

3. Lymphocytes

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The fundamental task of the immune system is to defend “self” from “nonself.” Lymphocytes are the primary cells of the immune system that developed one of the most sophisticated and comprehensive defense mechanisms in the biological system. T cells play a central role in orchestrating the immune response. Further, they are instrumental in eliminating intracellular pathogens (viruses, some bacteria) through the generation of cytotoxic T cells. B cells defend against extracellular pathogens by producing antibodies. Natural killer cells are an important component of innate immunity. Dendritic cells play a key role in initiating the immune response by presenting foreign antigens to T cells. The interaction among T cells, B cells, dendritic cells, and natural killer cells constitute the fundamental defense network of the host. The failure of any of these components severely jeopardizes the integrity of the immune system and its ability to mount the most appropriate immune response. (*J Allergy Clin Immunol* 2003;111:S476-85.)

Key words: *T cell, B cell, NK cell, dendritic cell, cytokines*

The immune system is divided into the adaptive system and the innate system. The adaptive immune system distinguishes itself from the innate system by the following features: specificity of antigen recognition, diversity of the antigen receptor repertoire, rapid clonal expansion, adaptiveness to the changing environment, and immunological memory.

Lymphocytes are the primary cells of adaptive immunity. Lymphocytes are divided into T cells, B cells, and natural killer (NK) cells. The description of these 3 individual cell types, as well as the system they form, will be the subject of this chapter.

BIRTH AND EDUCATION

Generation of antigen-specific receptors

The growth of B and, especially, T cells from pluripotent stem cells requires successive differentiation through a series of developmental stages that starts in the bone marrow and ends in the thymus (T cells) or peripheral lymphoid tissue (B cells).¹⁻³ During differentiation, lymphocytes are educated to recognize “self” and “nonself” antigens through the expression of antigen-specific receptors known as T-cell receptors (TCR) and B-cell receptors (BCR).⁴ TCR is comprised of $\alpha\beta$ or $\gamma\delta$ subunits, whereas BCR is composed of membrane-bound

Abbreviations used

BCR:	B-cell receptor
DC:	Dendritic cells
ICAM:	Intercellular adhesion molecule
IFN:	Interferon
MHC:	Major histocompatibility complex
NK:	Natural killer cells
Th:	T helper
TCR:	T-cell receptors

immunoglobulins (Figs 1 and 2). Approximately 90% of peripheral blood T cells are $\alpha\beta$ +, and the remaining cells are $\gamma\delta$ +, although the proportion of the latter reaches 25% to 30% in the gastrointestinal mucosa and skin.² The generation of TCR and BCR is a complex process that creates an impressive repertoire in the order of $>10^{14}$ through combinatorial joining of V, D, and J (β and δ chains) or V and J (α and γ chains) segments out of about a few hundred germline exons (200 for TCR and 700 for immunoglobulins) (Fig 3). The recombination process is triggered by IL-7 and involves many enzymes including 2 recombinases—recombination-activating gene-1 and -2 and a DNA repair enzyme (metallo- β -lactamase) encoded by the gene Artemis.⁵ The deficiency of the recombination-activating gene enzymes, IL-7 receptor and the Artemis gene product causes severe combined immunodeficiency. A partial deficiency of recombination-activating gene-1 and -2 causes Omenn syndrome (see chapter 12). BCR is capable of recognizing small and large peptides, including their secondary and tertiary structures (conformational antigens), as well as nonpeptide antigens. In contrast, TCR recognizes only small stretches of linear peptides of 10 to 12 amino-acid residues in length that are processed and presented in the antigen-presenting groove of major histocompatibility complex (MHC) molecules.⁶

Thymic selection of T cells

During random combinatorial generation of antigen receptors self MHC-reactive and nonreactive TCRs are generated.⁷ T cells require signaling through the TCR for survival and proliferation. Those T cells that are unable to recognize self-MHC or have very low avidity TCR for self-MHC die because of the lack of the TCR signal (death by neglect). T cells, which recognize self-MHC in conjunction with the self-peptide, are initially expanded (positive selection). Among the positively selected T cells, those with very high avidity for self-peptides (auto-reactive T cells) are killed (negative selection). Under normal circumstances, T cells with only moderate avidity for self-peptides survive and further differentiate. It is

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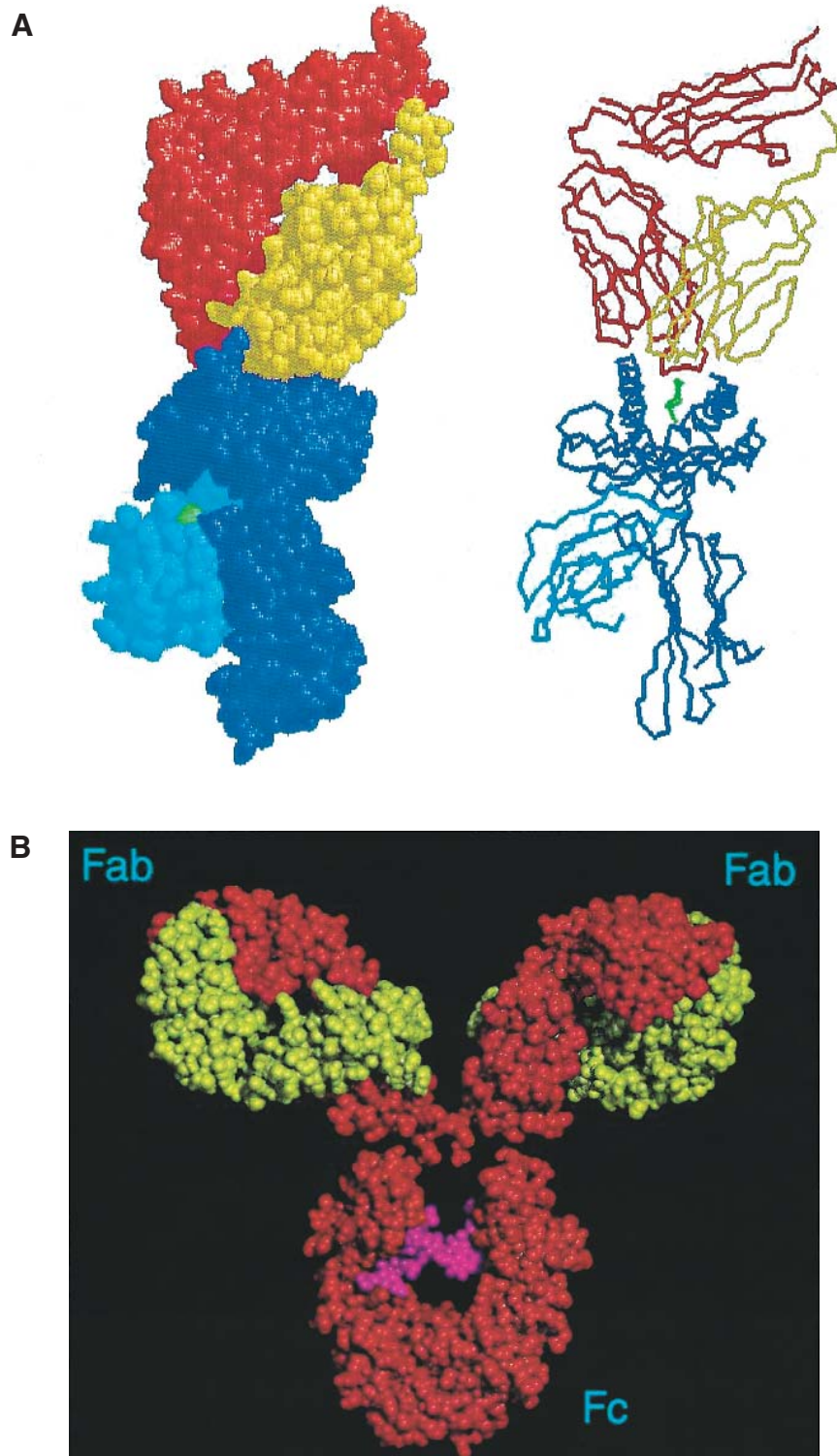


FIG 1. Crystallographic structure of the TCR interacting with the antigen-bound MHC class I molecule (**A**) and the IgG1 (**B**). **A**, The TCR (α chain in yellow and β chain in red) is on the top. The class I MHC molecule (α chain in dark blue and β 2 microglobulin in light blue) is at the bottom. The antigen (green) is presented in the antigen-presenting groove. The left panel shows a space-filling model, whereas the right panel is a ribbon structure. Note that the picture of the constant region for the TCR is incomplete (Based upon Garboczi DN, et al. Nature 1996;384:134-41.). **B**, IgG1 heavy chains are in red, light chains in yellow, and carbohydrate in pink. *Fc*, Constant fragment; *Fab*, antigen-binding fragment. Both pictures are courtesy of Mike Clark at <http://www.path.cam.ac.uk/~mrc7/>

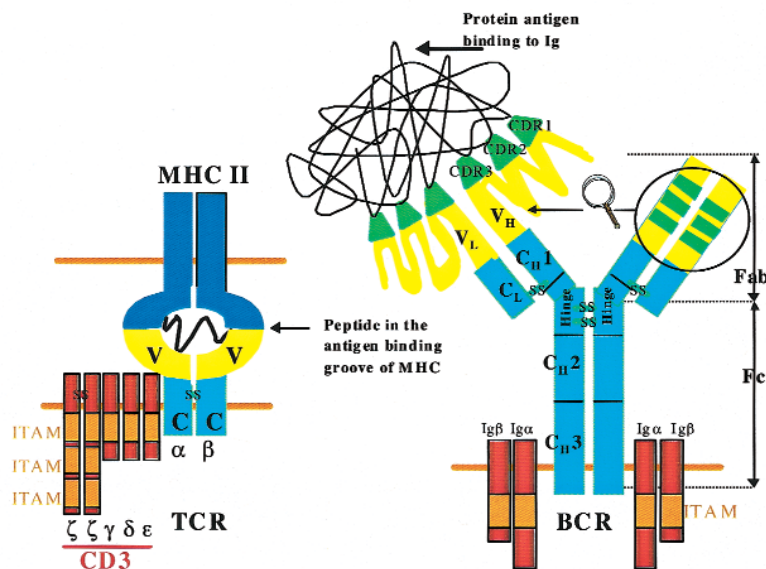


FIG 2. The composition of the TCR and the BCR. TCR is composed of the CD3 complex and $\alpha\beta$ (or $\gamma\delta$) subunits. $\alpha\beta$ (or $\gamma\delta$) subunits bind the MHC-bound antigenic epitope, the CD3 complex transduces intracellular signaling. BCR is composed of the surface immunoglobulin and the Ig α and Ig β accessory molecules. V, Variable region of the receptor; C, constant region of the receptor; $\zeta\gamma\delta\epsilon$, subunits of the CD3 complex; α and β , TCR subunits; Ig, membrane-bound immunoglobulin; Ig α and Ig β , BCR accessory BCR molecules; C_H and C_L , constant regions of the heavy (H) or light (L) chain of immunoglobulin; V_H and V_L , variable regions of the H or L chain of immunoglobulin; CDR, complementarity determining region; ITAM, immunoreceptor tyrosine-based activation motif; Fc, Constant fragment; Fab, antigen-binding fragment.

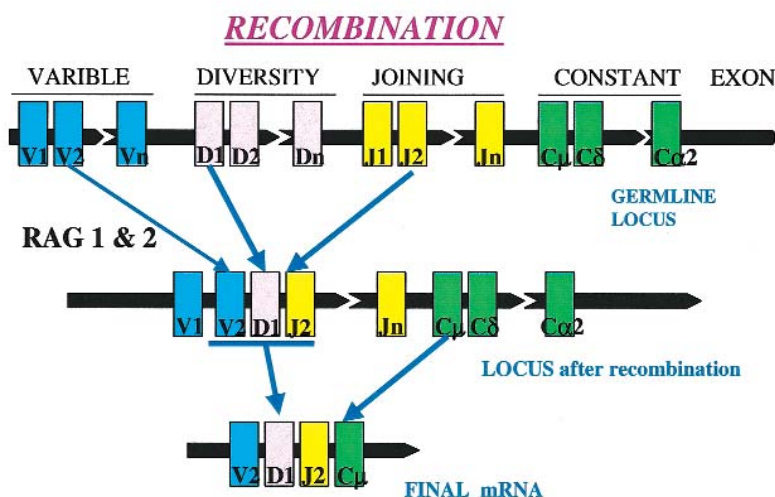


FIG 3. The immunoglobulin heavy chain locus as the example of genomic organization of antigen receptors. V, D, J exons encode the variable region of the immunoglobulin antigen-binding site and C exons encode the constant region. RAG, Recombination-activating gene.

estimated that 95% of T-cell precursors die due to negative selection or due to the failure to express appropriate TCR. In contrast to auto-reactive T cells, auto-reactive B-cell precursors are not subjected to extensive deletions. Instead, many self-reactive B cells undergo a second round of receptor gene rearrangement through a process called receptor editing and replace the auto-reactive BCR with a normal BCR. During the early phase of differentiation, immature T cells express both CD4 and CD8 core-

ceptors (double-positive cells).⁸ In the next phase, CD4 T cells are selected through interaction with the class II MHC molecules, whereas CD8 T cells are selected based upon their interaction with the class I MHC molecules. CD4 and CD8 T cells remain restricted by the MHC antigen for the duration of the lifespan.

Two tyrosine kinases—Lck and zeta-associated protein 70 (also known as ZAP-70)—play a critical role in the selection of CD4 and CD8 T cells, respectively.

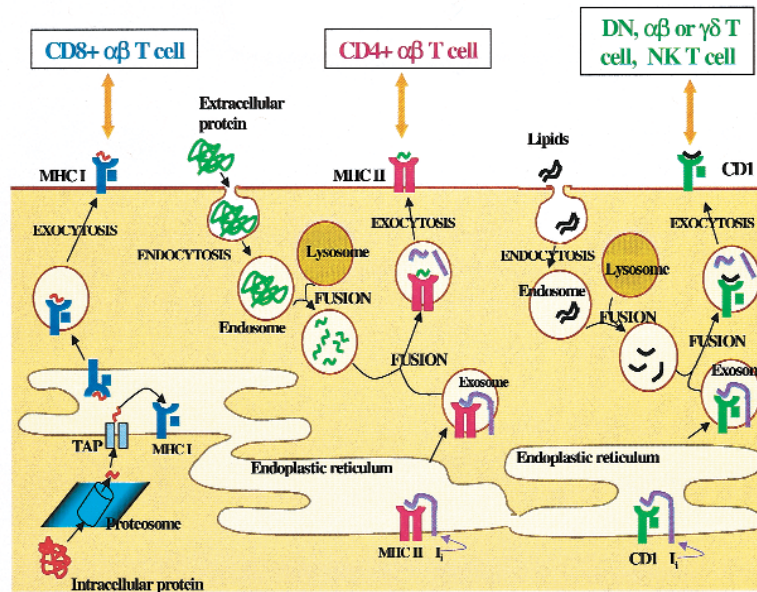


FIG 4. Antigen processing and presentation. Endogenously produced proteins (self-proteins and viral proteins) are degraded in the proteasome, transported by transporter-associated antigen processing to endoplasmic reticulum, where they are combined with class I molecules and transported to the cell surface for presentation to CD8 T cells. Unlike class I molecules, class II and CD1 molecules do not associate with endogenous proteins in the endoplasmic reticulum. Instead they associate with an invariant chain (Ii) and are transported to the exosome. Extracellular proteins and lipids are endocytosed and degraded by lysosomal enzymes. Subsequently, they are combined with class II and CD1 molecules, respectively, and the invariant chain is degraded. The exosome then transports the antigenic epitope-bound class II and CD1 molecules to the cell surface for presentation to CD4 $\alpha\beta$ T cells and double-negative or CD8 $\gamma\delta$ T cells, respectively.

Patients with congenital deficiency of Lck have severe combined immunodeficiency because of the failure of CD4 differentiation. Similarly, zeta-associated protein 70 deficiency results in the severe defect of CD8 T-cell differentiation (see Chapter 12). A small fraction of T cells, mostly of $\gamma\delta$ subtype, are negative for both CD4 and CD8 (double negative).²

THE ANTIGEN—NAÏVE LYMPHOCYTE

The frequency of an antigen-specific lymphocyte in the blood has been calculated to be less than 0.001%. In order to increase the probability of antigen encounter, lymphocytes continuously circulate across various tissues. Naïve T and B cells preferentially migrate to lymph nodes because of the presence of the appropriate set of homing receptors—L-selectin and CCR7.⁹ The corresponding ligands for the foregoing receptors, ie, peripheral node addressin, CC ligand 19 and CC ligand 21, are preferentially expressed on the high endothelial venules of lymph nodes. T cells stay in the lymph node for approximately 24 hours and then leave, but re-enter peripheral lymphoid organs many times.

ANTIGEN ENCOUNTER

Antigen presentation

Professional APC are those that express high levels of class II MHC molecules and possess the capacity to internalize, process, and present foreign antigens in the

MHC groove. Typical APC include dendritic cells (DC), B cells, monocytes/macrophages, and their tissue counterparts. Immature DCs, residing in the peripheral tissue, avidly phagocytose and process foreign antigens (Table I).¹⁰ Pathogen-derived molecules (ligands for toll-like receptors—lipopolysaccharide, flagellin, peptidoglycan, CpG oligodeoxynucleotides) and inflamed tissue-derived cytokines, eg, tumor necrosis factor and IL-1, trigger DC maturation and enhance antigen processing and expression of foreign-peptide-loaded MHC proteins. Mature DCs secrete cytokines and upregulate different costimulators and CCR7 (Table II). The expression of CCR7 enables them to migrate to the draining lymph nodes.

At least 3 distinct antigen-processing and presenting pathways have been delineated (Fig 4). Endogenously produced proteins (self-proteins and viral proteins) are degraded in the proteasome. The degraded peptides are transported to endoplasmic reticulum by the transporter-associated protein, then combined with the MHC class I molecule and transported to the cell surface for presentation to CD8 T cells. Proteins from extracellular pathogens are endocytosed, degraded to small peptides by lysosomal enzymes, then combined with MHC class II molecules in late endosomes and transported to the cell surface for presentation to CD4 T cells. Lipids and lipid derivatives are processed in a manner similar to extracellular proteins in endosomes, combined with CD1, an MHC-like molecule, and presented to double-negative or CD8 T cells frequently bearing $\gamma\delta$ receptors.

TABLE I. MHC molecules

Genes	MHC I	MHC II
	HLA-A, B, C	HLA-DP, DQ, DR
Structure	Transmembrane α chain bound to β 2-microglobulin, only the α chain interacts with the peptide	Transmembrane α and β chain, both chains interact with the antigenic peptide
Presented peptide	Peptides derived from self/nonself intracellular proteins, eg, viral peptides	Peptides derived from extracellular proteins, eg, bacterial peptide
Mechanism of presentation	Intracellular proteins are degraded by the ubiquitin/proteasome pathway in the cytosol; transported by TAP to endoplasmic reticulum and loaded onto MHC; the MHC/peptide complex translocates to the cell membrane	Extracellular proteins are endocytosed and degraded by lysosomal proteases; subsequently peptide-containing endosomes are fused to MHC-containing vesicles; peptides are loaded onto MHC and the complex translocates to the cell membrane
Presenting cells	All nucleated cells, including APC	APC: DC, B cells, macrophages
Interacting T cell	CD8 T cell	CD4 T cell

TAP, Transporter-associated antigen processing.

TABLE II. T-cell costimulatory molecules

Receptor	Expression	Ligand	Role	Knock-out mice
CD28 family members				
CD28	Constitutive	B7.1 (CD80) inducible (through CD40 stimulation), B7.2 (CD86) constitutive on APC	Essential for initiation of naïve T-cell response, enhances IL-2 production, protects from apoptosis, lack of CD28 signaling results in T-cell anergy	Diminished IL-2 production, CD25 expression, immunoglobulin secretion and class switching, deficient Th2 response, CD8 response largely intact
CD152 (CTLA-4)	Inducible on activated cells	B7.1/B7.2	Inhibits activated T-cell proliferation and IL-2 production, terminates T-cell response	Massive lymphoproliferation and multiple organ destruction, T cells are skewed towards Th2
ICOS	Inducible on activated cells	B7RP-1, constitutive, mainly B cells and macrophages	Important for differentiation and effector function of Th1/Th2 cells, enhances IL-4, 10, 13 but not IL-2 production, promotes T-cell memory generation	Impaired formation of germinal centers and class switching, low levels of immunoglobulins, T cells do not secrete IL-4 and IL-13. IFN γ and IL-5 production unperturbed
PD-1	Inducible on activated cells	B7-H1/B7-DC, inducible on APC	Inhibits activated T-cell proliferation and cytokine production	Lupus-like disorder, high titer of immunoglobulins
TNF family members				
CD134 (OX40)	Inducible, transiently expressed, within 24-120 h after T-cell stimulation	OX40L, inducible on APC	Promotes clonal expansion of activated T cells during primary response and enhances memory generation	CD4 T cells can not sustain IL-2 production and clonal expansion during the primary response; reduced number of memory T cells, impaired Th2 response and lung inflammation in the asthma model, CD8 response largely normal
CD27	Inducible on activated T cells	CD27L	As above (OX40), particularly important for CD8 T cells	Reduced clonal expansion and memory formation; CD8 T cells much more affected
CD137 (4-1BB)	Inducible	4-1BBL	Enhances CD8 cell function	Increased T-cell proliferation, reduced effector cell function
Light-R		Light	Enhances proliferation, cytokine production, and cytotoxicity of CD8 cells after superantigen stimulation	Impairment of CD8 response to superantigens, normal response to classic antigen stimulation

CTLA, Cytotoxic T lymphocyte antigen; ICOS, inducible costimulator; Light, lymphotoxins, exhibits inducible expression, and competes with herpes simplex virus glycoprotein D for herpes virus entry mediator, a receptor expressed by T lymphocytes; OX40, an antigen recognized by the monoclonal antibody OX; PD, programmed death; TNF, tumor necrosis factor.

Following processing antigens and migrating to the draining lymph node, the mature DC presents antigenic epitopes to a specific T cell. This process involves sustained physical contact between the 2 cells through the formation of the so-called "immunological synapse."¹¹ During the formation of the synapse, the initial contact is made through long extracellular ligands-counterligands (eg, CD11a-1-intercellular adhesion molecule [ICAM]1, DC-specific ICAM-3 grabbing nonintegrin-ICAM3). Following this initial contact, segregation of surface molecules takes place, allowing TCR and costimulatory molecules (eg, CD4/CD8, CD28) to localize to the center of the contact zone. TCR and the costimulatory molecules then engage with their counterligands on APC. This facilitated ligand-counterligand interaction in the immunological synapse lasts many hours. The TCR is associated with the CD3 complex, which is composed of γ , δ , ϵ , and 2 ζ subunits. Proper assembly of this receptor complex is important for TCR signaling. The lack of the CD3 γ subunit causes severe immunodeficiency, whereas that of the CD3 ϵ subunit induces a milder form of immunodeficiency (see Chapter 12). The binding of the TCR to the peptide-MHC complex results in the activation of CD4- and CD3-associated tyrosine kinases—Lck and Fyn. These kinases phosphorylate CD3-associated ζ chains at the so-called immunoreceptor tyrosine-based activation motifs, which allows docking of multiple signaling molecules and transduction of signals through downstream pathways.¹² Simultaneous signaling through the TCR and a costimulatory receptor are absolutely necessary for productive activation of T cells (2-signal paradigm).¹³ Such activation leads to IL-2 production and antigen-specific clonal expansion of T cells. The number of antigen-specific T cells may temporarily expand from less than 0.001% to >30% of the total population.¹⁴ Signaling through the TCR in the absence of costimulation leads to T-cell anergy (1-signal paradigm) indicated by a failure to produce IL-2 and to proliferate.

For naïve T cells, CD28 is the most important costimulatory molecule. CD28 binds to B7-family proteins, which are present on the APC surface. CD28 engagement triggers activation of rho family guanosine triphosphatases, which results in the enhancement of TCR-induced mitogen-activated protein kinase signaling. Mitogen-activated protein kinases are critically involved in the induction of IL-2 synthesis. CD28 activates anti-apoptotic molecules such as phosphatidylinositol-3 kinase and Akt (also known as protein kinase B), leading to increased T-cell survival. CD28 signaling is also necessary for the upregulation of additional costimulatory molecules, like CD40L and inducible costimulator resulting in the formation of a positive-feedback loop and enhancement of APC-T cell cross talk. Inducible costimulator increases cytokine secretion, and memory T-cell generation. CD40L has a crucial role in B cell activation.

Because of the low frequency of antigen-specific T cells, antigenic stimulation typically activates a small fraction of T cells. Superantigens are microbial products that bind to the TCR outside the antigen-binding site. Proto-

typic superantigens include staphylococcus exotoxins (induce toxic shock syndrome) and other bacterial products.¹⁵ They bind to certain V β families of the TCR and thereby activate all members of the given TCR V β family (5-10% of T cells). For example, staphylococcal enterotoxin B activates T cells expressing V β 3 and V β 8 families.

B CELLS

Binding of a foreign multivalent antigen by the BCR (Fig 1) triggers 4 processes: B-cell proliferation, differentiation into antibody-producing plasma cells, memory formation, and antigen presentation to T cells. Proliferating B cells form the germinal center in the lymph node. Like the TCR, the BCR engages Src-type kinases (Lyn, Fyn, Blk) to initiate signal transduction.¹⁶ This process is enhanced by the action of the costimulatory complex: CD21-CD19-CD81. CD21 is the receptor for the complement protein C3d. C3d interacts with CD21 and the BCR-bound pathogen. This interaction brings CD19 to the physical proximity of the BCR and augments intracellular signaling. In C3/C4-deficient mice B-cell proliferation and antibody secretion is impaired. Following binding of antigens to surface immunoglobulins, B cells internalize, process (Table I), express the antigenic epitopes in the MHC groove, and present them to T cells. T cells modulate B-cell functions in a number of ways. T-cell-derived cytokines IL-4, IL-5, IL-6, IL-2, and interferon (IFN)- γ enhance B-cell proliferation and differentiation into antibody-secreting plasma cells. The physical interaction of T cells with B cells allows signaling through the CD40L-CD40 coreceptors, which, in the presence of IL-4, plays an essential role in immunoglobulin class switching. Naïve B cells express IgM and IgD on the cell surface. Upon stimulation, the B cell switches immunoglobulin classes and produces IgG, IgA, or IgE. The function of different immunoglobulins is summarized in Table III. Patients deficient in CD40L suffer from the hyper IgM syndrome and are unable to class switch to IgG, IgA, or IgE (see Chapter 12). CD40 signaling involves the activation of NF- κ B pathway. Patients deficient in NF- κ B essential modulator, protein involved in nuclear factor κ B regulation, present with life-threatening infections due to low levels of IgG (accompanied by increased IgM levels). Promotion of class switching is only one of multiple CD40 functions. CD40 engagement facilitates B-cell proliferation, differentiation, memory, survival, somatic hypermutation (see below), and immunoglobulin secretion. In patients deficient in CD40L, the activation of B cells and formation of germinal centers are abolished. Class switching involves sequential recombination of constant segments in the heavy chain locus. The affinity for antigens is incrementally improved through a process called somatic hypermutation, in which DNA strand breaks are introduced followed by error-prone repair.¹⁷ The purpose is to create an antibody with the highest affinity for the antigen. Both class switching and somatic hypermutation seem to be facilitated by the RNA-editing enzyme acti-

TABLE III. Functions of immunoglobulins

Class	Immunoglobulin function	Fc receptor-bound function*
IgD	Forms BCR on B cells	None
IgM	Forms BCR on B cells, binds pathogens and toxins, activates complement	None
IgG (IgG ₁₋₄)	Directly neutralizes toxins, blocks pathogen adhesion, activates complement	Facilitates ADCC, acts as an opsonin, ie, activates phagocytes, inhibits lymphocyte function through FcγII (CD32)
IgA (IgA ₁₋₂)	Directly neutralizes toxins, blocks pathogen adhesion at mucosal sites	Facilitates ADCC
IgE	None	Induces mast cell and basophil degranulation, prolongs mast cell survival, facilitates eosinophil-mediated ADCC against parasites

ADCC, Antibody-dependent, cell-mediated cytotoxicity; Fc, constant fragment.

*Fc receptors are present on many immune cells and bind the constant region of immunoglobulins.

TABLE IV. Th cells

Subset	Differentiation factors		
	Cytokines	Transcriptional factors	Cytokines produced*
Th1	IL-12, IL-18, IL-27, IFNγ	T-bet, STAT-4, STAT-1, Hlx, NF-ATp	IFNγ, IL-2, TNF-β*
Th2	IL-4, IL-13	GATA-3, STAT-6, NF-ATc, c-maf, NF-κBp50, c-Rel	IL-3, 4, 5, 9, 10, 13, IL-25*

STAT, Signal transducer and activator of transcription; Hlx, H2.0-like homeobox gene; T-bet, T-box transcription factor expressed in T cells; TNF, tumor necrosis factor; NF-ATp, preexisting nuclear factor of activated T cells; NF-ATc, cytoplasmic nuclear factor of activated T cells; GATA, transcriptional factor binding to the nucleotide sequence element (A/T)GATA(AG); c-Maf, cellular homologue of the v-Maf oncogene; Maf, musculoaponeurotic fibrosarcoma; NF-κB, nuclear factor-κB; c-Rel, cellular homologue of the v-Rel oncogene; Rel, relish.

*Description of cytokine function can be found in the chapter "Cytokines."

TABLE V. Mechanisms of CD8 cell cytotoxicity

Group	Mediator	Function
Cytotoxic granular proteins	Perforin	Perforins insert themselves into the target cell membrane and form pores. CD8 T cells use these pores to inject the content of the granules directly into the cytosol of the target cell.
	Granzymes	Proteases inducing rapid target cell death through activation of pro-apoptotic molecules: caspases, BID, DFF45
Receptors	FasL	FasL associates with the Fas receptor on the target cell. Fas directly activates caspases and triggers apoptosis of the target cell
	TWEAK TRAIL	TWEAK and TRAIL induce apoptosis through similar mechanisms.
Cytokines	TNF-α	Activates caspases in target cells

BID, BH3-interacting death agonist; DFF45, DNA fragmentation factor-45; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; TWEAK, TNF-like weak inducer of apoptosis.

vation-induced cytidine deaminase. The role of immunoglobulins are provided (Table III).

EFFECTOR PHASE

T-cell phenotype

Activated T and B cells in the lymph node downregulate CCR7, begin to express receptors for chemokines that are preferentially expressed in the peripheral tissue (eg, CCR2, CCR4, CCR5, CXCR3), and migrate to the site of pathogen entry. Here they exert their effector functions (cytotoxicity or cytokine secretion by T cells, antibody formation by B cells). Following encounter with the specific antigen naïve CD4, T cells (also called Th0) differentiate into T helper 1 cells (Th1) and/or Th2 cells, whereas CD8 T cells differentiate into cytotoxic T cells (Tables IV and V). The differentiation of Th1 and Th2 cells is induced by the presence of IL-12 and IL-4, respectively, in the anti-

gen-presenting milieu.¹⁸ The foregoing cytokines are typically secreted by the APC and/or other accessory cells. The phenotypic differentiation of Th cells is a stepwise process and involves coordinated signaling events, cell divisions, and chromatin modulation, which finally lead to an epigenetic memory for production of specific cytokines. For Th1 differentiation IL-12 signaling via STAT-4 is essential. In addition, IL-27 signaling via STAT-1 plays an important role at an early stage of differentiation. The joint action of these signaling pathways activates the master Th1 regulator—T-box transcription factor expressed in T cells, which not only induces sustained production of Th1 cytokines but also blocks Th2 differentiation. On the other hand, IL-4 signaling via STAT-6 in conjunction with yet-to-be fully defined signaling molecules induces the master Th2 switch—GATA-3. Once activated, GATA-3 has an auto-inducing effect, stimulates Th2 cytokine production, and inhibits Th1 differentiation. The

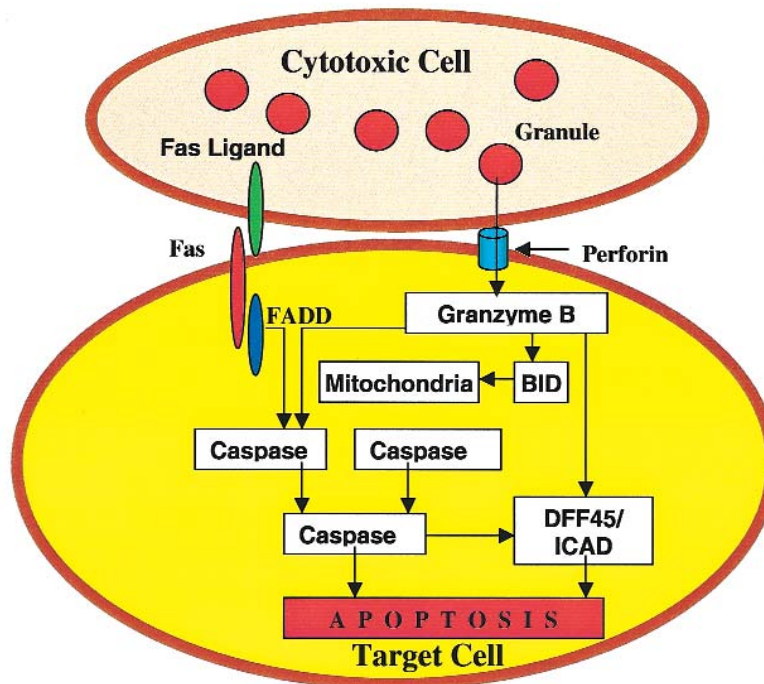


FIG 5. Cytotoxic mechanism of killer T cells and NK cells. Killer cells activate the exogenous apoptotic pathway through the engagement of FasL with Fas. Alternatively, they induce pore formation in the target cell membrane through the engagement of perforin. The pore formation allows penetration of cytotoxic enzymes such as granzyme B or granulysin. The former is capable of activating both exogenous and endogenous apoptotic pathways, which lead to the activation of caspases 8 and 9, respectively. The final common pathway is the activation of caspase 3 and the induction of apoptosis. Granzyme B can also directly activate the caspase-activated DNase (CAD) by degrading DNA fragmentation factor-(DFF45 /inhibitor CAD (ICAD). CAD is an endonuclease and cleaves DNA. BID, BH3-interacting death agonist.

list of cytokines produced by Th1 and Th2 cells are shown in Table IV. Th1 cells are primarily induced by and play a critical role in the defense against intracellular pathogens (viruses, *Mycobacterium*, *Listeria*, etc). Th2 cells are induced by extracellular pathogens/antigens—parasites, bacteria, and allergens.

A rapidly evolving field of DC research suggests that there are 2 major subtypes of DC—DC1 are strong IL-12 producers and favor Th1 differentiation, whereas DC2 are poor IL-12 producers and favor Th2 differentiation.¹⁹ In addition to Th1 and Th2, some investigators recognize another form of Th cells called Th3.²⁰ Chronic low doses of antigens administered orally preferentially induce CD4 Th3 cells, which produce large quantities of transforming growth factor- β and induce oral tolerance.

CD8 effector T cells exert their cytotoxicity upon recognition of nonself antigens in the context of class I MHC molecules. CD8 T cells have 2 distinct mechanisms of cytotoxicity: perforin and Fas ligand (Fig 5).²¹ Perforin is a membrane pore-forming molecule, which allows release of granular enzymes (eg, granzymes) directly into the cytosol of the target cell. Granzyme B induces rapid apoptosis of the target cell through caspase-dependent and caspase-independent manners. Some authors divide cytotoxic T cells into 2 populations—Tc1 and Tc2, apply-

ing the Th cell criteria. Tc1 secretes IFN- γ , and Tc2 secretes IL-4 and IL-5. Naïve CD8 T cells have a strong tendency to differentiate into Tc1 cells. IFN- γ and IL-12 encourage this process. Both subsets kill their targets equally well. Tc2 cells may provide help to B cells.

NK CELLS

NK cells constitute the CD56-positive (NK1.1+ in mice) subset of cytotoxic lymphocytes, which does not possess the antigen receptor, hence is a component of innate immunity.²² NK cells express receptors for MHC I molecules belonging to killer cell inhibitory receptor family. These receptors recruit phosphatases (Src homology domain containing protein tyrosine phosphatase 1) to transduce the inhibitory signal. NK cells are spontaneously active, unless they are inhibited by self-MHC molecules. Therefore, NK cells are well-suited to perform immunosurveillance for nonself MHC-bearing targets (eg, transplanted cells, tumors, virally-modified cells). NK cells also mediate antibody-dependent cell mediated cytotoxicity. In the latter process, NK cells release their toxic mediators upon binding to IgG-coated tumors or virally infected cells. The mechanism of cytotoxicity of NK cells is very similar to that of cytotoxic

CD8 T cells and is described in Table V. A subset of T cells also expresses NK1.1. This subpopulation is called NK T cells and is characterized by the expression of limited TCR repertoire ($V\alpha 14/V\beta 8.2$ in mice and $V\alpha 24/V\beta 11$ in humans).²³ These antigen receptors do not recognize peptides but glycolipids in the context of the nonclassic MHC I molecule CD1. Following stimulation, NK T cells produce high levels of IL-4, IFN- γ , and tumor necrosis factor- α .

IMMUNOLOGICAL TOLERANCE

The elimination of auto-reactive T and B cells in the thymus and bone marrow, respectively, through the negative selection is called central tolerance.²⁴ There is evidence that some self-reactive T and B cells manage to escape the negative selection. Their receptors may recognize MHC/self-peptide with the affinity, which is not high enough to elicit negative selection. Also, not all self-antigens are presented in the thymus or bone marrow. Despite the presence of auto-reactive cells and the presentation of self-antigens in lymphoid organs, autoimmunity does not develop, thanks to the presence of peripheral tolerance. There are few mechanisms responsible for peripheral tolerance. DCs expressing self-peptides are not activated and, therefore, have very low levels of costimulatory molecules. They also secrete IL-10. Under these conditions, the majority of lymphocytes are anergized instead of being activated. Presentation of self-antigens in the absence of costimulation may also lead to clonal deletion through apoptosis. Auto-reactive cells in the peripheral organs are suppressed by an unusual population of CD4+CD25+ effector cells, called regulatory T cells.²⁵ These regulatory cells secrete large amounts of IL-10 and transforming growth factor- β and block proliferation of auto-reactive lymphocytes. Transforming growth factor- β knock-out mice have diffuse inflammatory lesions in multiple organs. IL-10 deficient mice develop colitis resembling Crohn's Disease.

THE END OF THE BATTLE: RESTORATION OF HOMEOSTASIS

The immune system is characterized by enormous plasticity. After successful elimination of the pathogen, the lymphocyte compartment shrinks as quickly as it expanded. T-cell activation is programmed to be self-limited and is coupled to gradual upregulation and stimulation of inhibitory receptors. Cytotoxic T lymphocyte antigen-4, CD32 (Fc γ R2), paired Ig-like receptor-B, CD22, gp49B1, killer cell inhibitory receptor, and programmed death-1 are examples of a growing family of inhibitory receptors that downregulate immune response because of the presence of the so-called immunoreceptor inhibitory motif sequence on their cytosolic chain.²⁶ Activation-induced cell death is another mechanism, wherein upregulated CD95 interacts with the suicidal receptor Fas and eliminates Fas-expressing T cells.

SURVIVORS OF THE WAR: LINGERING MEMORIES

Some antigen-specific lymphocytes manage to survive the "shrinkage" phase of the immune response. They form the pool of long-lived memory cells. In the event of a second attack, pathogen-specific memory lymphocytes mount an accelerated and more vigorous immune response, which results in faster elimination of the invading target. The long-term survival of memory CD8 T cells is dependent upon the stromal cell-derived cytokine-IL-15. Memory T cells have increased levels of activation markers, eg, CD2, CD44, CD25, CD11a, and CD49d. These 2 populations of T cells also differ in the expression of isoforms of CD45 phosphatase. Naïve T cells express CD45RA, whereas memory T cells display CD45RO. There are 2 populations of memory T cells: effector memory T cells and central memory T cells.²⁷ The effector memory T cells do not express CCR7 and L-selectin, homes to peripheral tissues, and conveys immediate protection against ever-invading pathogens by rapidly producing cytokines. Central memory T cells home to lymph nodes due to the expression of CCR7 and L-selectin. Upon antigen stimulation, they can differentiate into effector cells and migrate to the peripheral tissue. Therefore, the central memory constitutes the back-up reservoir for the peripheral effector memory.

CONCLUSIONS

The immune system has evolved to combine many seemingly opposing virtues such as diversity and specificity, distinction between self and nonself, and preparedness for acute emergency and maintenance of a long-term memory. It has developed a unique genetic recombination mechanism that produces a virtually unlimited repertoire utilizing a limited number of genes. For versatility, adaptivity, and sophistication the immune system remains unparalleled to all other biological systems.

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