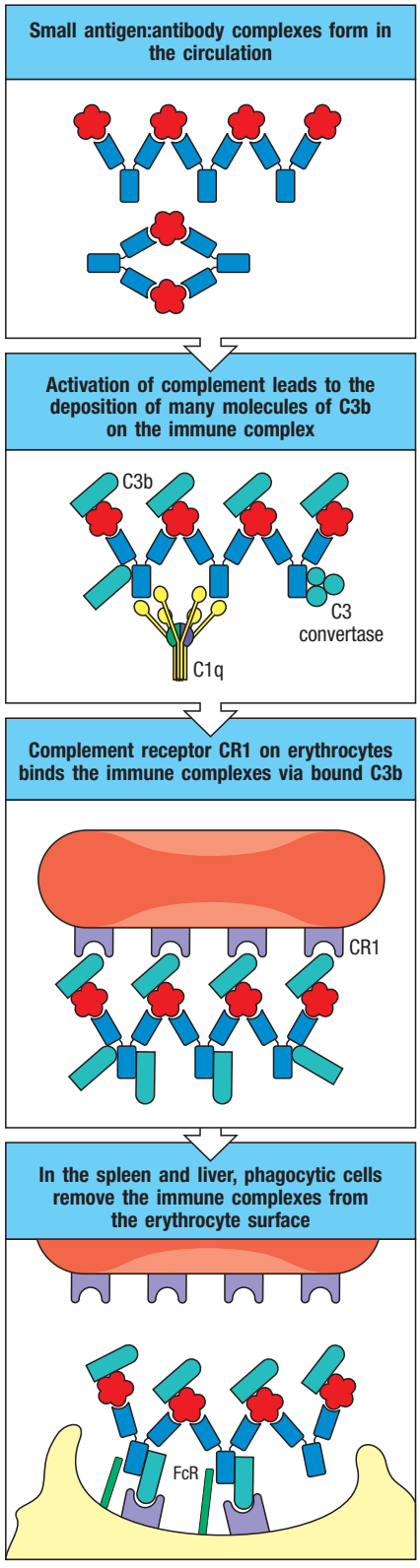


Fig. 10.37 Erythrocyte CR1 helps to clear immune complexes from the circulation. CR1 on the erythrocyte surface has an important role in the clearance of immune complexes from the circulation. Immune complexes bind to CR1 on erythrocytes, which transport them to the liver and spleen, where they are removed by macrophages expressing receptors for both Fc and bound complement components.

receptors remove the complexes from the erythrocyte surface without destroying the cell, and then degrade the complexes (Fig. 10.37). Even larger aggregates of particulate antigen, such as bacteria, viruses, and cell debris, can be coated with complement, picked up by erythrocytes, and transported to the spleen for destruction.

Complement-coated immune complexes that are not removed from the circulation tend to deposit in the basement membranes of small blood vessels, most notably those of the renal glomerulus, where the blood is filtered to form urine. Immune complexes that pass through the basement membrane of the glomerulus bind to CR1 present on the renal podocytes, cells that lie beneath the basement membrane. The functional significance of these receptors in the kidney is unknown; however, they have an important role in the pathology of some autoimmune diseases. In the autoimmune disease **systemic lupus erythematosus** (SLE) (see Section 15-16), excessive levels of circulating immune complexes lead to their deposition in large amounts on the podocytes, damaging the glomerulus; kidney failure is the principal danger in this disease. The strongest genetic risk factor for SLE is C1q deficiency, although this is very rare. Mutations in complement receptors 2 and 3 and the Fc receptor FcγRIIIa are also associated with increased susceptibility to develop lupus, implying the involvement of both complement receptors and FcR pathways in clearing immune complexes.

Antigen:antibody complexes can also be a cause of pathology in patients with deficiencies in the early components of complement (C1, C2, and C4). These deficiencies result in the classical complement pathway not being activated properly, and immune complexes not being cleared effectively because they do not become tagged with complement. These patients also suffer tissue damage as a result of immune-complex deposition, especially in the kidneys.

Summary.

The T-cell-dependent antibody response begins with IgM secretion but quickly progresses to the production of additional antibody classes. Each class is specialized both in its localization in the body and in the functions it can perform. IgM antibodies are found mainly in blood; they are pentameric in structure. IgM is specialized to activate complement efficiently upon binding antigen and to compensate for the low affinity of a typical IgM antigen-binding site. IgG antibodies are usually of higher affinity and are found in blood and in extracellular fluid, where they can neutralize toxins, viruses, and bacteria, opsonize them for phagocytosis, and activate the complement system. IgA antibodies are synthesized as monomers, which enter blood and extracellular fluids, or they are secreted as dimeric molecules by plasma cells in the lamina propria of various mucosal tissues. IgA dimers are selectively transported across the epithelial layer into sites such as the lumen of the gut, where they neutralize toxins and viruses and block the entry of bacteria across the intestinal epithelium. Most IgE antibody is bound to the surface of mast cells that reside mainly just below the body surface; antigen binding to this IgE triggers local defense reactions. Antibodies can defend the body against extracellular pathogens and their toxic products in several ways. The simplest is by direct interactions with pathogens or their products, for example, by binding to the active sites of toxins and neutralizing them or by blocking their ability to bind to host cells through specific receptors. When antibodies of the appropriate isotype bind to antigens, they can activate the classical pathway of complement, which leads

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to the elimination of the pathogen by the various mechanisms described in Chapter 2. Soluble immune complexes of antigen and antibody also fix complement and are cleared from the circulation via complement receptors on red blood cells.

The destruction of antibody-coated pathogens via Fc receptors.

The neutralization of toxins, viruses, or bacteria by high-affinity antibodies can protect against infection but does not, on its own, solve the problem of how to remove the pathogens and their products from the body. Moreover, many pathogens cannot be neutralized by antibody and must be destroyed by other means. Many pathogen-specific antibodies do not bind to neutralizing targets on pathogen surfaces and thus need to be linked to other effector mechanisms to play their part in host defense. We have already seen how the binding of antibody to antigen can activate complement. Another important defense mechanism is the activation of a variety of **accessory effector cells** bearing receptors called Fc receptors because they are specific for the Fc portion of antibodies. These receptors facilitate the phagocytosis of antibody-bound extracellular pathogens by macrophages, dendritic cells, and neutrophils. Other, nonphagocytic cells of the immune system—NK cells, eosinophils, basophils, and mast cells (see Fig. 1.8)—are triggered to secrete stored mediators when their Fc receptors are engaged by antibody-coated pathogens. These mechanisms maximize the effectiveness of all antibodies regardless of where they bind.

10-21 The Fc receptors of accessory cells are signaling receptors specific for immunoglobulins of different classes.

The **Fc receptors** are a family of cell-surface molecules that bind the Fc portion of immunoglobulins. Each member of the Fc family recognizes immunoglobulin of one or a few closely related heavy-chain isotypes through a recognition domain on the α chain of the Fc receptor. Most Fc receptors are themselves members of the immunoglobulin gene superfamily. Different cell types bear different sets of Fc receptors, and the isotype of the antibody thus determines which types of cells will be engaged in a given response. The different Fc receptors, the cells that express them, and their specificities for different antibody classes are shown in **Fig. 10.38**.

Most Fc receptors function as part of a multisubunit complex. Only the α chain is required for antibody recognition; the other chains are required for transport of the receptor to the cell surface and for signal transduction when an Fc region is bound. Some Fcγ receptors, the Fcα receptor I, and the high-affinity receptor for IgE (FcεRI) all use a γ chain for signaling. This chain, which is closely related to the ζ chain of the T-cell receptor complex (see Section 7-7), associates noncovalently with the Fc-binding α chain. The human FcγRII-A is a single-chain receptor in which the cytoplasmic domain of the α chain replaces the function of the γ chain. FcγRII-B1 and FcγRII-B2 are also single-chain receptors, but function as inhibitory receptors because they contain an ITIM that engages the inositol 5′-phosphatase SHIP (see Section 7-25). The most prominent function of Fc receptors is the activation of accessory cells to attack pathogens, but they also contribute in other ways to immune responses. For example, FcγRII-B receptors negatively regulate the activities of B cells, mast cells, macrophages, and neutrophils by adjusting the threshold at which immune complexes will activate these cells. Fc receptors expressed by dendritic cells enable them to ingest antigen:antibody complexes efficiently and thus process these antigens and present their peptides to T cells.

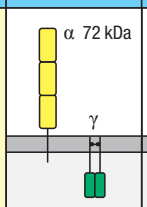
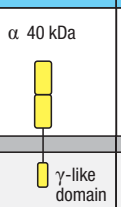
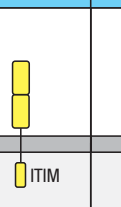
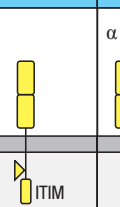
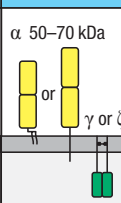
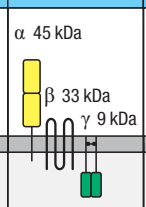
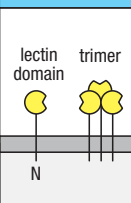
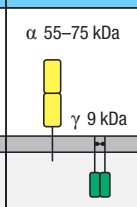
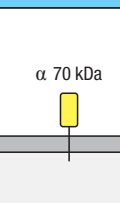
Receptor	FcγRI (CD64)	FcγRII-A (CD32)	FcγRII-B2 (CD32)	FcγRII-B1 (CD32)	FcγRIII (CD16)	FcεRI	FcεRII (CD23)	FcαRI (CD89)	Fcα/μR
Structure									
Binding Order of affinity	IgG1 $10^8\ M^{-1}$ 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 $2 \times 10^6\ M^{-1}$ 1) IgG1 2) IgG3=IgG2* 3) IgG4	IgG1 $2 \times 10^6\ M^{-1}$ 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 $2 \times 10^6\ M^{-1}$ 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 $5 \times 10^5\ M^{-1}$ IgG1=IgG3	IgE $10^{10}\ M^{-1}$	IgE $2–7 \times 10^7\ M^{-1}$ (trimer) $2–7 \times 10^6\ M^{-1}$ (monomer)	IgA1, IgA2 $10^7\ M^{-1}$ IgA1=IgA2	IgA, IgM $3 \times 10^9\ M^{-1}$ 1) IgM 2) IgA
Cell type	Macrophages Neutrophils Eosinophils	Macrophages Neutrophils Eosinophils Platelets Langerhans cells	Macrophages Neutrophils Eosinophils	B cells Mast cells	NK cells Eosinophils Macrophages Neutrophils Mast cells	Mast cells Basophils	Eosinophils B cells	Macrophages Eosinophils† Neutrophils	Macrophages B cells
Effect of ligation	Uptake Stimulation Activation of respiratory burst Induction of killing	Uptake Granule release (eosinophils)	Uptake Inhibition of stimulation	No uptake Inhibition of stimulation	Induction of killing (NK cells)	Secretion of granules	Degranulation	Uptake Induction of killing	Uptake

Fig. 10.38 Distinct receptors for the Fc region of the different immunoglobulin classes are expressed on different accessory cells. The subunit structure and binding properties of these receptors and the cell types expressing them are shown. All are immunoglobulin superfamily members except FcεRII, which is a lectin and can form trimers. The exact chain composition of any receptor can vary from one cell type to another. For example, FcγRIII in neutrophils is expressed as a molecule with a glycosylphosphatidylinositol membrane anchor without γ chains, whereas in NK cells it is a transmembrane molecule associated with γ chains. The FcγRII-B1 differs from the FcγRII-B2 by the presence of an additional exon in the intracellular region (indicated by yellow triangle). This exon prevents the FcγRII-B1 from being internalized after cross-linking. The binding affinities are taken from data on human receptors. *Only some allotypes of FcγRII-A bind IgG2. †In eosinophils, the molecular weight of the CD89α chain is 70–100 kDa.

Antibody-coated viruses that enter the cytoplasm are cleared by a system that employs a novel class of Fc receptor called **TRIM21** (tripartite motif-containing 21) that is expressed by a variety of immune and nonimmune cell types. TRIM21 is a cytosolic IgG receptor that has a higher affinity for IgG than any other Fc receptor, and it also has **E3 ligase** activity. When a virus that has bound IgG enters the cytoplasm, TRIM21 attaches to the antibody and uses its E3 ligase activity to ubiquitinate viral proteins. This leads to proteasomal degradation of virions in the cytosol before translation of virally encoded genes can occur.

10-22 Fc receptors on phagocytes are activated by antibodies bound to the surface of pathogens and enable the phagocytes to ingest and destroy pathogens.

The most important Fc-bearing cells in humoral immune responses are the phagocytic cells of the monocytic and myelocytic lineages, particularly macrophages and neutrophils. Many bacteria are directly recognized, ingested, and destroyed by phagocytes, and these bacteria are not pathogenic in normal individuals. However, some bacterial pathogens have **polysaccharide capsules**, a large structure that lies outside the bacterial cell membrane and resists direct engulfment by phagocytes. Such pathogens become susceptible to phagocytosis only when they are coated with antibodies and complement that engage the Fcγ or Fcα receptors and the complement receptor CR1 on phagocytic cells, triggering bacterial uptake (Fig. 10.39). The stimulation

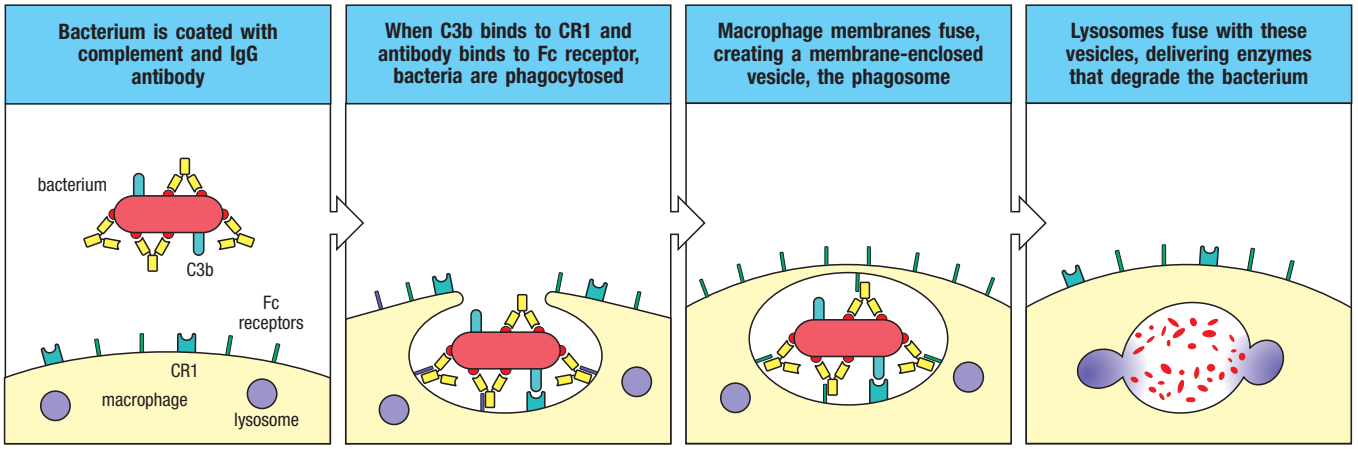


Fig. 10.39 Fc and complement receptors on phagocytes trigger the uptake and degradation of antibody-coated bacteria. Many bacteria resist phagocytosis by macrophages and neutrophils. Antibodies bound to these bacteria, however, enable the bacteria to be ingested and degraded through the interaction of the multiple Fc domains arrayed on the bacterial surface with Fc receptors on the phagocyte surface. Antibody coating also induces activation of the complement system and the binding of complement components to the bacterial surface. These can interact with complement receptors (for example, CR1) on the phagocyte. Fc receptors and complement receptors synergize in inducing phagocytosis. Bacteria coated with IgG antibody and complement are therefore more readily ingested than those coated with IgG alone. Binding of Fc and complement receptors signals the phagocyte to increase the rate of phagocytosis, to fuse lysosomes with phagosomes, and to increase its bactericidal activity.

of phagocytosis by complement-coated antigens binding to complement receptors is particularly important early in the immune response, before isotype-switched antibodies have been made. Capsular polysaccharides belong to the TI-2 class of thymus-independent antigens, and can therefore stimulate the early production of IgM antibodies, which are very effective at activating the complement system. IgM binding to encapsulated bacteria thus triggers the opsonization of these bacteria by complement and their prompt ingestion and destruction by phagocytes bearing complement receptors. Recently, Fcα/μR was discovered as a receptor that binds both IgA and IgM. Fcα/μR is expressed primarily on macrophages and B cells in the lamina propria of the intestine and in germinal centers. It is thought to have a role in the endocytosis of IgM antibody complexed with bacteria such as *Staphylococcus aureus*.

Phagocyte activation can initiate an inflammatory response that causes tissue damage, and so Fc receptors on phagocytes must be able to distinguish antibody molecules bound to a pathogen from the much larger number of free antibody molecules that are not bound to anything. This distinction is made possible by the aggregation of antibodies that occurs when they bind to multimeric antigens or to multivalent particulate antigens such as viruses and bacteria. Individual Fc receptors on a cell surface bind monomers of free antibody with low affinity, but when presented with an antibody-coated particle, the simultaneous binding by multiple Fc receptors results in binding of high avidity, and this is the principal mechanism by which bound antibodies are distinguished from free immunoglobulin (Fig. 10.40). The result is that Fc receptors enable cells to detect pathogens via the antibody molecules bound to them. Fc receptors therefore give phagocytic cells that lack intrinsic specificity the ability to identify and remove specific pathogens and their products from the extracellular spaces.

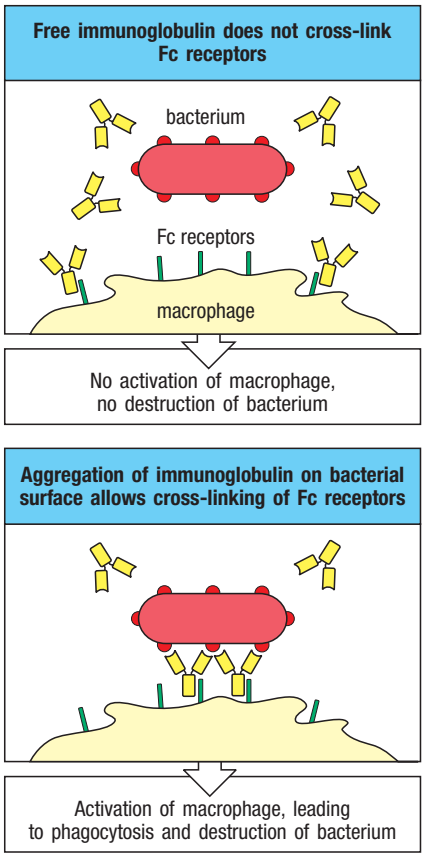


Fig. 10.40 Bound antibody is distinguishable from free immunoglobulin by its state of aggregation. Free immunoglobulin molecules bind most Fc receptors with very low affinity and cannot cross-link Fc receptors. Antigen-bound immunoglobulin, however, binds to Fc receptors with high avidity because several antibody molecules that are bound to the same surface bind to multiple Fc receptors on the surface of the accessory cell. This Fc receptor cross-linking sends a signal to activate the cell bearing it. With Fc receptors that have ITIMs, the result is inhibition.

Phagocytosis is greatly enhanced by interactions between the molecules coating an opsonized microorganism and receptors on the phagocyte surface. When an antibody-coated pathogen binds to Fcγ receptors, for example, the cell surface of the phagocyte extends around the surface of the pathogen through successive binding of the Fcγ receptors to the antibody Fc regions bound to the pathogen. This is an active process that is triggered by the stimulation of the Fcγ receptors. Phagocytosis leads to enclosure of the pathogen (or particle) in an acidified cytoplasmic vesicle—the phagosome. This then fuses with one or more lysosomes to generate a phagolysosome; lysosomal enzymes are released into the vesicle interior, where they destroy the bacterium (see Fig. 10.39). The process of intracellular killing by phagocytes was described in more detail in Chapter 3.

Some particles are too large for a phagocyte to ingest; parasitic worms are one example. In this case the phagocyte attaches to the surface of the antibody-coated parasite via its Fcγ, Fcα, or Fcε receptors, and the contents of the secretory granules or lysosomes of the phagocyte are released by exocytosis. The contents are discharged directly onto the surface of the parasite and damage it. Thus, stimulation of Fcγ and Fcα receptors can trigger either the internalization of external particles by phagocytosis or the externalization of internal vesicles by exocytosis. The principal leukocytes involved in the destruction of bacteria are macrophages and neutrophils, whereas large parasites such as helminths are usually attacked by eosinophils (**Fig. 10.41**), nonphagocytic cells that can bind antibody-coated parasites via several different Fc receptors, including the low-affinity Fcε receptor for IgE, CD23 (see Fig. 10.38). Cross-linking of these receptors by antibody-coated surfaces activates the eosinophil to release its granule contents, which include proteins toxic to parasites (see Fig. 14.10). Cross-linking by antigen of IgE bound to the high-affinity FcεRI on mast cells and basophils also results in exocytosis of their granule contents, as we describe below.

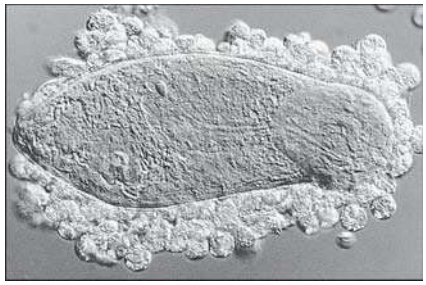


Fig. 10.41 Eosinophils attacking a schistosome larva in the presence of serum from an infected patient. Large parasites, such as worms, cannot be ingested by phagocytes; however, when the worm is coated with antibody, eosinophils can attack it through binding via their Fc receptors for IgG and IgA. Similar attacks on large targets can be mounted by other Fc receptor-bearing cells. These cells release the toxic contents of their granules directly onto the target, a process known as exocytosis. Photograph courtesy of A. Butterworth.

10-23 Fc receptors activate NK cells to destroy antibody-coated targets.

Virus-infected cells are usually destroyed by T cells that recognize virus-derived peptides bound to cell-surface MHC molecules. Cells infected by some viruses also signal the presence of intracellular infection by expressing on their surface proteins, such as viral envelope proteins, that can be recognized by antibodies originally produced against the virus particle. Host cells with antibodies bound to them can be killed by a specialized non-T, non-B cell of the lymphoid lineage called a natural killer cell (NK cell), which we met in Chapter 3. NK cells are large cells with prominent intracellular granules and make up a small fraction of peripheral blood lymphocytes. Although belonging to the lymphoid lineage, NK cells express a limited repertoire of invariant receptors recognizing a range of ligands that are induced on abnormal cells, such as those infected with viruses; NK cells are considered to be part of innate immunity (see Section 3-25). On recognition of a ligand, the NK cell kills the target cell directly without the need for antibody. Although first discovered for their ability to kill some tumor cells, NK cells play an important role in innate immunity in the early stages of virus infection.

As well as this innate function, NK cells can recognize and destroy antibody-coated target cells in a process called **antibody-dependent cell-mediated cytotoxicity** (**ADCC**). This is triggered when antibody bound to the surface of a cell interacts with Fc receptors on the NK cell (**Fig. 10.42**). NK cells express the receptor FcγRIII (CD16), which recognizes the IgG1 and IgG3 subclasses. The killing mechanism is analogous to that of cytotoxic T cells, involving the release of cytoplasmic granules containing perforin and granzymes (see Section 9-31). ADCC has been shown to have a role in the defense against

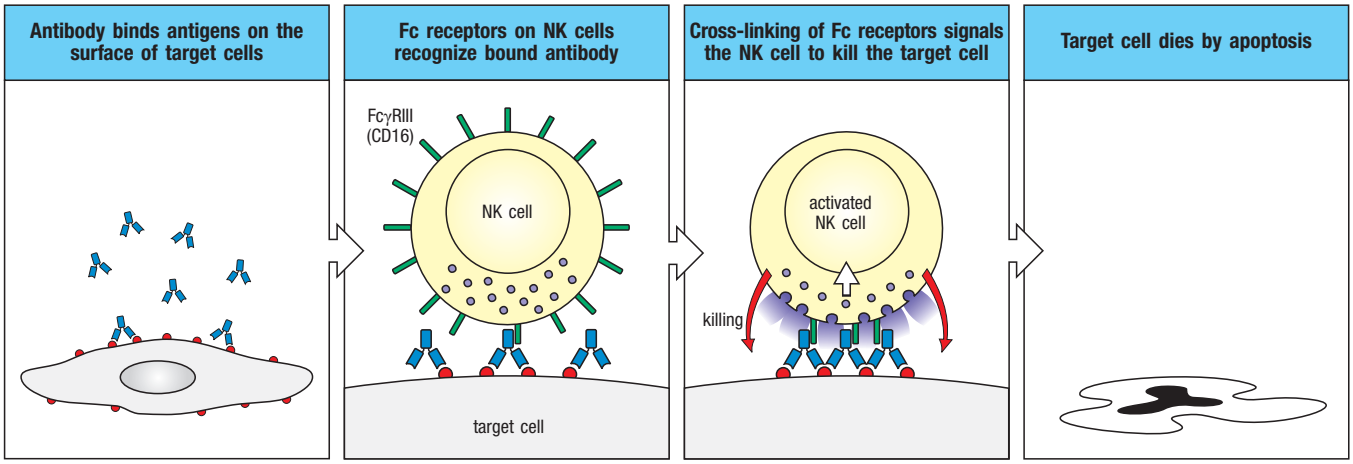


Fig. 10.42 Antibody-coated target cells can be killed by NK cells in antibody-dependent cell-mediated cytotoxicity (ADCC). NK cells (see Chapter 3) are large granular non-T, non-B lymphoid cells that have FcγRIII (CD16) on their surface. When these cells encounter cells coated with IgG antibody, they rapidly kill the target cell. ADCC is only one way in which NK cells can contribute to host defense.

infection by viruses, and represents another mechanism by which antibodies can direct an antigen-specific attack by an effector cell that itself lacks specificity for antigen.

10-24 Mast cells and basophils bind IgE antibody via the high-affinity Fcε receptor.

When pathogens cross epithelial barriers and establish a local focus of infection, the host must mobilize its defenses and direct them to the site of pathogen growth. One way in which this is achieved is to activate the cells known as **mast cells**. Mast cells are large cells containing distinctive cytoplasmic granules that contain a mixture of chemical mediators, including histamine, that act rapidly to make local blood vessels more permeable. Mast cells have a distinctive appearance after staining with the dye toluidine blue that makes them readily identifiable in tissues (see Fig. 1.8). They are found in particularly high concentrations in vascularized connective tissues just beneath epithelial surfaces, including the submucosal tissues of the gastrointestinal and respiratory tracts and the dermis of the skin.

Mast cells have Fc receptors specific for IgE (FcεRI) and IgG (FcγRIII), and can be activated to release their granules and to secrete lipid inflammatory mediators and cytokines via antibody bound to these receptors. Most Fc receptors bind stably to the Fc regions of antibodies only when the antibodies have themselves bound antigen, and cross-linking of multiple Fc receptors is needed for strong binding. In contrast, FcεRI binds IgE antibody monomers with a very high affinity—approximately 10^{10} M^{-1}. Thus, even at the low levels of circulating IgE present in normal individuals, a substantial portion of the total IgE is bound to the FcεRI on mast cells in tissues and on circulating basophils.

Although mast cells are usually stably associated with bound IgE, this on its own does not activate them, nor will the binding of monomeric antigen to the IgE. Mast-cell activation occurs only when the bound IgE is cross-linked by multivalent antigens. This signal activates the mast cell to release the contents of its granules, which occurs in seconds (Fig. 10.43), to synthesize and release lipid mediators such as prostaglandin D_2 and leukotriene C4, and to secrete cytokines such as TNF-α, thereby initiating a local inflammatory response. Degranulation also releases stored histamine, which increases local blood flow and vascular permeability; this quickly leads to an accumulation

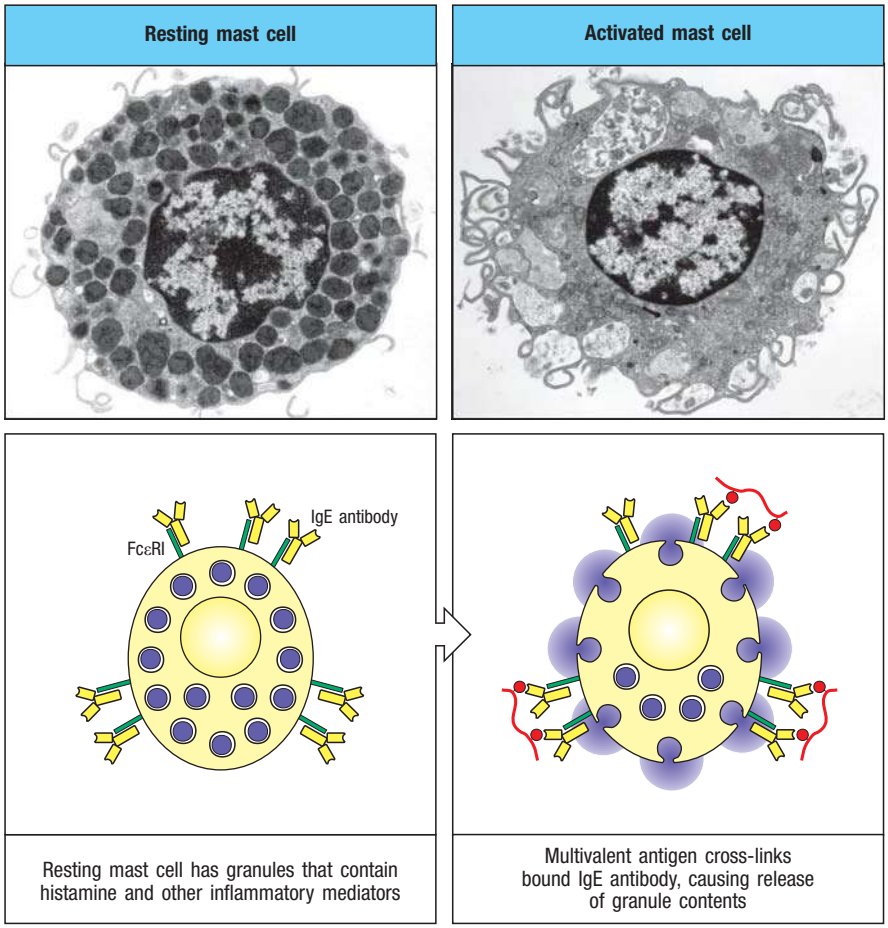


Fig. 10.43 IgE antibody cross-linking on mast-cell surfaces leads to a rapid release of inflammatory mediators. Mast cells are large cells found in connective tissue and can be distinguished by their secretory granules, which contain many inflammatory mediators. They bind stably to monomeric IgE antibodies through the very high-affinity receptor FcεRI. Antigen cross-linking of the bound IgE antibody molecules triggers rapid degranulation, releasing inflammatory mediators into the surrounding tissue. These mediators trigger local inflammation, which recruits cells and proteins required for host defense to sites of infection. These cells are also triggered during allergic reactions when allergens bind to IgE on mast cells. Photographs courtesy of A.M. Dvorak.

of fluid and blood proteins, including antibodies, in the surrounding tissue. Shortly afterward there is an influx of blood-borne cells such as neutrophils and, later, monocytes, eosinophils, and effector lymphocytes. This influx can last from a few minutes to a few hours and produces an inflammatory response at the site of infection. Thus, mast cells are part of the front-line host defenses against pathogens that enter the body across epithelial barriers. They are also of medical importance because of their involvement in IgE-mediated allergic responses, which are discussed in Chapter 14. In allergic responses, mast cells are activated in the way described above by exposure to normally innocuous antigens (allergens), such as pollen, to which the individual has previously mounted a sensitizing immune response that produces allergen-specific IgE.

10-25 IgE-mediated activation of accessory cells has an important role in resistance to parasite infection.

Mast cells are thought to serve at least three important functions in host defense. First, their location near body surfaces allows them to recruit both pathogen-specific elements, such as antigen-specific lymphocytes, and non-specific effector elements, such as neutrophils, macrophages, basophils, and eosinophils, to sites where infectious agents are most likely to enter the internal milieu. Second, the inflammation they cause increases the flow of lymph from sites of antigen deposition to the regional lymph nodes, where naive lymphocytes are first activated. Third, the ability of mast-cell products to trigger muscular contraction can contribute to the physical expulsion of pathogens from the lungs or the gut. Mast cells respond rapidly to the binding of antigen to surface-bound IgE antibodies, and their activation leads to the initiation of

an inflammatory response and the recruitment and activation of basophils and eosinophils, which contribute further to the inflammatory response (see Chapter 14). There is increasing evidence that such IgE-mediated responses are crucial to defense against parasite infestation.

A role for mast cells in the clearance of parasites is suggested by the accumulation of mast cells in the intestine, known as **mastocytosis**, that accompanies helminth infection, and by observations in W/W^V mutant mice, which have a profound mast-cell deficiency caused by a mutation in the gene c-*kit*. These mutant mice show impaired clearance of the intestinal nematodes *Trichinella spiralis* and *Strongyloides* species. Clearance of *Strongyloides* is even more impaired in W/W^V mice that lack IL-3 and so also fail to produce basophils. Thus, both mast cells and basophils seem to contribute to defense against these helminth parasites.

Other evidence points to the importance of IgE antibodies and eosinophils in the defense against parasites. Infection with certain types of multicellular parasites, particularly helminths, is strongly associated with the production of IgE antibodies and the presence of abnormally large numbers of eosinophils (eosinophilia) in blood and tissues. Furthermore, experiments in mice show that depletion of eosinophils by polyclonal anti-eosinophil antisera increases the severity of infection with the parasitic helminth *Schistosoma mansoni*. Eosinophils seem to be directly responsible for helminth destruction; examination of infected tissues shows degranulated eosinophils adhering to helminths, and experiments *in vitro* have shown that eosinophils can kill *S. mansoni* in the presence of anti-schistosome IgG or IgA antibodies (see Fig. 10.41).

The role of IgE, mast cells, basophils, and eosinophils can also be seen in resistance to the feeding of blood-sucking ixodid ticks. Skin at the site of a tick bite has degranulated mast cells and an accumulation of degranulated basophils and eosinophils, an indicator of recent activation. Subsequent resistance to feeding by these ticks develops after the first exposure, suggesting a specific immunological mechanism. Mice deficient in mast cells show no such acquired resistance to ticks, and in guinea pigs the depletion of either basophils or eosinophils by specific polyclonal antibodies also reduces resistance to tick feeding. Finally, experiments in mice showed that resistance to ticks is mediated by specific IgE antibody. Thus, many clinical studies and experiments support a role for this system of IgE bound to the high-affinity FcεRI in host resistance to pathogens that enter across epithelia or exoparasites such as ticks that breach it.

Summary.

Antibody-coated pathogens are recognized by effector cells through Fc receptors that bind to an array of constant regions (Fc portions) provided by the pathogen-bound antibodies. Binding activates the cell and triggers destruction of the pathogen, through either phagocytosis, granule release, or both. Fc receptors comprise a family of proteins, each of which recognizes immunoglobulins of particular isotypes. Fc receptors on macrophages and neutrophils recognize the constant regions of IgG or IgA antibodies bound to a pathogen and trigger the engulfment and destruction of such bacteria. Binding to the Fc receptor also induces the production of microbicidal agents in the intracellular vesicles of the phagocyte. Eosinophils are important in the elimination of parasites too large to be engulfed; they bear Fc receptors specific for the constant region of IgG, as well as receptors for IgE; aggregation of these receptors triggers the release of toxic substances onto the surface of the parasite. NK cells, tissue mast cells, and blood basophils also release their granule contents when their Fc receptors are engaged. The high-affinity receptor for

IgE is expressed constitutively by mast cells and basophils. It differs from other Fc receptors in that it can bind free monomeric antibody, thus enabling an immediate response to pathogens at their site of first entry into the tissues. When IgE bound to the surface of a mast cell is aggregated by binding to antigen, it triggers the release of histamine and many other mediators that increase the blood flow to sites of infection; it thereby recruits antibodies and effector cells to these sites. Mast cells are found principally below epithelial surfaces of the skin and beneath the basement membrane of the digestive and respiratory tracts. Their activation by innocuous substances is responsible for many of the symptoms of acute allergic reactions, as will be described in Chapter 14.

Summary to Chapter 10.

The humoral immune response to infection involves the production of antibody by plasma cells derived from B lymphocytes, the binding of this antibody to the pathogen, and the elimination of the pathogen by phagocytic cells and molecules of the humoral immune system. The production of antibody usually requires the action of helper T cells specific for a peptide fragment of the antigen recognized by the B cell, a phenomenon called linked recognition. An activated B cell first moves to the T-zone–B-zone boundary in secondary lymphoid tissues, where it may encounter its cognate T cell and begin to proliferate. Some B cells become plasmablasts, while others move to the germinal center, where somatic hypermutation and class switch recombination take place. B cells that bind antigen with the highest affinity are selected for survival and further differentiation, leading to affinity maturation of the antibody response. Cytokines made by helper T cells direct class switching, leading to the production of antibody of various classes that can be distributed to various body compartments.

IgM antibodies are produced early in an infection by conventional B cells and are also made in the absence of infection by subsets of nonconventional B cells in particular locations (as natural antibodies). IgM has a major role in protecting against infection in the bloodstream, whereas isotypes secreted later in an adaptive immune response, such as IgG, diffuse into the tissues. Antigens that have highly repeating antigenic determinants and that contain mitogens—called TI antigens—can elicit IgM and some IgG independently of T-cell help, and this provides an early protective immune response. Multimeric IgA is produced in the lamina propria and is transported across epithelial surfaces, whereas IgE is made in small amounts and binds avidly to receptors on the surface of basophils and mast cells.

Antibodies that bind with high affinity to critical sites on toxins, viruses, and bacteria can neutralize them. However, pathogens and their products are destroyed and removed from the body largely through uptake into phagocytes and degradation inside these cells. Antibodies that coat pathogens bind to Fc receptors on phagocytes, which are thereby triggered to engulf and destroy the pathogen. Binding of antibody C regions to Fc receptors on other cells leads to the exocytosis of stored mediators; this is particularly important in parasite infections, in which Fcε-expressing mast cells are triggered by the binding of antigen to IgE antibody to release inflammatory mediators directly onto parasite surfaces. Antibodies can also initiate the destruction of pathogens by activating the complement system. Complement components can opsonize pathogens for uptake by phagocytes, and recruit phagocytes to sites of infection. Receptors for complement components and Fc receptors often synergize in activating the uptake and destruction of pathogens and immune complexes. Thus, the humoral immune response is targeted to the infecting pathogen through the production of specific antibody; however, the effector actions of that antibody are determined by its heavy-chain isotype.

Questions.

10.1 Multiple Choice: Which of the following is not an antibody effector function?

A. Opsonization
B. Neutralization
C. Complement activation
D. Linked recognition
E. NK-cell cytotoxicity
F. Mast-cell degranulation

10.2 Short Answer: The *Haemophilus influenzae* type b (Hib) vaccine was initially composed only of the polysaccharide capsule of the organism, but this failed to mount potent antibody responses. Directly conjugating the Hib polysaccharide to a tetanus or diphtheria toxoid, however, yielded very potent antibody responses to Hib, and is the current vaccine formulation. Indicate which immunological phenomenon is taken advantage of by conjugating the Hib capsule-derived polysaccharide to a toxoid, and how it works to elicit a potent antibody response.

10.3 Matching: During T-dependent antibody responses, numerous receptor/ligand interactions and cytokine signaling events occur between T_{FH} cells and activated B cells. For the following list of surface receptors/ligands and cytokines, indicate whether they are produced by T cells (T), B cells (B), both (TB), or neither (N) in this context.

A. IL-21
B. ICOSL
C. CD40L
D. CD30L
E. Peptide:MHC II
F. CCL21
G. SLAM

10.4 Matching: Match the human disease to the associated genetic defect.

A. X-linked lymphoproliferative disorder	**i.** Translesion polymerase Polη
B. Hyper IgM type 2 immunodeficiency	**ii.** ATM (a DNA-PKcs-family kinase)
C. Xeroderma pigmentosum	**iii.** SLAM-associated protein (SAP)
D. Ataxia telangiectasia	**iv.** Activation-induced cytidine deaminase (AID)

10.5 Matching: Indicate whether the following properties apply to IgA, IgD, IgE, IgG, and/or IgM.

A. First produced during humoral response
B. Monomeric (predominantly)
C. Dimeric (predominantly)
D. Pentameric (predominantly)
E. Contains a J chain
F. Capable of eliciting complement deposition
G. Most abundant in mucosal surfaces and secretions
H. Low-affinity
I. Bound onto mast cells
J. Binds to polymeric immunoglobulin receptor (pIgR)
K. Binds the neonatal Fc receptor (FcRn)

10.6 Short Answer: How is TRIM21, a novel class of Fc receptor, different from other Fc receptors?

10.7 Multiple Choice: Which of the following functions is not elicited by antibody binding to Fcγ receptors?

A. Antibody-dependent cell-mediated cytotoxicity (ADCC) via NK cells
B. Phagocytosis by neutrophils
C. Mast-cell degranulation
D. Downregulation of B-cell activity
E. Ingestion of immune complexes by dendritic cells

10.8 Multiple Choice: Which of the following is a false statement?

A. Naive B-cell survival in follicles is dependent on BAFF, which signals through BAFF-R, TACI, and BCMA to induce Bcl-2 expression.
B. Subcapsular sinuses of lymph nodes and marginal sinuses of the spleen are functionally similar areas filled with specialized macrophages that retain but do not digest antigens.
C. ICOS signaling in T cells is essential for their completion of T_{FH} differentiation and expression of the transcription factors Bcl-6 and c-Maf.
D. Both plasmablasts and plasma cells express B7 co-stimulatory molecules, MHC class II molecules, and high levels of B-cell receptors.
E. T_{FH} cells determine the choice of isotype for class switching in T-dependent antibody responses.

10.9 True or False: Germinal centers contain a light and a dark zone. In the light zone, B cells proliferate extensively and are called centroblasts. They are maintained there by CXCL12–CXCR4 chemokine signaling and undergo somatic hypermutation leading to affinity maturation and class switching. In the dark zone, B cells cease proliferation and are called centrocytes. Here, they are maintained by CXCL13–CXCR5 chemokine signaling, express higher levels of B-cell receptor, and interact extensively with T_{FH} cells.

10.10 Multiple Choice: Choose the correct statement:

A. R-loops are structures formed during somatic hypermutation that promote accessibility of the immunoglobulin V regions to AID.
B. APE1 removes deaminated cytosine to create an abasic residue that results in the random insertion of a base during the next round of DNA replication.

C. Frameshift mutations during class switch recombination do not occur because switch regions lie in introns.

D. The error-prone MSH2/6 polymerase repairs DNA lesions and causes mutations that promote somatic hypermutation.

10.11 Fill-in-the-Blanks: Fc receptors diversify the effector functions of the distinct antibody isotypes. Most Fc receptors can bind the Fc regions of antibodies with _____ affinity. In contrast, FcεRI binds with _____ affinity. Multivalent antigen-bound IgE can bind _____ in mast cells and cause release of lipid mediators such as _____ and _____. Mast cells also degranulate in response to cross-linking of the FC receptor-bound IgE, which causes release of _____, and as a consequence local blood flow and _____ are increased, initiating an inflammatory response.

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