Adaptive immunity

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The innate immune system provides critical mechanisms for the rapid sensing and elimination of pathogens. Adaptive immunity has evolved to provide a broader and more finely tuned repertoire of recognition for both self- and nonself-antigens. Adaptive immunity involves a tightly regulated interplay between antigen-presenting cells and T and B lymphocytes, which facilitate pathogen-specific immunologic effector pathways, generation of immunologic memory, and regulation of host immune homeostasis. Lymphocytes develop and are activated within a series of lymphoid organs comprising the lymphatic system. During development, sets of gene segments are rearranged and assembled to create genes encoding the specific antigen receptors of T and B lymphocytes. The rearrangement mechanism generates a tremendously diverse repertoire of receptor specificities capable of recognizing components of all potential pathogens. In addition to specificity, another principal feature of adaptive immunity is the generation of immunologic memory. During the first encounter with an antigen (pathogen), sets of long-lived memory T and B cells are established. In subsequent encounters with the same pathogen, the memory cells are quickly activated to yield a more rapid and robust protective response. (J Allergy Clin Immunol 2010;125:S33-40.)

Although the innate immune system has evolved to rapidly sense and effect the elimination of a wide range of pathogens, the range of common pathogenic molecular patterns it can recognize is limited. The overwhelming variability of antigenic structures, as well as the ability of pathogens to mutate to avoid host detection, has driven the evolution of the adaptive immune system.[1] In contrast to the recognition receptors of the innate immune system, which are all encoded in their fully functional form in the germline genome, adaptive immune responses depend on receptors that are custom tailored and selected through a process of somatic recombination of a large array of gene segments. These arose by means of gene duplication early in the evolution of vertebrates to generate highly specific and flexible immune responses. After initial pathogen encounters, cells expressing these immune receptors can persist in the host for life, providing

Abbreviations used	
APC:	Antigen-presenting cell
CTL:	Cytolytic T lymphocyte
NK:	Natural killer
NKT:	Natural killer T
PLCγ1:	Phospholipase Cγ1
RAG:	Recombinase activating gene
SCID:	Severe combined immunodeficiency
SHM:	Somatic hypermutation
TACI:	Transmembrane activator and CamL interactor
TCR:	T-cell receptor
TI:	T independent
TLR:	Toll-like receptor
TREC:	T-cell receptor excision circle
ZAP-70:	ζ-Associated protein, 70 kd

immunologic memory and the capacity for rapid response in the event of re-exposure.

Cells of the adaptive immune system include the effectors of cellular immune responses, the T lymphocytes, which mature in the thymus, and antibody-producing cells, the B lymphocytes, which arise in the bone marrow. Lymphocytes are highly mobile. After developing in the primary lymphoid organs (thymus and bone marrow), they traffic to secondary lymphoid organs, including lymph nodes and the spleen, which serve to capture circulating antigens from lymph and blood, respectively. Adaptive immune responses originate in these areas, often under the influence of innate immune system signals provided either directly by circulating pathogens or indirectly by pathogen-activated cutaneous or mucosal antigen-presenting cells (APCs) migrating to the secondary lymphoid organs. Lymphocytes emigrating from the spleen and lymph nodes can then travel to many sites in the body to exert effector functions. This trafficking is regulated by an array of adhesion molecules and chemokine receptors; CLA-1^{+} CCR4-bearing lymphocytes traffic to skin, whereas cells bearing the α4β7 integrin which binds to mucosal addressin cellular adhesion molecule-1 (MadCAM-1) on gut endothelial cells preferentially home to the gastrointestinal tract.

T CELLS AND CELLULAR IMMUNITY
T-cell development

T cells develop in the thymus from common lymphoid progenitors coming from the bone marrow or fetal liver.[2-4] Seeding of the thymus is promoted by the interaction of platelet selectin glycoprotein 1 on the progenitors with the adhesion molecule P-selectin on thymic epithelium. Recently arrived cells rapidly expand under the influence of IL-7, the receptor of which signals through the common γ chain, which is encoded on the X-chromosome, and is shared by a number of other cytokine receptors (IL-2, IL-4, IL-9, IL-15, and IL-21). Mutations in this polypeptide underlie X-linked severe combined immunodeficiency (SCID), which is characterized by absent T cells. This early thymocyte expansion is accompanied by induction of Notch-1 and other

transcription factors, which commit precursors to the T-cell lineage and induce the expression of genes important in T-cell receptor (TCR) assembly. Subsequent differentiation of the expanded pool of T-cell progenitors or pro-T cells in the thymus involves an antigen-independent process in which a coordinated series of genomic rearrangements leads to the creation of functional genes encoding the α and β or γ and δ chains of the TCR.

In their germline configuration the TCR loci contain arrays of V (variable), D (diversity) and J (joining) segments. V and J segments are present at all TCR loci, whereas only the β and δ TCR loci contain D segments. In a spatially and sequentially ordered process, one V, one D (for β and δ) and one J segment are randomly spliced together (Fig 1). This is mediated by an enzymatic complex, the V(D)J recombinase composed of 2 proteins encoded by the recombinase-activating genes 1 and 2 (*RAG1* and *RAG2*). RAG1 and RAG2 bind to recombinase signal sequences flanking the borders of V-D-J segments. Recombination signal sequence accessibility is regulated by chromatin structure.[5] The V(D)J recombinase cleaves the DNA at these sites to give rise to hairpin structures. These, in turn, are substrates for cleavage by the nuclear enzyme Artemis, which is activated by DNA-dependent protein kinase catalytic subunit and exerts endonuclease activity on 5′ and 3′ overhangs and hairpins. Repair of the DNA breaks with resultant genomic juxtaposition of V, D, and J segments is effected by ubiquitous DNA repair enzymes including XRCC4 (X-ray repair cross-complementing protein 4) and Ligase IV in a process called nonhomologous end-joining. As would be predicted, null mutations in *RAG*, Artemis (DCLRE1C), DNA Ligase IV, and other enzymes involved in V(D)J recombination (including the XRCC4-like enzyme Cernunnos) give rise to SCID.

Each assembled V-D-J cassette represents one of a huge number of possible permutations of recombinations of the component V, D, and J segments, and the resulting structure dictates the amino acid sequence and binding specificity of the TCR. This is referred to as combinatorial diversity. Additional diversity, known as junctional diversity, is conferred by some inherent imprecision in the DNA-joining reactions involved in ligation of double-strand DNA breaks, resulting in some addition or removal of bases. Furthermore, the enzyme terminal deoxyribonucleotidyl transferase catalyzes the template-independent addition of several (generally 1-5) nucleotides at the joints. These junctional areas encode the third complementarity determining region of the antigen-binding pocket of the TCR, and this is the site of greatest variability.

In their germline configuration the component gene segments of the TCR are separated by large amounts of DNA. These intervening stretches of DNA are excised in the process of recombination but remain in the nucleus, where they circularize and are stable in an episomal form known as T-cell receptor excision circles (TRECs). TRECs are not duplicated during cell division, and therefore they dilute as newly formed T-cell clones expand. Measurement of TRECs in peripheral blood by means of PCR can be used to examine T-cell emigration from the thymus, and this approach is now in used in several states to analyze newborn blood spots in pilot screening programs for SCID.[6]

Gene-segment rearrangements are termed productive if they do not introduce stop codons and give rise to a gene encoding a full-length TCR protein. Sequential productive rearrangements of 2 TCR genes leading to surface expression of an αβ or γδ TCR marks the transition from a pre-T to a double-positive T cell; these
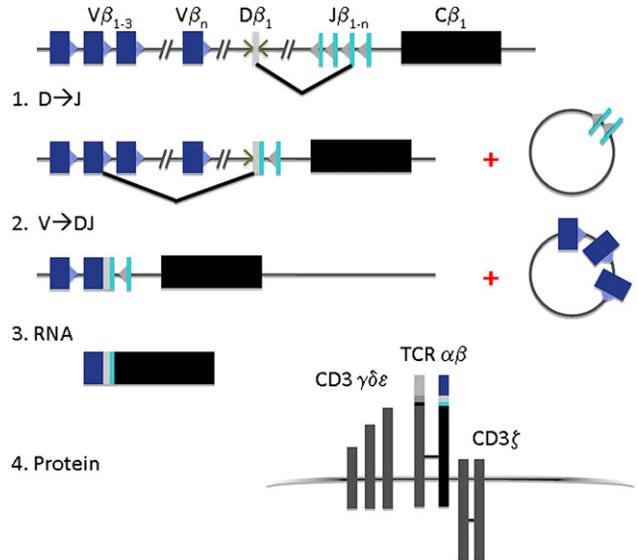

FIG 1. Sequential recombination of a random assortment of gene fragments dictates TCR structure and specificity. This schematic depiction of the TCR Vβ₁ locus indicates the relative locations of the Vβ, Dβ, and Jβ segments upstream of Cβ₁. *1*, The V(D)J recombinase recognizes signal sequences *(triangles)* upstream of one of many possible Jβ segments and introduces DNA breaks. The same process occurs at an upstream Dβ segment. Double-stranded DNA breaks are generated, and the 2 broken DNA ends are brought together and ligated by means of cellular DNA repair mechanisms (nonhomologous end-joining). The excised intervening DNA (the stretch between Dβ and Jβₙ) circularizes and remains in the nucleus as an episome known as a TREC. Such DNA circles are stable but are not replicated during cell division and dilute out during clonal expansion after T cells exit the thymus. *2*, By using the same mechanism, one of approximately 70 possible Vβ segments is brought into juxtaposition with the DJβ segment. A second excision product is generated. *3*, Transcripts of the rearranged TCRβ locus contain Vβ, Dβ, Jβ, and C cassettes. *4*, If this series of events has not introduced any stop codons, the rearrangement is termed productive, and a full functional TCRβ protein is translated. This event is permissive for subsequent TCRα rearrangement followed by expression of the complete TCR complex, including TCRαβ and CD3γδεζ chains at the T-cell surface. Rearrangement of α genes is the same as for β genes, except that the α gene is assembled only from Vα, Jα, and Cα. The γ chain of the TCR is similar to α and is also assembled from V, J, and C segments. The TCR δ chain is similar to the β chain and is comprised of V, D, J, and C segments. The α and δ gene loci are on chromosome 14. The β and γ loci are on chromosome 7.

cells express both CD4 and CD8. The TCR chains are assembled at the cell surface as a complex with the proteins constituting CD3, including the γ, δ, ε and ζ chains.

Further differentiation of these double-positive cells, which reside in the thymic cortex, to single-positive T cells, which are found in the medulla, is regulated by both positive and negative selection events involving antigens and molecules of the MHC. Positive selection occurs when the TCR of double-positive T cells binds with low avidity to self-MHC (complexed with self-peptides) on thymic epithelium. Double-positive cells bearing a TCR, which does not bind to self-MHC, are eliminated. Conversely, negative selection is exerted on double-positive T cells, the TCR of which binds with very high avidity to self-MHC/peptide, ensuring that autoreactive T-cell precursors are not permitted to mature (central tolerance). Deletion of T-cell clones interacting with peptides normally expressed in distant organs is facilitated by the function of the gene *AIRE* (autoimmune regulator), which stimulates expression of genes with wide tissue
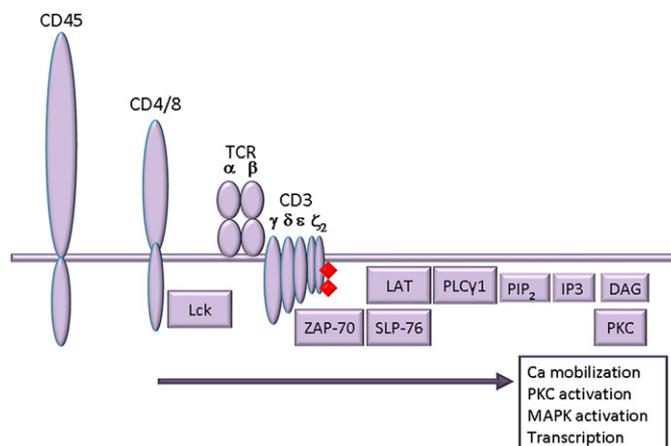
FIG 2. Signaling molecules in T-cell activation. The TCR α and β chains recognize peptide/MHC complexes expressed on APCs, an interaction that is stabilized by the simultaneous binding of T-cell CD8 to MHC class I or CD4 to MHC class II. Signaling is initiated by the CD3 chains (γ, δ, ϵ, and ζ) through cytoplasmic ITAMs *(red diamonds)*, which are phosphorylated by Src family kinases, including CD4/8-associated Lck, leading to recruitment of signaling molecules, including ZAP-70. The tyrosine phosphatase CD45 dephosphorylates inhibitory phosphotyrosines in Lck and is important for initiation of signaling. ZAP-70–mediated phosphorylation of downstream molecules, including the adapter proteins linker of activated T cells *(LAT)* and SH2-containing leukocyte protein, 76 kd *(SLP-76)*, drives the recruitment of PLCγ1, which hydrolyzes the membrane lipid phosphatidylinositol bisphosphate *(PIP$_2$)*, generating inositol-trisphosphate *(IP$_3$)* and diacylglycerol *(DAG)*. IP$_3$ increases intracellular calcium (Ca^{2+}) levels, and DAG activates protein kinase C, leading to the induction of nuclear factor κB *(NF-κB)*–mediated and mitogen-activated protein kinase *(MAPK)*–mediated gene transcription.

specificity in thymic epithelium.[7] Dysfunction of this gene is permissive for the escape of some self-reactive T cells and can give rise to autoimmune polyendocrine syndromes. Double-positive thymocytes that pass both positive and negative selection mature to CD8$^+$ single-positive T cells by means of further interaction with thymic epithelial MHC class I molecules, whereas those selected on MHC class II acquire a CD4$^+$ single-positive phenotype. Both CD4$^+$ and CD8$^+$ single-positive cells are found in the thymic medulla from which they exit to the circulation as fully differentiated but antigen-naive T cells.

T-cell activation

Mature T cells are activated on interaction of their TCRs with antigenic peptides complexed with MHC molecules. CD8$^+$ T cells can interact with peptides (9-11 amino acids in length) on almost any cell expressing MHC class I (HLA-A, HLA-B, and HLA-C). These MHC class I–restricted peptides are generally produced from proteins translated within the cell (endogenous antigens) encoded either in the host genome or by infecting viruses or other pathogens replicating intracellularly. In contrast, the TCRs of CD4$^+$ T cells engage peptides bearing MHC class II (HLA-DR, HLA-DQ, and HLA-DP). Unlike MHC class I expression, which is constitutive in all nucleated cells, MHC class II molecules are present on APCs and are inducible by innate immune stimuli, including ligands for Toll-like receptors (TLRs). APCs are specialized samplers of environmental antigens and danger signals (ligands for TLR and other systems of pattern-recognition receptors). They are present in large numbers in the skin and mucosal sites, where pathogen encounter is most likely, and they actively sample exogenous proteins by means of phagocytosis or endocytosis. Activation of these cells leads not only to induction of MHC class II expression but also to emigration from skin and mucosal

sites to regional lymph nodes, where interaction with T cells can occur, leading to initiation of immune responses.

T-cell activation is initiated when the TCR and associated proteins recognize a peptide/MHC complex on an APC, leading to a rapid clustering of TCR-associated molecules at the physical interface between T cells and APCs and the formation of a so-called immunologic synapse.[8] This is also called a supramolecular activation complex. The T-cell side of the synapse is focused around a central cluster of CD3 (γ, δ, ϵ, and ζ) and TCR (α and β), which bind specifically to the peptide/MHC complex, as well as CD4/CD8 molecules, which stabilize this interaction by binding to nonpolymorphic regions of MHC class I or MHC class II, respectively. The synapse is stabilized by adhesion molecules known as integrins. The aggregation of these molecules in the synapse facilitates the early events in TCR signaling (Fig 2). Simultaneous binding to MHC/peptide on the APCs by TCRs and CD4/CD8 in the synapse brings the cytosolic domains of these molecules into proximity. As a result, the CD4- and CD8-associated Src family protein tyrosine kinase Lck is able to phosphorylate tyrosine residues contained in cytoplasmic immunoreceptor tyrosine-based activation motifs of the TCR-associated CD3 chains. This results in the recruitment of the critical adaptor molecule, ζ-associated protein, 70 kd (ZAP-70), which binds to immunoreceptor tyrosine-based activation motif phosphotyrosines and phosphorylates a number of cytosolic proteins triggering the assembly of an intracellular complex of scaffolding and activated signaling proteins, including linker of activated T cells and SH2-containing leukocyte protein, 76 kd. The CD45 transmembrane protein, which contains 2 tyrosine phosphatase domains and is ubiquitous in lymphoid cells, might play a critical role in TCR-triggered activation of this kinase cascade by dephosphorylating inhibitory phosphotyrosine residues in Src family kinases, such as Lck. Mutations in *CD45* give rise to a SCID phenotype.

One of the active signaling enzymes recruited to linker of activated T cells and phosphorylated by ZAP-70 is phospholipase Cγ1 (PLCγ1). PLCγ1 mediates hydrolysis of the membrane inositol phospholipid phosphatidylinositol bisphosphate, generating inositol-trisphosphate and diacylglycerol. Inositol-trisphosphate induces a rapid increase in intracellular calcium (Ca^{2+}) levels by means of activation of stores contained within the endoplasmic reticulum. This calcium flux activates a calcium release–activated calcium channel facilitating the influx of extracellular calcium.[9] Calcium entering the cytosol from the endoplasmic reticulum or extracellular space binds to the regulatory protein calmodulin, which in turn activates the phosphatase calcineurin, which dephosphorylates nuclear factor of activated T cells in the cytosol, generating the active form of this critical transcription factor, which then translocates to the nucleus.

In a simultaneous, parallel pathway triggered by diacylglycerol, the other product of PLCγ1-mediated hydrolysis of phosphatidylinositol bisphosphate, protein kinase C is activated. This leads, through intermediates, to the activation of nuclear factor κB, another critical transcription factor in T-cell activation. Activation of the mitogen-activated protein kinase pathway, which is initiated by recruitment of RasGTP to the supramolecular activation complex, leads to the generation of the activator protein 1 transcription factor. The coordinated action of this series of transcription factors (nuclear factor of activated T cells, nuclear factor κB, and activator protein 1), as well as others, induces a constellation of gene expression important for the function of activated T cells.

T-cell effector subsets

Although the basic principles of thymic development and the mechanisms of activation are shared by all T cells, there is a remarkable diversity of effector functions that are elicited in response to activation. T cells can play direct roles in elimination of pathogens by killing infected target cells. They can function as helper cells, providing cognate (involving direct cellular contact) or cytokine signals to enhance both B- and T-cell responses, as well as causing activation of mononuclear phagocytes. Finally, T cells regulate immune responses, limiting tissue damage incurred by means of autoreactive or overly inflammatory immune responses.

The largest group of T cells in the body is the $CD4^+$ $\alpha\beta$ TCR population. Most of these cells serve a helper function and have been designated T_H cells. On activation, T_H cells produce a range of cytokines. About 20 years ago, immunologists Robert Coffman and Tim Mossman first discovered that not every individual $CD4^+$ T_H cell has the capacity to produce the full range of cytokines known to be in the T-cell repertoire.[10] Instead, by means of analysis of T-cell clones, they demonstrated 2 main categories of T_H cells, both T_H1 and T_H2 cells, each producing (mostly) mutually exclusive panels of cytokines. T_H1 cells were characterized by their capacity to make IFN-γ and IL-2 and were shown to differentiate from naive T_H0 precursors under the influence of IL-12 and IFN-γ and the T-box expressed in T cells transcription factor (T-bet) (Fig 3). In contrast, T_H2 cells are producers of IL-4, IL-5, IL-10, and IL-13, and their development is driven by IL-4 and the transcription factor GATA-3. T_H1 cell cytokines drive cell-mediated responses, activating mononuclear phagocytes, natural killer (NK) cells, and cytolytic T cells for killing of intracellular microbes and virally infected targets. The T_H2 cytokine profile enhances antibody production, as well as a number of aspects of hypersensitivity and parasite-induced immune responses, including eosinophilopoiesis. In some cases there is more plasticity to
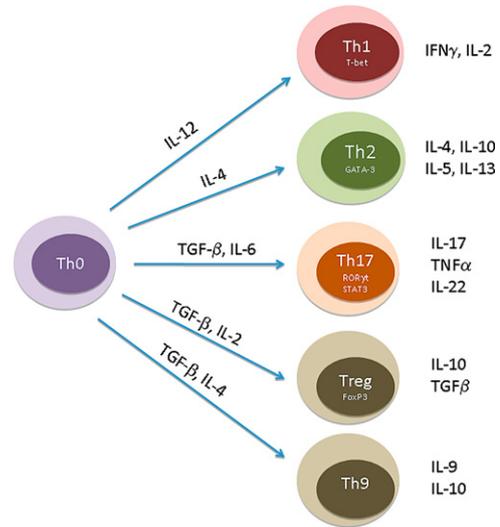

FIG 3. $CD4^+$ T_H cell subsets. Antigen-specific naive T_H0 T cells are stimulated to expand on interaction with APCs expressing MHC class II/peptide complexes. Depending on the type of APC and the cytokine milieu *(arrows)* at the site of antigen encounter, T_H0 cells can be driven down one of several differentiation pathways. The T_H populations that arise retain the TCR specificity of the parent T_H0 cell but secrete unique constellations of cytokine products that mediate distinct effector functions, including activation for killing of microbes (T_H1), production of antibodies and expulsion of helminths (T_H2), induction of inflammatory responses (T_H17), and dampening of immune activation (regulatory T *[Treg]* cells). Specific transcription factors (indicated in the nuclei) stabilize lineage commitments and dictate the specific cytokine secretion profiles. *FoxP3,* Forkhead box protein 3; *RORγt,* (retinoic acid receptor related orphan receptor γt); *STAT3,* signal transducer and activator of transcription 3; *T-bet,* T-box expressed in T cells.

T-cell production of T_H1 and T_H2 cytokine production than the constraints of the T_H1/T_H2 paradigm would suggest; overlapping cytokine expression profiles are possible. For example, it was recently shown that T-box transcription factor expression, along with IFN-γ production, can be induced in some T_H2 cells.[11]

Over the 2 decades since their discovery, the relationship between T_H1 and T_H2 cells has been viewed as a Yin-Yang paradigm, and immune responses to pathogens or immunologically mediated disease processes have been considered as primarily T_H1 or T_H2 mediated. However, inconsistencies between the T_H1/T_H2 model and clinical observations and animal data suggested that not all $CD4^+$-driven processes could be attributed to cytokines predicted to arise from T_H1 or T_H2 responses. In the past 2 years, strong evidence for additional T_H diversity has arisen.[12] T_H17 cells are induced by IL-6 and TGF-β and express the transcription factor RORγt (retinoic acid receptor related orphan receptor γt). T_H17 cells produce IL-17, a group of 5 homologous molecules designated IL-17A-F. T_H17 cells produce mainly IL-17A and IL-17F, and IL-17E is now called IL-25. IL-17A and IL-17F are potent proinflammatory cytokines capable of inducing IL-6 and TNF production, as well as driving granulocyte recruitment and tissue damage. T_H17 cells are thought to be important in autoimmunity; IL-17 is present in the inflamed tissues of patients with arthritis, multiple sclerosis, and systemic lupus erythematosus. In animal models genetic deletion or antibody inhibition of IL-17 blocks experimental autoimmune diseases, such as experimental autoimmune encephalomyelitis. T_H17 cells are also prominent in chronic allergic inflammatory processes, such as asthma.[13] Defects that impair T_H17 production in human
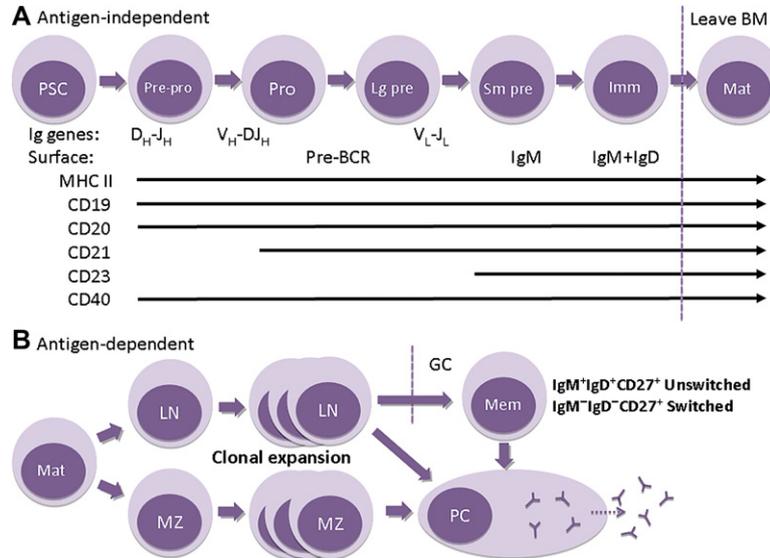
FIG 4. A, Antigen-independent B-cell development in the bone marrow. The earliest recognized committed stage is the pre-/pro-B cell, where immunoglobulin D_H and J_H genes rearrange. In the pro-B cell stage, a V_H segment is joined to the DJ_H unit. At this point, if heavy chain gene rearrangement on at least 1 chromosome has been successful, an IgM heavy chain might form and pair with the surrogate light chain heterodimer (lambda 5 and VpreB) to make the surface-expressed pre-B cell receptor *(BCR)* at the large pre-B cell *(Lg pre)* stage. Subsequent to signaling through the receptor, precursors undergo expansion through proliferation (not shown) and light chain genes rearrange (κ chains first and λ chains next, if κ rearrangement is unsuccessful) at the small pre-B cell *(Sm pre)* stage. If light chain assembly is successful, the cell might express a fully formed IgM receptor on its surface at the immature *(Imm)* stage and leave the bone marrow *(BM)*. Subsequently, the cell also expresses IgD on the surface in addition to IgM and becomes a mature *(Mat)* B cell. **B**, Antigen-dependent B-cell development in the periphery. Many B cells recirculate through the lymphatic system and lymph nodes *(LN)*, where they can encounter antigen. When activated, these cells proliferate and might become short-lived antibody-secreting plasma cells *(PC)*. Alternatively, they might enter follicles and establish germinal centers *(GC)*. Memory B cells are mainly formed in GCs. Memory cells can be subsequently activated and become long-lived plasma cells at some time in the future. Cells that have been activated acquire the CD27 surface marker. Cells that retain surface expression of IgM and IgD are called unswitched, whereas cells that have undergone immunoglobulin class-switching and have lost expression of IgM and IgD are called switched memory B cells. Cells can also enter the splenic marginal zone *(MZ)*, where they do not actively recirculate. If they are activated here in the absence of cognate T-cell help (see text), they also undergo clonal expansion and form plasma cells. However, little B-cell memory is generated in this pathway.

subjects, such as signal transducer and activator of transcription 3 mutations in the hyper-IgE syndrome, are associated with decreased inflammatory response and recurrent infections. It is likely that future investigations will uncover further diversity of T_H subsets. The existence of IL-9–producing T_H9 cells has recently been suggested by the observation that exposure of T_H2 cells to a combination of IL-4 and TGF-β reprograms them to produce IL-9, a potent mast cell growth factor and mediator of helminthic immunity.[14,15] A specialized subset of T_H cells, follicular T helper (T_{FH}) cells resides in lymph nodes and the spleen. T_{FH} cells are memory $CD4^+$ cells expressing the chemokine receptor CXCR5, which mediates their recruitment to follicles. These cells trigger B-cell activation, leading to germinal center formation.

The critical function of regulation of T-cell responses also resides within the $CD4^+$ αβ TCR subset of lymphocytes and is likely effected by several regulatory cell types. IL-10–producing regulatory T (T_R1) cells, as well as both naturally occurring and inducible $CD25^+CD4^+$ T cells expressing the transcription factor forkhead box protein 3, have been shown to quell T-cell responses. Absence of forkhead box protein 3, which is encoded on the X-chromosome, gives rise to a severe multisystem inflammatory disorder (immune dysregulation, polyendocrinopathy, X-linked syndrome). The complexity of the regulatory T-cell system has recently been well reviewed.[16]

$CD8^+$ T cells represent a major fraction of circulating T cells and act to remove both cells harboring intracellular pathogens, including viruses and transformed cells. Because CD8 serves as a coreceptor for MHC class I and $CD8^+$ thymocytes are selected on MHC class I, $CD8^+$ T cells primarily recognize antigenic peptides derived from cytosolic proteins. Cytolytic T lymphocytes (CTLs) kill target host cells in a contact-dependent mechanism. Recognition of foreign cytosolic peptides of the target cell in the context of host MHC class I by the CTL TCR leads to the formation of a conjugate with an immunologic synapse. Within minutes, the CTL activates apoptotic cell death in the target cell. This process is mediated by rapid mobilization of CTL granules to the synapse followed by fusion of granule membranes with the target cell plasma membrane and exocytosis of granule contents, including granzymes and perforin. The granzymes are serine proteases that target a number of proteins in the host cell, leading to activation of apoptosis. In a parallel proapoptotic pathway, TCR activation in the immune synapse drives expression of Fas ligand on the CTL. This in turn engages Fas (CD95) on the target cell membrane, again triggering apoptosis.

A small subset of T cells expresses a γδ TCR, and most are double negative (expressing neither CD4 nor CD8), with some variably $CD4^+$ or $CD8^+$. In human subjects these represent less than 5% of lymphocytes in most tissues but are found in higher
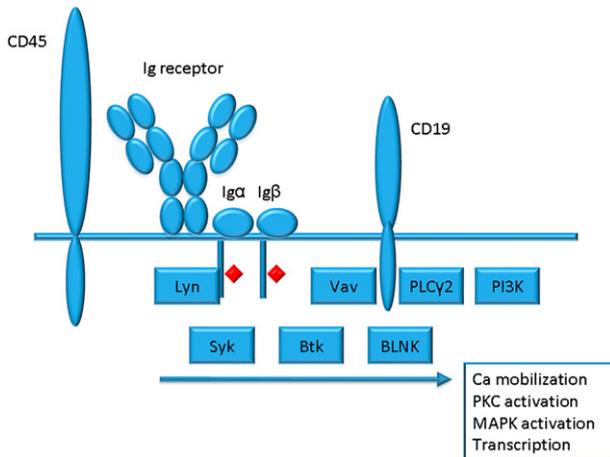
FIG 5. Signaling molecules in B-cell activation. The immunoglobulin receptor contains 2 signaling molecules called Ig-α and Ig-β (encoded by the *CD79A* and *CD79B* genes, respectively). The cytoplasmic domains of these molecules contain ITAMs (*red diamonds*), which recruit signaling molecules to clusters of cross-linked or immobilized receptors on the cell surface. The tyrosine phosphatase CD45 is important for initiation of signaling, and the coreceptor molecule CD19 also assists in the recruitment of signaling molecules to the complex. Some of the proximal signaling molecules include the tyrosine kinases Lyn, Syk, and Btk; the adaptor protein B-cell linker protein (*BLNK*); the guanine nucleotide exchange factor Vav; phospholipase Cγ2 (*PLCγ2*); and phosphoinositide 3′ kinase (*PI3 K*). Additional downstream signaling events include the release of intracellular calcium stores, the influx of extracellular calcium, activation of protein kinase C (*PKC*), activation of mitogen-activated protein kinases (*MAPK*), and activation of transcription regulated by a variety of factors, including OCA-B/OBF-1 (Oct binding factor 1, also called POU domain class 2 associating factor 1) and Pip/IRF-4 (interferon regulatory factor 4).

TABLE I. B-cell subpopulations in peripheral blood

Surface phenotype	B-cell subset
IgM$^+$IgD$^-$CD27$^-$	Immature
IgM$^+$IgD$^+$CD27$^-$	Naive
IgM$^+$IgD$^+$CD27$^+$	Marginal zone (unswitched memory)
IgM$^-$IgD$^-$CD27$^+$	Germinal center (switched memory)*
CD38^{low}CD21^{low}	Uncharacterized†
CD38^{high}IgM^{high}	Transitional (activated)
CD38^{high}IgM$^-$	Plasmablast

*Reduction in this population is associated with several complications of common variable immunodeficiency.
†Expansion of this (thus far) otherwise uncharacterized population is seen in patients with autoimmune diseases, such as lupus, and in patients with common variable immunodeficiency with autoimmune complications.

numbers in the gastrointestinal epithelium. Unlike $\alpha\beta$ T cells, $\gamma\delta$ cells recognize antigens not in the context of MHC class I or MHC class II molecules but rather as presented by nonclassical MHC molecules of the CD1 family. The $\gamma\delta$ subset is expanded in the setting of mycobacterial infection, and it is thought that these T cells might respond to mycobacterial antigens. In addition to recognizing peptide antigens, the $\gamma\delta$ TCR can bind to small molecules, including phospholipids and alkyl amines.

Natural killer T (NKT) cells represent another subset of T cells, which, like $\gamma\delta$ T cells, recognize nonpeptide antigens presented by nonclassical MHC molecules of the CD1 family. NKT cells are defined by their simultaneous expression of T-cell (CD3, TCR$\alpha\beta$) and NK cell antigens (CD56). A large fraction of NKT cells is characterized by the expression of a single unique TCRα rearrangement, Vα24-Jα18 with Vβ11, and are referred to as invariant NKT cells. Activated NKT cells are capable of rapid and substantial production of cytokines, including IL-4, and have been implicated in allergic pathogenesis.[17] A currently very active area of research is the identification of endogenous and pathogen-derived ligands that might stimulate NKT expansion and activation.

B CELLS AND HUMORAL IMMUNITY
B-cell development

Adaptive humoral immunity is mediated by antibodies produced by plasma cells that develop from B cells under the direction of signals received from T cells and other cells, such as dendritic cells. B cells arise from hemopoietic stem cells in the bone marrow. Commitment to the B-cell lineage is under the

control of several transcription factors, such as PU.1, IKAROS (IKAROS family zinc finger 1), E2A, EBF (early B cell factor 1), PAX5 (paired box gene 5) and IRF8 (interferon regulatory factor 8).[18-20] In the bone marrow B cells pass through several distinct developmental stages, during which they acquire their antigen specificity (Fig 4, *A*). Reaching the immature stage, B cells exit the marrow and complete development to the mature or naive stage. This is signaled by the appearance of IgD in addition to IgM on the cell surface. This entire developmental sequence occurs in the absence of any contact with exogenous antigen. Thus it is called antigen-independent B-cell development. Any genetic mutations affecting components of the pre-B cell receptor or the signaling pathways connected to it (Fig 5) lead to immunodeficiency with agammaglobulinemia and absence of B cells.[21]

The genes encoding immunoglobulins are assembled from segments in a manner entirely analogous to the process for TCR genes. Heavy chains are assembled from 4 segments (V$_H$, D, J$_H$ and C$_H$); light chains are assembled from 3 segments (V$_L$, J$_L$, and C$_L$). There are 9 different heavy chain types (IgM, IgD, IgG1-4, IgA1 and IgA2, and IgE) and 2 light chain types (κ and λ). The heavy chain genes are on chromosome 14, and the κ and λ genes are on chromosomes 2 and 22, respectively. Immunoglobulin structure is considered in detail in the chapter "Structure and function of immunoglobulins."

B-cell subsets

In mice the presence of the surface marker CD5 distinguishes a population of B1 B cells with distinct characteristics: they develop early in ontogeny, they tend not to undergo somatic hypermutation (SHM; see below), and they secrete IgM antibody with polyspecificity, including binding to self-antigens.[22] The CD5$^-$ population is called B2 or conventional B cells. B cells expressing CD5 also exist in human subjects, and at least a subset of these cells might have characteristics similar to those of murine B1 cells. However, clear-cut phenotypically and functionally distinct B1- and B2-cell sublineages are not well described in human subjects. Furthermore, the marginal zone of the periarteriolar lymphoid sheath in the murine spleen contains B cells with a particular role in responding to so-called T-independent type 2 antigens (see below).[23] The histologic structure of the human spleen is distinct, and it is not yet clear whether an identical distinct population of marginal-zone B cells exists in human subjects.

Several subpopulations of B cells in peripheral blood can be distinguished based on surface-marker expression (Table I).
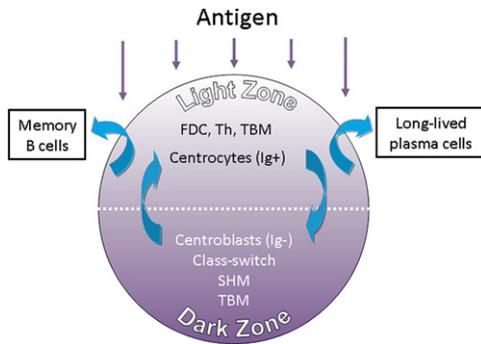
FIG 6. Diagram of a germinal center. Cells (and antigens) enter the light zone, which is positioned to facilitate exposure to sources of antigen (eg, intestinal lumen, splenic arterioles, and subcapsular sinus in lymph nodes). This area has a high concentration of follicular dendritic cells *(FDC)*, T_H cells, and tingible body macrophages *(TBM)*, which are engulfing apoptotic B cells. Light zone B cells (centrocytes) that interact effectively with FDCs and T_H cells lose expression of immunoglobulin and migrate to the dark zone and become centroblasts. Here they undergo immunoglobulin class-switching and SHM. If these processes destroy the ability to express immunoglobulin, the cells die by means of apoptosis and are engulfed by TBM. If they succeed in expressing immunoglobulin again, they migrate back to the light zone and interact with antigen on FDCs. If antigen specificity has been lost because of SHM, the cells die by means of apoptosis and are engulfed by TBM. The B cells with the highest affinity for antigen receive the most effective activating signals and are able to express more peptides for recognition by T_H cells. These cells might become memory cells or long-lived plasma cells (whereupon they leave the germinal center), or they might re-enter the dark zone and repeat the cycle.

These mainly represent different developmental stages and pathways, as described above (and below) and in Fig 4. Alterations of some of these populations have been associated with clinical phenotypes in immunodeficiency and autoimmune disease.[24]

B-cell activation

The second phase of B-cell development occurs after encounter with antigen and activation and is called the antigen-dependent phase (Fig 4, *B*). Depending on the various contacts and cytokine stimuli received by the activated cell (discussed below), it will become either a memory cell to be activated once again in the future or it will become a plasma cell producing large amounts of antibody.

T-independent antigens. Some antigens elicit antibody formation in the absence of T cells, and are called T-independent (TI) antigens. Although the phenomenon is most clearly seen in murine models, similar mechanisms of B-cell activation exist in human subjects. Certain molecules, such as some plant lectins (eg, pokeweed mitogen), are alone capable of inducing proliferation and antibody production from mature B cells. These are called TI type 1 antigens.[25]

Some macromolecules, such as polymerized proteins or polysaccharides, possess repeating molecular patterns that can interact with multiple immunoglobulin receptors on the cell surface and cross-link them. This might deliver a partially activating signal that can progress to memory or plasma cell development with only the additional signals provided by cytokines or other cell contacts provided by dendritic cells.[26] These are called TI type 2 antigens. In many cases the antigens themselves might also provide more than 1 activating signal because some might interact with other receptor systems, such as TLR.[27]

Another important signaling system in direct dendritic cell–B-cell interactions involves transmembrane activator and CamL

interactor (TACI, also TNFRSF13B), which is expressed on activated B cells.[28] One TACI ligand, a proliferation inducing ligand (APRIL, also TNFSF13), is expressed on a broad range of leukocytes. Another TACI ligand, B cell–activating factor (BAFF, also TNFSF13B) is expressed on dendritic cells and myeloid cells. In combination with the signals described above, this system can promote immunoglobulin isotype switching (see below) independently of T cells. This process could underlie some rapid responses to polysaccharide antigens to provide adequate immunity before the recruitment of effective T-cell help.

T-dependent antigens. The vast majority of antibody responses to proteins and glycoproteins require participation of T cells, and these antigens are called T dependent. Mature B cells recirculate through secondary lymphoid organs, including lymph nodes, the spleen, and mucosal-associated lymphoid tissues. In the lymph nodes B cells are concentrated in the cortex in primary follicles in contact with follicular dendritic cells. T cells are in the paracortical areas. Low-molecular-weight antigens might diffuse directly into B-cell areas in secondary lymphoid tissues. Larger molecules require transport by means of cellular mechanisms that are still being elucidated.[29] Antigens complexed to varying to degrees with IgM, IgG, and complement might be carried on the surfaces of specialized macrophages, follicular dendritic cells, or even B cells themselves, all of which have receptors for IgG Fc and complement fragments. Antigen presented on these surfaces can stimulate B cells through immunoglobulin receptor cross-linking, expression of other interacting surface molecules, and cytokine secretion.

B cells require 2 principal types of signals to become activated. Signal 1 is delivered by cross-linking of the immunoglobulin receptor, as described above. This cross-linking leads to activation of intracellular signaling pathways (Fig 5) that render the cell capable of interacting with T cells and thereby receiving signal 2. B cells are active as APCs and express peptides along with MHC class II on their surface. These peptides can arise from processed antigen that was internalized after binding to the B-cell surface immunoglobulin receptor. When the B cell contacts a $CD4^+$ T cell specific for such a peptide with self-MHC class II and having been previously activated by an APC, the T cell is able to provide cognate (direct cellular contact) help and activate the B cell for further differentiation into memory cells or plasma cells.

The cognate interaction between T cells and B cells is analogous to the interaction between T cells and dendritic cells. B cells express many of the same costimulating molecules found on dendritic cells, such as CD40, B7-1 (CD80), and B7-2 (CD86). T cells and B cells form an analogous immunologic synapse, and the signaling pathways involved are similar. This initial interaction takes place at the margin between primary follicles and T-cell areas in secondary lymphoid tissues. The activated B cells enter one of 2 pathways. Either they immediately become short-lived plasma cells secreting low-affinity antibody without somatic mutation, or they enter a follicle to establish a germinal center (Fig 4).[30]

In the germinal center (Fig 6) B cells can change from the production of IgM and IgD to other isotypes, such as IgG, IgA, and IgE. This is called class-switching.[31] This process occurs through a mechanism of gene rearrangement somewhat analogous to the process of TCR and B-cell receptor gene segment rearrangement described above. In class-switching a DNA sequence between the VDJ unit and the genes encoding IgM and IgD is cut and ligated to a similar sequence in front of another immunoglobulin C-region gene encoding any of the subclasses of IgG, IgA, or IgE. The

result is the loss of the intervening DNA and the production of an antibody with the same specificity (same VDJ unit) with a new C-region isotype. The process of class-switching is partly under cytokine control. For example, IL-4 and IL-13 promote switching to IgE.[32] IFN-γ can antagonize this effect. IL-10 and TGF-β promote switching to IgA.[33]

At the same time that class-switching is occurring, a mechanism of nucleotide substitution is activated, leading to the accumulation of point mutations in the immunoglobulin heavy and light chain variable regions. This process is known as SHM.[34,35] The enzymes activation-induced cytidine deaminase and uracil nucleoside glycosylase, among others, are important for the DNA cutting and splicing events of class-switching, as well as for the nucleotide substitutions leading to SHM. Lack of either activation-induced cytidine deaminase or uracil nucleoside glycosylase enzymes leads to immunodeficiency (forms of hyper-IgM syndrome). As a result of the selection mechanisms operating in the germinal center, SHM leads to the production of antibodies with higher affinity for antigen. This is known as affinity maturation.

The immune response to the first exposure to an antigen is called the primary response. It is relatively slow (it takes a few weeks to develop fully) and leads to production of predominantly IgM antibody of relatively low affinity. Other isotypes, such as IgG, IgA, or IgE, appear relatively late (2 weeks or longer) and show higher affinity (affinity maturation). During the primary response, memory T cells and B cells are generated. In a subsequent exposure to the same antigens (pathogen), these cells are activated more quickly in comparison with a primary response, so that production of high-affinity IgG (or IgA or IgE) is established quickly (within 1 week). This is called a secondary response.

CONCLUSION

Phylogenetically ancient mechanisms of innate immunity are still critical for the protection of more highly evolved organisms from many pathogens. The evolution of pathogens that themselves had the capacity to alter their molecular patterns to evade innate immune mechanisms drove the counter-evolution of the mechanisms of adaptive immunity briefly reviewed above. The key feature of adaptive immunity is the vast repertoire of T- and B-lymphocyte receptor specificities generated through the somatic recombination of gene segments. Another important feature is the generation of immunologic memory or the ability of the system to learn or record its experiences of encounters with various pathogens in a manner leading to even more effective and rapid responses with subsequent challenges with the same or similar infections. The ability to generate such a wide repertoire of specificities vastly increases the opportunities for inappropriate attack against self-components. Thus a third principal feature of adaptive immunity is the requirement for complex and robust regulatory systems to prevent such attack.

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