

# immuno biology®

THE IMMUNE SYSTEM IN HEALTH AND DISEASE

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## Part I

## AN INTRODUCTION TO IMMUNO-BIOLOGY AND INNATE IMMUNITY

### Chapter 1 Basic Concepts in Immunology

#### The components of the immune system.

- 1-1 The white blood cells of the immune system derive from precursors in the bone marrow.
  - 1-2 Lymphocytes mature in the bone marrow or the thymus.
  - 1-3 The peripheral lymphoid organs are specialized to trap antigen, to allow the initiation of adaptive immune responses, and to provide signals that sustain recirculating lymphocytes.
  - 1-4 Lymphocytes circulate between blood and lymph.
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#### Principles of innate and adaptive immunity.

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  - 1-6 Activation of specialized antigen-presenting cells is a necessary first step for induction of adaptive immunity.
  - 1-7 Lymphocytes activated by antigen give rise to clones of antigen-specific cells that mediate adaptive immunity.
  - 1-8 Clonal selection of lymphocytes is the central principle of adaptive immunity.
  - 1-9 The structure of the antibody molecule illustrates the central puzzle of adaptive immunity.
  - 1-10 Each developing lymphocyte generates a unique antigen receptor by rearranging its receptor genes.
  - 1-11 Lymphocyte development and survival are determined by signals received through their antigen receptors.
  - 1-12 Lymphocytes proliferate in response to antigen in peripheral lymphoid organs, generating effector cells and immunological memory.
  - 1-13 Interaction with other cells as well as with antigen is necessary for lymphocyte activation.
- Summary.

#### The recognition and effector mechanisms of adaptive immunity.

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- 1-15 T cells are needed to control intracellular pathogens and to activate B-cell responses to most antigens.
- 1-16 T cells are specialized to recognize foreign antigens as peptide fragments bound to proteins of the major histocompatibility complex.
- 1-17 Two major types of T cell recognize peptides bound to proteins of two different classes of MHC molecule.
- 1-18 Defects in the immune system result in increased susceptibility to infection.
- 1-19 Understanding adaptive immune responses is important for the

control of allergies, autoimmune disease, and organ graft rejection.

- 1-20 Vaccination is the most effective means of controlling infectious diseases.

Summary.

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  - 2-13 The terminal complement proteins polymerize to form pores in membranes that can kill certain pathogens.
  - 2-14 Complement control proteins regulate all three pathways of complement activation and protect the host from its destructive effects.
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- 2-16 Receptors on phagocytes can signal the presence of pathogens.
- 2-17 The effects of bacterial lipopolysaccharide on macrophages are mediated by CD14 binding to Toll-like receptor 4.

- 2-18 Activation of Toll-like receptors triggers the production of pro-inflammatory cytokines and chemokines, and the expression of co-stimulatory molecules.  
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Summary to Chapter 2.

## Part II THE RECOGNITION OF ANTIGEN

### Chapter 3 Antigen Recognition by B-cell and T-cell Receptors

#### The structure of a typical antibody molecule.

- 3-1 IgG antibodies consist of four polypeptide chains.  
3-2 Immunoglobulin heavy and light chains are composed of constant and variable regions.  
3-3 The antibody molecule can readily be cleaved into functionally distinct fragments.  
3-4 The immunoglobulin molecule is flexible, especially at the hinge region.  
3-5 The domains of an immunoglobulin molecule have similar structures.  
Summary.

#### The interaction of the antibody molecule with specific antigen.

- 3-6 Localized regions of hypervariable sequence form the antigen-binding site.  
3-7 Antibodies bind antigens via contacts with amino acids in CDRs, but the details of binding depend upon the size and shape of the antigen.  
3-8 Antibodies bind to conformational shapes on the surfaces of antigens.  
3-9 Antigen-antibody interactions involve a variety of forces.  
Summary.

#### Antigen recognition by T cells.

- 3-10 The antigen receptor on T cells is very similar to a Fab fragment of immunoglobulin.  
3-11 A T-cell receptor recognizes antigen in the form of a complex of a foreign peptide bound to an MHC molecule.  
3-12 T cells with different functions are distinguished by CD4 and CD8 cell-surface proteins and recognize peptides bound to different classes of MHC molecule.  
3-13 The two classes of MHC molecule are expressed differentially on cells.  
3-14 The two classes of MHC molecule have distinct subunit structures but similar three-dimensional structures.  
3-15 Peptides are stably bound to MHC molecules, and also serve to stabilize the MHC molecule on the cell surface.  
3-16 MHC class I molecules bind short peptides of 8–10 amino acids by both ends.  
3-17 The length of the peptides bound by MHC class II molecules is not constrained.  
3-18 The crystal structures of several MHC:peptide:T-cell receptor complexes all show the same T-cell receptor orientation over the MHC:peptide complex.  
3-19 A distinct subset of T cells bears an alternative receptor made up of  $\gamma$  and  $\delta$  chains.  
Summary.  
Summary to Chapter 3.

### Chapter 4 The Generation of Lymphocyte Antigen Receptors

#### The generation of diversity in immunoglobulins.

- 4-1 Immunoglobulin genes are rearranged in antibody-producing cells.  
4-2 The DNA sequence encoding a complete V region is generated by the somatic recombination of separate gene segments.  
4-3 There are multiple different V-region gene segments.  
4-4 Rearrangement of V, D, and J gene segments is guided by flanking DNA sequences.  
4-5 The reaction that recombines V, D, and J gene segments involves both lymphocyte-specific and ubiquitous DNA-modifying enzymes.  
4-6 The diversity of the immunoglobulin repertoire is generated by four main processes.  
4-7 The multiple inherited gene segments are used in different combinations.  
4-8 Variable addition and subtraction of nucleotides at the junctions between gene segments contributes to diversity in the third hypervariable region.  
4-9 Rearranged V genes are further diversified by somatic hypermutation.  
4-10 In some species most immunoglobulin gene diversification occurs after gene rearrangement.  
Summary.

#### T-cell receptor gene rearrangement.

- 4-11 The T-cell receptor loci comprise sets of gene segments and are rearranged by the same enzymes as the immunoglobulin loci.  
4-12 T-cell receptors concentrate diversity in the third hypervariable region.  
4-13  $\gamma\delta$  T-cell receptors are also generated by gene rearrangement.

- 4-14 Somatic hypermutation does not generate diversity in T-cell receptors.  
Summary.

#### Structural variation in immunoglobulin constant regions.

- 4-15 The immunoglobulin heavy-chain isotypes are distinguished by the structure of their constant regions.  
4-16 The same  $V_H$  exon can associate with different  $C_H$  genes in the course of an immune response.  
4-17 Transmembrane and secreted forms of immunoglobulin are generated from alternative heavy-chain transcripts.  
4-18 Antibody C regions confer functional specialization.  
4-19 IgM and IgA can form polymers.  
4-20 Various differences between immunoglobulins can be detected by antibodies.  
Summary.  
Summary to Chapter 4.

### Chapter 5 Antigen Presentation to T Lymphocytes

#### The generation of T-cell receptor ligands.

- 5-1 The MHC class I and class II molecules deliver peptides to the cell surface from two distinct intracellular compartments.  
5-2 Peptides that bind to MHC class I molecules are actively transported from the cytosol to the endoplasmic reticulum.  
5-3 Peptides for transport into the endoplasmic reticulum are generated in the cytosol.  
5-4 Newly synthesized MHC class I molecules are retained in the endoplasmic reticulum until they bind peptide.  
5-5 Peptides presented by MHC class II molecules are generated in acidified endocytic vesicles.  
5-6 The invariant chain directs newly synthesized MHC class II molecules to acidified intracellular vesicles.  
5-7 A specialized MHC class II-like molecule catalyzes loading of MHC class II molecules with peptides.  
5-8 Stable binding of peptides by MHC molecules provides effective antigen presentation at the cell surface.  
Summary.

#### The major histocompatibility complex and its functions.

- 5-9 Many proteins involved in antigen processing and presentation are encoded by genes within the major histocompatibility complex.  
5-10 A variety of genes with specialized functions in immunity are also encoded in the MHC.  
5-11 Specialized MHC class I molecules act as ligands for activation and inhibition of NK cells.  
5-12 The protein products of MHC class I and class II genes are highly polymorphic.  
5-13 MHC polymorphism affects antigen recognition by T cells by influencing both peptide binding and the contacts between T-cell receptor and MHC molecule.  
5-14 Nonself MHC molecules are recognized by 1–10% of T cells.  
5-15 Many T cells respond to superantigens.  
5-16 MHC polymorphism extends the range of antigens to which the immune system can respond.  
5-17 Multiple genetic processes generate MHC polymorphism.  
5-18 Some peptides and lipids generated in the endocytic pathway can be bound by MHC class I-like molecules that are encoded outside the MHC.  
Summary.  
Summary to Chapter 5.

## Part III

## THE DEVELOPMENT OF MATURE LYMPHOCYTE RECEPTOR REPERTOIRES

### Chapter 6 Signaling Through Immune System Receptors

#### General principles of transmembrane signaling.

- 6-1 Binding of antigen leads to clustering of antigen receptors on lymphocytes.  
6-2 Clustering of antigen receptors leads to activation of intracellular signal molecules.  
6-3 Phosphorylation of receptor cytoplasmic tails by tyrosine kinases concentrates intracellular signaling molecules around the receptors.  
6-4 Intracellular signaling components recruited to activated receptors transmit the signal onward from the membrane and amplify it.  
6-5 Small G proteins activate a protein kinase cascade that transmits the signal to the nucleus.  
Summary.

#### Antigen receptor structure and signaling pathways.

- 6-6 The variable chains of lymphocyte antigen receptors are associated with invariant accessory chains that carry out the signaling function of the receptor.  
6-7 The ITAMs associated with the B-cell and T-cell receptors are phosphorylated by protein tyrosine kinases of the Src family.  
6-8 Antigen receptor signaling is enhanced by co-receptors that bind the same ligand.  
6-9 Fully phosphorylated ITAMs bind the protein tyrosine kinases Syk and ZAP-70 and enable them to be activated.  
6-10 Downstream events are mediated by proteins that associate with the phosphorylated tyrosines and bind to and activate other proteins.  
6-11 Antigen recognition leads ultimately to the induction of new gene synthesis by activating transcription factors.  
6-12 Not all ligands for the T-cell receptor produce a similar response.  
6-13 Other receptors on leukocytes also use ITAMs to signal activation.  
6-14 Antigen-receptor signaling can be inhibited by receptors associated with ITIMs.  
Summary.

#### Other signaling pathways that contribute to lymphocyte behavior.

- 6-15 Microbes and their products release NF $\kappa$ B from its site in the cytosol through an ancient pathway of host defense against infection.  
6-16 Bacterial peptides, mediators of inflammatory responses, and chemokines signal through members of the seven-transmembrane-domain, trimeric G protein-coupled receptor family.  
6-17 Cytokines signal lymphocytes by binding to cytokine receptors and triggering Janus kinases to phosphorylate and activate STAT proteins.  
6-18 Programmed cell death of activated lymphocytes is triggered mainly through the receptor Fas.

- 6-19 Lymphocyte survival is maintained by a balance between death-promoting and death-inhibiting members of the Bcl-2 family of proteins.
- 6-20 Homeostasis of lymphocyte populations is maintained by signals that lymphocytes are continually receiving through their antigen receptors.
- Summary.
- Summary to Chapter 6.

## Chapter 7 The Development and Survival of Lymphocytes

### Generation of lymphocytes in bone marrow and thymus.

- 7-1 Lymphocyte development occurs in specialized environments and is regulated by the somatic rearrangement of the antigen-receptor genes.
- 7-2 B cells develop in the bone marrow with the help of stromal cells and achieve maturity in peripheral lymphoid organs.
- 7-3 Stages in B-cell development are distinguished by the expression of immunoglobulin chains and particular cell-surface proteins.
- 7-4 T cells also originate in the bone marrow, but all the important events in their development occur in the thymus.
- 7-5 Most developing T cells die in the thymus.
- 7-6 Successive stages in the development of thymocytes are marked by changes in cell-surface molecules.
- 7-7 Thymocytes at different developmental stages are found in distinct parts of the thymus.
- Summary.

### The rearrangement of antigen-receptor gene segments controls lymphocyte development.

- 7-8 B cells undergo a strictly programmed series of gene rearrangements in the bone marrow.
- 7-9 Successful rearrangement of heavy-chain immunoglobulin gene segments leads to the formation of a pre-B-cell receptor that halts further  $V_H$  to  $DJ_H$  rearrangement and triggers the cell to divide.
- 7-10 Rearrangement at the immunoglobulin light-chain locus leads to cell-surface expression of the B-cell receptor.
- 7-11 The expression of proteins regulating immunoglobulin gene rearrangement and function is developmentally programmed.
- 7-12 T cells in the thymus undergo a series of gene segment rearrangements similar to those of B cells.
- 7-13 T cells with  $\alpha:\beta$  or  $\gamma:\delta$  receptors arise from a common progenitor.
- 7-14 T cells expressing particular  $\gamma$ - and  $\delta$ -chain V regions arise in an ordered sequence early in life.
- 7-15 Rearrangement of the  $\beta$ -chain locus and production of a  $\beta$  chain trigger several events in developing thymocytes.
- 7-16 T-cell  $\alpha$ -chain genes undergo successive rearrangements until positive selection or cell death intervenes.
- Summary.

### Interaction with self antigens selects some lymphocytes for survival but eliminates others.

- 7-17 Immature B cells that bind self antigens undergo further receptor rearrangement, or die, or are inactivated.
- 7-18 Mature B cells can also be rendered self-tolerant.
- 7-19 Only thymocytes whose receptors can interact with self MHC:peptide complexes can survive and mature.

- 7-20 Most thymocytes express receptors that cannot interact with self MHC and these cells die in the thymus.
- 7-21 Positive selection acts on a repertoire of receptors with inherent specificity for MHC molecules.
- 7-22 Positive selection coordinates the expression of CD4 or CD8 with the specificity of the T-cell receptor and the potential effector functions of the cell.
- 7-23 Thymic cortical epithelial cells mediate positive selection of developing thymocytes.
- 7-24 T cells that react strongly with ubiquitous self antigens are deleted in the thymus.
- 7-25 Negative selection is driven most efficiently by bone marrow-derived antigen-presenting cells.
- 7-26 Endogenous superantigens mediate negative selection of T-cell receptors derived from particular  $V_\beta$  gene segments.
- 7-27 The specificity and strength of signals for negative and positive selection must differ.
- 7-28 The B-1 subset of B cells has a distinct developmental history and expresses a distinctive repertoire of receptors.
- Summary.

### Survival and maturation of lymphocytes in peripheral lymphoid tissues.

- 7-29 Newly formed lymphocytes home to particular locations in peripheral lymphoid tissues.
- 7-30 The development and organization of peripheral lymphoid tissues is controlled by cytokines and chemokines.
- 7-31 Only a small fraction of immature B cells mature and survive in peripheral lymphoid tissues.
- 7-32 The life-span of naive T cells in the periphery is determined by ongoing contact with self peptide:self MHC complexes similar to those that initially selected them.
- 7-33 B-cell tumors often occupy the same site as their normal counterparts.
- 7-34 A range of tumors of immune system cells throws light on different stages of T-cell development.
- 7-35 Malignant lymphocyte tumors frequently carry chromosomal translocations that join immunoglobulin loci to genes regulating cell growth.
- Summary.
- Summary to Chapter 7.

## Part IV

## THE ADAPTIVE IMMUNE RESPONSE

### Chapter 8 T Cell-Mediated Immunity

#### The production of armed effector T cells.

- 8-1 T-cell responses are initiated in peripheral lymphoid organs by activated antigen-presenting cells.
- 8-2 Naive T cells sample the MHC:peptide complexes on the surface of antigen-presenting cells as they migrate through peripheral lymphoid tissue.
- 8-3 Lymphocyte migration, activation, and effector function depend on cell-cell interactions mediated by cell-adhesion molecules.
- 8-4 The initial interaction of T cells with antigen-presenting cells is mediated by cell-adhesion molecules.

- 8-5 Both specific ligand and co-stimulatory signals provided by an antigen-presenting cell are required for the clonal expansion of naive T cells.
  - 8-6 Dendritic cells specialize in taking up antigen and activating naive T cells.
  - 8-7 Macrophages are scavenger cells that can be induced by pathogens to present foreign antigens to naive T cells.
  - 8-8 B cells are highly efficient at presenting antigens that bind to their surface immunoglobulin.
  - 8-9 Activated T cells synthesize the T-cell growth factor interleukin-2 and its receptor.
  - 8-10 The co-stimulatory signal is necessary for the synthesis and secretion of IL-2.
  - 8-11 Antigen recognition in the absence of co-stimulation leads to T-cell tolerance.
  - 8-12 Proliferating T cells differentiate into armed effector T cells that do not require co-stimulation to act.
  - 8-13 The differentiation of CD4 T cells into  $T_H1$  or  $T_H2$  cells determines whether humoral or cell-mediated immunity will predominate.
  - 8-14 Naive CD8 T cells can be activated in different ways to become armed cytotoxic effector cells.
- Summary.

#### General properties of armed effector T cells.

- 8-15 Effector T-cell interactions with target cells are initiated by antigen-nonspecific cell-adhesion molecules.
  - 8-16 Binding of the T-cell receptor complex directs the release of effector molecules and focuses them on the target cell.
  - 8-17 The effector functions of T cells are determined by the array of effector molecules they produce.
  - 8-18 Cytokines can act locally or at a distance.
  - 8-19 Cytokines and their receptors fall into distinct families of structurally related proteins.
  - 8-20 The TNF family of cytokines are trimeric proteins that are often associated with the cell surface.
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- 8-21 Cytotoxic T cells can induce target cells to undergo programmed cell death.
  - 8-22 Cytotoxic effector proteins that trigger apoptosis are contained in the granules of CD8 cytotoxic T cells.
  - 8-23 Activated CD8 T cells and some CD4 effector T cells express Fas ligand, which can also activate apoptosis.
  - 8-24 Cytotoxic T cells are selective and serial killers of targets expressing specific antigen.
  - 8-25 Cytotoxic T cells also act by releasing cytokines.
- Summary.

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  - 8-28 Activation of macrophages by armed  $T_H1$  cells promotes microbial killing and must be tightly regulated to avoid tissue damage.
  - 8-29  $T_H1$  cells coordinate the host response to intracellular pathogens.
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## Chapter 9 The Humoral Immune Response

### B-cell activation by armed helper T cells.

- 9-1 The humoral immune response is initiated when B cells that bind antigen are signaled by helper T cells or by certain microbial antigens alone.
  - 9-2 Armed helper T cells activate B cells that recognize the same antigen.
  - 9-3 Antigenic peptides bound to self MHC class II molecules trigger armed helper T cells to make membrane-bound and secreted molecules that can activate a B cell.
  - 9-4 Isotype switching requires expression of CD40L by the helper T cell and is directed by cytokines.
  - 9-5 Antigen-binding B cells are trapped in the T-cell zone of secondary lymphoid tissues and are activated by encounter with armed helper T cells.
  - 9-6 The second phase of the primary B-cell immune response occurs when activated B cells migrate to follicles and proliferate to form germinal centers.
  - 9-7 Germinal center B cells undergo V-region somatic hypermutation and cells with mutations that improve affinity for antigen are selected.
  - 9-8 Ligation of the B-cell receptor and CD40, together with direct contact with T cells, are all required to sustain germinal center B cells.
  - 9-9 Surviving germinal center B cells differentiate into either plasma cells or memory cells.
  - 9-10 B-cell responses to bacterial antigens with intrinsic ability to activate B cells do not require T-cell help.
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- 9-12 Antibodies of different isotype operate in distinct places and have distinct effector functions.
  - 9-13 Transport proteins that bind to the Fc regions of antibodies carry particular isotypes across epithelial barriers.
  - 9-14 High-affinity IgG and IgA antibodies can neutralize bacterial toxins.
  - 9-15 High-affinity IgG and IgA antibodies can inhibit the infectivity of viruses.
  - 9-16 Antibodies can block the adherence of bacteria to host cells.
  - 9-17 Antibody:antigen complexes activate the classical pathway of complement by binding to C1q.
  - 9-18 Complement receptors are important in the removal of immune complexes from the circulation.
- Summary.

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- 9-19 The Fc receptors of accessory cells are signaling receptors specific for immunoglobulins of different isotypes.
  - 9-20 Fc receptors on phagocytes are activated by antibodies bound to the surface of pathogens and enable the phagocytes to ingest and destroy pathogens.
  - 9-21 Fc receptors activate natural killer cells to destroy antibody-coated targets.
  - 9-22 Mast cells, basophils, and activated eosinophils bind IgE antibody via the high-affinity Fc $\epsilon$  receptor.
  - 9-23 IgE-mediated activation of accessory cells has an important role in resistance to parasite infection.
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## Chapter 10 Adaptive Immunity to Infection

### Infectious agents and how they cause disease.

- 10-1 The course of an infection can be divided into several distinct phases.
  - 10-2 Infectious diseases are caused by diverse living agents that replicate in their hosts.
- Summary.

### The course of the adaptive response to infection.

- 10-3 The nonspecific responses of innate immunity are necessary for an adaptive immune response to be initiated.
  - 10-4 An adaptive immune response is initiated when circulating T cells encounter their corresponding antigen in draining lymphoid tissues and become activated.
  - 10-5 Cytokines made in the early phases of an infection influence the functional differentiation of CD4 T cells.
  - 10-6 Distinct subsets of T cells can regulate the growth and effector functions of other T-cell subsets.
  - 10-7 The nature and amount of antigenic peptide can also affect the differentiation of CD4 T cells.
  - 10-8 Armed effector T cells are guided to sites of infection by chemokines and newly expressed adhesion molecules.
  - 10-9 Antibody responses develop in lymphoid tissues under the direction of armed helper T cells.
  - 10-10 Antibody responses are sustained in medullary cords and bone marrow.
  - 10-11 The effector mechanisms used to clear an infection depend on the infectious agent.
  - 10-12 Resolution of an infection is accompanied by the death of most of the effector cells and the generation of memory cells.
- Summary.

### The mucosal immune system.

- 10-13 Mucosa-associated lymphoid tissue is located in anatomically defined microcompartments throughout the gut.
  - 10-14 The mucosal immune system contains a distinctive repertoire of lymphocytes.
  - 10-15 Secretory IgA is the antibody isotype associated with the mucosal immune system.
  - 10-16 Most antigens presented to the mucosal immune system induce tolerance.
  - 10-17 The mucosal immune system can mount an immune response to the normal bacterial flora of the gut.
  - 10-18 Enteric pathogens cause a local inflammatory response and the development of protective immunity.
  - 10-19 Infection by *Helicobacter pylori* causes a chronic inflammatory response, which may cause peptic ulcers, carcinoma of the stomach, and unusual lymphoid tumors.
  - 10-20 In the absence of inflammatory stimuli, the normal response of the mucosal immune system to foreign antigens is tolerance.
- Summary.

### Immunological memory.

- 10-21 Immunological memory is long-lived after infection or vaccination.
- 10-22 Both clonal expansion and clonal differentiation contribute to immunological memory in B cells.
- 10-23 Repeated immunizations lead to increasing affinity of antibody owing to somatic hypermutation and selection by antigen in germinal centers.

- 10-24 Memory T cells are increased in frequency and have distinct activation requirements and cell-surface proteins that distinguish them from armed effector T cells.
  - 10-25 In immune individuals, secondary and subsequent responses are mediated solely by memory lymphocytes and not by naive lymphocytes.
- Summary.
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## Part V

## THE IMMUNE SYSTEM IN HEALTH AND DISEASE

### Chapter 11 Failures of Host Defense Mechanisms

#### Pathogens have evolved various means of evading or subverting normal host defenses.

- 11-1 Antigenic variation allows pathogens to escape from immunity.
  - 11-2 Some viruses persist *in vivo* by ceasing to replicate until immunity wanes.
  - 11-3 Some pathogens resist destruction by host defense mechanisms or exploit them for their own purposes.
  - 11-4 Immunosuppression or inappropriate immune responses can contribute to persistent disease.
  - 11-5 Immune responses can contribute directly to pathogenesis.
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#### Inherited immunodeficiency diseases.

- 11-6 A history of repeated infections suggests a diagnosis of immunodeficiency.
  - 11-7 Inherited immunodeficiency diseases are caused by recessive gene defects.
  - 11-8 The main effect of low levels of antibody is an inability to clear extracellular bacteria.
  - 11-9 T-cell defects can result in low antibody levels.
  - 11-10 Defects in complement components cause defective humoral immune function.
  - 11-11 Defects in phagocytic cells permit widespread bacterial infections.
  - 11-12 Defects in T-cell function result in severe combined immunodeficiencies.
  - 11-13 Defective T-cell signaling, cytokine production, or cytokine action can cause immunodeficiency.
  - 11-14 The normal pathways for host defense against intracellular bacteria are illustrated by genetic deficiencies of IFN- $\gamma$  and IL-12 and their receptors.
  - 11-15 X-linked lymphoproliferative syndrome is associated with fatal infection by Epstein-Barr virus and with the development of lymphomas.
  - 11-16 Bone marrow transplantation or gene therapy can be useful to correct genetic defects.
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#### Acquired immune deficiency syndrome.

- 11-17 Most individuals infected with HIV progress over time to AIDS.
- 11-18 HIV is a retrovirus that infects CD4 T cells, dendritic cells, and macrophages.

- 11-19 Genetic deficiency of the macrophage chemokine co-receptor for HIV confers resistance to HIV infection *in vivo*.
  - 11-20 HIV RNA is transcribed by viral reverse transcriptase into DNA that integrates into the host cell genome.
  - 11-21 Transcription of the HIV provirus depends on host cell transcription factors induced upon the activation of infected T cells.
  - 11-22 Drugs that block HIV replication lead to a rapid decrease in titer of infectious virus and an increase in CD4 T cells.
  - 11-23 HIV accumulates many mutations in the course of infection in a single individual and drug treatment is soon followed by the outgrowth of drug-resistant variants of the virus.
  - 11-24 Lymphoid tissue is the major reservoir of HIV infection.
  - 11-25 An immune response controls but does not eliminate HIV.
  - 11-26 HIV infection leads to low levels of CD4 T cells, increased susceptibility to opportunistic infection, and eventually to death.
  - 11-27 Vaccination against HIV is an attractive solution but poses many difficulties.
  - 11-28 Prevention and education are one way in which the spread of HIV and AIDS can be controlled.
- Summary.  
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## Chapter 12 Allergy and Hypersensitivity

### The production of IgE.

- 12-1 Allergens are often delivered transmucosally at low dose, a route that favors IgE production.
  - 12-2 Enzymes are frequent triggers of allergy.
  - 12-3 Class switching to IgE in B lymphocytes is favored by specific signals.
  - 12-4 Genetic factors contribute to the development of IgE-mediated allergy, but environmental factors may also be important.
- Summary.

### Effector mechanisms in allergic reactions.

- 12-5 Most IgE is cell-bound and engages effector mechanisms of the immune system by different pathways from other antibody isotypes.
  - 12-6 Mast cells reside in tissues and orchestrate allergic reactions.
  - 12-7 Eosinophils are normally under tight control to prevent inappropriate toxic responses.
  - 12-8 Eosinophils and basophils cause inflammation and tissue damage in allergic reactions.
  - 12-9 An allergic reaction is divided into an immediate response and a late-phase response.
  - 12-10 The clinical effects of allergic reactions vary according to the site of mast-cell activation.
  - 12-11 Allergen inhalation is associated with the development of rhinitis and asthma.
  - 12-12 Skin allergy is manifest as urticaria or chronic eczema.
  - 12-13 Allergy to foods causes symptoms limited to the gut and systemic reactions.
  - 12-14 Allergy can be treated by inhibiting either IgE production or the effector pathways activated by cross-linking of cell-surface IgE.
- Summary.

### Hypersensitivity diseases.

- 12-15 Innocuous antigens can cause type II hypersensitivity reactions in susceptible individuals by binding to the surfaces of circulating blood cells.

- 12-16 Systemic disease caused by immune complex formation can follow the administration of large quantities of poorly catabolized antigens.
  - 12-17 Delayed-type hypersensitivity reactions are mediated by T<sub>H</sub>1 cells and CD8 cytotoxic T cells.
- Summary.  
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## Chapter 13 Autoimmunity and Transplantation

### Autoimmune responses are directed against self antigens.

- 13-1 Specific adaptive immune responses to self antigens can cause autoimmune disease.
  - 13-2 Autoimmune diseases can be classified into clusters that are typically either organ-specific or systemic.
  - 13-3 Susceptibility to autoimmune disease is controlled by environmental and genetic factors, especially MHC genes.
  - 13-4 The genes that have been associated with the development of systemic lupus erythematosus provide important clues to the etiology of the disease.
  - 13-5 Antibody and T cells can cause tissue damage in autoimmune disease.
  - 13-6 Autoantibodies against blood cells promote their destruction.
  - 13-7 The fixation of sublytic doses of complement to cells in tissues stimulates a powerful inflammatory response.
  - 13-8 Autoantibodies against receptors cause disease by stimulating or blocking receptor function.
  - 13-9 Autoantibodies against extracellular antigens cause inflammatory injury by mechanisms akin to type II and type III hypersensitivity reactions.
  - 13-10 Environmental cofactors can influence the expression of autoimmune disease.
  - 13-11 The pattern of inflammatory injury in autoimmunity can be modified by anatomical constraints.
  - 13-12 The mechanism of autoimmune tissue damage can often be determined by adoptive transfer.
  - 13-13 T cells specific for self antigens can cause direct tissue injury and have a role in sustained autoantibody responses.
  - 13-14 Autoantibodies can be used to identify the target of the autoimmune process.
  - 13-15 The target of T cell-mediated autoimmunity is difficult to identify owing to the nature of T-cell ligands.
- Summary.

### Responses to alloantigens and transplant rejection.

- 13-16 Graft rejection is an immunological response mediated primarily by T cells.
- 13-17 Matching donor and recipient at the MHC improves the outcome of transplantation.
- 13-18 In MHC-identical grafts, rejection is caused by peptides from other alloantigens bound to graft MHC molecules.
- 13-19 There are two ways of presenting alloantigens on the transplant to the recipient's T lymphocytes.
- 13-20 Antibodies reacting with endothelium cause hyperacute graft rejection.
- 13-21 The converse of graft rejection is graft-versus-host disease.
- 13-22 Chronic organ rejection is caused by inflammatory vascular injury to the graft.
- 13-23 A variety of organs are transplanted routinely in clinical medicine.

- 13-24 The fetus is an allograft that is tolerated repeatedly.  
Summary.

#### Self-tolerance and its loss.

- 13-25 Many autoantigens are not so abundantly expressed that they induce clonal deletion or anergy but are not so rare as to escape recognition entirely.
- 13-26 The induction of a tissue-specific response requires the presentation of antigen by antigen-presenting cells with co-stimulatory activity.
- 13-27 In the absence of co-stimulation, tolerance is induced.
- 13-28 Dominant immune suppression can be demonstrated in models of tolerance and can affect the course of autoimmune disease.
- 13-29 Antigens in immunologically privileged sites do not induce immune attack but can serve as targets.
- 13-30 B cells with receptors specific for peripheral autoantigens are held in check by a variety of mechanisms.
- 13-31 Autoimmunity may sometimes be triggered by infection.  
Summary.  
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## Chapter 14 Manipulation of the Immune Response

### Extrinsic regulation of unwanted immune responses.

- 14-1 Corticosteroids are powerful anti-inflammatory drugs that alter the transcription of many genes.
- 14-2 Cytotoxic drugs cause immunosuppression by killing dividing cells and have serious side-effects.
- 14-3 Cyclosporin A, FK506 (tacrolimus), and rapamycin (sirolimus) are powerful immunosuppressive agents that interfere with T-cell signaling.
- 14-4 Immunosuppressive drugs are valuable probes of intracellular signaling pathways in lymphocytes.
- 14-5 Antibodies against cell-surface molecules have been used to remove specific lymphocyte subsets or to inhibit cell function.
- 14-6 Antibodies can be engineered to reduce their immunogenicity in humans.
- 14-7 Monoclonal antibodies can be used to inhibit allograft rejection.
- 14-8 Antibodies can be used to alleviate and suppress autoimmune disease.
- 14-9 Modulation of the pattern of cytokine expression by T lymphocytes can inhibit autoimmune disease.
- 14-10 Controlled administration of antigen can be used to manipulate the nature of an antigen-specific response.  
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- 14-11 The development of transplantable tumors in mice led to the discovery that mice could mount a protective immune response against tumors.
- 14-12 T lymphocytes can recognize specific antigens on human tumors.
- 14-13 Tumors can escape rejection in many ways.
- 14-14 Monoclonal antibodies against tumor antigens, alone or linked to toxins, can control tumor growth.
- 14-15 Enhancing the immunogenicity of tumors holds promise for cancer therapy.  
Summary.

### Manipulating the immune response to fight infection.

- 14-16 There are several requirements for an effective vaccine.

- 14-17 The history of vaccination against *Bordetella pertussis* illustrates the importance of developing an effective vaccine that is perceived to be safe.
- 14-18 Conjugate vaccines have been developed as a result of understanding how T and B cells collaborate in an immune response.
- 14-19 The use of adjuvants is another important approach to enhancing the immunogenicity of vaccines.
- 14-20 Live-attenuated viral vaccines are usually more potent than 'killed' vaccines and can be made safer by using recombinant DNA technology.
- 14-21 Live-attenuated bacterial vaccines can be developed by selecting nonpathogenic or disabled mutants.
- 14-22 Attenuated microorganisms can serve as vectors for vaccination against many pathogens.
- 14-23 Synthetic peptides of protective antigens can elicit protective immunity.
- 14-24 The route of vaccination is an important determinant of success.
- 14-25 Protective immunity can be induced by injecting DNA encoding microbial antigens and human cytokines into muscle.
- 14-26 The effectiveness of a vaccine can be enhanced by targeting it to sites of antigen presentation.
- 14-27 An important question is whether vaccination can be used therapeutically to control existing chronic infections.
- 14-28 Modulation of the immune system might be used to inhibit immunopathological responses to infectious agents.  
Summary.  
Summary to Chapter 14.

## Afterword Evolution of the Immune System: Past, Present, and Future, by Charles A. Janeway, Jr.

### Evolution of the innate immune system.

Innate immunity has its origins in early eukaryotes such as the amoeba.

Sophisticated means of host defense were hard-wired in the genome by the time organisms diverged into plants and animals.

Fruit flies illustrate the virtues of a nonclonal system of host defense.

Many genes that operate in fruit fly immunity also operate in humans and plants and appear to be universal components of host defense.  
Summary.

### Evolution of the adaptive immune response.

Adaptive immunity appears abruptly in the cartilaginous fish.

Gene rearrangement is used to control gene expression.

Animals generate antigen receptor diversity in many different ways.  
Summary.

### The importance of immunological memory in fixing adaptive immunity in the genome.

Immunological memory is the hallmark of adaptive immunity.

Immunological memory allows survival in a world filled with pathogens.

Immunological memory for self proteins leads to autoimmune disease.  
Summary.

## Future directions of research in immunobiology

Future studies should vastly expand our knowledge of innate immunity.

Future studies should refine our knowledge of adaptive immunity.

Future studies of tumor immunity hold great promise for an immunological cure for cancer.

Future vaccine development should greatly increase our ability to prevent infectious disease.

Future studies of autoimmunity and graft rejection should allow control of immune responses to one's own body or to a piece borrowed from someone else.

Summary.

Summary of the Afterword.

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- A-2 Routes of immunization.
- A-3 Effects of antigen dose.
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### The detection, measurement, and characterization of antibodies and their use as research and diagnostic tools.

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- A-8 Precipitin reaction.
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# Basic Concepts in Immunology

# 1

Immunology is a relatively new science. Its origin is usually attributed to **Edward Jenner** (Fig. 1.1), who discovered in 1796 that cowpox, or vaccinia, induced protection against human smallpox, an often fatal disease. Jenner called his procedure vaccination, and this term is still used to describe the inoculation of healthy individuals with weakened or attenuated strains of disease-causing agents to provide protection from disease. Although Jenner's bold experiment was successful, it took almost two centuries for smallpox vaccination to become universal, an advance that enabled the World Health Organization to announce in 1979 that smallpox had been eradicated (Fig. 1.2), arguably the greatest triumph of modern medicine.

When Jenner introduced vaccination he knew nothing of the infectious agents that cause disease: it was not until late in the 19th century that **Robert Koch** proved that infectious diseases are caused by **microorganisms**, each one responsible for a particular disease, or **pathology**. We now recognize four broad categories of disease-causing microorganisms, or **pathogens**: these are **viruses**, **bacteria**, pathogenic **fungi**, and other relatively large and complex eukaryotic organisms collectively termed **parasites**.

The discoveries of Koch and other great 19th century microbiologists stimulated the extension of Jenner's strategy of vaccination to other diseases. In the 1880s, **Louis Pasteur** devised a vaccine against cholera in chickens, and developed a rabies vaccine that proved a spectacular success upon its first trial in a boy bitten by a rabid dog. These practical triumphs led to a search for the mechanism of protection and to the development of the science of immunology. In 1890, **Emil von Behring** and **Shibasaburo Kitasato** discovered that the serum of vaccinated individuals contained substances—which they called **antibodies**—that specifically bound to the relevant pathogen.

A specific **immune response**, such as the production of antibodies against a particular pathogen, is known as an **adaptive immune response**, because it occurs during the lifetime of an individual as an adaptation to infection with that pathogen. In many cases, an adaptive immune response confers lifelong **protective immunity** to reinfection with the same pathogen. This



**Fig. 1.1 Edward Jenner.** Portrait by John Raphael Smith. Reproduced courtesy of Yale University, Harvey Cushing/John Hay Whitney Medical Library.

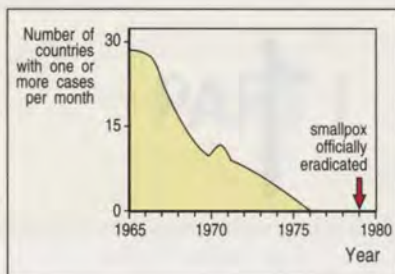


**Fig. 1.2 The eradication of smallpox by vaccination.** After a period of 3 years in which no cases of smallpox were recorded, the World Health Organization was able to announce in 1979 that smallpox had been eradicated.

distinguishes such responses from **innate immunity**, which, at the time that von Behring and Kitasato discovered antibodies, was known chiefly through the work of the great Russian immunologist **Elie Metchnikoff**. Metchnikoff discovered that many microorganisms could be engulfed and digested by **phagocytic cells**, which he called **macrophages**. These cells are immediately available to combat a wide range of pathogens without requiring prior exposure and are a key component of the innate immune system. Antibodies, by contrast, are produced only after infection, and are specific for the infecting pathogen. The antibodies present in a given person therefore directly reflect the infections to which he or she has been exposed.

Indeed, it quickly became clear that specific antibodies can be induced against a vast range of substances. Such substances are known as **antigens** because they can stimulate the *generation* of *antibodies*. We shall see, however, that not all adaptive immune responses entail the production of antibodies, and the term antigen is now used in a broader sense to describe any substance that can be recognized by the adaptive immune system.

Both innate immunity and adaptive immune responses depend upon the activities of white blood cells, or **leukocytes**. Innate immunity largely involves **granulocytes** and macrophages. Granulocytes, also called polymorphonuclear leukocytes, are a diverse collection of white blood cells whose prominent granules give them their characteristic staining patterns; they include the **neutrophils**, which are phagocytic. The macrophages of humans and other vertebrates are presumed to be the direct evolutionary descendants of the phagocytic cells present in simpler animals, such as those that Metchnikoff observed in sea stars. Adaptive immune responses depend upon **lymphocytes**, which provide the lifelong immunity that can follow exposure to disease or vaccination. The innate and adaptive immune systems together provide a remarkably effective defense system. It ensures that although we spend our lives surrounded by potentially pathogenic microorganisms, we become ill only relatively rarely. Many infections are handled successfully by the innate immune system and cause no disease; others that cannot be resolved by innate immunity trigger adaptive immunity and are then overcome successfully, followed by lasting immunological memory.

The main focus of this book will be on the diverse mechanisms of adaptive immunity, whereby specialized classes of lymphocytes recognize and target pathogenic microorganisms or the cells infected with them. We shall see, however, that all the cells involved in innate immune responses also participate in adaptive immune responses. Indeed, most of the effector actions that the adaptive immune system uses to destroy invading microorganisms depend upon linking antigen-specific recognition to the activation of effector mechanisms that are also used in innate host defense.

In this chapter, we first introduce the cells of the immune system, and the tissues in which they develop and through which they circulate or migrate. In later sections, we outline the specialized functions of the different types of cells and the mechanisms by which they eliminate infection.

### The components of the immune system.

The cells of the immune system originate in the **bone marrow**, where many of them also mature. They then migrate to guard the peripheral tissues, circulating in the blood and in a specialized system of vessels called the **lymphatic system**.

### 1-1 The white blood cells of the immune system derive from precursors in the bone marrow.

All the cellular elements of blood, including the red blood cells that transport oxygen, the platelets that trigger blood clotting in damaged tissues, and the white blood cells of the immune system, derive ultimately from the same **progenitor** or precursor cells—the **hematopoietic stem cells** in the bone marrow. As these stem cells can give rise to all of the different types of blood cells, they are often known as pluripotent hematopoietic stem cells. Initially, they give rise to stem cells of more limited potential, which are the immediate progenitors of red blood cells, platelets, and the two main categories of white blood cells. The different types of blood cell and their lineage relationships are summarized in Fig. 1.3. We shall be concerned here with all the cells derived from the common lymphoid progenitor and the myeloid progenitor, apart from the megakaryocytes and red blood cells.

The **myeloid progenitor** is the precursor of the granulocytes, macrophages, dendritic cells, and mast cells of the immune system. Macrophages are one of the three types of phagocyte in the immune system and are distributed widely in the body tissues, where they play a critical part in innate immunity. They are the mature form of **monocytes**, which circulate in the blood and differentiate continuously into macrophages upon migration into the tissues. **Dendritic cells** are specialized to take up antigen and display it for recognition by lymphocytes. Immature dendritic cells migrate from the blood to reside in the tissues and are both phagocytic and macropinocytic, ingesting large amounts of the surrounding extracellular fluid. Upon encountering a pathogen, they rapidly mature and migrate to lymph nodes.

**Mast cells**, whose blood-borne precursors are not well defined, also differentiate in the tissues. They mainly reside near small blood vessels and, when activated, release substances that affect vascular permeability. Although best known for their role in orchestrating allergic responses, they are believed to play a part in protecting mucosal surfaces against pathogens.

The **granulocytes** are so called because they have densely staining granules in their cytoplasm; they are also sometimes called **polymorphonuclear leukocytes** because of their oddly shaped nuclei. There are three types of granulocyte, all of which are relatively short lived and are produced in increased numbers during immune responses, when they leave the blood to migrate to sites of infection or inflammation. **Neutrophils**, which are the third phagocytic cell of the immune system, are the most numerous and most important cellular component of the innate immune response: hereditary deficiencies in neutrophil function lead to overwhelming bacterial infection, which is fatal if untreated. **Eosinophils** are thought to be important chiefly in defense against parasitic infections, because their numbers increase during a parasitic infection. The function of **basophils** is probably similar and complementary to that of eosinophils and mast cells; we shall discuss the functions of these cells in Chapter 9 and their role in allergic inflammation in Chapter 12. The cells of the myeloid lineage are shown in Fig. 1.4.

The **common lymphoid progenitor** gives rise to the lymphocytes, with which most of this book will be concerned. There are two major types of lymphocyte: **B lymphocytes** or **B cells**, which when activated differentiate into **plasma cells** that secrete antibodies; and **T lymphocytes** or **T cells**, of which there are two main classes. One class differentiates on activation into **cytotoxic T cells**, which kill cells infected with viruses, whereas the second class of T cells differentiates into cells that activate other cells such as B cells and macrophages.

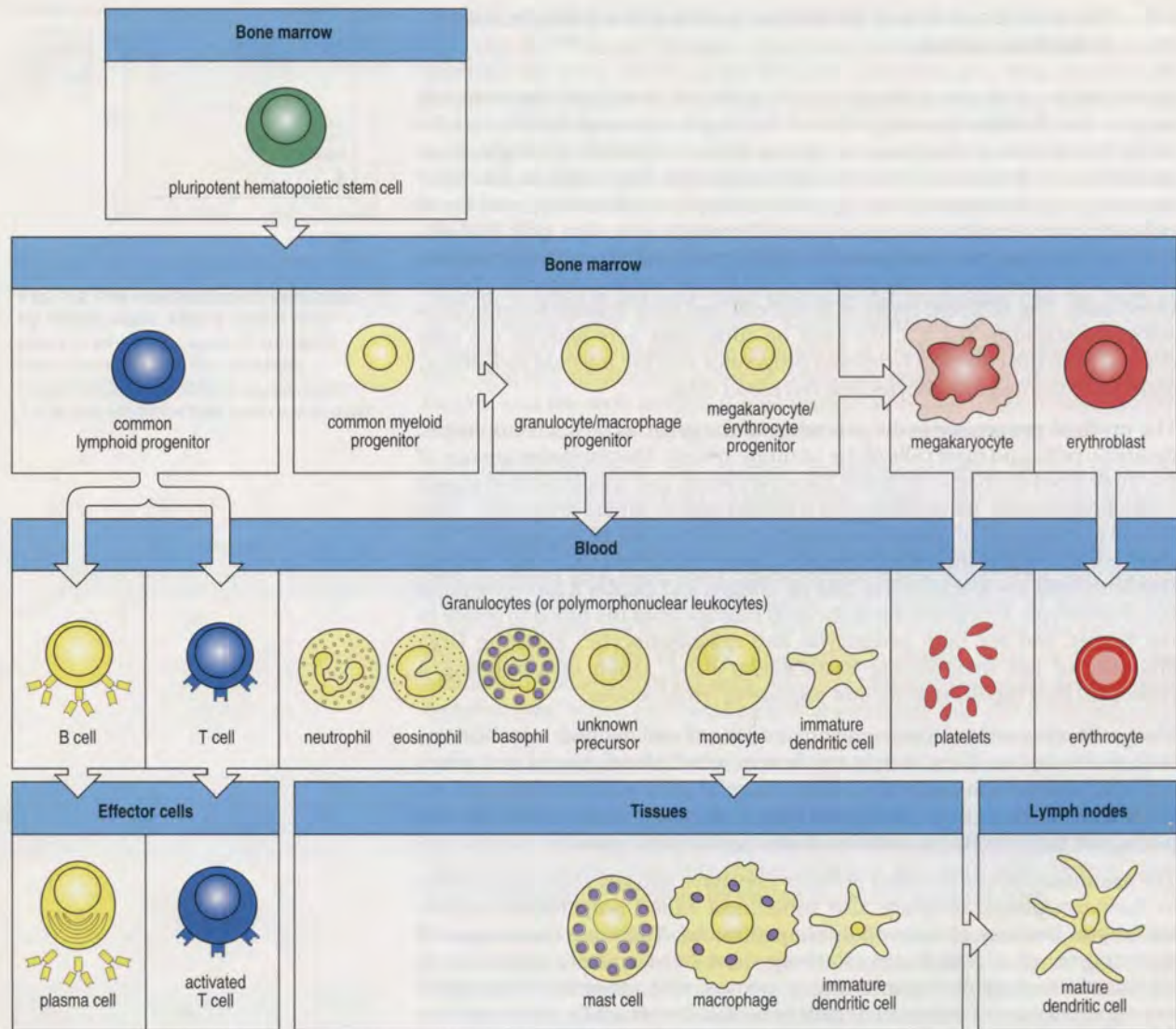


**Fig. 1.3 All the cellular elements of blood, including the lymphocytes of the adaptive immune system, arise from hematopoietic stem cells in the bone marrow.** These pluripotent cells divide to produce two more specialized types of stem cells, a common lymphoid progenitor that gives rise to the T and B lymphocytes responsible for adaptive immunity, and a common myeloid progenitor that gives rise to different types of leukocytes (white blood cells), erythrocytes (red blood cells that carry oxygen), and the megakaryocytes that produce platelets that are important in blood clotting. The existence of a common lymphoid progenitor for T and B lymphocytes is strongly supported by current data. T and B lymphocytes are distinguished by their sites of differentiation—T cells in the thymus and B cells in the bone marrow—and by their antigen receptors. Mature T and B lymphocytes circulate between the blood and peripheral lymphoid tissues. After encounter with antigen, B cells differentiate into antibody-secreting plasma cells, whereas T cells differentiate into effector T cells with a variety of functions. A third lineage of lymphoid-like cells, the natural killer cells, derive from the same progenitor cell but lack the antigen-specificity that is the hallmark of the adaptive

immune response (not shown). The leukocytes that derive from the myeloid stem cell are the monocytes, the dendritic cells, and the basophils, eosinophils, and neutrophils. The latter three are collectively termed either granulocytes, because of the cytoplasmic granules whose characteristic staining gives them a distinctive appearance in blood smears, or polymorphonuclear leukocytes, because of their irregularly shaped nuclei. They circulate in the blood and enter the tissues only when recruited to sites of infection or inflammation where neutrophils are recruited to phagocytose bacteria. Eosinophils and basophils are recruited to sites of allergic inflammation, and appear to be involved in defending against parasites. Immature dendritic cells travel via the blood to enter peripheral tissues, where they ingest antigens. When they encounter a pathogen, they mature and migrate to lymphoid tissues, where they activate antigen-specific T lymphocytes. Monocytes enter tissues, where they differentiate into macrophages; these are the main tissue-resident phagocytic cells of the innate immune system. Mast cells arise from precursors in bone marrow but complete their maturation in tissues; they are important in allergic responses.

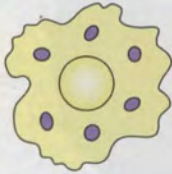
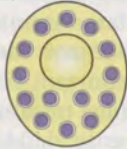
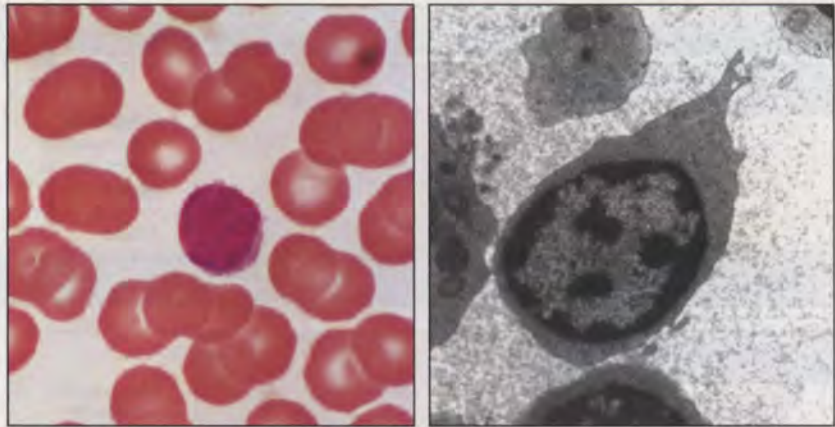
| Cell           |                                                                                     | Activated function                                                             |
|----------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Macrophage     |    | Phagocytosis and activation of bactericidal mechanisms<br>Antigen presentation |
| Dendritic cell |    | Antigen uptake in peripheral sites<br>Antigen presentation in lymph nodes      |
| Neutrophil     |   | Phagocytosis and activation of bactericidal mechanisms                         |
| Eosinophil     |  | Killing of antibody-coated parasites                                           |
| Basophil       |  | Unknown                                                                        |
| Mast cell      |  | Release of granules containing histamine and other active agents               |

**Fig. 1.4 Myeloid cells in innate and adaptive immunity.** Cells of the myeloid lineage perform various important functions in the immune response. The cells are shown schematically in the left column in the form in which they will be represented throughout the rest of the book. A photomicrograph of each cell type is shown in the center column. Macrophages and neutrophils are primarily phagocytic cells that engulf pathogens and destroy them in intracellular vesicles, a function they perform in both innate and adaptive immune responses. Dendritic cells are phagocytic when they are immature and take up pathogens; after maturing they act as antigen-presenting cells to T cells, initiating adaptive immune responses. Macrophages can also present antigens to T cells and can activate them. The other myeloid cells are primarily secretory cells that release the contents of their prominent granules upon activation via antibody during an adaptive immune response. Eosinophils are thought to be involved in attacking large antibody-coated parasites such as worms, whereas the function of basophils is less clear. Mast cells are tissue cells that trigger a local inflammatory response to antigen by releasing substances that act on local blood vessels. Photographs courtesy of N. Rooney and B. Smith.

**Fig. 1.5 Lymphocytes are mostly small and inactive cells.** The left panel shows a light micrograph of a small lymphocyte surrounded by red blood cells. Note the condensed chromatin of the nucleus, indicating little transcriptional activity, the relative absence of cytoplasm, and the small size. The right panel shows a transmission electron micrograph of a small lymphocyte. Note the condensed chromatin, the scanty cytoplasm and the absence of rough endoplasmic reticulum and other evidence of functional activity. Photographs courtesy of N. Rooney.



Most lymphocytes are small, featureless cells with few cytoplasmic organelles and much of the nuclear chromatin inactive, as shown by its condensed state (Fig. 1.5). This appearance is typical of inactive cells and it is not surprising that, as recently as the early 1960s, textbooks could describe these cells, now the central focus of immunology, as having no known function. Indeed, these small lymphocytes have no functional activity until they encounter antigen, which is necessary to trigger their proliferation and the differentiation of their specialized functional characteristics.

Lymphocytes are remarkable in being able to mount a specific immune response against virtually any foreign antigen. This is possible because each individual lymphocyte matures bearing a unique variant of a prototype antigen receptor, so that the population of T and B lymphocytes collectively bear a huge repertoire of receptors that are highly diverse in their antigen-binding sites. The **B-cell antigen receptor (BCR)** is a membrane-bound form of the antibody that the B cell will secrete after activation and differentiation to plasma cells. Antibody molecules as a class are known as **immunoglobulins**, usually shortened to **Ig**, and the antigen receptor of B lymphocytes is therefore also known as **membrane immunoglobulin (mIg)**. The **T-cell antigen receptor (TCR)** is related to immunoglobulin but is quite distinct from it, as it is specially adapted to detect antigens derived from foreign proteins or pathogens that have entered into host cells. We shall describe the structures of these lymphocyte antigen receptors in detail in Chapters 3, 4, and 5, and the way in which their diversity of binding sites is created as lymphocytes develop in Chapter 7.

A third lineage of lymphoid cells, called **natural killer cells**, lack antigen-specific receptors and are part of the innate immune system. These cells circulate in the blood as large lymphocytes with distinctive cytotoxic granules (Fig. 1.6). They are able to recognize and kill some abnormal cells, for example some tumor cells and virus-infected cells, and are thought to be important in the innate immune defense against intracellular pathogens.

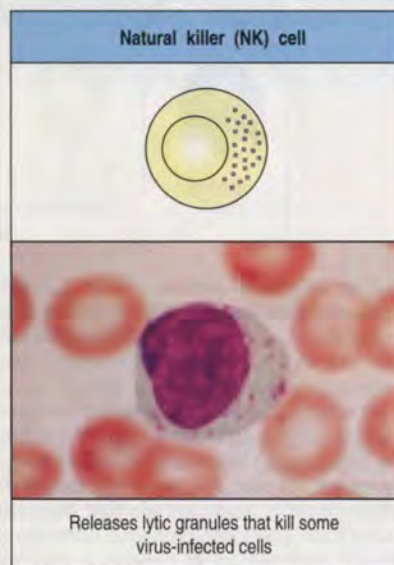


**Fig. 1.6 Natural killer (NK) cells.** These are large granular lymphocyte-like cells with important functions in innate immunity. Although lacking antigen-specific receptors, they can detect and attack certain virus-infected cells. Photograph courtesy of N. Rooney and B. Smith.

## 1-2 Lymphocytes mature in the bone marrow or the thymus.

The **lymphoid organs** are organized tissues containing large numbers of lymphocytes in a framework of nonlymphoid cells. In these organs, the interactions lymphocytes make with nonlymphoid cells are important either to lymphocyte development, to the initiation of adaptive immune responses, or to the sustenance of lymphocytes. Lymphoid organs can be divided broadly into **central or primary lymphoid organs**, where lymphocytes are generated,

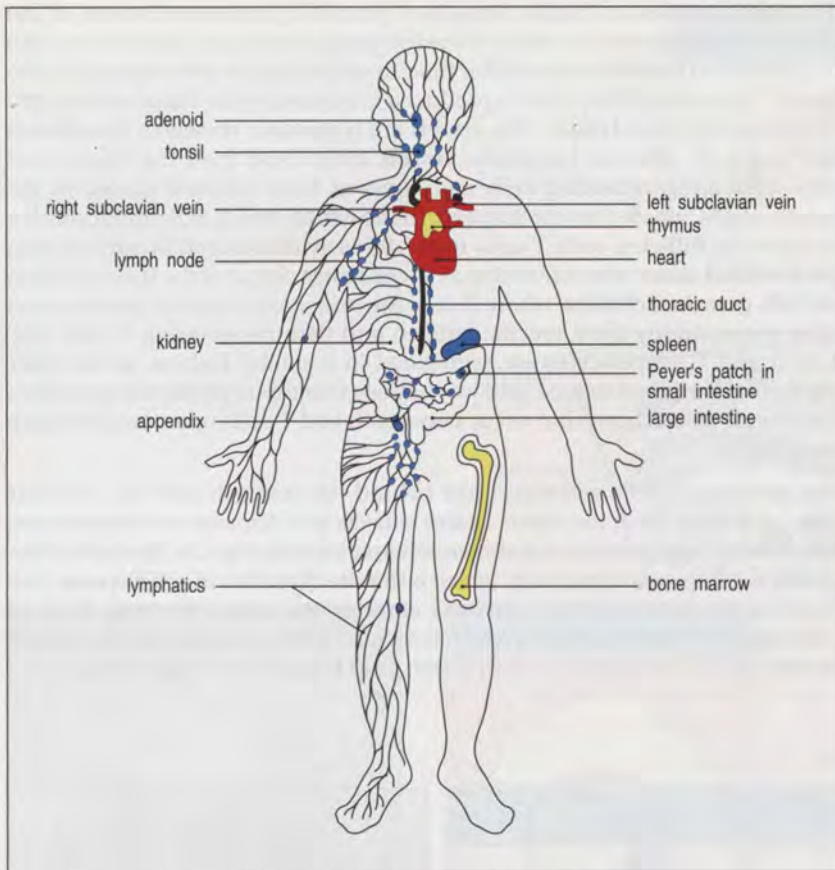


**Fig. 1.7 The distribution of lymphoid tissues in the body.** Lymphocytes arise from stem cells in bone marrow, and differentiate in the central lymphoid organs (yellow), B cells in bone marrow and T cells in the thymus. They migrate from these tissues and are carried in the bloodstream to the peripheral or secondary lymphoid organs (blue), the lymph nodes, the spleen, and lymphoid tissues associated with mucosa, like the gut-associated tonsils, Peyer's patches, and appendix. The peripheral lymphoid organs are the sites of lymphocyte activation by antigen, and lymphocytes recirculate between the blood and these organs until they encounter antigen. Lymphatics drain extracellular fluid from the peripheral tissues, through the lymph nodes and into the thoracic duct, which empties into the left subclavian vein. This fluid, known as lymph, carries antigen to the lymph nodes and recirculating lymphocytes from the lymph nodes back into the blood. Lymphoid tissue is also associated with other mucosa such as the bronchial linings (not shown).

and **peripheral or secondary lymphoid organs**, where adaptive immune responses are initiated and where lymphocytes are maintained. The central lymphoid organs are the bone marrow and the **thymus**, a large organ in the upper chest; the location of the thymus, and of the other lymphoid organs, is shown schematically in Fig. 1.7.

Both B and T lymphocytes originate in the bone marrow but only B lymphocytes mature there; T lymphocytes migrate to the thymus to undergo their maturation. Thus B lymphocytes are so-called because they are bone marrow derived, and T lymphocytes because they are thymus derived. Once they have completed their maturation, both types of lymphocyte enter the bloodstream, from which they migrate to the peripheral lymphoid organs.

**1-3 The peripheral lymphoid organs are specialized to trap antigen, to allow the initiation of adaptive immune responses, and to provide signals that sustain recirculating lymphocytes.**

Pathogens can enter the body by many routes and set up an infection anywhere, but antigen and lymphocytes will eventually encounter each other in the peripheral lymphoid organs—the lymph nodes, the spleen, and the mucosal lymphoid tissues (see Fig. 1.7). Lymphocytes are continually recirculating through these tissues, to which antigen is also carried from sites of infection, primarily within macrophages and dendritic cells. Within the lymphoid organs, specialized cells such as mature dendritic cells display the antigen to lymphocytes.

The **lymph nodes** are highly organized lymphoid structures located at the points of convergence of vessels of the lymphatic system, an extensive system of vessels that collects extracellular fluid from the tissues and returns it to the blood. This extracellular fluid is produced continuously by filtration from the blood, and is called **lymph**. The vessels are **lymphatic vessels** or **lymphatics** (see Fig. 1.7). **Afferent lymphatic vessels** drain fluid from the tissues and also carry antigen-bearing cells and antigens from infected tissues to the lymph nodes, where they are trapped. In the lymph nodes, B lymphocytes are localized in **follicles**, with T cells more diffusely distributed in surrounding **paracortical areas**, also referred to as **T-cell zones**. Some of the B-cell follicles include **germinal centers**, where B cells are undergoing intense proliferation after encountering their specific antigen and their cooperating T cells (Fig. 1.8). B and T lymphocytes are segregated in a similar fashion in the other peripheral lymphoid tissues, and we shall see that this organization promotes the crucial interactions that occur between B and T cells upon encountering antigen.

The **spleen** is a fist-sized organ just behind the stomach (see Fig. 1.7) that collects antigen from the blood. It also collects and disposes of senescent red blood cells. Its organization is shown schematically in Fig. 1.9. The bulk of the spleen is composed of **red pulp**, which is the site of red blood cell disposal. The lymphocytes surround the arterioles entering the organ, forming areas of **white pulp**, the inner region of which is divided into a **periarteriolar lymphoid sheath (PALS)**, containing mainly T cells, and a flanking **B-cell corona**.

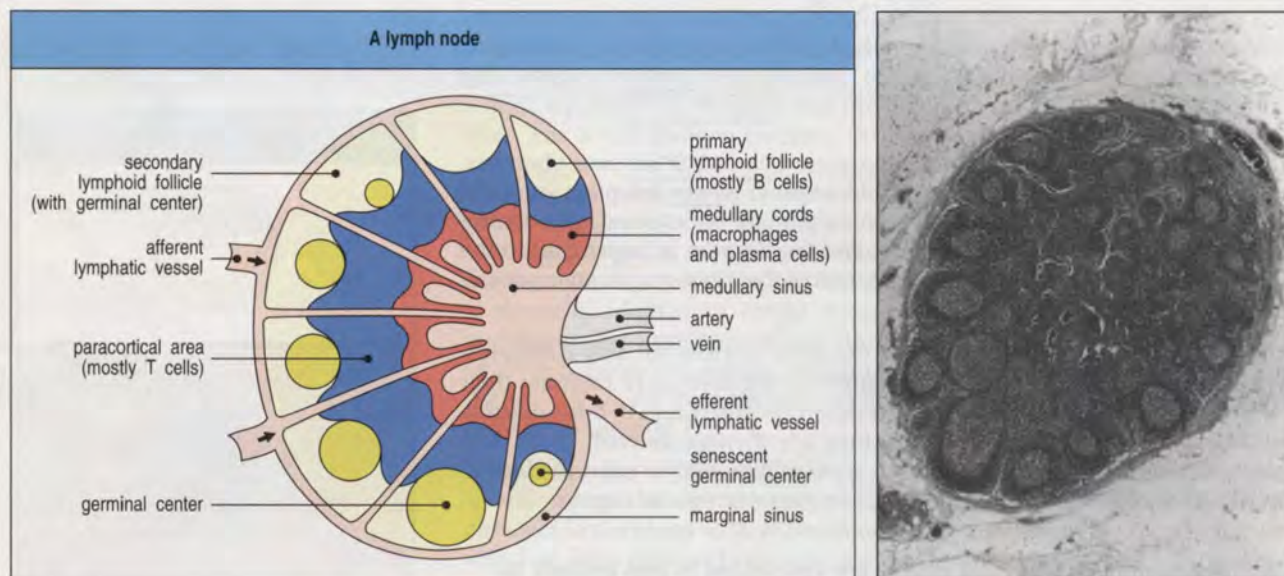


**Fig. 1.8 Organization of a lymph node.** As shown in the diagram on the left, a lymph node consists of an outermost cortex and an inner medulla. The cortex is composed of an outer cortex of B cells organized into lymphoid follicles, and deep, or paracortical, areas made up mainly of T cells and dendritic cells. When an immune response is underway, some of the follicles contain central areas of intense B-cell proliferation called germinal centers and are known as secondary lymphoid follicles. These reactions are very dramatic, but eventually die out as senescent germinal centers. Lymph draining from the extracellular spaces of the body carries antigens in phagocytic dendritic cells and

macrophages from the tissues to the lymph node via the afferent lymphatics. Lymph leaves by the efferent lymphatic in the medulla. The medulla consists of strings of macrophages and antibody-secreting plasma cells known as the medullary cords. Naive lymphocytes enter the node from the bloodstream through specialized postcapillary venules (not shown) and leave with the lymph through the efferent lymphatic. The light micrograph shows a section through a lymph node, with prominent follicles containing germinal centers. Magnification  $\times 7$ . Photograph courtesy of N. Rooney.

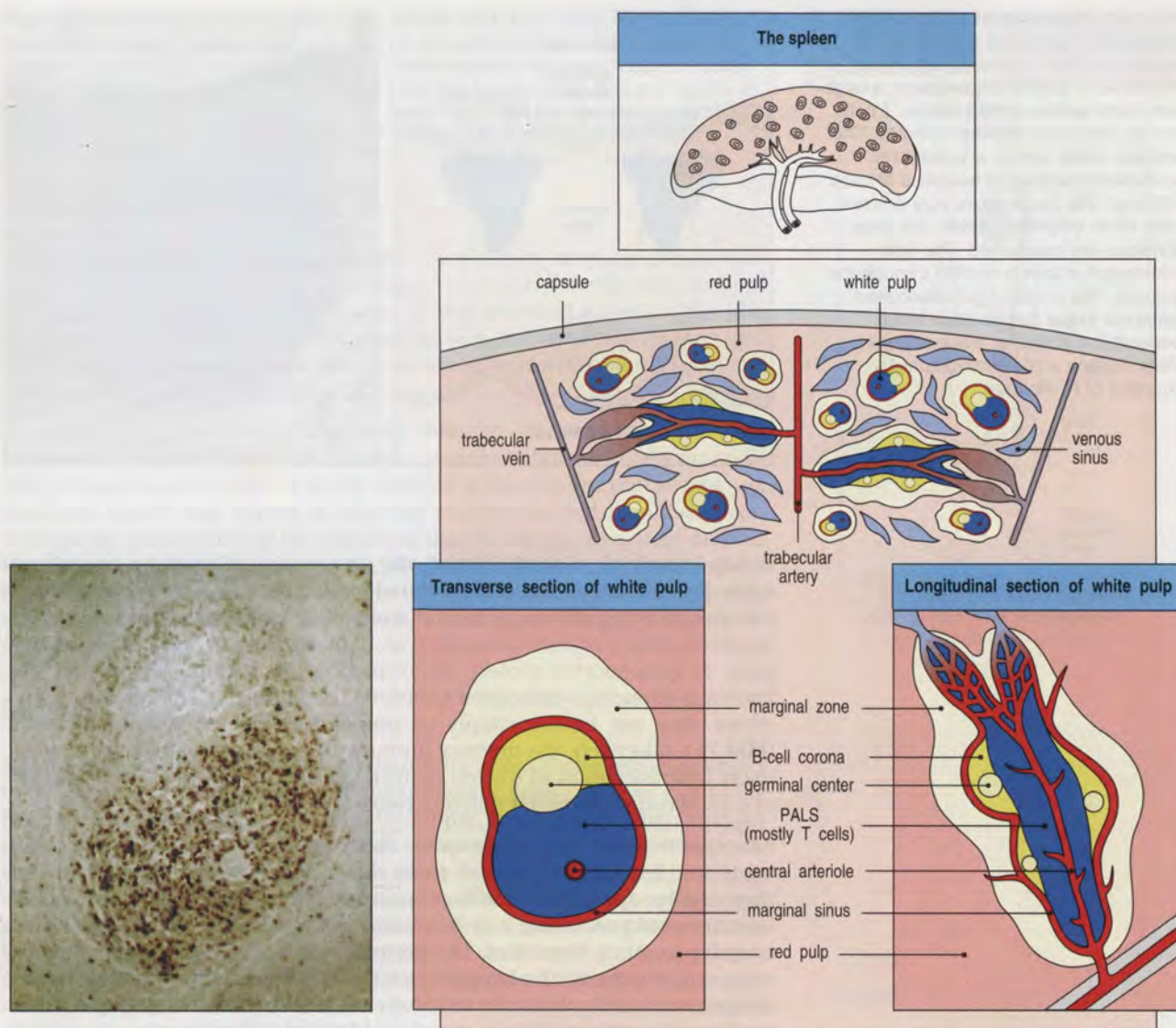


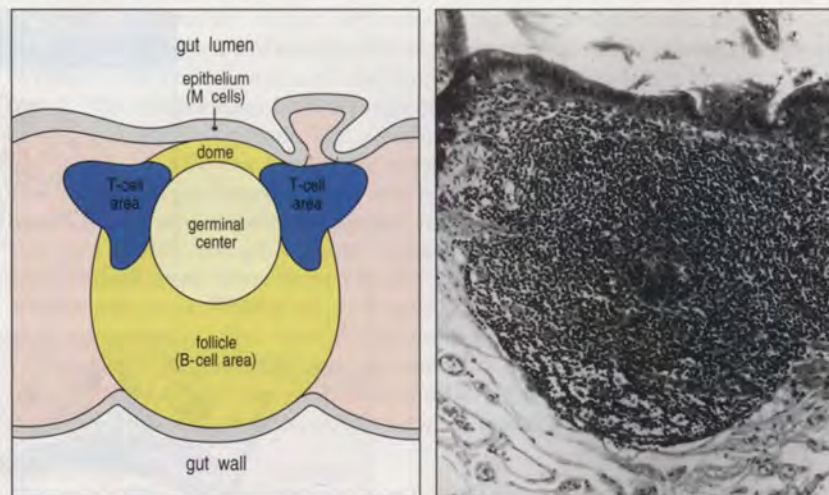
**Fig. 1.9 Organization of the lymphoid tissues of the spleen.**

The schematic at top right shows that the spleen consists of red pulp (pink areas in the top panel), which is a site of red blood cell destruction, interspersed with lymphoid white pulp. An enlargement of a small section of the spleen (center) shows the arrangement of discrete areas of white pulp (yellow and blue) around central arterioles. Lymphocytes and antigen-loaded dendritic cells come together in the periarteriolar lymphoid sheath. Most of the white pulp is shown in transverse section, with two portions in longitudinal section. The bottom two schematics show enlargements of a transverse section (lower left) and longitudinal section (lower right) of white pulp. In each area of white pulp, blood carrying lymphocytes and antigen flows from a trabecular artery into a central arteriole. Cells and

antigen then pass into a marginal sinus and drain into a trabecular vein. The marginal sinus is surrounded by a marginal zone of lymphocytes. Within the marginal sinus and surrounding the central arteriole is the periarteriolar lymphoid sheath (PALS), made up of T cells. The follicles consist mainly of B cells; in secondary follicles a germinal center is surrounded by a B-cell corona. The light micrograph at bottom left shows a transverse section of white pulp stained with hematoxylin and eosin. The T cells of the PALS stain darkly, while the B-cell corona is lightly stained. The unstained cells lying between the B- and T-cell areas represent a germinal center. Although the organization of the spleen is similar to that of a lymph node, antigen enters the spleen from the blood rather than from the lymph. Photograph courtesy of J.C. Howard.

The gut-associated lymphoid tissues (GALT), which include the tonsils, adenoids, and appendix, and specialized structures called Peyer's patches in the small intestine, collect antigen from the epithelial surfaces of the gastrointestinal tract. In Peyer's patches, which are the most important and

**Fig. 1.10 Organization of typical gut-associated lymphoid tissue.** As the diagram on the left shows, the bulk of the tissue is B cells, organized in a large and highly active domed follicle. T cells occupy the areas between follicles. The antigen enters across a specialized epithelium made up of so-called M cells. Although this tissue looks very different from other lymphoid organs, the basic divisions are maintained. The light micrograph shows a section through the gut wall. The dome of gut-associated lymphoid tissue can be seen lying beneath the epithelial tissues. Magnification  $\times 16$ . Photograph courtesy of N. Rooney.



highly organized of these tissues, the antigen is collected by specialized epithelial cells called **multi-fenestrated** or **M cells**. The lymphocytes form a follicle consisting of a large central dome of B lymphocytes surrounded by smaller numbers of T lymphocytes (Fig. 1.10). Similar but more diffuse aggregates of lymphocytes protect the respiratory epithelium, where they are known as **bronchial-associated lymphoid tissue (BALT)**, and other mucosa, where they are known simply as **mucosal-associated lymphoid tissue (MALT)**. Collectively, the mucosal immune system is estimated to contain as many lymphocytes as all the rest of the body, and they form a specialized set of cells obeying somewhat different rules.

Although very different in appearance, the lymph nodes, spleen, and mucosal-associated lymphoid tissues all share the same basic architecture. Each of these tissues operates on the same principle, trapping antigen from sites of infection and presenting it to migratory small lymphocytes, thus inducing adaptive immune responses. The peripheral lymphoid tissues also provide sustaining signals to the lymphocytes that do not encounter their specific antigen, so that they continue to survive and recirculate until they encounter their specific antigen. This is important in maintaining the correct numbers of circulating T and B lymphocytes, and ensures that only those lymphocytes with the potential to respond to foreign antigen are sustained.

#### 1-4 Lymphocytes circulate between blood and lymph.

Small B and T lymphocytes that have matured in the bone marrow and thymus but have not yet encountered antigen are referred to as **naïve lymphocytes**. These cells circulate continually from the blood into the peripheral lymphoid tissues, which they enter by squeezing between the cells of capillary walls. They are then returned to the blood via the lymphatic vessels (Fig. 1.11) or, in the case of the spleen, return directly to the blood. In the event of an infection, lymphocytes that recognize the infectious agent are arrested in the lymphoid tissue, where they proliferate and differentiate into **effector cells** capable of combating the infection.

When an infection occurs in the periphery, for example, large amounts of antigen are taken up by dendritic cells which then travel from the site of infection through the afferent lymphatic vessels into the **draining lymph nodes** (see Fig. 1.11). In the lymph nodes, these cells display the antigen to

**Fig. 1.11 Circulating lymphocytes encounter antigen in peripheral lymphoid organs.** Naive lymphocytes recirculate constantly through peripheral lymphoid tissue, here illustrated as a lymph node behind the knee, a popliteal

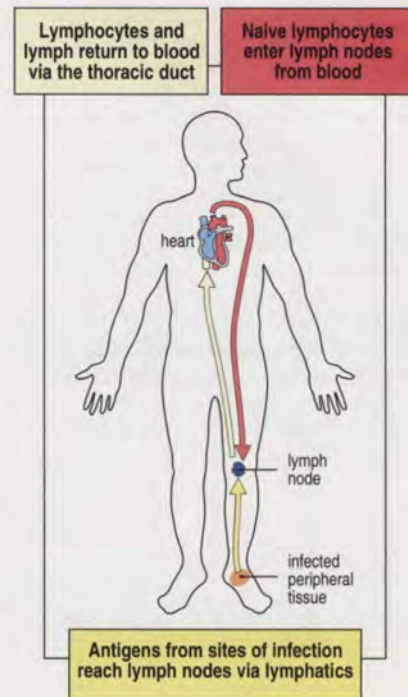
lymph node. Here, they may encounter their specific antigen, draining from an infected site in the foot. These are called draining lymph nodes, and are the site at which lymphocytes may become activated by encountering their specific ligand.

recirculating T lymphocytes, which they also help to activate. B cells that encounter antigen as they migrate through the lymph node are also arrested and activated, with the help of some of the activated T cells. Once the antigen-specific lymphocytes have undergone a period of proliferation and differentiation, they leave the lymph nodes as effector cells through the **efferent lymphatic vessel** (see Fig. 1.8).

Because they are involved in initiating adaptive immune responses, the peripheral lymphoid tissues are not static structures but vary quite dramatically depending upon whether or not infection is present. The diffuse mucosal lymphoid tissues may appear in response to infection and then disappear, whereas the architecture of the organized tissues changes in a more defined way during an infection. For example, the B-cell follicles of the lymph nodes expand as B lymphocytes proliferate to form germinal centers (see Fig. 1.8), and the entire lymph node enlarges, a phenomenon familiarly known as swollen glands.

### Summary.

Immune responses are mediated by leukocytes, which derive from precursors in the bone marrow. A pluripotent hematopoietic stem cell gives rise to the lymphocytes responsible for adaptive immunity, and also to myeloid lineages that participate in both innate and adaptive immunity. Neutrophils, eosinophils, and basophils are collectively known as granulocytes; they circulate in the blood unless recruited to act as effector cells at sites of infection and inflammation. Macrophages and mast cells complete their differentiation in the tissues where they act as effector cells in the front line of host defense and initiate inflammation. Macrophages phagocytose bacteria, and recruit other phagocytic cells, the neutrophils, from the blood. Mast cells are exocytic and are thought to orchestrate the defense against parasites as well as triggering allergic inflammation; they recruit eosinophils and basophils, which are also exocytic. Dendritic cells enter the tissues as immature phagocytes where they specialize in ingesting antigens. These antigen-presenting cells subsequently migrate into lymphoid tissue. There are two major types of lymphocyte: B lymphocytes, which mature in the bone marrow; and T lymphocytes, which mature in the thymus. The bone marrow and thymus are thus known as the central or primary lymphoid organs. Mature lymphocytes recirculate continually from the bloodstream through the peripheral or secondary lymphoid organs, returning to the bloodstream through the lymphatic vessels. Most adaptive immune responses are triggered when a recirculating T cell recognizes its specific antigen on the surface of a dendritic cell. The three major types of peripheral lymphoid tissue are the spleen, which collects antigens from the blood; the lymph nodes, which collect antigen from sites of infection in the tissues; and the mucosal-associated lymphoid tissues (MALT), which collect antigens from the epithelial surfaces of the body. Adaptive immune responses are initiated in these peripheral lymphoid tissues: T cells that encounter antigen proliferate and differentiate into antigen-specific effector cells, while B cells proliferate and differentiate into antibody-secreting cells.



## Principles of innate and adaptive immunity.

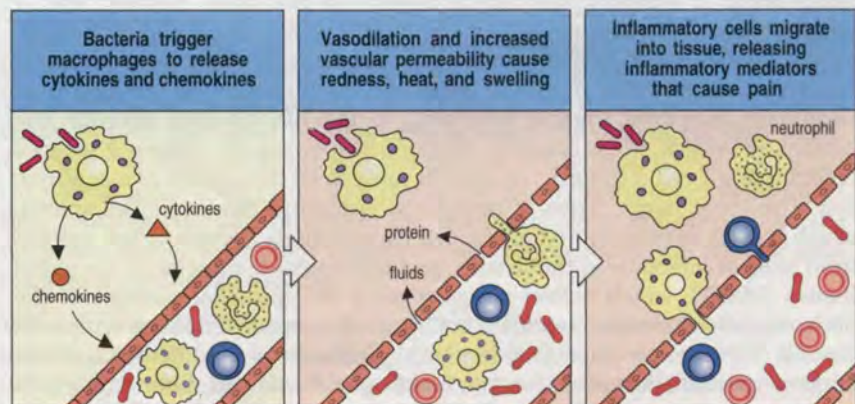
The macrophages and neutrophils of the innate immune system provide a first line of defense against many common microorganisms and are essential for the control of common bacterial infections. However, they cannot always eliminate infectious organisms, and there are some pathogens that they cannot recognize. The lymphocytes of the adaptive immune system have evolved to provide a more versatile means of defense which, in addition, provides increased protection against subsequent reinfection with the same pathogen. The cells of the innate immune system, however, play a crucial part in the initiation and subsequent direction of adaptive immune responses, as well as participating in the removal of pathogens that have been targeted by an adaptive immune response. Moreover, because there is a delay of 4–7 days before the initial adaptive immune response takes effect, the innate immune response has a critical role in controlling infections during this period.

### 1-5 Most infectious agents induce inflammatory responses by activating innate immunity.

Microorganisms such as bacteria that penetrate the epithelial surfaces of the body for the first time are met immediately by cells and molecules that can mount an innate immune response. Phagocytic macrophages conduct the defense against bacteria by means of surface receptors that are able to recognize and bind common constituents of many bacterial surfaces. Bacterial molecules binding to these receptors trigger the macrophage to engulf the bacterium and also induce the secretion of biologically active molecules. Activated macrophages secrete **cytokines**, which are defined as proteins released by cells that affect the behavior of other cells that bear receptors for them. They also release proteins known as **chemokines** that attract cells with chemokine receptors such as neutrophils and monocytes from the bloodstream (Fig. 1.12). The cytokines and chemokines released by macrophages in response to bacterial constituents initiate the process known as **inflammation**. Local inflammation and the phagocytosis of invading bacteria may also be triggered as a result of the activation of **complement** on the bacterial cell surface. Complement is a system of plasma proteins that activates a cascade of proteolytic reactions on microbial surfaces but not on host cells, coating these surfaces with fragments that are recognized and bound by phagocytic receptors on macrophages. The cascade of reactions also releases small peptides that contribute to inflammation.

**Fig. 1.12 Bacterial infection triggers an inflammatory response.**

Macrophages encountering bacteria in the tissues are triggered to release cytokines that increase the permeability of blood vessels, allowing fluid and proteins to pass into the tissues. They also produce chemokines that direct the migration of neutrophils to the site of infection. The stickiness of the endothelial cells of the blood vessels is also changed, so that cells adhere to the blood vessel wall and are able to crawl through it; first neutrophils and then monocytes are shown entering the tissue from a blood vessel. The accumulation of fluid and cells at the site of infection causes the redness, swelling, heat, and pain, known collectively as inflammation. Neutrophils and macrophages are the principal inflammatory cells. Later in an immune response, activated lymphocytes may also contribute to inflammation.



Inflammation is traditionally defined by the four Latin words *calor*, *dolor*, *rubor*, and *tumor*, meaning heat, pain, redness, and swelling, all of which reflect the effects of cytokines and other inflammatory mediators on the local blood vessels. Dilation and increased permeability of the blood vessels during inflammation lead to increased local blood flow and the leakage of fluid, and account for the heat, redness, and swelling. Cytokines and complement fragments also have important effects on the adhesive properties of the endothelium, causing circulating leukocytes to stick to the endothelial cells of the blood vessel wall and migrate between them to the site of infection, to which they are attracted by chemokines. The migration of cells into the tissue and their local actions account for the pain. The main cell types seen in an inflammatory response in its initial phases are neutrophils, which are recruited into the inflamed, infected tissue in large numbers. Like macrophages, they have surface receptors for common bacterial constituents and complement, and they are the principal cells that engulf and destroy the invading microorganisms. The influx of neutrophils is followed a short time later by monocytes that rapidly differentiate into macrophages. Macrophages and neutrophils are thus also known as **inflammatory cells**. Inflammatory responses later in an infection also involve lymphocytes, which have meanwhile been activated by antigen that has drained from the site of infection via the afferent lymphatics.

The innate immune response makes a crucial contribution to the activation of adaptive immunity. The inflammatory response increases the flow of lymph containing antigen and antigen-bearing cells into lymphoid tissue, while complement fragments on microbial surfaces and induced changes in cells that have taken up microorganisms provide signals that synergize in activating lymphocytes whose receptors bind to specific microbial antigens. Macrophages that have phagocytosed bacteria and become activated can also activate T lymphocytes. However, the cells that specialize in presenting antigen to T lymphocytes and initiating adaptive immunity are the dendritic cells.

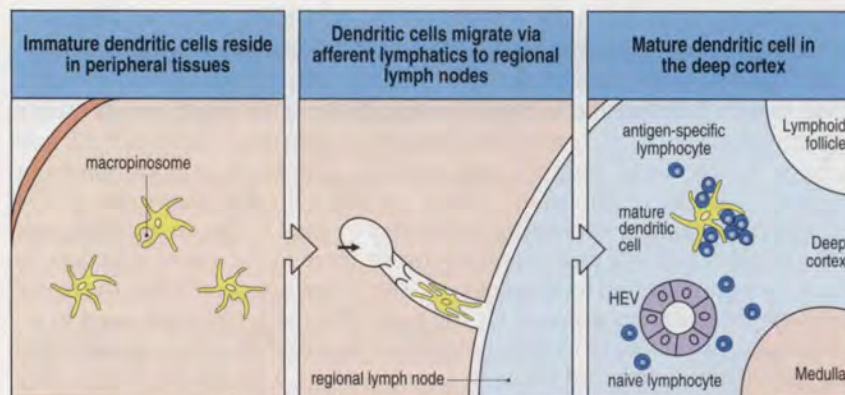
#### 1-6 Activation of specialized antigen-presenting cells is a necessary first step for induction of adaptive immunity.

The induction of an adaptive immune response begins when a pathogen is ingested by an immature dendritic cell in the infected tissue. These specialized phagocytic cells are resident in most tissues and are relatively long-lived, turning over at a slow rate. They derive from the same bone marrow precursor as macrophages, and migrate from the bone marrow to their peripheral stations, where their role is to survey the local environment for pathogens. Eventually, all tissue-resident dendritic cells migrate through the lymph to the regional lymph nodes where they interact with recirculating naive lymphocytes. If the dendritic cells fail to be activated, they induce tolerance to the antigens of self that they bear.

The immature dendritic cell carries receptors on its surface that recognize common features of many pathogens, such as bacterial cell wall proteoglycans. As with macrophages and neutrophils, binding of a bacterium to these receptors stimulates the dendritic cell to engulf the pathogen and degrade it intracellularly. Immature dendritic cells are also continually taking up extracellular material, including any virus particles or bacteria that may be present, by the receptor-independent mechanism of **macropinocytosis**. The function of dendritic cells, however, is not primarily to destroy pathogens but to carry pathogen antigens to peripheral lymphoid organs and there present them to T lymphocytes. When a dendritic cell takes up a pathogen in infected tissue, it becomes activated, and travels to a nearby lymph node. On activation,

**Fig. 1.13 Dendritic cells initiate adaptive immune responses.**

Immature dendritic cells resident in infected tissues take up pathogens and their antigens by macropinocytosis and receptor-mediated phagocytosis. They are stimulated by recognition of the presence of pathogens to migrate via the lymphatics to regional lymph nodes, where they arrive as fully mature nonphagocytic dendritic cells. Here the mature dendritic cell encounters and activates antigen-specific naive T lymphocytes, which enter lymph nodes from the blood via a specialized vessel known from its cuboidal endothelial cells as a high endothelial venule (HEV).



the dendritic cell matures into a highly effective **antigen-presenting cell (APC)** and undergoes changes that enable it to activate pathogen-specific lymphocytes that it encounters in the lymph node (Fig. 1.13). Activated dendritic cells secrete cytokines that influence both innate and adaptive immune responses, making these cells essential gatekeepers that determine whether and how the immune system responds to the presence of infectious agents. We shall consider the maturation of dendritic cells and their central role in presenting antigens to T lymphocytes in Chapter 8.

**1-7 Lymphocytes activated by antigen give rise to clones of antigen-specific cells that mediate adaptive immunity.**

The defense systems of innate immunity are effective in combating many pathogens. They are constrained, however, by relying on germline-encoded receptors to recognize microorganisms that can evolve more rapidly than the hosts they infect. This explains why they can only recognize microorganisms bearing surface molecules that are common to many pathogens and that have been conserved over the course of evolution. Not surprisingly, many pathogenic bacteria have evolved a protective capsule that enables them to conceal these molecules and thereby avoid being recognized and phagocytosed. Viruses carry no invariant molecules similar to those of bacteria and are rarely recognized directly by macrophages. Viruses and encapsulated bacteria can, however, still be taken up by dendritic cells through the nonreceptor-dependent process of macropinocytosis. Molecules that reveal their infectious nature may then be unmasked, and the dendritic cell activated to present their antigens to lymphocytes. The recognition mechanism used by the lymphocytes of the adaptive immune response has evolved to overcome the constraints faced by the innate immune system, and enables recognition of an almost infinite diversity of antigens, so that each different pathogen can be targeted specifically.

Instead of bearing several different receptors, each recognizing a different surface feature shared by many pathogens, each naive lymphocyte entering the bloodstream bears antigen receptors of a single specificity. The specificity of these receptors is determined by a unique genetic mechanism that operates during lymphocyte development in the bone marrow and thymus to generate millions of different variants of the genes encoding the receptor molecules. Thus, although an individual lymphocyte carries receptors of only one specificity, the specificity of each lymphocyte is different. This ensures that the millions of lymphocytes in the body collectively carry millions of

**Fig. 1.14 Clonal selection.** Each lymphocyte progenitor gives rise to many lymphocytes, each bearing a distinct antigen receptor. Lymphocytes with receptors that bind ubiquitous self antigens are eliminated before they become fully mature, ensuring tolerance to such self antigens. When antigen interacts with the receptor on a mature

naive lymphocyte, that cell is activated and starts to divide. It gives rise to a clone of identical progeny, all of whose receptors bind the same antigen. Antigen specificity is thus maintained as the progeny proliferate and differentiate into effector cells. Once antigen has been eliminated by these effector cells, the immune response ceases.

different antigen receptor specificities—the **lymphocyte receptor repertoire** of the individual. During a person's lifetime these lymphocytes undergo a process akin to natural selection; only those lymphocytes that encounter an antigen to which their receptor binds will be activated to proliferate and differentiate into effector cells.

This selective mechanism was first proposed in the 1950s by **Macfarlane Burnet** to explain why antibodies, which can be induced in response to virtually any antigen, are produced in each individual only to those antigens to which he or she is exposed. He postulated the preexistence in the body of many different potential antibody-producing cells, each having the ability to make antibody of a different specificity and displaying on its surface a membrane-bound version of the antibody that served as a receptor for antigen. On binding antigen, the cell is activated to divide and produce many identical progeny, known as a **clone**; these cells can now secrete **clonotypic** antibodies with a specificity identical to that of the surface receptor that first triggered activation and clonal expansion (Fig. 1.14). **Burnet** called this the **clonal selection theory**.

### 1-8 Clonal selection of lymphocytes is the central principle of adaptive immunity.

Remarkably, at the time that **Burnet** formulated his theory, nothing was known of the antigen receptors of lymphocytes; indeed the function of lymphocytes themselves was still obscure. Lymphocytes did not take center stage until the early 1960s, when **James Gowans** discovered that removal of the small lymphocytes from rats resulted in the loss of all known adaptive immune responses. These immune responses were restored when the small lymphocytes were replaced. This led to the realization that lymphocytes must be the units of clonal selection, and their biology became the focus of the new field of **cellular immunology**.

Clonal selection of lymphocytes with diverse receptors elegantly explained adaptive immunity but it raised one significant intellectual problem. If the antigen receptors of lymphocytes are generated randomly during the lifetime of an individual, how are lymphocytes prevented from recognizing antigens on the tissues of the body and attacking them? **Ray Owen** had shown in the late 1940s that genetically different twin calves with a common placenta were immunologically **tolerant** of one another's tissues, that is, they did not make an immune response against each other. **Sir Peter Medawar** then showed in 1953 that if exposed to foreign tissues during embryonic development, mice become immunologically tolerant to these tissues. **Burnet** proposed that developing lymphocytes that are potentially self-reactive are removed before they can mature, a process known as **clonal deletion**. He has since been proved right in this too, although the mechanisms of tolerance are still being worked out, as we shall see when we discuss the development of lymphocytes in Chapter 7.

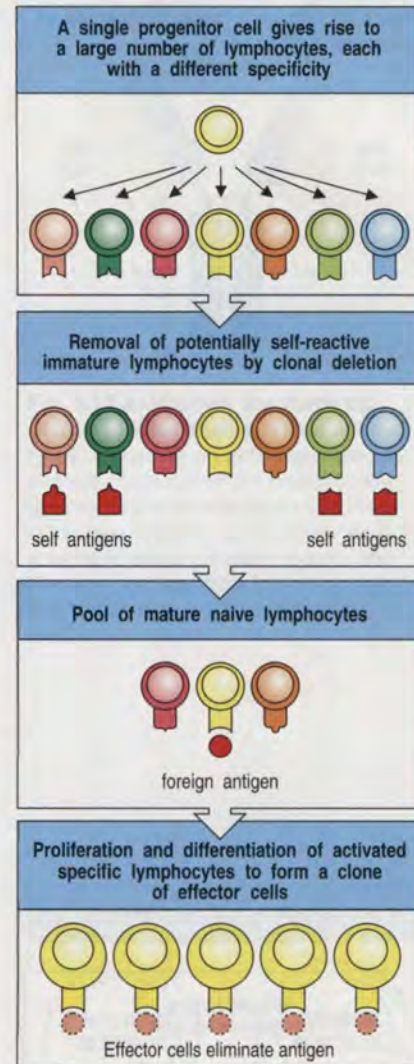


**Fig. 1.15** The four basic principles of clonal selection.

| Postulates of the clonal selection hypothesis                                                                                                                                                      |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Each lymphocyte bears a single type of receptor with a unique specificity                                                                                                                          |
| Interaction between a foreign molecule and a lymphocyte receptor capable of binding that molecule with high affinity leads to lymphocyte activation                                                |
| The differentiated effector cells derived from an activated lymphocyte will bear receptors of identical specificity to those of the parental cell from which that lymphocyte was derived           |
| Lymphocytes bearing receptors specific for ubiquitous self molecules are deleted at an early stage in lymphoid cell development and are therefore absent from the repertoire of mature lymphocytes |

Clonal selection of lymphocytes is the single most important principle in adaptive immunity. Its four basic postulates are listed in Fig. 1.15. The last of the problems posed by the clonal selection theory—how the diversity of lymphocyte antigen receptors is generated—was solved in the 1970s when advances in molecular biology made it possible to clone the genes encoding antibody molecules.

### 1-9 The structure of the antibody molecule illustrates the central puzzle of adaptive immunity.

Antibodies, as discussed above, are the secreted form of the B-cell antigen receptor or BCR. Because they are produced in very large quantities in response to antigen, they can be studied by traditional biochemical techniques; indeed, their structure was understood long before recombinant DNA technology made it possible to study the membrane-bound antigen receptors of lymphocytes. The startling feature that emerged from the biochemical studies was that an antibody molecule is composed of two distinct regions. One is a **constant region** that can take one of only four or five biochemically distinguishable forms; the other is a **variable region** that can take an apparently infinite variety of subtly different forms that allow it to bind specifically to an equally vast variety of different antigens.

This division is illustrated in the simple schematic diagram in Fig. 1.16, where the antibody is depicted as a Y-shaped molecule, with the constant region shown in blue and the variable region in red. The two variable regions, which are identical in any one antibody molecule, determine the antigen-binding specificity of the antibody; the constant region determines how the antibody disposes of the pathogen once it is bound.

Each antibody molecule has a twofold axis of symmetry and is composed of two identical heavy chains and two identical light chains (Fig. 1.17). Heavy and light chains both have variable and constant regions; the variable regions of a heavy and a light chain combine to form an antigen-binding site, so that both chains contribute to the antigen-binding specificity of the antibody molecule. The structure of antibody molecules will be described in detail in Chapter 3, and the functional properties of antibodies conferred by their constant regions will be considered in Chapters 4 and 9. For the time being we are concerned only with the properties of immunoglobulin molecules as antigen receptors, and how the diversity of the variable regions is generated.

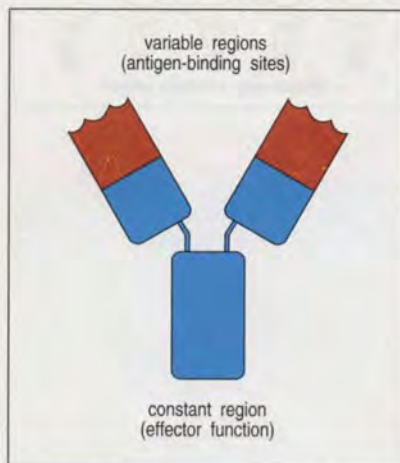


**Fig. 1.16** Schematic structure of an antibody molecule. The two arms of the Y-shaped antibody molecule contain the variable regions that form the two identical antigen-binding sites. The stem can take one of only a limited number of forms and is known as the constant region. It is the region that engages the effector mechanisms that antibodies activate to eliminate pathogens.

### 1-10 Each developing lymphocyte generates a unique antigen receptor by rearranging its receptor genes.

How are antigen receptors with an almost infinite range of specificities encoded by a finite number of genes? This question was answered in 1976, when **Susumu Tonegawa** discovered that the genes for immunoglobulin variable regions are inherited as sets of **gene segments**, each encoding a part of the variable region of one of the immunoglobulin polypeptide chains (Fig. 1.18). During B-cell development in the bone marrow, these gene segments are irreversibly joined by DNA recombination to form a stretch of DNA encoding a complete variable region. Because there are many different gene segments in each set, and different gene segments are joined together in different cells, each cell generates unique genes for the variable regions of the heavy and light chains of the immunoglobulin molecule. Once these recombination events have succeeded in producing a functional receptor, further rearrangement is prohibited. Thus each lymphocyte expresses only one receptor specificity.

This mechanism has three important consequences. First, it enables a limited number of gene segments to generate a vast number of different proteins. Second, because each cell assembles a different set of gene segments, each cell expresses a unique receptor specificity. Third, because gene rearrangement involves an irreversible change in a cell's DNA, all the progeny of that cell will inherit genes encoding the same receptor specificity. This general scheme was later also confirmed for the genes encoding the antigen receptor on T lymphocytes. The main distinctions between B- and T-lymphocyte receptors are that the immunoglobulin that serves as the B-cell antigen receptor has two identical antigen-recognition sites and can also be secreted, whereas the T-cell antigen receptor has a single antigen-recognition site and is always a cell-surface molecule. We shall see later that these receptors also recognize antigen in very different ways.

The potential diversity of lymphocyte receptors generated in this way is enormous. Just a few hundred different gene segments can combine in different ways to generate thousands of different receptor chains. The diversity of lymphocyte receptors is further amplified by junctional diversity, created by adding or subtracting nucleotides in the process of joining the gene segments, and by the fact that each receptor is made by pairing two different variable chains, each encoded in distinct sets of gene segments. A thousand different chains of each type could thus generate  $10^6$  distinct antigen receptors through this **combinatorial diversity**. Thus a small amount of genetic material can encode a truly staggering diversity of receptors. Only a subset of these randomly generated receptor specificities survive the selective processes that shape the peripheral lymphocyte repertoire; nevertheless, there are lymphocytes of at least  $10^8$  different specificities in an individual at any one time. These provide the raw material on which clonal selection acts.

**Fig. 1.18 The diversity of lymphocyte antigen receptors is generated by somatic gene rearrangements.** Different parts of the variable regions of antigen receptors are encoded by sets of gene segments. During a lymphocyte's development, one member of each set of gene segments is joined randomly to the others by an irreversible process of DNA recombination. The juxtaposed gene segments make up a complete gene that

encodes the variable part of one chain of the receptor, and is unique to that cell. This random rearrangement is repeated for the set of gene segments encoding the other chain. The rearranged genes are expressed to produce the two types of polypeptide chain. These come together to form a unique antigen receptor on the lymphocyte surface. Each lymphocyte bears many copies of its unique receptor.

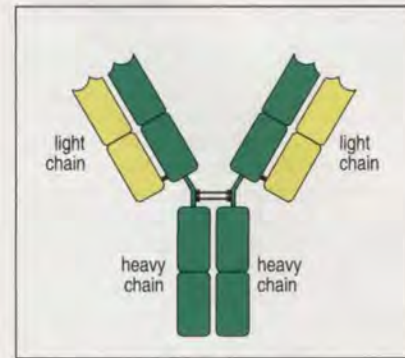
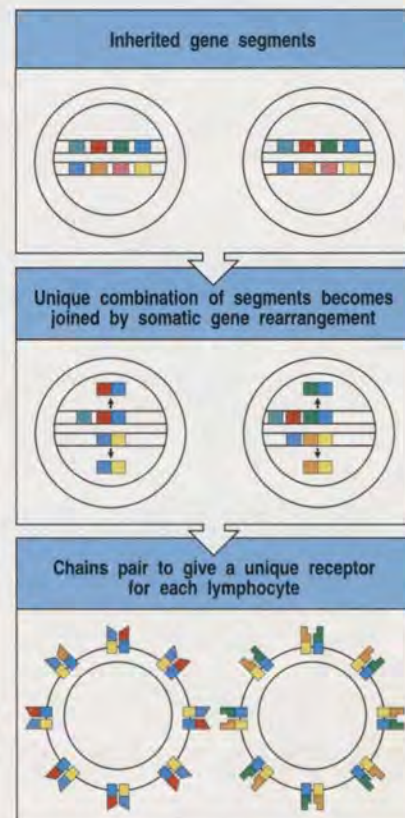


**Fig. 1.17 Antibodies are made up of four protein chains.** There are two types of chain in an antibody molecule: a larger chain called the heavy chain (green), and a smaller one called the light chain (yellow). Each chain has both a variable and a constant region, and there are two identical light chains and two identical heavy chains in each antibody molecule.



### 1-11 Lymphocyte development and survival are determined by signals received through their antigen receptors.

Equally amazing as the generation of millions of specificities of lymphocyte antigen receptors is the shaping of this repertoire during lymphocyte development and the homeostatic maintenance of such an extensive repertoire in the periphery. How are the most useful receptor specificities selected, and how are the numbers of peripheral lymphocytes, and the percentages of B cells and T cells kept relatively constant? The answer seems to be that lymphocyte maturation and survival are regulated by signals received through their antigen receptors. Strong signals received through the antigen receptor by an immature lymphocyte cause it to die or undergo further receptor rearrangement, and in this way self-reactive receptor specificities are deleted from the repertoire. However, a complete absence of signals from the antigen receptor can also lead to cell death. It seems that in order to survive, lymphocytes must periodically receive certain signals from their environment via their antigen receptors. In this way, the body can ensure that each receptor is functional and regulate the number and type of lymphocytes in the population at any given time. These survival signals appear to be delivered by other cells in the lymphoid organs and must derive, at least in part, from the body's own molecules, the **self antigens**, as altering the self environment alters the lifespan of lymphocytes in that environment. Developing B cells in the bone marrow interact with stromal cells, while their final maturation and continued recirculation appears to depend on survival signals received from the B-cell follicles of peripheral lymphoid tissue. T lymphocytes receive survival signals from self molecules on specialized epithelial cells in the thymus during development, and from the same molecules expressed by dendritic cells in the lymphoid tissues in the periphery. The self ligands that interact with the T-cell receptor to deliver these signals are partially defined, being composed of known cell-surface molecules complexed with undefined peptides from other self proteins in the cell. These same cell-surface molecules function to present foreign intracellular antigens to T cells, as we shall explain in Section 1-16, and in Chapter 5. They select only a subset of T-cell receptors for survival, but these are the receptors most likely to be useful in responding to foreign antigens, as we shall see in Chapter 7.

Lymphocytes that fail to receive survival signals, and those that are clonally deleted because they are self-reactive, undergo a form of cell suicide called **apoptosis** or **programmed cell death**. Apoptosis, derived from a Greek word meaning the falling of leaves from the trees, occurs in all tissues, at a relatively constant rate in each tissue, and is a means of regulating the number of cells in the body. It is responsible, for example, for the death and shedding of skin cells, the turnover of liver cells, and the death of the oldest intestinal epithelial cells that are constantly replaced by new cells. Thus, it should come as no surprise that immune system cells are regulated through the same mechanism. Each day the bone marrow produces many millions of new neutrophils, monocytes, red blood cells, and lymphocytes, and this production must be balanced by an equal loss of these cells. Regulated loss of all these blood cells occurs by apoptosis, and the dying cells are finally phagocytosed by specialized macrophages in the liver and spleen. Lymphocytes are a special case, because the loss of an individual naive lymphocyte means the loss of a receptor specificity from the repertoire, while each newly matured cell that survives will contribute a different specificity. The survival signals received through the antigen receptors appear to regulate this process by inhibiting the apoptosis of individual lymphocytes, thus regulating the maintenance and composition of the lymphocyte repertoire. We shall return to the question of which ligands deliver these signals, and how they contribute to shaping and maintaining the receptor repertoire, in Chapter 7.

### 1-12 Lymphocytes proliferate in response to antigen in peripheral lymphoid organs, generating effector cells and immunological memory.

The large diversity of lymphocyte receptors means that there will usually be at least a few that can bind to any given foreign antigen. However, because each lymphocyte has a different receptor, the numbers of lymphocytes that can bind and respond to any given antigen is very small. To generate sufficient antigen-specific effector lymphocytes to fight an infection, a lymphocyte with an appropriate receptor specificity must be activated to proliferate before its progeny finally differentiate into effector cells. This **clonal expansion** is a feature common to all adaptive immune responses.

As we have seen, lymphocyte activation and proliferation is initiated in the draining lymphoid tissues, where naive lymphocytes and activated antigen-presenting cells can come together. Antigens are thus presented to the naive recirculating lymphocytes as they migrate through the lymphoid tissue before returning to the bloodstream via the efferent lymph. On recognizing its specific antigen, a small lymphocyte stops migrating and enlarges. The chromatin in its nucleus becomes less dense, nucleoli appear, the volume of both the nucleus and the cytoplasm increases, and new RNAs and proteins are synthesized. Within a few hours, the cell looks completely different and is known as a **lymphoblast** (Fig. 1.19).

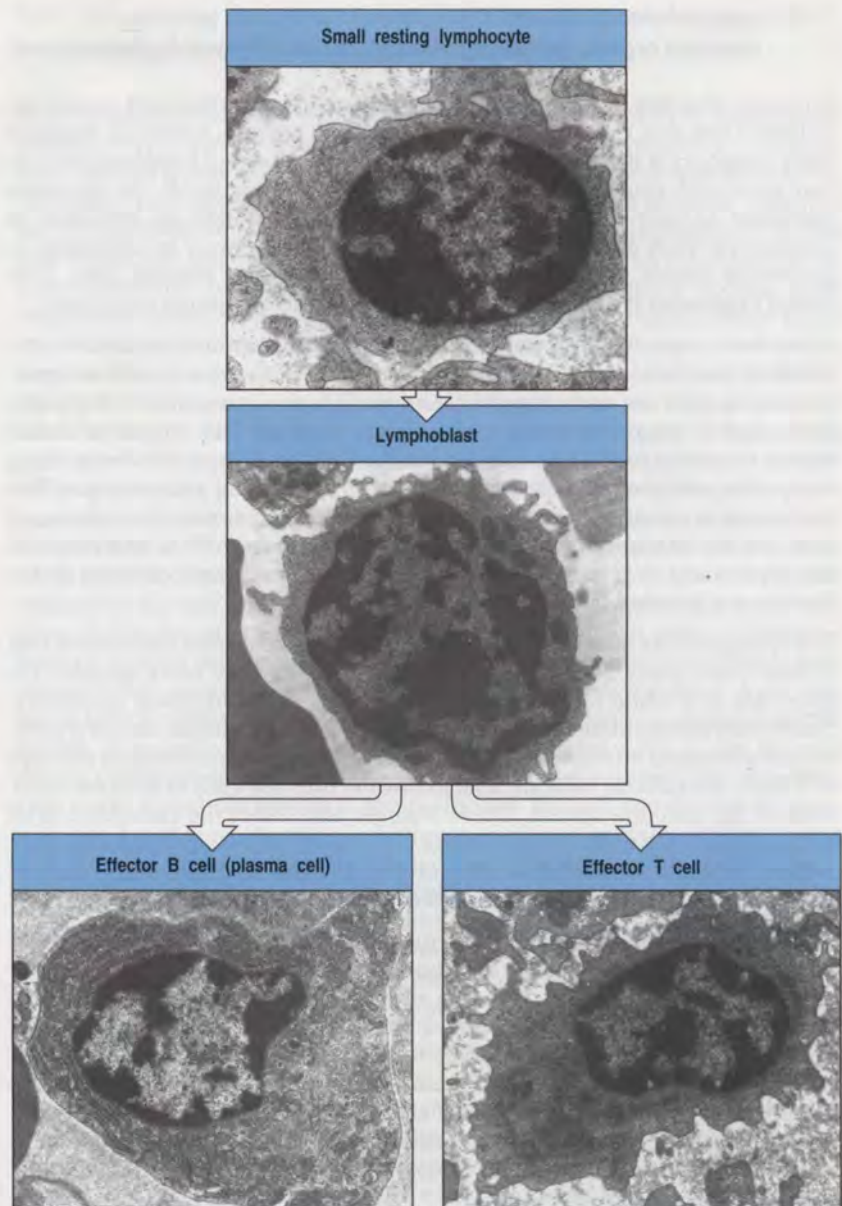
The lymphoblasts now begin to divide, normally duplicating themselves two to four times every 24 hours for 3 to 5 days, so that one naive lymphocyte gives rise to a clone of around 1000 daughter cells of identical specificity. These then differentiate into effector cells (see Fig. 1.19). In the case of B cells, the differentiated effector cells, the plasma cells, secrete antibody; in the case of T cells, the effector cells are able to destroy infected cells or activate other cells of the immune system. These changes also affect the recirculation of antigen-specific lymphocytes. Changes in the cell-adhesion molecules they express on their surface allow effector lymphocytes to migrate into sites of infection or stay in the lymphoid organs to activate B cells.

After a naive lymphocyte has been activated, it takes 4 to 5 days before clonal expansion is complete and the lymphocytes have differentiated into effector cells. That is why adaptive immune responses occur only after a delay of several days. Effector cells have only a limited life-span and, once antigen is removed, most of the antigen-specific cells generated by the clonal expansion of small lymphocytes undergo apoptosis. However, some persist after the antigen has been eliminated. These cells are known as **memory cells** and form the basis of **immunological memory**, which ensures a more rapid and effective response on a second encounter with a pathogen and thereby provides lasting protective immunity.

The characteristics of immunological memory are readily observed by comparing the antibody response of an individual to a first or **primary immunization** with the response elicited in the same individual by a **secondary** or **booster immunization** with the same antigen. As shown in Fig. 1.20, the **secondary antibody response** occurs after a shorter lag phase, achieves a markedly higher level, and produces antibodies of higher **affinity**, or strength of binding, for the antigen. We shall describe the mechanisms of these remarkable changes in Chapters 9 and 10. The cellular basis of immunological memory is the clonal expansion and clonal differentiation of cells specific for the eliciting antigen, and it is therefore entirely antigen specific.

It is immunological memory that enables successful vaccination and prevents reinfection with pathogens that have been repelled successfully by an adaptive immune response. Immunological memory is the most important biological consequence of the development of adaptive immunity, although its cellular and molecular basis is still not fully understood, as we shall see in Chapter 10.

**Fig. 1.19 Transmission electron micrographs of lymphocytes at various stages of activation to effector function.** Small resting lymphocytes (top panel) have not yet encountered antigen. Note the scanty cytoplasm, the absence of rough endoplasmic reticulum, and the condensed chromatin, all indicative of an inactive cell. This could be either a T cell or a B cell. Small circulating lymphocytes are trapped in lymph nodes when their receptors encounter antigen on antigen-presenting cells. Stimulation by antigen induces the lymphocyte to become an active lymphoblast (center panel). Note the large size, the nucleoli, the enlarged nucleus with diffuse chromatin, and the active cytoplasm; again, T and B lymphoblasts are similar in appearance. This cell undergoes repeated division, which is followed by differentiation to effector function. The bottom panels show effector T and B lymphocytes. Note the large amount of cytoplasm, the nucleus with prominent nucleoli, abundant mitochondria, and the presence of rough endoplasmic reticulum, all hallmarks of active cells. The rough endoplasmic reticulum is especially prominent in plasma cells (effector B cells), which are synthesizing and secreting very large amounts of protein in the form of antibody. Photographs courtesy of N. Rooney.



### 1-13 Interaction with other cells as well as with antigen is necessary for lymphocyte activation.

Peripheral lymphoid tissues are specialized not only to trap phagocytic cells that have ingested antigen (see Sections 1-3 and 1-6) but also to promote their interactions with lymphocytes that are needed to initiate an adaptive immune response. The spleen and lymph nodes in particular are highly organized for the latter function.

All lymphocyte responses to antigen require not only the signal that results from antigen binding to their receptors, but also a second signal, which is delivered by another cell. Naive T cells are generally activated by activated dendritic cells (Fig. 1.21, left panel) but for B cells (Fig. 1.21, right panel), the

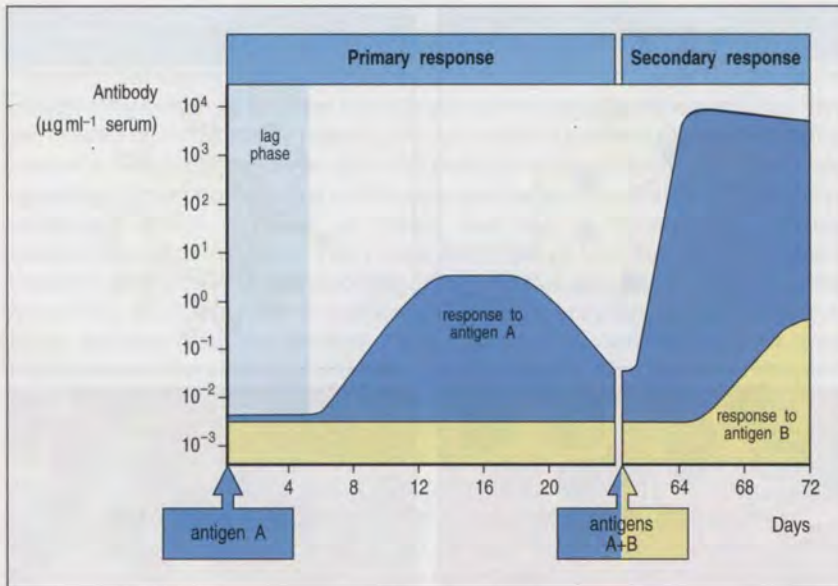


**Fig. 1.20 The course of a typical antibody response.** First encounter with an antigen produces a primary response. Antigen A introduced at time zero encounters little specific antibody in the serum. After a lag phase, antibody against antigen A (blue) appears; its concentration rises to a plateau, and then declines. When the serum is tested for antibody against another antigen, B (yellow), there is none present, demonstrating the specificity of the antibody response. When the animal is later challenged with a mixture of antigens A and B, a very rapid and intense secondary response to A occurs. This illustrates immunological memory, the ability of the immune system to make a second response to the same antigen more efficiently and effectively, providing the host with a specific defense against infection. This is the main reason for giving booster injections after an initial vaccination. Note that the response to B resembles the initial or primary response to A, as this is the first encounter of the animal with antigen B.

second signal is delivered by an armed effector T cell. Because of their ability to deliver activating signals, these three cell types are known as **professional antigen-presenting cells**, or often just **antigen-presenting cells**. They are illustrated in Fig. 1.22. Dendritic cells are the most important antigen-presenting cell of the three, with a central role in the initiation of adaptive immune responses (see Section 1-6). Macrophages can also mediate innate immune responses directly and make a crucial contribution to the effector phase of the adaptive immune response. B cells contribute to adaptive immunity by presenting peptides from antigens they have ingested and by secreting antibody.

Thus, the final postulate of adaptive immunity is that it occurs on a cell that also presents the antigen. This appears to be an absolute rule *in vivo*, although exceptions have been observed in *in vitro* systems. Nevertheless, what we are attempting to define is what does happen, not what can happen.

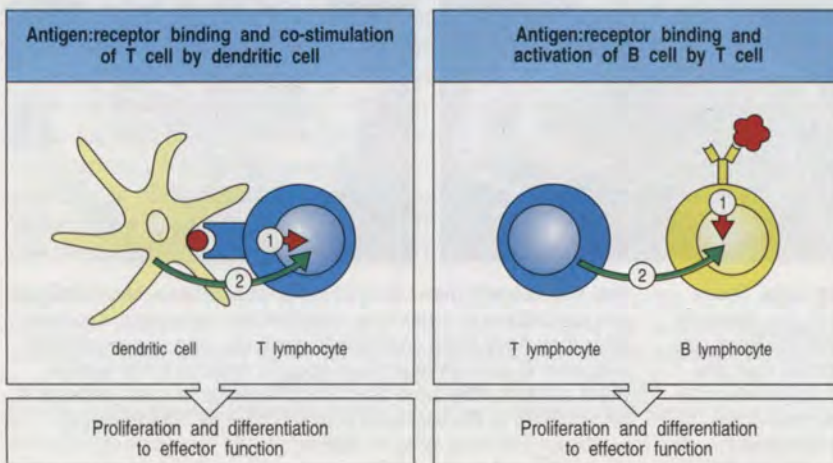


**Fig. 1.21 Two signals are required for lymphocyte activation.** As well as receiving a signal through their antigen receptor, mature naive lymphocytes must also receive a second signal to become activated. For T cells (left panel) it is delivered by a professional antigen-presenting cell such as the dendritic cell shown here. For B cells (right panel), the second signal is usually delivered by an activated T cell.

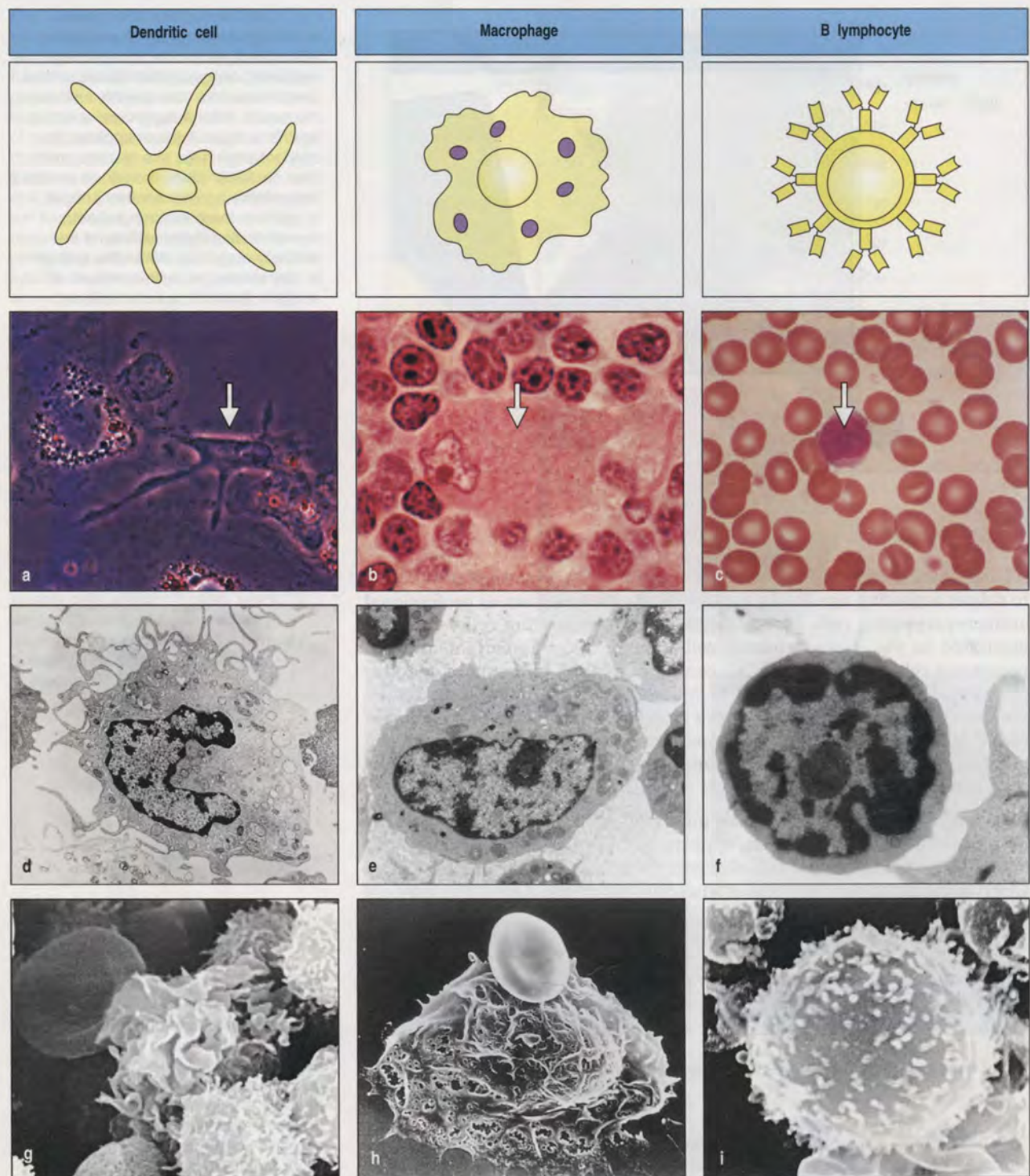


**Fig. 1.22 The professional antigen-presenting cells.** The three types of professional antigen-presenting cell are shown in the form in which they will be depicted throughout this book (top row), as they appear in the light microscope (second row; the relevant cell is indicated by an arrow), by transmission electron microscopy (third row) and by scanning electron microscopy (bottom row). Mature dendritic cells are found in lymphoid tissues and are derived from immature tissue dendritic cells

that interact with many distinct types of pathogen. Macrophages are specialized to internalize extracellular pathogens, especially after they have been coated with antibody, and to present their antigens. B cells have antigen-specific receptors that enable them to internalize large amounts of specific antigen, process it, and present it. Photographs courtesy of R.M. Steinman (a); N. Rooney (b, c, e, f); S. Knight (d, g); P.F. Heap (h, i).

## Summary.

The early innate systems of defense, which depend on invariant receptors recognizing common features of pathogens, are crucially important, but they are evaded or overcome by many pathogens and do not lead to immunological memory. The abilities to recognize all pathogens specifically and to provide enhanced protection against reinfection are the unique features of adaptive immunity, which is based on clonal selection of lymphocytes bearing antigen-specific receptors. The clonal selection of lymphocytes provides a theoretical framework for understanding all the key features of adaptive immunity. Each lymphocyte carries cell-surface receptors of a single specificity, generated by the random recombination of variable receptor gene segments and the pairing of different variable chains. This produces lymphocytes, each bearing a distinct receptor, so that the total repertoire of receptors can recognize virtually any antigen. If the receptor on a lymphocyte is specific for a ubiquitous self antigen, the cell is eliminated by encountering the antigen early in its development, while survival signals received through the antigen receptor select and maintain a functional lymphocyte repertoire. Adaptive immunity is initiated when an innate immune response fails to eliminate a new infection, and antigen and activated antigen-presenting cells are delivered to the draining lymphoid tissues. When a recirculating lymphocyte encounters its specific foreign antigen in peripheral lymphoid tissues, it is induced to proliferate and its progeny then differentiate into effector cells that can eliminate the infectious agent. A subset of these proliferating lymphocytes differentiate into memory cells, ready to respond rapidly to the same pathogen if it is encountered again. The details of these processes of recognition, development, and differentiation form the main material of the middle three parts of this book.

## The recognition and effector mechanisms of adaptive immunity.

Clonal selection describes the basic operating principle of the adaptive immune response but not how it defends the body against infection. In the last part of this chapter, we outline the mechanisms by which pathogens are detected by lymphocytes and are eventually destroyed in a successful adaptive immune response. The distinct lifestyles of different pathogens require different response mechanisms, not only to ensure their destruction but also for their detection and recognition (Fig. 1.23). We have already seen that there are two different kinds of antigen receptor: the surface immunoglobulin of B cells, and the smaller antigen receptor of T cells. These surface receptors are adapted to recognize antigen in two different ways: B cells recognize antigen that is present outside the cells of the body, where, for example, most bacteria are found; T cells, by contrast, can detect antigens generated inside infected cells, for example those due to viruses.

The **effector mechanisms** that operate to eliminate pathogens in an adaptive immune response are essentially identical to those of innate immunity. Indeed, it seems likely that specific recognition by clonally distributed receptors evolved as a late addition to existing innate effector mechanisms to produce the present-day adaptive immune response. We begin by outlining the effector actions of antibodies, which depend almost entirely on recruiting cells and molecules of the innate immune system.

Fig. 1.23 The major pathogen types confronting the immune system and some of the diseases that they cause.

| The immune system protects against four classes of pathogen |                                                                                                                          |                                                           |
|-------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------|
| Type of pathogen                                            | Examples                                                                                                                 | Diseases                                                  |
| Extracellular bacteria, parasites, fungi                    | <i>Streptococcus pneumoniae</i><br><i>Clostridium tetani</i><br><i>Trypanosoma brucei</i><br><i>Pneumocystis carinii</i> | Pneumonia<br>Tetanus<br>Sleeping sickness<br>PC Pneumonia |
| Intracellular bacteria, parasites                           | <i>Mycobacterium leprae</i><br><i>Leishmania donovani</i><br><i>Plasmodium falciparum</i>                                | Leprosy<br>Leishmaniasis<br>Malaria                       |
| Viruses (intracellular)                                     | Variola<br>Influenza<br>Varicella                                                                                        | Smallpox<br>Flu<br>Chickenpox                             |
| Parasitic worms (extracellular)                             | <i>Ascaris</i><br><i>Schistosoma</i>                                                                                     | Ascariasis<br>Schistosomiasis                             |

#### 1-14 Antibodies deal with extracellular forms of pathogens and their toxic products.

Antibodies, which were the first specific product of the adaptive immune response to be identified, are found in the fluid component of blood, or **plasma**, and in extracellular fluids. Because body fluids were once known as humors, immunity mediated by antibodies is known as **humoral immunity**.

As we have seen in Fig. 1.16, antibodies are Y-shaped molecules whose arms form two identical antigen-binding sites. These are highly variable from one molecule to another, providing the diversity required for specific antigen recognition. The stem of the Y, which defines the **class** of the antibody and determines its functional properties, takes one of only five major forms, or **isotypes**. Each of the five antibody classes engages a distinct set of effector mechanisms for disposing of antigen once it is recognized. We shall describe the isotypes and their actions in detail in Chapters 4 and 9.

The simplest and most direct way in which antibodies can protect from pathogens or their toxic products is by binding to them and thereby blocking their access to cells that they might infect or destroy (Fig. 1.24, left panels). This is known as **neutralization** and is important for protection against bacterial toxins and against pathogens such as viruses, which can thus be prevented from entering cells and replicating.

Binding by antibodies, however, is not sufficient on its own to arrest the replication of bacteria that multiply outside cells. In this case, one role of antibody is to enable a phagocytic cell to ingest and destroy the bacterium. This is important for the many bacteria that are resistant to direct recognition by phagocytes; instead, the phagocytes recognize the constant region of the antibodies coating the bacterium (see Fig. 1.24, middle panels). The coating of pathogens and foreign particles in this way is known as **opsonization**.

The third function of antibodies is to activate a system of plasma proteins known as **complement**. The complement system, which we shall discuss in detail in Chapter 2, can also be activated without the help of antibodies on many microbial surfaces, and therefore contributes to innate as well as adaptive immunity. The pores formed by activated complement components directly destroy bacteria, and this is important in a few bacterial infections (see Fig. 1.24, right panels). However, the main function of complement, like that of the antibodies themselves, is to coat the surface of pathogens and

enable phagocytes to engulf and destroy bacteria that they would otherwise not recognize. Complement also enhances the bactericidal actions of phagocytes; indeed it is so-called because it 'complements' the activities of antibodies.

Antibodies of different isotypes are found in different compartments of the body and differ in the effector mechanisms that they recruit, but all pathogens and particles bound by antibody are eventually delivered to phagocytes for ingestion, degradation, and removal from the body (see Fig. 1.24, bottom panels). The complement system and the phagocytes that antibodies recruit are not themselves antigen-specific; they depend upon antibody molecules to mark the particles as foreign. Antibodies are the sole contribution of B cells to the adaptive immune response. T cells, by contrast, have a variety of effector actions.

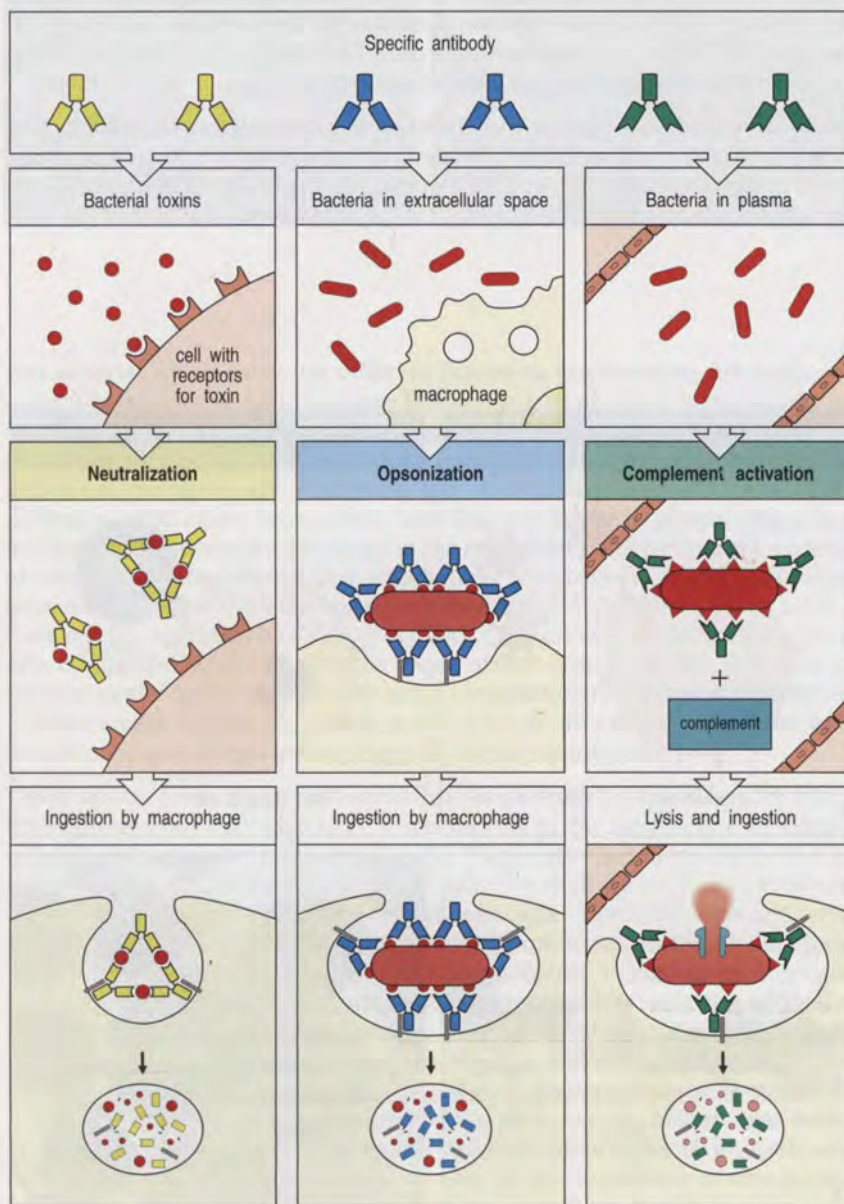


**Fig. 1.24 Antibodies can participate in host defense in three main ways.**

The left panels show antibodies binding to and neutralizing a bacterial toxin, thus preventing it from interacting with host cells and causing pathology. Unbound toxin can react with receptors on the host cell, whereas the toxin:antibody complex cannot. Antibodies also neutralize complete virus particles and bacterial cells by binding to them and inactivating them. The antigen:antibody complex is eventually scavenged and degraded by macrophages. Antibodies coating an antigen render it recognizable as foreign by phagocytes (macrophages and neutrophils), which then ingest and destroy it; this is called opsonization. The middle panels show opsonization and phagocytosis of a bacterial cell. The right panels show activation of the complement system by antibodies coating a bacterial cell. Bound antibodies form a receptor for the first protein of the complement system, which eventually forms a protein complex on the surface of the bacterium that, in some cases, can kill the bacterium directly. More generally, complement coating favors the uptake and destruction of the bacterium by phagocytes. Thus, antibodies target pathogens and their toxic products for disposal by phagocytes.

### 1-15 T cells are needed to control intracellular pathogens and to activate B-cell responses to most antigens.

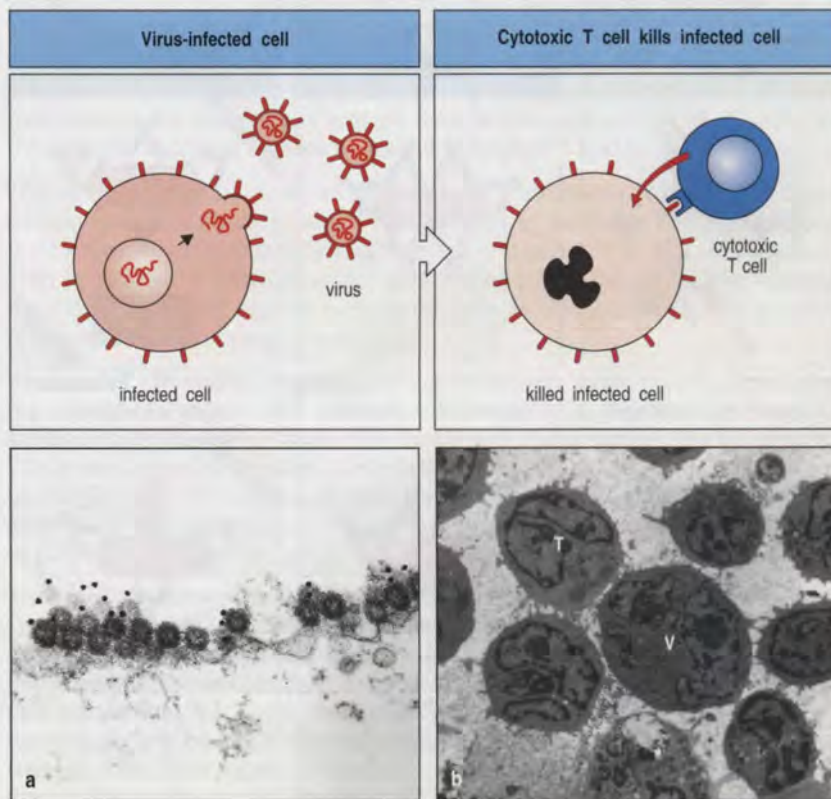
Pathogens are accessible to antibodies only in the blood and the extracellular spaces. However, some bacterial pathogens and parasites, and all viruses, replicate inside cells where they cannot be detected by antibodies. The destruction of these invaders is the function of the T lymphocytes, or T cells, which are responsible for the **cell-mediated immune responses** of adaptive immunity.

Cell-mediated reactions depend on direct interactions between T lymphocytes and cells bearing the antigen that the T cells recognize. The actions of cytotoxic T cells are the most direct. These recognize any of the body's cells that are infected with viruses, which replicate inside cells, using the biosynthetic machinery of the cell itself. The replicating virus eventually kills the cell, releasing new virus particles. Antigens derived from the replicating virus are, however, displayed on the surface of infected cells, where they are recognized by cytotoxic T cells. These cells can then control the infection by killing the infected cell before viral replication is complete (Fig. 1.25). Cytotoxic T cells typically express the molecule CD8 on their cell surfaces.

Other T lymphocytes that activate the cells they recognize are marked by the expression of the cell-surface molecule CD4 instead of CD8. Such T cells are often generically called helper T, or  $T_H$  cells, but this is a term that we will use for a specific subset of CD4 T cells. CD4 T lymphocytes can be divided into

**Fig. 1.25 Mechanism of host defense against intracellular infection by viruses.**

Cells infected by viruses are recognized by specialized T cells called cytotoxic T cells, which kill the infected cells directly. The killing mechanism involves the activation of enzymes known as caspases, which cleave after aspartic acid. These in turn activate a cytosolic nuclease in the infected cell, which cleaves host and viral DNA. Panel a is a transmission electron micrograph showing the plasma membrane of a cultured CHO cell (the Chinese hamster ovary cell line) infected with influenza virus. Many virus particles can be seen budding from the cell surface. Some of these have been labeled with a monoclonal antibody that is specific for a viral protein and is coupled to gold particles, which appear as the solid black dots in the micrograph. Panel b is a transmission electron micrograph of a virus-infected cell (V) surrounded by cytotoxic T lymphocytes. Note the close apposition of the membranes of the virus-infected cell and the T cell (T) in the upper left corner of the micrograph, and the clustering of the cytoplasmic organelles in the T cell between the nucleus and the point of contact with the infected cell. Panel a courtesy of M. Bui and A. Helenius; panel b courtesy of N. Rooney.



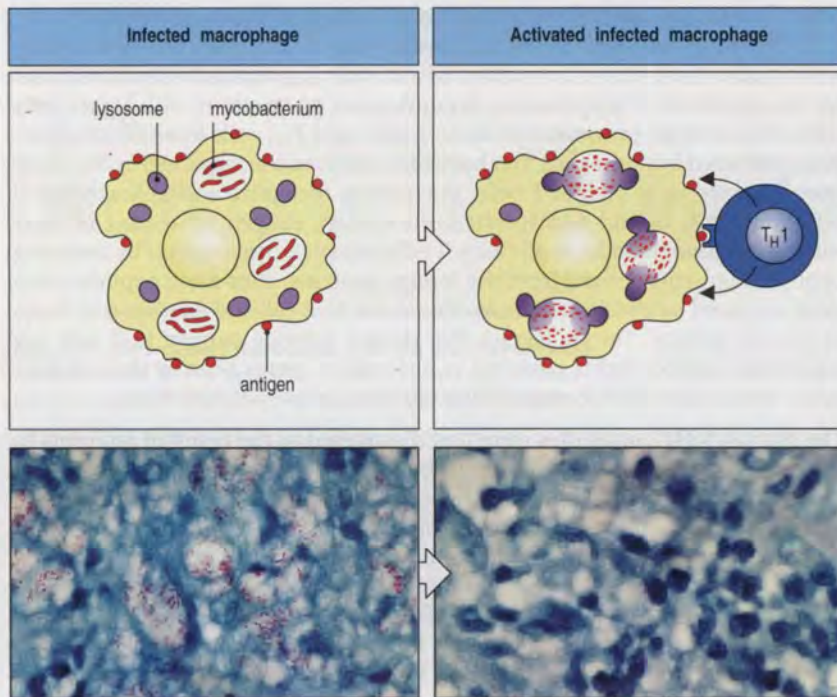


**Fig. 1.26 Mechanism of host defense against intracellular infection by mycobacteria.** Mycobacteria are engulfed by macrophages but resist being destroyed by preventing the fusion of the intracellular vesicles in which they reside with the lysosomes containing bactericidal agents; instead they persist and replicate in these vesicles. However, when a specific  $T_H1$  cell recognizes an infected macrophage, it releases cytokines that activate the macrophage and induce lysosomal fusion and macrophage bactericidal activity. The light micrographs (bottom row) show resting (left) and activated (right) macrophages infected with mycobacteria. The cells have been stained with an acid-fast red dye to reveal mycobacteria. These are prominent as red-staining rods in the resting macrophages but have been eliminated from the activated macrophages. Photographs courtesy of G. Kaplan.

two subsets, which carry out different functions in defending the body, in particular from bacterial infections. The first subset of CD4 T lymphocytes is important in the control of intracellular bacterial infections. Some bacteria grow only in the intracellular membrane-bounded vesicles of macrophages; important examples are *Mycobacterium tuberculosis* and *M. leprae*, the pathogens that cause tuberculosis and leprosy. Bacteria phagocytosed by macrophages are usually destroyed in the lysosomes, which contain a variety of enzymes and antimicrobial substances. Intracellular bacteria survive because the vesicles they occupy do not fuse with the lysosomes. These infections can be controlled by a subset of CD4 T cells, known as a  $T_H1$  cells, which activate macrophages, inducing the fusion of their lysosomes with the vesicles containing the bacteria and at the same time stimulating other antibacterial mechanisms of the phagocyte (Fig. 1.26).  $T_H1$  cells also release cytokines and chemokines that attract macrophages to the site of infection.

T cells destroy intracellular pathogens by killing infected cells and by activating macrophages but they also have a central role in the destruction of extracellular pathogens by activating B cells. This is the specialized role of the second subset of CD4 T cells, called  $T_H2$  cells. We shall see in Chapter 9, when we discuss the humoral immune response in detail, that only a few antigens with special properties can activate naive B lymphocytes on their own. Most antigens require an accompanying signal from helper T cells before they can stimulate B cells to proliferate and differentiate into cells secreting antibody (see Fig. 1.21). The ability of T cells to activate B cells was discovered long before it was recognized that a functionally distinct class of T cells activates macrophages, and the term helper T cell was originally coined to describe T cells that activate B cells. Although the designation 'helper' was later extended to T cells that activate macrophages (hence the H in  $T_H1$ ), we consider this usage confusing and we will, in the remainder of this book, reserve the term helper T cells for all T cells that activate B cells.

### 1-16 T cells are specialized to recognize foreign antigens as peptide fragments bound to proteins of the major histocompatibility complex.

All the effects of T lymphocytes depend upon interactions with target cells containing foreign proteins. Cytotoxic T cells and  $T_H1$  cells interact with antigens produced by pathogens that have infected the target cell or that have been ingested by it. Helper T cells, in contrast, recognize and interact with B cells that have bound and internalized foreign antigen by means of their surface immunoglobulin. In all cases, T cells recognize their targets by detecting peptide fragments derived from the foreign proteins, after these peptides have been captured by specialized molecules in the host cell and displayed by them at the cell surface. The molecules that display peptide antigen to T cells are membrane glycoproteins encoded in a cluster of genes bearing the cumbersome name **major histocompatibility complex**, abbreviated to **MHC**.

The human MHC molecules were first discovered as the result of attempts to use skin grafts from donors to repair badly burned pilots and bomb victims during World War II. The patients rejected the grafts, which were recognized as being 'foreign.' It was soon appreciated from studies in mice that rejection was due to an immune response, and eventually genetic experiments using inbred strains of mice showed that rapid rejection of skin grafts is caused by differences in a single genetic region. Because they control the compatibility of tissue grafts, these genes became known as 'histocompatibility genes.' Later, it was found that several closely linked, and highly **polymorphic** genes specify histocompatibility, which led to the term major histocompatibility complex. The central role of the MHC in antigen recognition by T cells, which we shall discuss in Chapter 5, was discovered later still, revealing the true physiological function of the proteins encoded by the MHC. This, in turn, led to an explanation for the major effect on tissue compatibility for which they were named. We shall discuss these diverse functions of MHC molecules in Chapters 4, 5, 7, 8, and 13.

### 1-17 Two major types of T cell recognize peptides bound to proteins of two different classes of MHC molecule.

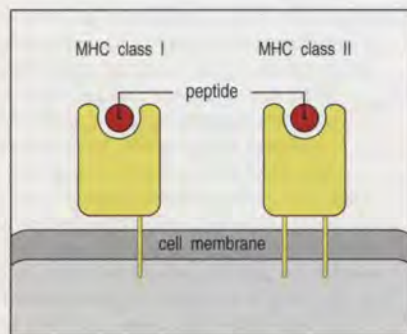


**Fig. 1.27 MHC molecules on the cell surface display peptide fragments of antigens.** MHC molecules are membrane proteins whose outer extracellular domains form a cleft in which a peptide fragment is bound. These fragments, which are derived from proteins degraded inside the cell, including foreign protein antigens, are bound by the newly synthesized MHC molecule before it reaches the surface. There are two kinds of MHC molecule—MHC class I and MHC class II—with related but distinct structures and functions.

There are two types of MHC molecule, called MHC class I and MHC class II. These differ in several subtle ways but share most of their major structural features. The most important of these is formed by the two outer extracellular domains of the molecule, which combine to create a long cleft in which a single peptide fragment is trapped during the synthesis and assembly of the MHC molecule inside the cell. The MHC molecule bearing its cargo of peptide is then transported to the cell surface, where it displays the peptide to T cells (Fig. 1.27). The antigen receptors of T lymphocytes are specialized to recognize a foreign antigenic peptide fragment bound to an MHC molecule. A T cell with a receptor specific for the complex formed between that particular foreign peptide and MHC molecule can then recognize and respond to the antigen-presenting cell.

The most important differences between the two classes of MHC molecule lie not in their structure but in the source of the peptides that they trap and carry to the cell surface. MHC class I molecules collect peptides derived from proteins synthesized in the cytosol, and are thus able to display fragments of viral proteins on the cell surface (Fig. 1.28). MHC class II molecules bind peptides derived from proteins in intracellular vesicles, and thus display peptides derived from pathogens living in macrophage vesicles or internalized by phagocytic cells and B cells (Fig. 1.29). We shall see in Chapter 5 exactly how peptides from these different sources are made accessible to the two types of MHC molecule.

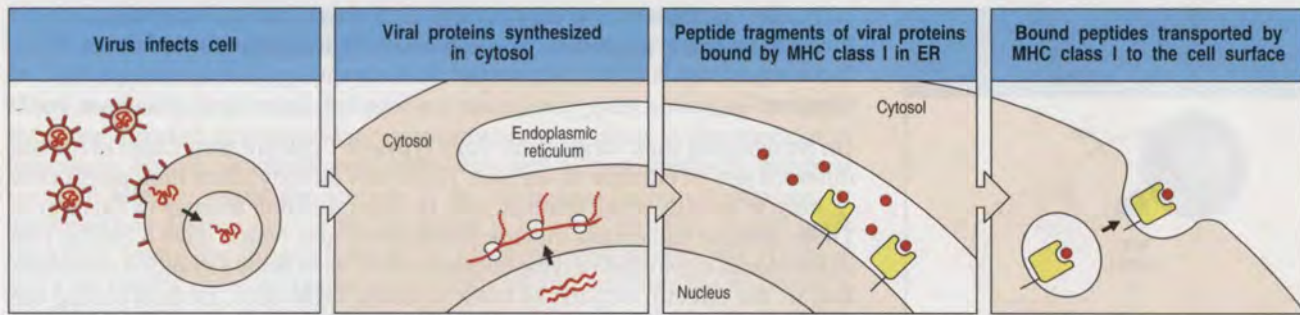


**Fig. 1.28 MHC class I molecules present antigen derived from proteins in the cytosol.** In cells infected with viruses, viral proteins are synthesized in the cytosol. Peptide fragments of

viral proteins are transported into the endoplasmic reticulum (ER) where they are bound by MHC class I molecules, which then deliver the peptides to the cell surface.

Having reached the cell surface with their peptide cargo, the two classes of MHC molecule are recognized by different functional classes of T cell. MHC class I molecules bearing viral peptides are recognized by cytotoxic T cells, which then kill the infected cell (Fig. 1.30); MHC class II molecules bearing peptides derived from pathogens taken up into vesicles are recognized by  $T_H1$  or  $T_H2$  cells (Fig. 1.31).

The antigen-specific activation of these effector T cells is aided by co-receptors that distinguish between the two classes of MHC molecule; cytotoxic cells express the CD8 co-receptor that binds MHC class I molecules, whereas  $T_H1$  and  $T_H2$  cells express the CD4 co-receptor with specificity for MHC class II molecules. However, even before T cells have encountered the specific foreign antigen that activates them to differentiate into effector cells, they express the appropriate co-receptor to match their receptor specificity. The maturation of T cells into either CD8 or CD4 T cells reflects the testing of T-cell receptor specificity that occurs during development, and the selection

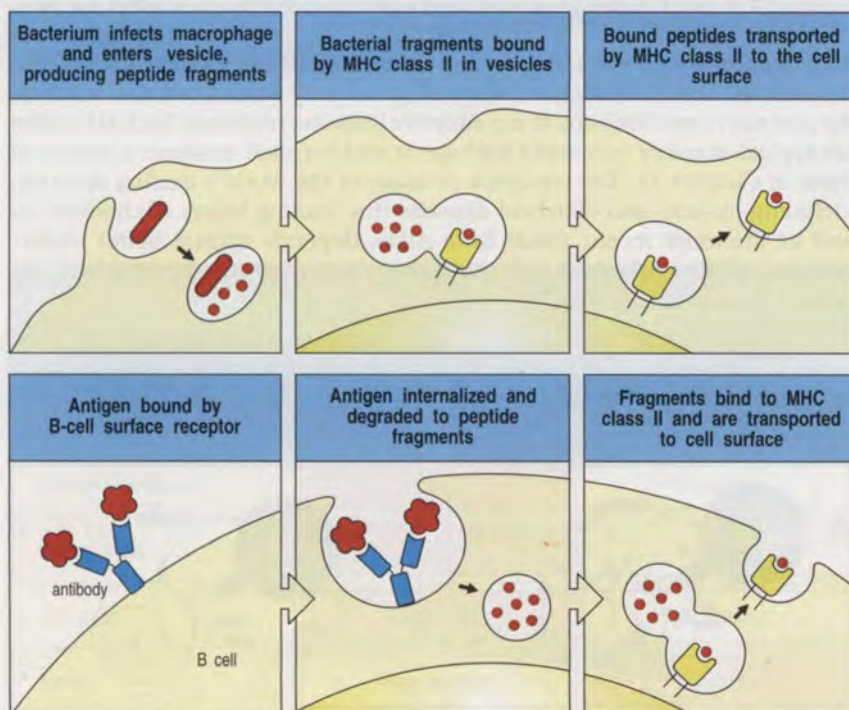


**Fig. 1.29 MHC class II molecules present antigen originating in intracellular vesicles.** Some bacteria infect cells and grow in intracellular vesicles. Peptides derived from such bacteria are bound by MHC class II molecules and transported to the cell surface (top row). MHC class II molecules also bind and transport peptides derived from antigen that has been bound and internalized by B-cell antigen receptor-mediated uptake into intracellular vesicles (bottom row).

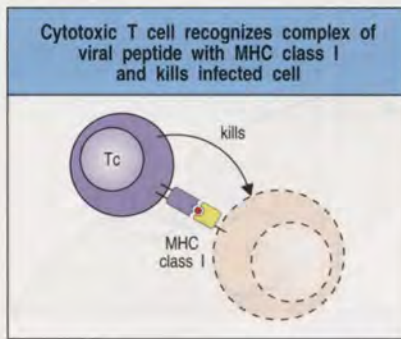


**Fig. 1.30 Cytotoxic T cells recognize antigen presented by MHC class I molecules and kill the cell.** The peptide:MHC class I complex on virus-infected cells is detected by antigen-specific cytotoxic T cells. Cytotoxic T cells are preprogrammed to kill the cells they recognize.

of T cells that can receive survival signals from self MHC molecules. Exactly how this selective process works, and how it maximizes the usefulness of the T cell repertoire is a central question in immunology and is a major topic of Chapter 7.

On recognizing their targets, the three types of T cell are stimulated to release different sets of effector molecules. These can directly affect their target cells or help to recruit other effector cells in ways we shall discuss in Chapter 8. These effector molecules include many cytokines, which have a crucial role in the clonal expansion of lymphocytes as well as in innate immune responses and in the effector actions of most immune cells; thus, understanding the actions of cytokines is central to understanding the various behaviors of the immune system.

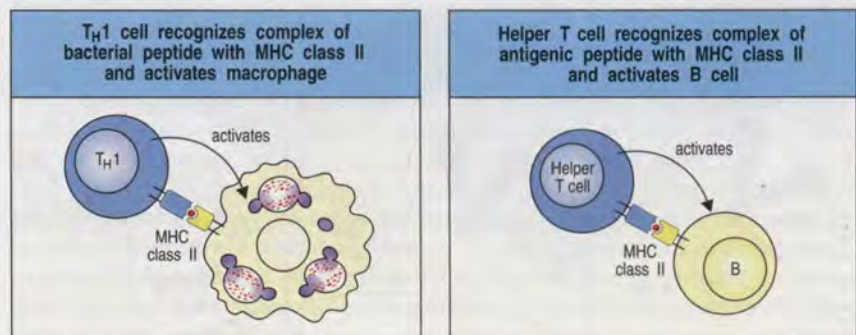
### 1-18 Defects in the immune system result in increased susceptibility to infection.

We tend to take for granted the ability of our immune systems to free our bodies of infection and prevent its recurrence. In some people, however, parts of the immune system fail. In the most severe of these **immunodeficiency diseases**, adaptive immunity is completely absent, and death occurs in infancy from overwhelming infection unless heroic measures are taken. Other less catastrophic failures lead to recurrent infections with particular types of pathogen, depending on the particular deficiency. Much has been learned about the functions of the different components of the human immune system through the study of these immunodeficiencies.

More than twenty-five years ago, a devastating form of immunodeficiency appeared, the **acquired immune deficiency syndrome**, or **AIDS**, which is itself caused by an infectious agent. This disease destroys the T cells that activate macrophages, leading to infections caused by intracellular bacteria and other pathogens normally controlled by these T cells. Such infections are the major cause of death from this increasingly prevalent immunodeficiency disease, which is discussed fully in Chapter 11 together with inherited immunodeficiencies.

AIDS is caused by a virus, the **human immunodeficiency virus**, or **HIV**, that has evolved several strategies by which it not only evades but also subverts the protective mechanisms of the adaptive immune response. Such strategies are typical of many successful pathogens and we shall examine a variety of them in Chapter 11. The conquest of many of the world's leading diseases, including malaria and diarrheal diseases (the leading killers of children) as well as the more recent threat from AIDS, depends upon a better understanding of the pathogens that cause them and their interactions with the cells of the immune system.

**Fig. 1.31  $T_H1$  and helper T cells recognize antigen presented by MHC class II molecules.** On recognition of their specific antigen on infected macrophages,  $T_H1$  cells activate the macrophage, leading to the destruction of the intracellular bacteria (left panel). When helper T cells recognize antigen on B cells, they activate these cells to proliferate and differentiate into antibody-producing plasma cells (right panel).



### 1-19 Understanding adaptive immune responses is important for the control of allergies, autoimmune disease, and organ graft rejection.

Many medically important diseases are associated with a normal immune response directed against an inappropriate antigen, often in the absence of infectious disease. Immune responses directed at noninfectious antigens occur in **allergy**, where the antigen is an innocuous foreign substance, in **autoimmune disease**, where the response is to a self antigen, and in **graft rejection**, where the antigen is borne by a transplanted foreign cell. What we call a successful immune response or a failure, and whether the response is considered harmful or beneficial to the host, depends not on the response itself but rather on the nature of the antigen (Fig. 1.32).

Allergic diseases, which include asthma, are an increasingly common cause of disability in the developed world, and many other important diseases are now recognized as autoimmune. An autoimmune response directed against pancreatic  $\beta$  cells is the leading cause of diabetes in the young. In allergies and autoimmune diseases, the powerful protective mechanisms of the adaptive immune response cause serious damage to the patient.

Immune responses to harmless antigens, to body tissues, or to organ grafts are, like all other immune responses, highly specific. At present, the usual way to treat these responses is with **immunosuppressive drugs**, which inhibit all immune responses, desirable or undesirable. If it were possible to suppress only those lymphocyte clones responsible for the unwanted response, the disease could be cured or the grafted organ protected without impeding protective immune responses. There is hope that this dream of antigen-specific **immunoregulation** to control unwanted immune responses could become a reality, since antigen-specific suppression of immune responses can be induced experimentally, although the molecular basis of this suppression is not fully understood. We shall see in Chapter 10 how the mechanisms of immune regulation are beginning to emerge from a better understanding of the functional subsets of lymphocytes and the cytokines that control them, and we shall discuss the present state of understanding of allergies, autoimmune disease, graft rejection, and immunosuppressive drugs in Chapters 12, 13, and 14.

| Antigen             | Effect of response to antigen |                     |
|---------------------|-------------------------------|---------------------|
|                     | Normal response               | Deficient response  |
| Infectious agent    | Protective immunity           | Recurrent infection |
| Innocuous substance | Allergy                       | No response         |
| Grafted organ       | Rejection                     | Acceptance          |
| Self organ          | Autoimmunity                  | Self tolerance      |
| Tumor               | Tumor immunity                | Cancer              |

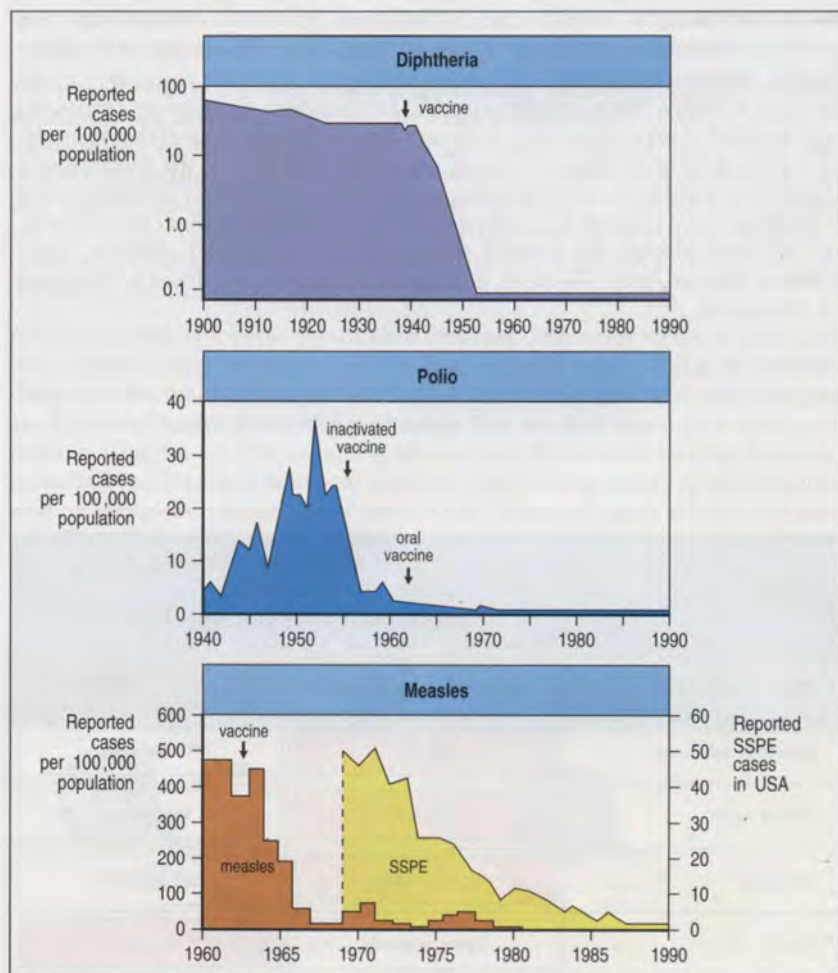
**Fig. 1.32 Immune responses can be beneficial or harmful depending on the nature of the antigen.** Beneficial responses are shown in white, harmful responses in shaded boxes. Where the response is beneficial, its absence is harmful.

### 1-20 Vaccination is the most effective means of controlling infectious diseases.

Although the specific suppression of immune responses must await advances in basic research on immune regulation and its application, the deliberate stimulation of an immune response by immunization, or vaccination, has achieved many successes in the two centuries since Jenner's pioneering experiment.

Mass immunization programs have led to the virtual eradication of several diseases that used to be associated with significant morbidity (illness) and mortality (Fig. 1.33). Immunization is considered so safe and so important that most states in the United States require children to be immunized against up to seven common childhood diseases. Impressive as these accomplishments are, there are still many diseases for which we lack effective vaccines. And even where vaccines for diseases such as measles or polio can be used effectively in developed countries, technical and economic problems can prevent their widespread use in developing countries, where mortality from these diseases is still high. The tools of modern immunology and molecular biology are being applied to develop new vaccines and improve old

**Fig. 1.33 Successful vaccination campaigns.** Diphtheria, polio, and measles and its consequences have been virtually eliminated in the United States, as shown in these three graphs. SSPE stands for subacute sclerosing panencephalitis, a brain disease that is a late consequence of measles infection in a few patients. When measles was prevented, SSPE disappeared 15–20 years later. However, as these diseases have not been eradicated worldwide, immunization must be maintained in a very high percentage of the population to prevent their reappearance.



ones, and we shall discuss these advances in Chapter 14. The prospect of controlling these important diseases is tremendously exciting. The guarantee of good health is a critical step toward population control and economic development. At a cost of pennies per person, great hardship and suffering can be alleviated.

### Summary.

Lymphocytes have two distinct recognition systems specialized for detection of extracellular and intracellular pathogens. B cells have cell-surface immunoglobulin molecules as receptors for antigen and, upon activation, secrete the immunoglobulin as soluble antibody that provides defense against pathogens in the extracellular spaces of the body. T cells have receptors that recognize peptide fragments of intracellular pathogens transported to the cell surface by the glycoproteins of the major histocompatibility complex (MHC). Two classes of MHC molecule transport peptides from different intracellular compartments to present them to distinct types of effector T cell: cytotoxic T cells that kill infected target cells, and  $T_H1$  cells and helper T cells that mainly activate macrophages and B cells, respectively. Thus, T cells are crucially important for both the humoral and cell-mediated responses of adaptive immunity. The adaptive immune response seems to have engrafted specific antigen recognition by highly diversified receptors onto innate defense systems, which have a central role in the effector actions of both B and T lymphocytes. The vital role of adaptive immunity in fighting infection is illustrated by the immunodeficiency diseases and the problems caused by pathogens that succeed in evading or subverting an adaptive immune response. The antigen-specific suppression of adaptive immune responses is the goal of treatment for important human diseases involving inappropriate activation of lymphocytes, whereas the specific stimulation of an adaptive immune response is the basis of successful vaccination.

### Summary to Chapter 1.

The immune system defends the host against infection. Innate immunity serves as a first line of defense but lacks the ability to recognize certain pathogens and to provide the specific protective immunity that prevents reinfection. Adaptive immunity is based on clonal selection from a repertoire of lymphocytes bearing highly diverse antigen-specific receptors that enable the immune system to recognize any foreign antigen. In the adaptive immune response, antigen-specific lymphocytes proliferate and differentiate into effector cells that eliminate pathogens. Host defense requires different recognition systems and a wide variety of effector mechanisms to seek out and destroy the wide variety of pathogens in their various habitats within the body and at its surface. Not only can the adaptive immune response eliminate a pathogen but, in the process, it also generates increased numbers of differentiated memory lymphocytes through clonal selection, and this allows a more rapid and effective response upon reinfection. The regulation of immune responses, whether to suppress them when unwanted or to stimulate them in the prevention of infectious disease, is the major medical goal of research in immunology.

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International Immunology  
Journal of Autoimmunity  
Journal of Experimental Medicine  
Journal of Immunology  
Nature Immunology  
Regional Immunology  
Thymus

### Primary journals with frequent papers in immunology

Cell  
Current Biology  
EMBO Journal  
Journal of Biological Chemistry  
Journal of Cell Biology  
Journal of Clinical Investigation  
Molecular Cell Biology  
Nature  
Nature Cell Biology  
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Science

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# Antigen Recognition by B-cell and T-cell Receptors

## 3

We have learned in Chapter 2 that the body is defended by innate immune responses, but these will only work to control pathogens that have certain molecular patterns or that induce interferons and other secreted yet non-specific defenses. Most crucially, they do not allow memory to form as they operate by receptors that are coded in the genome. Thus, innate immunity is good for preventing pathogens from growing freely in the body, but it does not lead to the most important feature of adaptive immunity, which is long-lasting memory of specific pathogen.

To recognize and fight the wide range of pathogens an individual will encounter, the lymphocytes of the adaptive immune system have evolved to recognize a great variety of different antigens from bacteria, viruses, and other disease-causing organisms. The antigen-recognition molecules of B cells are the **immunoglobulins**, or **Ig**. These proteins are produced by B cells in a vast range of antigen specificities, each B cell producing immunoglobulin of a single specificity (see Sections 1-8 to 1-10). Membrane-bound immunoglobulin on the B-cell surface serves as the cell's receptor for antigen, and is known as the **B-cell receptor (BCR)**. Immunoglobulin of the same antigen specificity is secreted as **antibody** by terminally differentiated B cells—the plasma cells. The secretion of antibodies, which bind pathogens or their toxic products in the extracellular spaces of the body, is the main effector function of B cells in adaptive immunity.

Antibodies were the first molecules involved in specific immune recognition to be characterized and are still the best understood. The antibody molecule has two separate functions: one is to bind specifically to molecules from the pathogen that elicited the immune response; the other is to recruit other cells and molecules to destroy the pathogen once the antibody is bound to it. For example, binding by antibody neutralizes viruses and marks pathogens for destruction by phagocytes and complement, as described in Section 1-14. These functions are structurally separated in the antibody molecule, one part of which specifically recognizes and binds to the pathogen or antigen whereas the other engages different effector mechanisms. The antigen-binding region varies extensively between antibody molecules and is thus known as the **variable region** or **V region**. The variability of antibody molecules allows each antibody to bind a different specific antigen, and the total repertoire of antibodies made by a single individual is large enough to ensure that virtually any structure can be recognized. The region of the antibody molecule that engages the effector functions of the immune system does not vary in the same way and is thus known as the **constant region** or **C region**. It comes in five main forms, which are each specialized for activating different effector mechanisms. The membrane-bound B-cell receptor does not have these effector functions, as the C region remains inserted in the membrane of the B cell. Its function is as a receptor that recognizes and binds antigen by the V regions exposed on the surface of the cell, thus transmitting a signal that causes B-cell activation leading to clonal expansion and specific antibody production.

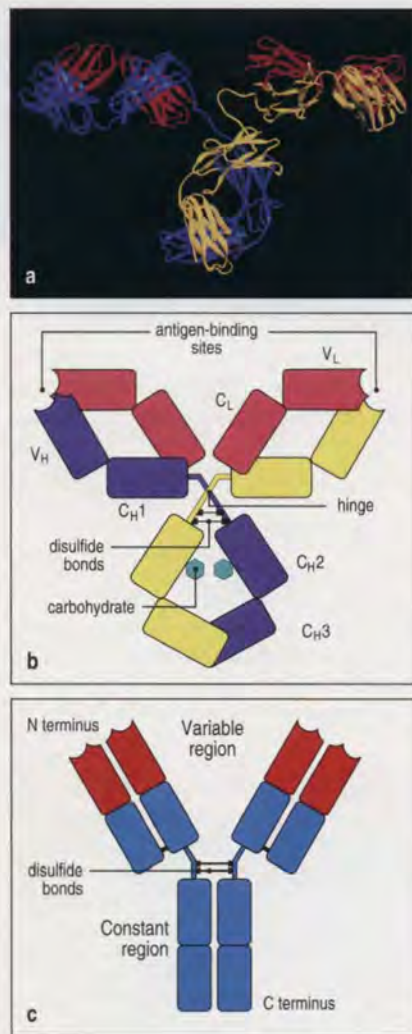


**Fig. 3.1 Structure of an antibody molecule.** Panel a illustrates a ribbon diagram based on the X-ray crystallographic structure of an IgG antibody, showing the course of the backbones of the polypeptide chains. Three globular regions form a Y. The two antigen-binding sites are at the tips of the arms, which are tethered to the trunk of the Y by a flexible hinge region. A schematic representation of the structure in a is given in panel b, illustrating the four-chain composition and the separate domains comprising each chain. Panel c shows a simplified schematic representation of an antibody molecule that will be used throughout this book. Photograph courtesy of A. McPherson and L. Harris.

The antigen-recognition molecules of T cells are made solely as membrane-bound proteins and only function to signal T cells for activation. These **T-cell receptors (TCRs)** are related to immunoglobulins both in their protein structure—having both V and C regions—and in the genetic mechanism that produces their great variability (see Section 1-10 and Chapter 4). However, the T-cell receptor differs from the B-cell receptor in an important way: it does not recognize and bind antigen directly, but instead recognizes short peptide fragments of pathogen protein antigens, which are bound to **MHC molecules** on the surfaces of other cells.

The MHC molecules are glycoproteins encoded in the large cluster of genes known as the **major histocompatibility complex (MHC)** (see Sections 1-16 and 1-17). Their most striking structural feature is a cleft running across their outermost surface, in which a variety of peptides can be bound. As we shall discuss further in Chapter 5, MHC molecules show great genetic variation in the population, and each individual carries up to 12 of the possible variants, which increases the range of pathogen-derived peptides that can be bound. T-cell receptors recognize features both of the peptide antigen and of the MHC molecule to which it is bound. This introduces an extra dimension to antigen recognition by T cells, known as **MHC restriction**, because any given T-cell receptor is specific not simply for a foreign peptide antigen, but for a unique combination of a peptide and a particular MHC molecule. The ability of T-cell receptors to recognize MHC molecules, and their selection during T-cell development for the ability to recognize the particular MHC molecules expressed by an individual, are topics we shall return to in Chapters 5 and 7.

In this chapter we focus on the structure and antigen-binding properties of immunoglobulins and T-cell receptors. Although B cells and T cells recognize foreign molecules in two distinct fashions, the receptor molecules they use for this task are very similar in structure. We will see how this basic structure can accommodate great variability in antigen specificity, and how it enables immunoglobulins and T-cell receptors to carry out their functions as the antigen-recognition molecules of the adaptive immune response.

### The structure of a typical antibody molecule.

Antibodies are the secreted form of the B-cell receptor. An antibody is identical to the B-cell receptor of the cell that secretes it except for a small portion of the C-terminus of the heavy-chain constant region. In the case of the B-cell receptor the C-terminus is a hydrophobic membrane-anchoring sequence, and in the case of antibody it is a hydrophilic sequence that allows secretion. Since they are soluble, and secreted in large quantities, antibodies are easily obtainable and easily studied. For this reason, most of what we know about the B-cell receptor comes from the study of antibodies.

Antibody molecules are roughly Y-shaped molecules consisting of three equal-sized portions, loosely connected by a flexible tether. Three schematic representations of antibody structure, which has been determined by X-ray crystallography, are shown in Fig. 3.1. The aim of this part of the chapter is to explain how this structure is formed and how it allows antibody molecules to carry out their dual tasks—binding on the one hand to a wide variety of antigens, and on the other hand to a limited number of effector molecules and cells. As we will see, each of these tasks is carried out by separable parts of the molecule. The two arms of the Y end in regions that vary between different

antibody molecules, the V regions. These are involved in antigen binding, whereas the stem of the Y, or the C region, is far less variable and is the part that interacts with effector cells and molecules.

All antibodies are constructed in the same way from paired heavy and light polypeptide chains, and the generic term immunoglobulin is used for all such proteins. Within this general category, however, five different **classes** of immunoglobulins—IgM, IgD, IgG, IgA, and IgE—can be distinguished by their C regions, which will be described more fully in Chapter 4. More subtle differences confined to the V region account for the specificity of antigen binding. We will use the IgG antibody molecule as an example to describe the general structural features of immunoglobulins.

### 3-1 IgG antibodies consist of four polypeptide chains.

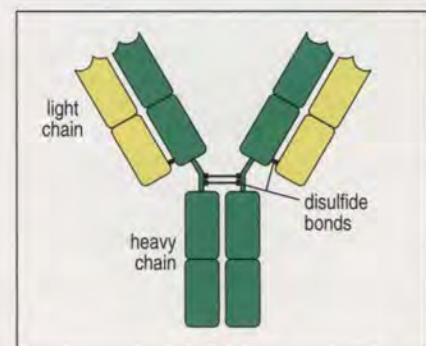
IgG antibodies are large molecules, having a molecular weight of approximately 150 kDa, composed of two different kinds of polypeptide chain. One, of approximately 50 kDa, is termed the **heavy** or **H chain**, and the other, of 25 kDa, is termed the **light** or **L chain** (Fig. 3.2). Each IgG molecule consists of two heavy chains and two light chains. The two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. In any given immunoglobulin molecule, the two heavy chains and the two light chains are identical, giving an antibody molecule two identical antigen-binding sites (see Fig. 3.1), and thus the ability to bind simultaneously to two identical structures.

Two types of light chain, termed **lambda** ( $\lambda$ ) and **kappa** ( $\kappa$ ), are found in antibodies. A given immunoglobulin either has  $\kappa$  chains or  $\lambda$  chains, never one of each. No functional difference has been found between antibodies having  $\lambda$  or  $\kappa$  light chains, and either type of light chain may be found in antibodies of any of the five major classes. The ratio of the two types of light chain varies from species to species. In mice, the average  $\kappa$  to  $\lambda$  ratio is 20:1, whereas in humans it is 2:1 and in cattle it is 1:20. The reason for this variation is unknown. Distortions of this ratio can sometimes be used to detect the abnormal proliferation of a clone of B cells. These would all express the identical light chain, and thus an excess of  $\lambda$  light chains in a person might indicate the presence of a B-cell tumor producing  $\lambda$  chains.

By contrast, the class, and thus the effector function, of an antibody, is defined by the structure of its heavy chain. There are five main **heavy-chain classes** or **isotypes**, some of which have several subtypes, and these determine the functional activity of an antibody molecule. The five major classes of immunoglobulin are **immunoglobulin M (IgM)**, **immunoglobulin D (IgD)**, **immunoglobulin G (IgG)**, **immunoglobulin A (IgA)**, and **immunoglobulin E (IgE)**. Their heavy chains are denoted by the corresponding lower-case Greek letter ( $\mu$ ,  $\delta$ ,  $\gamma$ ,  $\alpha$ , and  $\epsilon$ , respectively). IgG is by far the most abundant immunoglobulin and has several subclasses (IgG1, 2, 3, and 4 in humans). Their distinctive functional properties are conferred by the carboxy-terminal part of the heavy chain, where it is not associated with the light chain. We will describe the structure and functions of the different heavy-chain isotypes in Chapter 4. The general structural features of all the isotypes are similar and we will consider IgG, the most abundant isotype in plasma, as a typical antibody molecule.

**Fig. 3.2 Immunoglobulin molecules are composed of two types of protein chain: heavy chains and light chains.** Each immunoglobulin molecule is made up of two heavy chains (green) and two

light chains (yellow) joined by disulfide bonds so that each heavy chain is linked to a light chain and the two heavy chains are linked together.



### 3-2 Immunoglobulin heavy and light chains are composed of constant and variable regions.

The amino acid sequences of many immunoglobulin heavy and light chains have been determined and reveal two important features of antibody molecules. First, each chain consists of a series of similar, although not identical, sequences, each about 110 amino acids long. Each of these repeats corresponds to a discrete, compactly folded region of protein structure known as a protein domain. The light chain is made up of two such **immunoglobulin domains**, whereas the heavy chain of the IgG antibody contains four (see Fig. 3.1a). This suggests that the immunoglobulin chains have evolved by repeated duplication of an ancestral gene corresponding to a single domain.

The second important feature revealed by comparisons of amino acid sequences is that the amino-terminal sequences of both the heavy and light chains vary greatly between different antibodies. The variability in sequence is limited to approximately the first 110 amino acids, corresponding to the first domain, whereas the remaining domains are constant between immunoglobulin chains of the same isotype. The amino-terminal variable or **V domains** of the heavy and light chains ( $V_H$  and  $V_L$ , respectively) together make up the V region of the antibody and confer on it the ability to bind specific antigen, while the constant domains (**C domains**) of the heavy and light chains ( $C_H$  and  $C_L$ , respectively) make up the C region (see Fig. 3.1b, c). The multiple heavy-chain C domains are numbered from the amino-terminal end to the carboxy terminus, for example  $C_{H1}$ ,  $C_{H2}$ , and so on.

### 3-3 The antibody molecule can readily be cleaved into functionally distinct fragments.

The protein domains described above associate to form larger globular domains. Thus, when fully folded and assembled, an antibody molecule comprises three equal-sized globular portions joined by a flexible stretch of polypeptide chain known as the **hinge region** (see Fig. 3.1b). Each arm of this Y-shaped structure is formed by the association of a light chain with the amino-terminal half of a heavy chain, whereas the trunk of the Y is formed by the pairing of the carboxy-terminal halves of the two heavy chains. The association of the heavy and light chains is such that the  $V_H$  and  $V_L$  domains are paired, as are the  $C_{H1}$  and  $C_L$  domains. The  $C_{H3}$  domains pair with each other but the  $C_{H2}$  domains do not interact; carbohydrate side chains attached to the  $C_{H2}$  domains lie between the two heavy chains. The two antigen-binding sites are formed by the paired  $V_H$  and  $V_L$  domains at the ends of the two arms of the Y (see Fig. 3.1b).

Proteolytic enzymes (proteases) that cleave polypeptide sequences have been used to dissect the structure of antibody molecules and to determine which parts of the molecule are responsible for its various functions. Limited digestion with the protease papain cleaves antibody molecules into three fragments (Fig. 3.3). Two fragments are identical and contain the antigen-binding activity. These are termed the **Fab fragments**, for Fragment antigen binding. The Fab fragments correspond to the two identical arms of the antibody molecule, which contain the complete light chains paired with the  $V_H$  and  $C_{H1}$  domains of the heavy chains. The other fragment contains no antigen-binding activity but was originally observed to crystallize readily, and for this reason was named the **Fc fragment**, for Fragment crystallizable. This fragment corresponds to the paired  $C_{H2}$  and  $C_{H3}$  domains and is the part of the antibody molecule that interacts with effector molecules and cells. The functional differences between heavy-chain isotypes lie mainly in the Fc fragment.

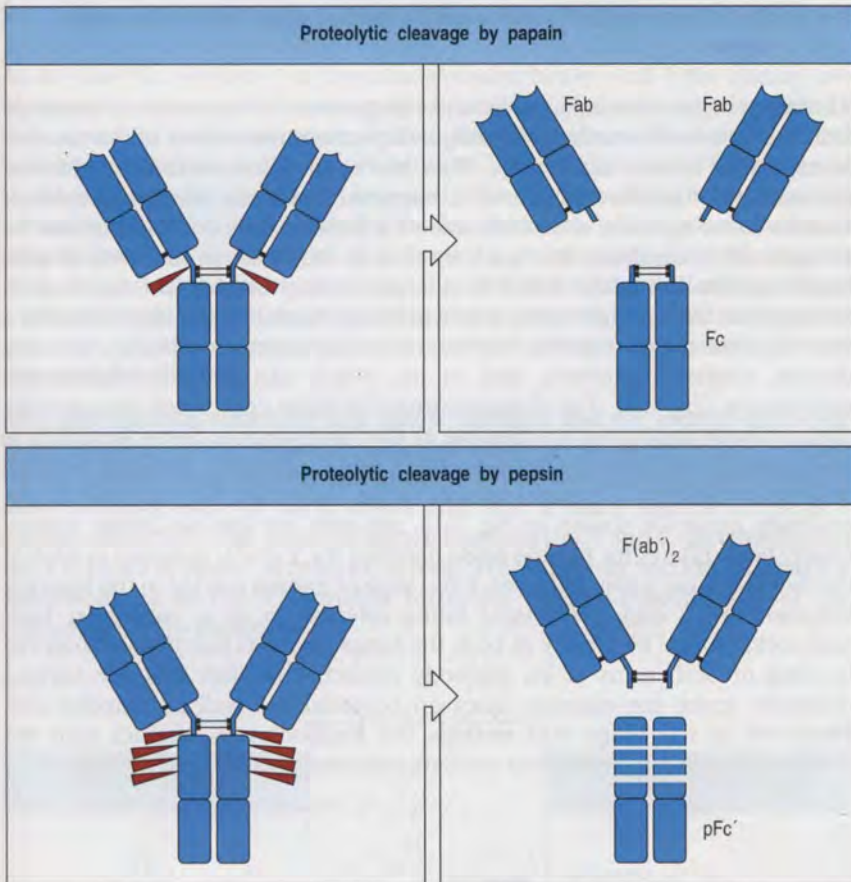


**Fig. 3.3 The Y-shaped immunoglobulin molecule can be dissected by partial digestion with proteases.**

Papain cleaves the immunoglobulin molecule into three pieces, two Fab fragments and one Fc fragment (upper panels). The Fab fragment contains the V regions and binds antigen. The Fc fragment is crystallizable and contains C regions. Pepsin cleaves immunoglobulin to yield one F(ab')<sub>2</sub> fragment and many small pieces of the Fc fragment, the largest of which is called the pFc' fragment (lower panels). F(ab')<sub>2</sub> is written with a prime because it contains a few more amino acids than Fab, including the cysteines that form the disulfide bonds.

The protein fragments obtained after proteolysis are determined by where the protease cuts the antibody molecule in relation to the disulfide bonds that link the two heavy chains. These lie in the hinge region between the C<sub>H</sub>1 and C<sub>H</sub>2 domains and, as illustrated in Fig. 3.3, papain cleaves the antibody molecule on the amino-terminal side of the disulfide bonds. This releases the two arms of the antibody as separate Fab fragments, whereas in the Fc fragment the carboxy-terminal halves of the heavy chains remain linked.

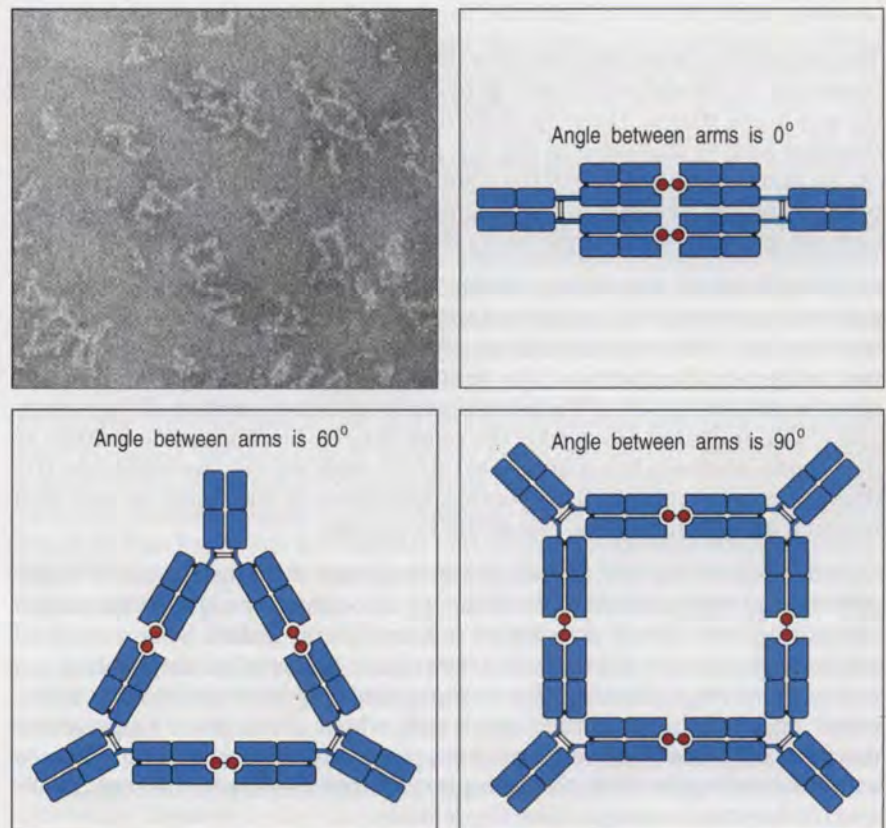
Another protease, pepsin, cuts in the same general region of the antibody molecule as papain but on the carboxy-terminal side of the disulfide bonds (see Fig. 3.3). This produces a fragment, the F(ab')<sub>2</sub> fragment, in which the two antigen-binding arms of the antibody molecule remain linked. In this case the remaining part of the heavy chain is cut into several small fragments. The F(ab')<sub>2</sub> fragment has exactly the same antigen-binding characteristics as the original antibody but is unable to interact with any effector molecule. It is thus of potential value in therapeutic applications of antibodies as well as in research into the functional role of the Fc portion.

Genetic engineering techniques also now permit the construction of many different antibody-related molecules. One important type is a truncated Fab comprising only the V domain of a heavy chain linked by a stretch of synthetic peptide to a V domain of a light chain. This is called **single-chain Fv**, named from Fragment variable. Fv molecules may become valuable therapeutic agents because of their small size, which allows them to penetrate tissues readily. They can be coupled to protein toxins to yield immunotoxins with potential application, for example, in tumor therapy in the case of a Fv specific for a tumor antigen (see Chapter 14).

### 3-4 The immunoglobulin molecule is flexible, especially at the hinge region.

The hinge region that links the Fc and Fab portions of the antibody molecule is in reality a flexible tether, allowing independent movement of the two Fab arms, rather than a rigid hinge. This has been demonstrated by electron microscopy of antibodies bound to **haptens**. These are small molecules of various sorts, typically about the size of a tyrosine side chain. They can be recognized by antibody but are only able to stimulate production of anti-hapten antibodies when linked to a larger protein **carrier** (see Appendix I, Section A-1). An antigen made of two identical hapten molecules joined by a short flexible region can link two or more anti-hapten antibodies, forming dimers, trimers, tetramers, and so on, which can be seen by electron microscopy (Fig. 3.4). The shapes formed by these complexes demonstrate that antibody molecules are flexible at the hinge region. Some flexibility is also found at the junction between the V and C domains, allowing bending and rotation of the V domain relative to the C domain. For example, in the antibody molecule shown in Fig. 3.1a, not only are the two hinge regions clearly bent differently, but the angle between the V and C domains in each of the two Fab arms is also different. This range of motion has led to the junction between the V and C domains being referred to as a 'molecular ball-and-socket joint.' Flexibility at both the hinge and V-C junction enables the binding of both arms of an antibody molecule to sites that are various distances apart, for example, sites on bacterial cell-wall polysaccharides. Flexibility at the hinge also enables the antibodies to interact with the antibody-binding proteins that mediate immune effector mechanisms.

**Fig. 3.4 Antibody arms are joined by a flexible hinge.** An antigen consisting of two hapten molecules (red balls in diagrams) that can cross-link two antigen-binding sites is used to create antigen:antibody complexes, which can be seen in the electron micrograph. Linear, triangular, and square forms are seen, with short projections or spikes. Limited pepsin digestion removes these spikes (not shown in the figure), which therefore correspond to the Fc portion of the antibody; the F(ab')<sub>2</sub> pieces remain cross-linked by antigen. The interpretation of the complexes is shown in the diagrams. The angle between the arms of the antibody molecules varies, from 0° in the antibody dimers, through 60° in the triangular forms, to 90° in the square forms, showing that the connections between the arms are flexible. Photograph (× 300,000) courtesy of N.M. Green.



### 3-5 The domains of an immunoglobulin molecule have similar structures.

As we saw in Section 3-2, immunoglobulin heavy and light chains are composed of a series of discrete protein domains. These protein domains all have a similar folded structure. Within this basic three-dimensional structure, there are distinct differences between V and C domains. The structural similarities and differences can be seen in the diagram of a light chain in Fig. 3.5. Each domain is constructed from two  $\beta$  sheets, which are elements of protein structure made up of strands of the polypeptide chain ( $\beta$  strands) packed together; the sheets are linked by a disulfide bridge and together form a roughly barrel-shaped structure, known as a  $\beta$  barrel. The distinctive folded structure of the immunoglobulin protein domain is known as the **immunoglobulin fold**.

Both the essential similarity of V and C domains and the critical difference between them are most clearly seen in the bottom panels of Fig. 3.5, where the cylindrical domains are opened out to reveal how the polypeptide chain folds to create each of the  $\beta$  sheets and how it forms flexible loops as it changes direction. The main difference between the V and C domains is that the V domain is larger, with an extra loop. We will see in Section 3-6 that the flexible loops of the V domains form the antigen-binding site of the immunoglobulin molecule.

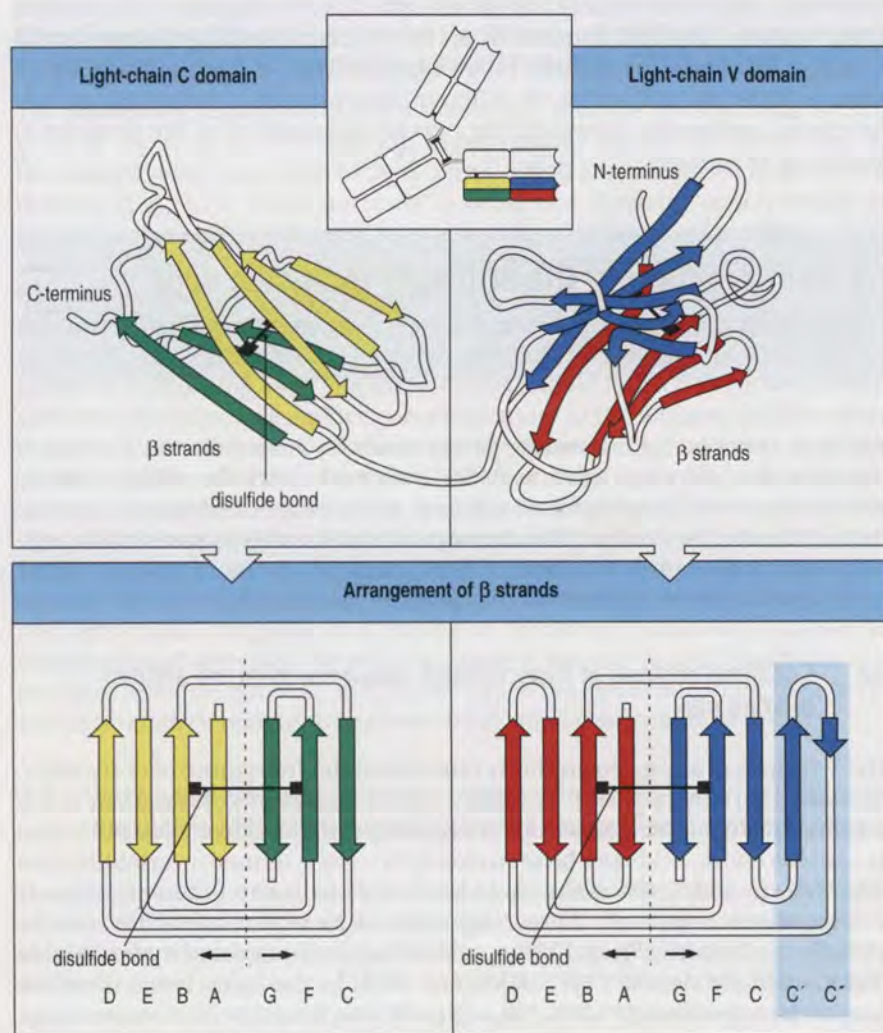


**Fig. 3.5 The structure of immunoglobulin constant and variable domains.** The upper panels show schematically the folding pattern of the constant (C) and variable (V) domains of an immunoglobulin light chain. Each domain is a barrel-shaped structure in which strands of polypeptide chain ( $\beta$  strands) running in opposite directions (antiparallel) pack together to form two  $\beta$  sheets (shown in yellow and green in the diagram of the C domain), which are held together by a disulfide bond. The way the polypeptide chain folds to give the final structure can be seen more clearly when the sheets are opened out, as shown in the lower panels. The  $\beta$  strands are lettered sequentially with respect to the order of their occurrence in the amino acid sequence of the domains; the order in each  $\beta$  sheet is characteristic of immunoglobulin domains. The  $\beta$  strands C' and C'' that are found in the V domains but not in the C domains are indicated by a blue shaded background. The characteristic four-strand plus three-strand (C-region type domain) or four-strand plus five-strand (V-region type domain) arrangements are typical immunoglobulin superfamily domain building blocks, found in a whole range of other proteins as well as antibodies and T-cell receptors.

Many of the amino acids that are common to C and V domains of immunoglobulin chains lie in the core of the immunoglobulin fold and are critical to its stability. For that reason, other proteins having sequences similar to those of immunoglobulins are believed to form domains of similar structure, and in many cases this has been demonstrated by crystallography. These **immunoglobulin-like domains** are present in many other proteins of the immune system, and in proteins involved in cell-cell recognition in the nervous system and other tissues. Together with the immunoglobulins and the T-cell receptors, they make up the extensive **immunoglobulin superfamily**.

### Summary.

The IgG antibody molecule is made up of four polypeptide chains, comprising two identical light chains and two identical heavy chains, and can be thought of as forming a flexible Y-shaped structure. Each of the four chains has a variable (V) region at its amino terminus, which contributes to the antigen-binding site, and a constant (C) region, which determines the isotype. The isotype of the heavy chain determines the functional properties of the antibody. The light chains are bound to the heavy chains by many non-covalent interactions and by disulfide bonds, and the V regions of the heavy and light chains pair in each arm of the Y to generate two identical antigen-binding sites, which lie at the tips of the arms of the Y. The possession of two antigen-binding sites allows antibody molecules to cross-link antigens and to bind them much more stably. The trunk of the Y, or Fc fragment, is composed of the carboxy-terminal domains of the heavy chains. Joining the arms of the Y to the trunk are the flexible hinge regions. The Fc fragment and hinge regions differ in antibodies of different isotypes, thus determining their functional properties. However, the overall organization of the domains is similar in all isotypes.

### The interaction of the antibody molecule with specific antigen.

We have described the structure of the antibody molecule and how the V regions of the heavy and light chains fold and pair to form the antigen-binding site. In this part of the chapter we will look at the antigen-binding site in more detail. We will discuss the different ways in which antigens can bind to antibody and address the question of how variation in the sequences of the antibody V domains determines the specificity for antigen.

#### 3-6 Localized regions of hypervariable sequence form the antigen-binding site.

The V regions of any given antibody molecule differ from those of every other. Sequence variability is not, however, distributed evenly throughout the V regions but is concentrated in certain segments of the V region. The distribution of variable amino acids can be seen clearly in what is termed a **variability plot** (Fig. 3.6), in which the amino acid sequences of many different antibody V regions are compared. Three segments of particular variability can be identified in both the  $V_H$  and  $V_L$  domains. They are designated **hypervariable regions** and are denoted HV1, HV2, and HV3. In the light chains these are roughly from residues 28 to 35, from 49 to 59, and from 92 to 103, respectively.

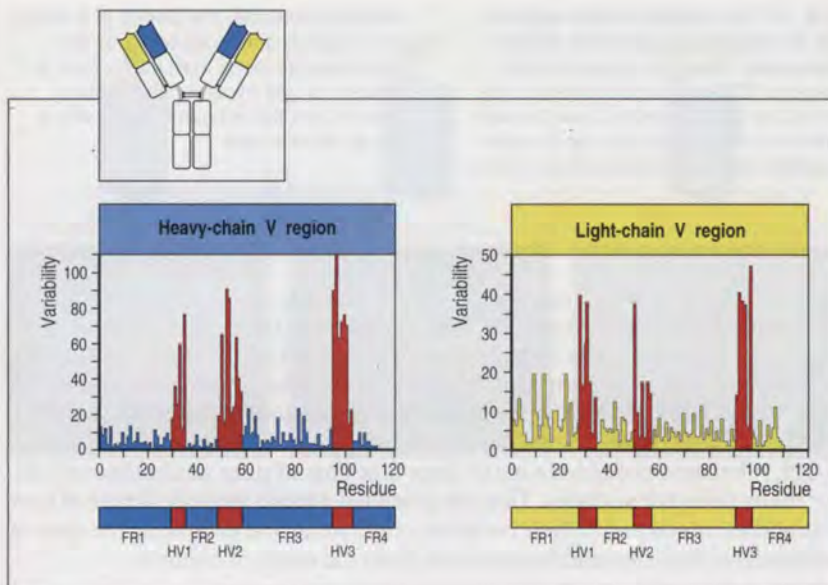


**Fig. 3.6** There are discrete regions of hypervariability in V domains. A variability plot derived from comparison of the amino acid sequences of several dozen heavy-chain and light-chain V domains. At each amino acid position the degree of variability is the ratio of the number of different amino acids seen in all of the sequences together to the frequency of the most common amino acid. Three hypervariable regions (HV1, HV2, and HV3) are indicated in red and are also known as the complementarity-determining regions, CDR1, CDR2, and CDR3. They are flanked by less variable framework regions (FR1, FR2, FR3, and FR4, shown in blue or yellow).

The most variable part of the domain is in the HV3 region. The regions between the hypervariable regions, which comprise the rest of the V domain, show less variability and are termed the **framework regions**. There are four such regions in each V domain, designated FR1, FR2, FR3, and FR4.

The framework regions form the  $\beta$  sheets that provide the structural framework of the domain, whereas the hypervariable sequences correspond to three loops at the outer edge of the  $\beta$  barrel, which are juxtaposed in the folded domain (Fig. 3.7). Thus, not only is sequence diversity concentrated in particular parts of the V domain but it is localized to a particular region on the surface of the molecule. When the  $V_H$  and  $V_L$  domains are paired in the antibody molecule, the hypervariable loops from each domain are brought together, creating a single hypervariable site at the tip of each arm of the molecule. This is the binding site for antigen, the **antigen-binding site** or **antibody combining site**. The three hypervariable loops determine antigen specificity by forming a surface complementary to the antigen, and are more commonly termed the **complementarity-determining regions**, or CDRs (CDR1, CDR2, and CDR3). Because CDRs from both  $V_H$  and  $V_L$  domains contribute to the antigen-binding site, it is the combination of the heavy and the light chain, and not either alone, that determines the final antigen specificity. Thus, one way in which the immune system is able to generate antibodies of different specificities is by generating different combinations of heavy- and light-chain V regions. This means of producing variability is known as **combinatorial diversity**; we will encounter a second form of combinatorial diversity when we consider in Chapter 4 how the genes encoding the heavy- and light-chain V regions are created from smaller segments of DNA.

### 3-7 Antibodies bind antigens via contacts with amino acids in CDRs, but the details of binding depend upon the size and shape of the antigen.

In early investigations of antigen binding to antibodies, the only available sources of large quantities of a single type of antibody molecule were tumors of antibody-secreting cells. The antigen specificities of the tumor-derived antibodies were unknown, so many compounds had to be screened to identify ligands that could be used to study antigen binding. In general, the substances

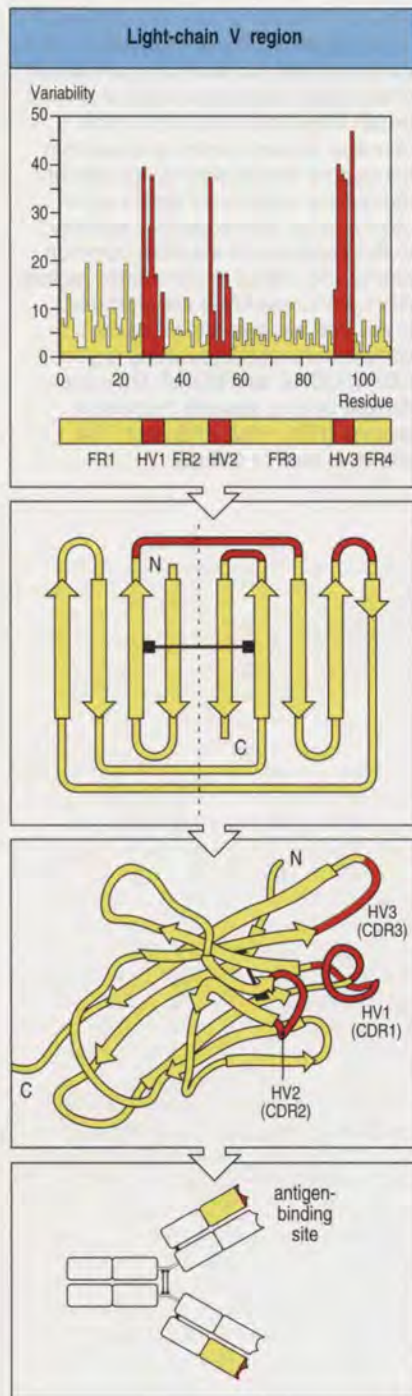


**Fig. 3.7 The hypervariable regions lie in discrete loops of the folded structure.** When the hypervariable regions (CDRs) are positioned on the structure of a V domain it can be seen that they lie in loops that are brought together in the folded structure. In the

antibody molecule, the pairing of a heavy and a light chain brings together the hypervariable loops from each chain to create a single hypervariable surface, which forms the antigen-binding site at the tip of each arm.

found to bind to these antibodies were haptens (see Section 3-4) such as phosphorylcholine or vitamin K<sub>1</sub>. Structural analysis of complexes of antibodies with their hapten ligands provided the first direct evidence that the hypervariable regions form the antigen-binding site, and demonstrated the structural basis of specificity for the hapten. Subsequently, with the discovery of methods of generating monoclonal antibodies (see Appendix I, Section A-12), it became possible to make large amounts of pure antibodies specific for many different antigens. This has provided a more general picture of how antibodies interact with their antigens, confirming and extending the view of antibody-antigen interactions derived from the study of haptens.

The surface of the antibody molecule formed by the juxtaposition of the CDRs of the heavy and light chains creates the site to which an antigen binds. Clearly, as the amino acid sequences of the CDRs are different in different antibodies, so are the shapes of the surfaces created by these CDRs. As a general principle, antibodies bind ligands whose surfaces are complementary to that of the antibody. A small antigen, such as a hapten or a short peptide, generally binds in a pocket or groove lying between the heavy- and light-chain V domains (Fig. 3.8, left and center panels). Other antigens, such as a protein molecule, can be of the same size as, or larger than, the antibody molecule itself, and cannot fit into a groove or pocket. In these cases, the interface between the two molecules is often an extended surface that involves all of the CDRs and, in some cases, part of the framework region of the antibody (Fig. 3.8, right panel). This surface need not be concave but can be flat, undulating, or even convex.

### 3-8 Antibodies bind to conformational shapes on the surfaces of antigens.

The biological function of antibodies is to bind to pathogens and their products, and to facilitate their removal from the body. An antibody generally recognizes only a small region on the surface of a large molecule such as a polysaccharide or protein. The structure recognized by an antibody is called an **antigenic determinant** or **epitope**. Some of the most important pathogens have polysaccharide coats, and antibodies that recognize epitopes formed by the sugar subunits of these molecules are essential in providing immune protection from such pathogens. In many cases, however, the antigens that provoke an immune response are proteins. For example, protective antibodies against viruses recognize viral coat proteins. In such cases, the structures recognized by the antibody are located on the surface of the protein. Such sites are likely to be composed of amino acids from different parts of the polypeptide chain that have been brought together by protein folding. Antigenic determinants of this kind are known as **conformational** or **discontinuous epitopes** because the structure recognized is composed of segments of the protein that are discontinuous in the amino acid sequence of the antigen but are brought together in the three-dimensional structure. In contrast, an epitope composed of a single segment of polypeptide chain is termed a **continuous** or **linear epitope**. Although most antibodies raised

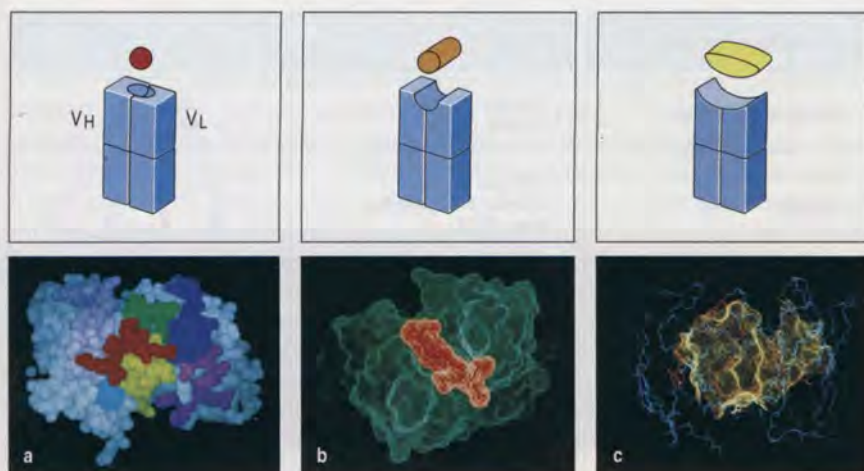


**Fig. 3.8 Antigens can bind in pockets or grooves, or on extended surfaces in the binding sites of antibodies.**

The panels in the top row show schematic representations of the different types of binding site in a Fab fragment of an antibody: left, pocket; center, groove; right, extended surface. Below are examples of each type. Panel a: space-filling representation of the interaction of a small peptide antigen with the complementarity-determining regions (CDRs) of a Fab fragment as viewed looking into the antigen-binding site. Seven amino acid residues of the antigen, shown in red, are bound in the antigen-binding pocket. Five of the six CDRs (H1, H2, H3, L1, and L3) interact with the peptide, whereas L2 does not. The CDR loops are colored as follows: L2, magenta; L3, green; H1, blue; H2, pale purple; H3, yellow. Panel b: in a complex of an antibody with a peptide from the human immunodeficiency virus, the peptide (orange) binds along a groove formed between the heavy- and light-chain V domains (green). Panel c: complex between hen egg-white lysozyme and the Fab fragment of its corresponding antibody (HyHel5). Two extended surfaces come into contact, as can be seen from this computer-generated image, where the surface contour of the lysozyme molecule (yellow dots) is superimposed on the antigen-binding site. Residues in the antibody that make contact with the lysozyme are shown in full (red); for the rest of the Fab fragment only the peptide backbone is shown (blue). All six CDRs of the antibody are involved in the binding. Photographs a and b courtesy of I.A. Wilson and R.L. Stanfield. Photograph c courtesy of S. Sheriff.

against intact, fully folded proteins recognize discontinuous epitopes, some will bind peptide fragments of the protein. Conversely, antibodies raised against peptides of a protein or against synthetic peptides corresponding to part of its sequence are occasionally found to bind to the natural folded protein. This makes it possible, in some cases, to use synthetic peptides in vaccines that aim at raising antibodies against a pathogen protein.

### 3-9 Antigen-antibody interactions involve a variety of forces.

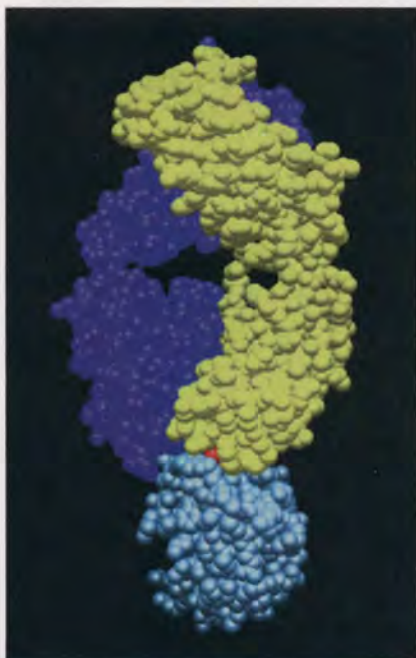
The interaction between an antibody and its antigen can be disrupted by high salt concentrations, extremes of pH, detergents, and sometimes by competition with high concentrations of the pure epitope itself. The binding is therefore a reversible noncovalent interaction. The forces, or bonds, involved in these noncovalent interactions are outlined in Fig. 3.9.

Electrostatic interactions occur between charged amino acid side chains, as in salt bridges. Interactions also occur between electric dipoles, as in hydrogen bonds, or can involve short-range van der Waals forces. High salt concentrations and extremes of pH disrupt antigen-antibody binding by weakening electrostatic interactions and/or hydrogen bonds. This principle is employed in the purification of antigens using affinity columns of immobilized antibodies, and vice versa for antibody purification (see Appendix I, Section A-5). Hydrophobic interactions occur when two hydrophobic surfaces come together to exclude water. The strength of a hydrophobic interaction is proportional to the surface area that is hidden from water. For some antigens, hydrophobic interactions probably account for most of the binding energy. In some cases, water molecules are trapped in pockets in the interface between antigen and antibody. These trapped water molecules may also contribute to binding, especially between polar amino acid residues.

The contribution of each of these forces to the overall interaction depends on the particular antibody and antigen involved. A striking difference between antibody interactions with protein antigens and most other natural protein-protein interactions is that antibodies possess many aromatic amino acids in their antigen-binding sites. These amino acids participate mainly in van der Waals and hydrophobic interactions, and sometimes in hydrogen bonds. In general, the hydrophobic and van der Waals forces operate over very

**Fig. 3.9 The noncovalent forces that hold together the antigen:antibody complex.** Partial charges found in electric dipoles are shown as  $\delta^+$  or  $\delta^-$ . Electrostatic forces diminish as the inverse square of the distance separating the charges, whereas van der Waals forces, which are more numerous in most antigen–antibody contacts, fall off as the sixth power of the separation and therefore operate only over very short ranges. Covalent bonds never occur between antigens and naturally produced antibodies.

| Noncovalent forces   | Origin                                                                                                                                                     |                                                                                                                                                                                                         |
|----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Electrostatic forces | Attraction between opposite charges                                                                                                                        | $-\text{NH}_3^+ \quad \text{OOC}^-$                                                                                                                                                                     |
| Hydrogen bonds       | Hydrogen shared between electronegative atoms (N,O)                                                                                                        | $\begin{array}{c} > \text{N} - \text{H} - - \text{O} = \text{C} < \\ \delta^- \quad \delta^+ \quad \delta^- \end{array}$                                                                                |
| Van der Waals forces | Fluctuations in electron clouds around molecules oppositely polarize neighboring atoms                                                                     | $\begin{array}{cc} \delta^+ & \longleftrightarrow & \delta^- \\ \delta^- & \longleftrightarrow & \delta^+ \end{array}$                                                                                  |
| Hydrophobic forces   | Hydrophobic groups interact unfavorably with water and tend to pack together to exclude water molecules. The attraction also involves van der Waals forces | $\begin{array}{c} \text{H} > \text{O} & \text{H} & \text{O} & \text{H} \\ & \delta^+ & & \delta^- & \\ & \delta^- & & \delta^+ & \\ & \text{O} & & \text{O} & \\ & \text{H} & & \text{H} & \end{array}$ |



short ranges and serve to pull together two surfaces that are complementary in shape: hills on one surface must fit into valleys on the other for good binding to occur. In contrast, electrostatic interactions between charged side chains, and hydrogen bonds bridging oxygen and/or nitrogen atoms, accommodate specific features or reactive groups while strengthening the interaction overall.

For example, in the complex of hen egg-white lysozyme with the antibody D1.3 (Fig. 3.10), strong hydrogen bonds are formed between the antibody and a particular glutamine in the lysozyme molecule that protrudes between the  $V_H$  and  $V_L$  domains. Lysozymes from partridge and turkey have another amino acid in place of the glutamine and do not bind to the antibody. In the high-affinity complex of hen egg-white lysozyme with another antibody, HyHel5 (see Fig. 3.8c), two salt bridges between two basic arginines on the surface of the lysozyme interact with two glutamic acids, one each from the  $V_H$  CDR1 and CDR2 loops. Again, lysozymes that lack one of the two arginine residues show a 1000-fold decrease in affinity. Although overall surface complementarity must play an important part in antigen–antibody interactions, specific electrostatic and hydrogen-bonding interactions appear to determine antibody affinity. In most antibodies that have been studied at this level of detail, only a few residues make a major contribution to the binding energy. Genetic engineering by site-directed mutagenesis can further tailor an antibody's binding to its complementary epitope.

**Fig. 3.10 The complex of lysozyme with the antibody D1.3.** The interaction of the Fab fragment of D1.3 with hen egg-white lysozyme is shown, with the lysozyme in blue, the heavy chain in purple and the light chain in yellow.

A glutamine residue of lysozyme, shown in red, protrudes between the two V domains of the antigen-binding site and makes hydrogen bonds important to the antigen–antibody binding. Original photograph courtesy of R.J. Poljak.

## Summary.

X-ray crystallographic analysis of antigen:antibody complexes has demonstrated that the hypervariable loops (complementarity-determining regions) of immunoglobulin V regions determine the specificity of antibodies. With protein antigens, the antibody molecule contacts the antigen over a broad area of its surface that is complementary to the surface recognized on the antigen. Electrostatic interactions, hydrogen bonds, van der Waals forces, and hydrophobic interactions can all contribute to binding. Amino acid side chains in most or all of the hypervariable loops make contact with antigen and determine both the specificity and the affinity of the interaction. Other parts of the V region play little part in the direct contact with the antigen but provide a stable structural framework for the hypervariable loops and help determine their position and conformation. Antibodies raised against intact proteins usually bind to the surface of the protein and make contact with residues that are discontinuous in the primary structure of the molecule; they may, however, occasionally bind peptide fragments of the protein, and antibodies raised against peptides derived from a protein can sometimes be used to detect the native protein molecule. Peptides binding to antibodies usually bind in the cleft between the V regions of the heavy and light chains, where they make specific contact with some, but not necessarily all, of the hypervariable loops. This is also the usual mode of binding for carbohydrate antigens and small molecules such as haptens.

## Antigen recognition by T cells.

In contrast to the immunoglobulins, which interact with pathogens and their toxic products in the extracellular spaces of the body, T cells only recognize foreign antigens that are displayed on the surfaces of the body's own cells. These antigens can derive from pathogens such as viruses or intracellular bacteria, which replicate within cells, or from pathogens or their products that cells have internalized by endocytosis from the extracellular fluid.

T cells can detect the presence of an intracellular pathogen because infected cells display on their surface peptide fragments derived from the pathogen's proteins. These foreign peptides are delivered to the cell surface by specialized host-cell glycoproteins. These are encoded in a large cluster of genes that were first identified by their powerful effects on the immune response to transplanted tissues. For that reason, the gene complex was called the major histocompatibility complex (MHC), and the peptide-binding glycoproteins are still known as MHC molecules. The recognition of antigen as a small peptide fragment bound to an MHC molecule and displayed at the cell surface is one of the most distinctive features of T cells, and will be the focus of this part of the chapter. How peptide fragments of antigen become complexed with MHC molecules will be considered in Chapter 5.

In this part of the chapter we will describe the structure and properties of the T-cell antigen receptor, T-cell receptor, or TCR for short. As might be expected from their function as highly variable antigen-recognition structures, T-cell receptors are closely related to antibody molecules in the structure of their genes. There are, however, important differences between T-cell receptors and immunoglobulins that reflect the special features of antigen recognition by the T-cell receptor, and its lack of effector functions.

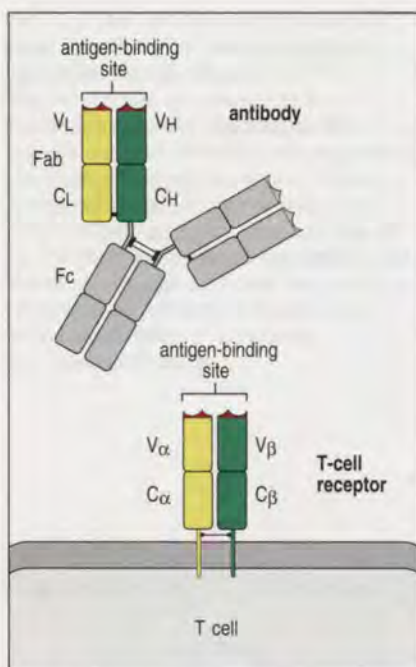
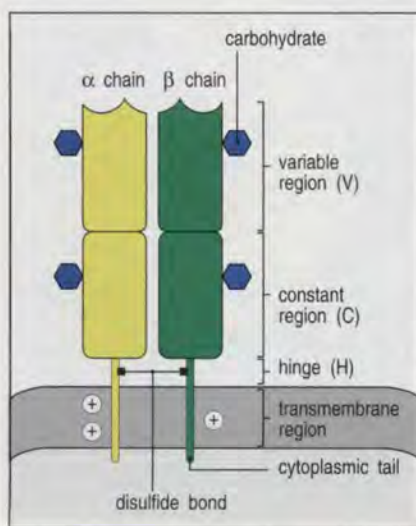


**Fig. 3.11 The T-cell receptor resembles a membrane-bound Fab fragment.** The Fab fragment of antibody molecules is a disulfide-linked heterodimer, each chain of which contains one immunoglobulin C domain and one V domain; the juxtaposition of the V domains forms the antigen-binding site (see Section 3-6). The T-cell receptor is also a disulfide-linked heterodimer, with each chain containing an immunoglobulin C-like domain and an immunoglobulin V-like domain. As in the Fab fragment, the juxtaposition of the V domains forms the site for antigen recognition.



### 3-10 The antigen receptor on T cells is very similar to a Fab fragment of immunoglobulin.

T-cell receptors were first identified using monoclonal antibodies that bound only one cloned T-cell line but not others and that could specifically inhibit antigen recognition by that clone of T cells, or specifically activate them (see Appendix I, Section A-19). These **clonotypic** antibodies were then used to show that each T cell bears about 30,000 antigen-receptor molecules on its surface, each receptor consisting of two different polypeptide chains, termed the **T-cell receptor  $\alpha$  (TCR $\alpha$ )** and  **$\beta$  (TCR $\beta$ ) chains**, linked by a disulfide bond. These  $\alpha\beta$  heterodimers are very similar in structure to the Fab fragment of an immunoglobulin molecule (Fig. 3.11), and they account for antigen recognition by most T cells. A minority of T cells bear an alternative, but structurally similar, receptor made up of a different pair of polypeptide chains designated  $\gamma$  and  $\delta$ .  **$\gamma\delta$  T-cell receptors** appear to have different antigen-recognition properties from the  $\alpha\beta$  T-cell receptors, and the function of  $\gamma\delta$  T cells in immune responses is not yet entirely clear. In the rest of this chapter, we shall use the term T-cell receptor to mean the  $\alpha\beta$  receptor, except where specified otherwise. Both types of T-cell receptor differ from the membrane-bound immunoglobulin that serves as the B-cell receptor: a T-cell receptor has only one antigen-binding site, whereas a B-cell receptor has two, and T-cell receptors are **never secreted**, whereas immunoglobulin can be secreted as antibody.

Our initial insights into the structure and function of the  $\alpha\beta$  T-cell receptor came from studies of cloned cDNA encoding the receptor chains. The amino acid sequences predicted from T-cell receptor cDNAs show clearly that both chains of the T-cell receptor have an amino-terminal variable (V) region with homology to an immunoglobulin V domain, a constant (C) region with homology to an immunoglobulin C domain, and a short hinge region containing a cysteine residue that forms the interchain disulfide bond (Fig. 3.12). Each chain spans the lipid bilayer by a hydrophobic transmembrane domain, and ends in a short cytoplasmic tail. These close similarities of T-cell receptor chains to the heavy and light immunoglobulin chains first enabled prediction of the structural resemblance of the T-cell receptor heterodimer to a Fab fragment of immunoglobulin.

Recently, the three-dimensional structure of the T-cell receptor has been determined. The structure is indeed similar to that of an antibody Fab fragment, as was suspected from earlier studies on the genes that encoded it. The T-cell receptor chains fold in much the same way as those of a Fab fragment (Fig. 3.13a), although the final structure appears a little shorter and wider. There are, however, some distinct differences between T-cell receptors and Fab fragments. The most striking difference is in the  **$C_\alpha$  domain**, where the fold is unlike that of any other immunoglobulin-like domain. The half of the domain that is juxtaposed with the  $C_\beta$  domain forms a  $\beta$  sheet similar to that

**Fig. 3.12 Structure of the T-cell receptor.** The T-cell receptor heterodimer is composed of two transmembrane glycoprotein chains,  $\alpha$  and  $\beta$ . The extracellular portion of each chain consists of two domains, resembling immunoglobulin V and C domains, respectively. Both chains have carbohydrate side chains attached to each domain. A short segment, analogous to an immunoglobulin hinge region,

connects the immunoglobulin-like domains to the membrane and contains the cysteine residue that forms the interchain disulfide bond. The transmembrane helices of both chains are unusual in containing positively charged (basic) residues within the hydrophobic transmembrane segment. The  $\alpha$  chains carry two such residues; the  $\beta$  chains have one.

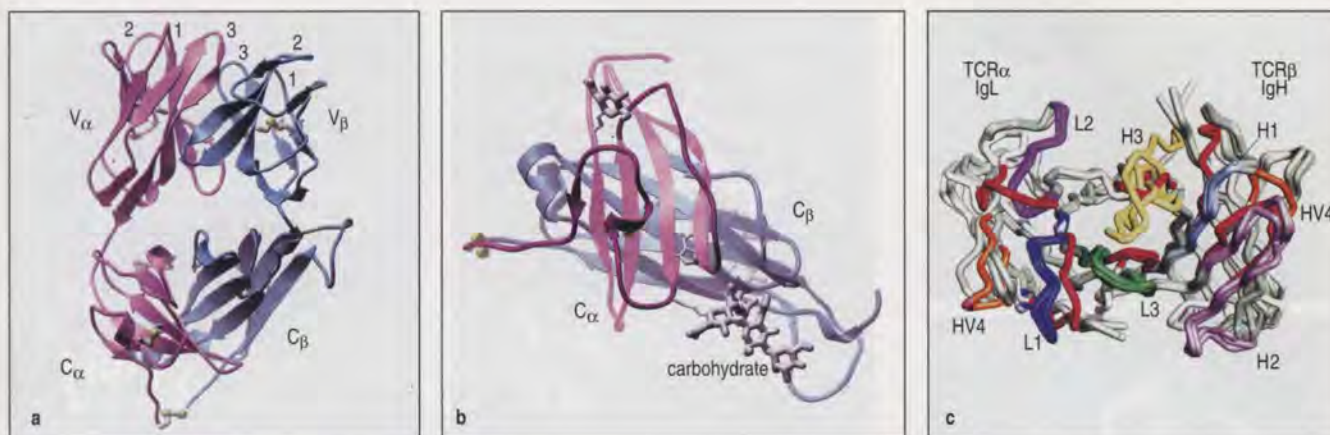


**Fig. 3.13 The crystal structure of an  $\alpha\beta$  T-cell receptor resolved at 2.5 Å.**

In panels a and b the  $\alpha$  chain is shown in pink and the  $\beta$  chain in blue. Disulfide bonds are shown in green. In panel a, the T-cell receptor is viewed from the side as it would sit on a cell surface, with the CDR loops that form the antigen-binding site (labeled 1, 2, and 3) arrayed across its relatively flat top. In panel b, the  $C_\alpha$  and  $C_\beta$  domains are shown. The  $C_\alpha$  domain does not fold into a typical immunoglobulin-like domain; the face of the domain away from the  $C_\beta$  domain is mainly composed of irregular strands of polypeptide rather than  $\beta$  sheet. The intramolecular disulfide bond joins a  $\beta$  strand to this segment of  $\alpha$  helix. The interaction between the  $C_\alpha$  and  $C_\beta$  domains is assisted by carbohydrate (colored grey and labeled on the figure), with a sugar group from the  $C_\alpha$  domain making hydrogen bonds to the  $C_\beta$  domain. In panel c, the T-cell receptor is shown aligned with the antigen-binding sites from three different antibodies. This view is looking down into the binding site. The  $V_\alpha$  domain of the T-cell receptor is aligned with the  $V_L$  domains of the antigen-binding sites of the antibodies, and the  $V_\beta$  domain is aligned with the  $V_H$  domains. The CDRs of the T-cell receptor and immunoglobulin molecules are colored, with CDRs 1, 2, and 3 of the TCR shown in red and the HV4 loop in orange. For the immunoglobulin V domains, the CDR1 loops of the heavy chain (H1) and light chain (L1) are shown in light and dark blue, respectively, and the CDR2 loops (H2, L2) in light and dark purple, respectively. The heavy-chain CDR3 loops (H3) are in yellow; the light-chain CDR3s (L3) are in bright green. The HV4 loops of the TCR (orange) have no hypervariable counterparts in immunoglobulins. Photographs courtesy of I.A. Wilson.

found in other immunoglobulin-like domains, but the other half of the domain is formed of loosely packed strands and a short segment of  $\alpha$  helix (Fig. 3.13b). The intramolecular disulfide bond, which in immunoglobulin-like domains normally joins two  $\beta$  strands, in a  $C_\alpha$  domain joins a  $\beta$  strand to this segment of  $\alpha$  helix.

There are also differences in the way in which the domains interact. The interface between the V and C domains of both T-cell receptor chains is more extensive than in antibodies, which may make the hinge joint between the domains less flexible. And the interaction between the  $C_\alpha$  and  $C_\beta$  domains is distinctive in being assisted by carbohydrate, with a sugar group from the  $C_\alpha$  domain making a number of hydrogen bonds to the  $C_\beta$  domain (see Fig. 3.13b). Finally, a comparison of the variable binding sites shows that, although the complementarity-determining region (CDR) loops align fairly closely with those of antibody molecules, there is some displacement relative to those of the antibody molecule (Fig. 3.13c). This displacement is particularly marked in the  $V_\alpha$  CDR2 loop, which is oriented at roughly right angles to the equivalent loop in antibody V domains, as a result of a shift in the  $\beta$  strand that anchors one end of the loop from one face of the domain to the other. A strand displacement also causes a change in the orientation of the  $V_\beta$  CDR2 loop in two of the seven  $V_\beta$  domains whose structures are known. As yet, the crystallographic structures of only seven T-cell receptors have been solved to this level of resolution, so it remains to be seen to what degree all T-cell receptors share these features, and whether there is more variability to be discovered.

### 3-11 A T-cell receptor recognizes antigen in the form of a complex of a foreign peptide bound to an MHC molecule.

Antigen recognition by T-cell receptors clearly differs from recognition by B-cell receptors and antibodies. Antigen recognition by B cells involves direct binding of immunoglobulin to the intact antigen and, as discussed in Section 3-8, antibodies typically bind to the surface of protein antigens, contacting amino acids that are discontinuous in the primary structure but are brought together in the folded protein. T cells, on the other hand, were found to respond to short contiguous amino acid sequences in proteins. These sequences were often buried within the native structure of the protein and thus could not be recognized directly by T-cell receptors unless some unfolding of the protein antigen and its 'processing' into peptide fragments had occurred (Fig. 3.14).

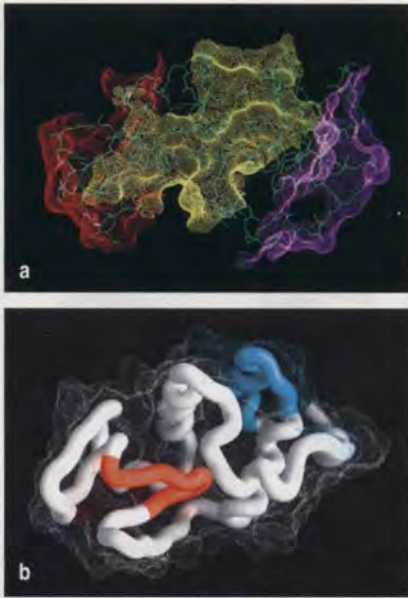


**Fig. 3.14 Differences in the recognition of hen egg-white lysozyme by immunoglobulins and T-cell receptors.** Antibodies can be shown by X-ray crystallography to bind epitopes on the surface of proteins, as shown in panel a, where the epitopes for three antibodies are shown on the surface of hen egg lysozyme (see also Fig. 3.10). In contrast, the epitopes recognized by T-cell receptors need not lie on the surface of the molecule, as the T-cell receptor

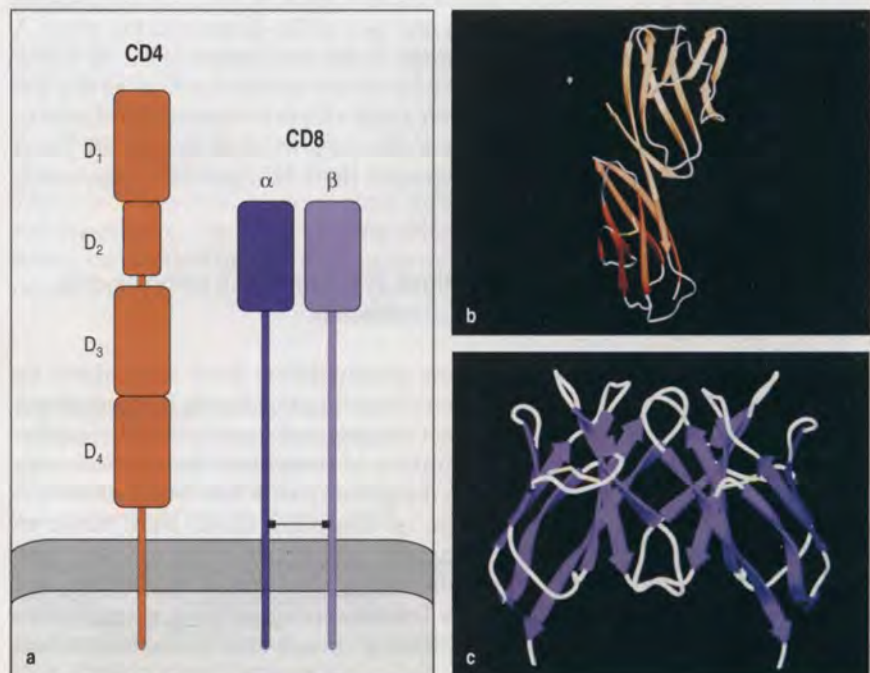
recognizes not the antigenic protein itself but a peptide fragment of the protein. The peptides corresponding to two T-cell epitopes of lysozyme are shown in panel b, one epitope, shown in blue, lies on the surface of the protein but a second, shown in red, lies mostly within the core and is inaccessible in the folded protein. For this residue to be accessible to the T-cell receptor, the protein must be unfolded and processed. Panel a courtesy of S. Sheriff.

The nature of the antigen recognized by T cells became clear with the realization that the peptides that stimulate T cells are recognized only when bound to an MHC molecule. These cell-surface glycoproteins are encoded by genes within the major histocompatibility complex (MHC). The ligand recognized by the T cell is thus a complex of peptide and MHC molecule. The evidence for involvement of the MHC in T-cell recognition of antigen was at first indirect, but it has recently been proved conclusively by stimulating T cells with purified peptide:MHC complexes. The T-cell receptor interacts with this ligand by making contacts with both the MHC molecule and the antigen peptide.

### 3-12 T cells with different functions are distinguished by CD4 and CD8 cell-surface proteins and recognize peptides bound to different classes of MHC molecule.

T cells fall into two major classes that have different effector functions. The two classes are distinguished by the expression of the cell-surface proteins **CD4** and **CD8**. These two types of T cell differ in the class of MHC molecule they recognize. There are two classes of MHC molecule—**MHC class I** and **MHC class II**—which differ in their structure and expression pattern on

**Fig. 3.15 The outline structures of the CD4 and CD8 co-receptor molecules.** The CD4 molecule contains four immunoglobulin-like domains, as shown in diagrammatic form in panel a, and as a ribbon diagram of the structure in panel b. The amino-terminal domain, D<sub>1</sub>, is similar in structure to an immunoglobulin V domain. The second domain, D<sub>2</sub>, although clearly related to an immunoglobulin domain, is different from both V and C domains and has been termed a C2 domain. The first two domains of CD4 form a rigid rodlike structure that is linked to the two carboxy-terminal domains by a flexible link. The binding site for MHC class II molecules is thought to involve both the D<sub>1</sub> and D<sub>2</sub> domains. The CD8 molecule (panels a and c) is a heterodimer of an  $\alpha$  and a  $\beta$  chain covalently linked by a disulfide bond; an alternative form of CD8 exists as a homodimer of  $\alpha$  chains. The heterodimer is depicted in panel a, while the ribbon diagram in panel c is of the homodimer. CD8 $\alpha$  and CD8 $\beta$  chains have very similar structures, each having a single domain resembling an immunoglobulin V domain and a stretch of polypeptide chain, believed to be in a relatively extended conformation, that anchors the V-like domain to the cell membrane.



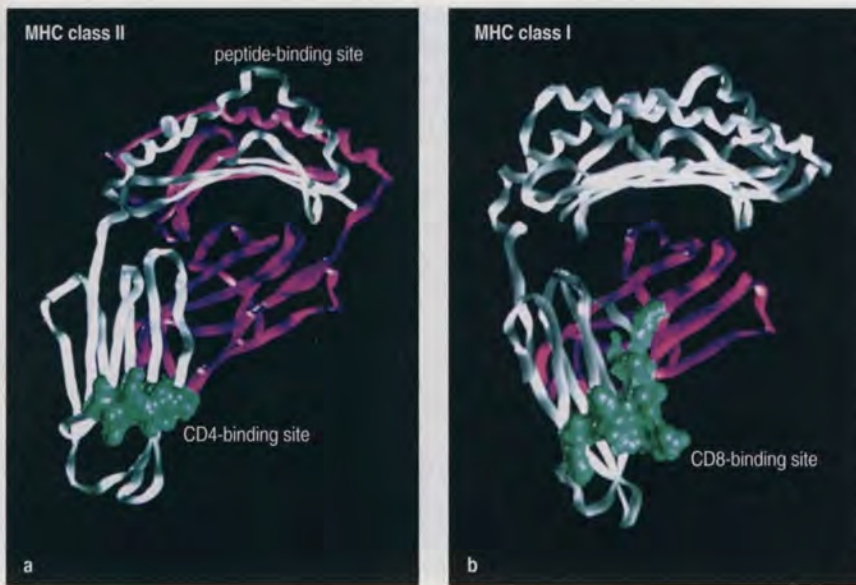


**Fig. 3.16** The binding sites for CD4 and CD8 on MHC class II and class I molecules lie in the immunoglobulin-like domains. The binding sites for CD4 and CD8 on the MHC class II and class I molecules, respectively, lie in the immunoglobulin-like domains nearest to the membrane and distant from the peptide-binding cleft. In panel a, the binding site for CD4 is shown as a bright green surface. It lies at the base of the  $\beta_2$  domain of an MHC class II molecule and is distant from the peptide-binding site at the top of the molecule. The  $\alpha$  chain of the MHC class II molecule is purple, and the  $\beta$  chain is white. In panel b, the binding site for CD8 is also shown as a green surface, at the base of the  $\alpha_3$  domain of the MHC class I molecule. The  $\alpha$  chain of the class I molecule is white, and the  $\beta_2$ -microglobulin is purple. Photographs courtesy of C. Thorpe.

tissues of the body (see Section 3-13). CD4 and CD8 were known as markers for different functional sets of T cells for some time before it became clear that they play an important part in the direct recognition of MHC class II and MHC class I molecules, respectively. CD4 binds to the MHC class II molecule and CD8 to the MHC class I molecule. During antigen recognition, depending on the type of T-cell, CD4 or CD8 molecules associate on the T-cell surface with the T-cell receptor and bind to invariant sites on the MHC portion of the composite MHC:peptide ligand. This binding is required for the T cell to make an effective response, and so CD4 and CD8 are called **co-receptors**.

CD4 is a single-chain molecule composed of four immunoglobulin-like domains (Fig. 3.15). The first two domains ( $D_1$  and  $D_2$ ) of the CD4 molecule are packed tightly together to form a rigid rod some 60 Å long, which is joined by a flexible hinge to a similar rod formed by the third and fourth domains ( $D_3$  and  $D_4$ ). CD4 binds MHC class II molecules through a region that is located mainly on a lateral face of the first domain,  $D_1$ . Because CD4 binds to a site on the  $\beta_2$  domain of the MHC class II molecule that is well away from the site where the T-cell receptor binds (Fig. 3.16a), the CD4 molecule and the T-cell receptor can bind the same peptide:MHC class II complex. CD4 interacts strongly with a cytoplasmic tyrosine kinase called *Lck* and can deliver this tyrosine kinase into close proximity with the signaling components of the T-cell receptor complex. This results in enhancement of the signal that is generated when the T-cell receptor binds its peptide:MHC class II ligand, as we will discuss further in Chapter 6. When CD4 and the T-cell receptor can simultaneously bind to the same MHC class II:peptide complex, the sensitivity of a T cell to antigen presented by MHC class II molecules is markedly increased; the T-cell in this case requires 100-fold less antigen for activation.

CD4 binding to an MHC class II molecule on its own is weak, and it is not clear whether such binding would be able to transmit a signal to the interior of the T cell. As shown in Fig. 3.17, CD4 can form homodimers through a site in the  $D_4$  domain, which leaves the MHC-binding site free to interact with an MHC class II molecule. Thus, the CD4 dimer could cross-link two MHC class II molecules and thus the two T-cell receptors bound to them. Whether the dimerization of CD4 is important in its co-receptor function is not known at present.



**Fig. 3.17** CD4 is capable of forming dimers. The structure of the extra-cellular domains of the CD4 molecule has been determined by X-ray crystallography. Two molecules of CD4 can interact with each other through their  $D_4$  domains, forming homodimers. The site that binds MHC class II molecules remains available in such dimers.



**Fig. 3.18 CD8 binds to a site on MHC class I molecules distant from that to which the T-cell receptor binds.** The relative positions of the T-cell receptor and CD8 molecules bound to the same MHC class I molecule can be seen in this hypothetical reconstruction of the interaction of an MHC class I molecule (the  $\alpha$  chain is shown in green;  $\beta_2$ -microglobulin (dull yellow) can be seen faintly in the background) with a T-cell receptor and CD8. The  $\alpha$  and  $\beta$  chains of the T-cell receptor are shown in pink and purple, respectively. The CD8 structure is that of a CD8 $\alpha$  homodimer, but is colored to represent the likely orientation of the subunits in the heterodimer, with the CD8 $\alpha$  subunit in red and the CD8 $\beta$  subunit in blue. Photograph courtesy of G. Gao.

Although CD4 and CD8 both function as co-receptors, their structures are quite distinct. The CD8 molecule is a disulfide-linked heterodimer consisting of an  $\alpha$  and a  $\beta$  chain, each containing a single immunoglobulin-like domain linked to the membrane by a segment of extended polypeptide chain (see Fig. 3.15). This segment is extensively glycosylated, which is thought to be important in maintaining this polypeptide in an extended conformation and protecting it from cleavage by proteases. CD8 $\alpha$  chains can also form homodimers, although these are not found when the CD8 $\beta$  chains are present.

CD8 binds weakly to an invariant site in the  $\alpha_3$  domain of an MHC class I molecule (Fig. 3.16b), which is equivalent to the site in MHC class II molecules to which CD4 binds. Although only the interaction of the CD8 $\alpha$  homodimer with MHC class I is so far known in detail, it is clear from this that the MHC class I binding site of the CD8  $\alpha\beta$  heterodimer will be formed by the interaction of the CD8 $\alpha$  and  $\beta$  chains. In addition, CD8 (most probably through the  $\alpha$  chain) interacts with residues in the base of the  $\alpha_2$  domain of the MHC class I molecule. Binding in this way, CD8 leaves the upper surface of the MHC class I molecule exposed and free to interact simultaneously with a T-cell receptor, as shown in Fig. 3.18. Like CD4, CD8 also binds Lck through the cytoplasmic tail of the  $\alpha$  chain and brings it into close proximity with the T-cell receptor. And as with CD4, the presence of CD8 increases the sensitivity of T cells to antigen presented by MHC class I molecules by about 100-fold. Thus, CD4 and CD8 have similar functions and bind to the same approximate location in MHC class I and MHC class II molecules even though the structures of the two co-receptor proteins are only distantly related.

### 3-13 The two classes of MHC molecule are expressed differentially on cells.

MHC class I and MHC class II molecules have a distinct distribution among cells that reflects the different effector functions of the T cells that recognize them (Fig. 3.19). MHC class I molecules present peptides from pathogens, commonly viruses, to CD8 cytotoxic T cells, which are specialized to kill any cell that they specifically recognize. As viruses can infect any nucleated cell, almost all such cells express MHC class I molecules, although the level of constitutive expression varies from one cell type to the next. For example, cells of the immune system express abundant MHC class I on their surface, whereas liver cells (hepatocytes) express relatively low levels (see Fig. 3.19). Nonnucleated cells, such as mammalian red blood cells, express little or no MHC class I, and thus the interior of red blood cells is a site in which an infection can go undetected by cytotoxic T cells. As red blood cells cannot support viral replication, this is of no great consequence for viral infection, but it may be the absence of MHC class I that allows the *Plasmodium* species that cause malaria to live in this privileged site.

In contrast, the main function of the CD4 T cells that recognize MHC class II molecules is to activate other effector cells of the immune system. Thus MHC class II molecules are normally found on B lymphocytes, dendritic cells, and macrophages—cells that participate in immune responses—but not on other tissue cells (see Fig. 3.19). When CD4 T cells recognize peptides bound to MHC class II molecules on B cells, they stimulate the B cells to produce antibody. Likewise, CD4 T cells recognizing peptides bound to MHC class II molecules on macrophages activate these cells to destroy the pathogens in their vesicles. We shall see in Chapter 8 that MHC class II molecules are also expressed on specialized antigen-presenting cells in lymphoid tissues where naive T cells encounter antigen and are first activated. The expression of both MHC class I and MHC class II molecules is regulated by cytokines, in particular interferons, released in the course of immune responses. Interferon- $\gamma$  (IFN- $\gamma$ ), for

| Tissue                                                   | MHC class I | MHC class II |
|----------------------------------------------------------|-------------|--------------|
| <b>Lymphoid tissues</b>                                  |             |              |
| T cells                                                  | +++         | +*           |
| B cells                                                  | +++         | +++          |
| Macrophages                                              | +++         | ++           |
| Other antigen-presenting cells<br>(eg Langerhans' cells) | +++         | +++          |
| Epithelial cells of the thymus                           | +           | +++          |
| <b>Other nucleated cells</b>                             |             |              |
| Neutrophils                                              | +++         | —            |
| Hepatocytes                                              | +           | —            |
| Kidney                                                   | +           | —            |
| Brain                                                    | +           | —†           |
| <b>Non-nucleated cells</b>                               |             |              |
| Red blood cells                                          | —           | —            |

**Fig. 3.19 The expression of MHC molecules differs between tissues.** MHC class I molecules are expressed on all nucleated cells, although they are most highly expressed in hematopoietic cells. MHC class II molecules are normally expressed only by a subset of hematopoietic cells and by thymic stromal cells, although they may be expressed by other cell types on exposure to the inflammatory cytokine interferon- $\gamma$ . \*In humans, activated T cells express MHC class II molecules, whereas in mice, all T cells are MHC class II-negative. †In the brain, most cell types are MHC class II-negative but microglia, which are related to macrophages, are MHC class II-positive.

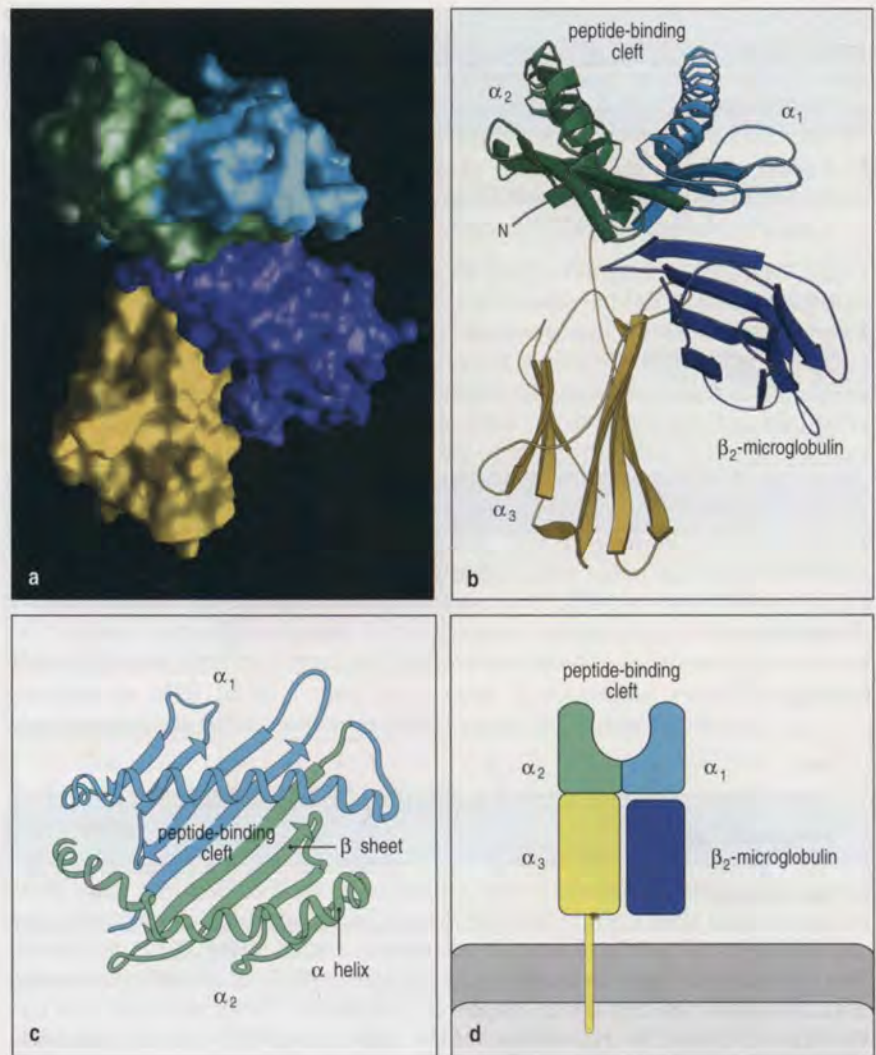
example, increases the expression of MHC class I and MHC class II molecules, and can induce the expression of MHC class II molecules on certain cell types that do not normally express them. Interferons also enhance the antigen-presenting function of MHC class I molecules by inducing the expression of key components of the intracellular machinery that enables peptides to be loaded onto the MHC molecules.

### 3-14 The two classes of MHC molecule have distinct subunit structures but similar three-dimensional structures.

The two classes of MHC molecule differ from each other in their structure and also have different distributions on the cells of the body. Their different structures enable the two classes of MHC molecules to serve distinct functions in antigen presentation, binding peptides from different intracellular sites and activating different subsets of T cells, as we will see in Chapter 5. Despite their differences in subunit structure, however, MHC class I and class II molecules are closely related in overall structure. In both classes, the two paired protein domains nearest the membrane resemble immunoglobulin domains, whereas the two domains distal to the membrane fold together to create a long cleft, or groove, which is the site at which a peptide binds. Purified peptide:MHC class I and peptide:MHC class II complexes have been characterized structurally, allowing us to describe in detail both the MHC molecules themselves and the way in which they bind peptides.

**Fig. 3.20 The structure of an MHC class I molecule determined by X-ray crystallography.**

Panel a shows a computer graphic representation of a human MHC class I molecule, HLA-A2, which has been cleaved from the cell surface by the enzyme papain. The surface of the molecule is shown, colored according to the domains shown in panels b–d and described below. Panels b and c show a ribbon diagram of that structure. Shown schematically in panel d, the MHC class I molecule is a heterodimer of a membrane-spanning  $\alpha$  chain (molecular weight 43 kDa) bound noncovalently to  $\beta_2$ -microglobulin (12 kDa), which does not span the membrane. The  $\alpha$  chain folds into three domains:  $\alpha_1$ ,  $\alpha_2$ , and  $\alpha_3$ . The  $\alpha_3$  domain and  $\beta_2$ -microglobulin show similarities in amino acid sequence to immunoglobulin C domains and have similar folded structures, whereas the  $\alpha_1$  and  $\alpha_2$  domains fold together into a single structure consisting of two segmented  $\alpha$  helices lying on a sheet of eight antiparallel  $\beta$  strands. The folding of the  $\alpha_1$  and  $\alpha_2$  domains creates a long cleft or groove, which is the site at which peptide antigens bind to the MHC molecules. The transmembrane region and the short stretch of peptide that connects the external domains to the cell surface are not seen in panels a and b as they have been removed by the papain digestion. As can be seen in panel c, looking down on the molecule from above, the sides of the cleft are formed from the inner faces of the two  $\alpha$  helices; the  $\beta$ -pleated sheet formed by the pairing of the  $\alpha_1$  and  $\alpha_2$  domains creates the floor of the cleft. We shall use the schematic representation in panel d throughout this text.



MHC class I structure is outlined in Fig. 3.20. MHC class I molecules consist of two polypeptide chains, a larger  $\alpha$  chain encoded in the MHC genetic locus, and a smaller noncovalently associated chain,  $\beta_2$ -microglobulin, which is not polymorphic and is not encoded in the MHC locus. Only the class I  $\alpha$  chain spans the membrane. The complete molecule has four domains, three formed from the MHC-encoded  $\alpha$  chain, and one contributed by  $\beta_2$ -microglobulin. The  $\alpha_3$  domain and  $\beta_2$ -microglobulin have a folded structure that closely resembles that of an immunoglobulin domain. The most remarkable feature of MHC class I molecules is the structure of the folded  $\alpha_1$  and  $\alpha_2$  domains. These two domains form the walls of a cleft on the surface of the molecule; this is the site of peptide binding. They also are sites of polymorphisms that determine T-cell antigen recognition (see Chapter 5).

An MHC class II molecule consists of a noncovalent complex of two chains,  $\alpha$  and  $\beta$ , both of which span the membrane (Fig. 3.21). The MHC class II  $\alpha$  and  $\beta$  chains are both encoded within the MHC. The crystallographic structure of the MHC class II molecule shows that it is folded very much like the MHC class I molecule. The major differences lie at the ends of the peptide-binding cleft, which are more open in MHC class II molecules compared with MHC

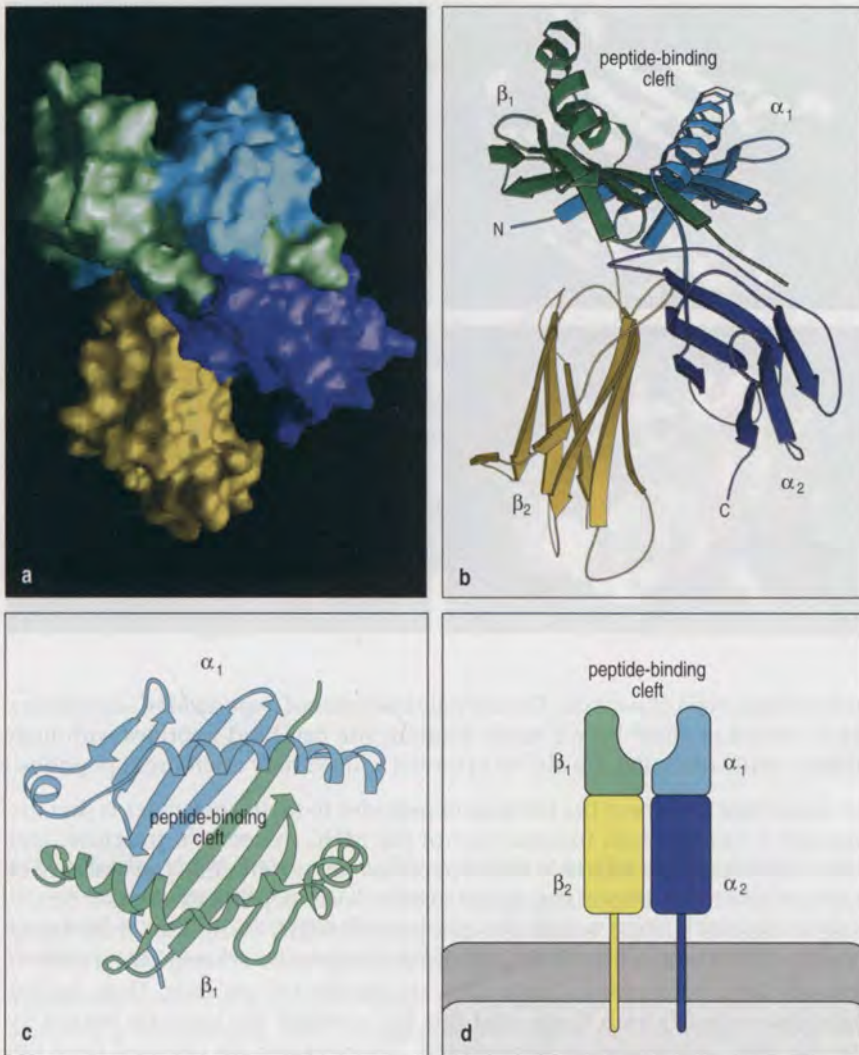


**Fig. 3.21 MHC class II molecules resemble MHC class I molecules in overall structure.** The MHC class II molecule is composed of two trans-membrane glycoprotein chains,  $\alpha$  (34 kDa) and  $\beta$  (29 kDa), as shown schematically in panel d. Each chain has two domains, and the two chains together form a compact four-domain structure similar to that of the MHC class I molecule (compare with panel d of Fig. 3.20). Panel a shows a computer graphic representation of the surface of the MHC class II molecule, in this case the human protein HLA-DR1, and panel b shows the equivalent ribbon diagram. The  $\alpha_2$  and  $\beta_2$  domains, like the  $\alpha_3$  and  $\beta_2$ -microglobulin domains of the MHC class I molecule, have amino acid sequence and structural similarities to immunoglobulin C domains; in the MHC class II molecule, the two domains forming the peptide-binding cleft are contributed by different chains and are therefore not joined by a covalent bond (see panels c and d). Another important difference, not apparent in this diagram, is that the peptide-binding groove of the MHC class II molecule is open at both ends.

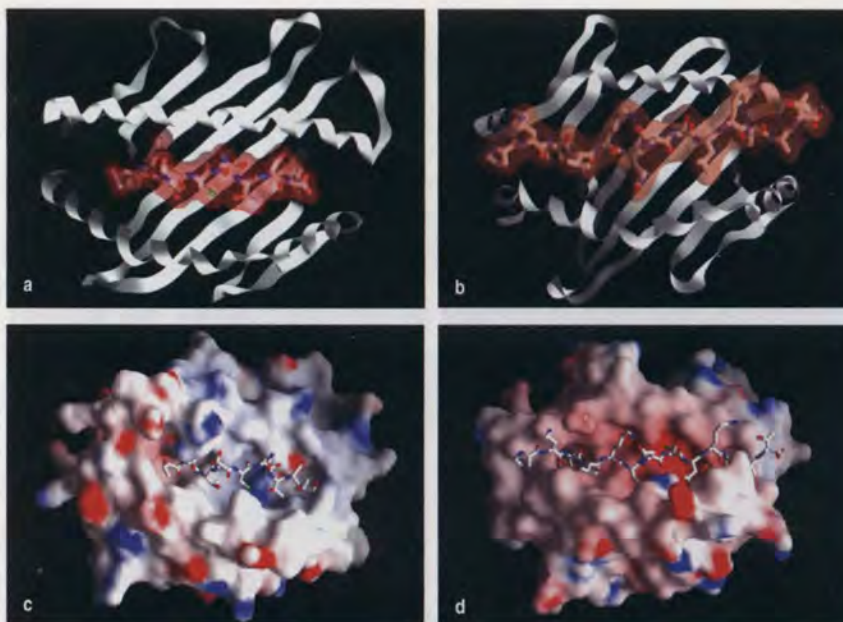
class I molecules. The main consequence of this is that the ends of a peptide bound to an MHC class I molecule are substantially buried within the molecule, whereas the ends of peptides bound to MHC class II molecules are not. Again, the sites of major polymorphism are located in the peptide-binding cleft, which in the case of an MHC class II molecule are formed by the  $\alpha_1$ , and  $\beta_1$  domains (see Chapter 5).

In both MHC class I and class II molecules, bound peptides are sandwiched between the two  $\alpha$ -helical segments of the MHC molecule (Fig. 3.22). The T-cell receptor interacts with this compound ligand, making contacts with both the MHC molecule and with the peptide fragment of antigen.

### 3-15 Peptides are stably bound to MHC molecules, and also serve to stabilize the MHC molecule on the cell surface.

An individual can be infected by a wide variety of different pathogens the proteins of which will not generally have peptide sequences in common. If T cells are to be alerted to all possible infections, then the MHC molecules on each cell (both class I and class II) must be able to bind stably to many different peptides. This behavior is quite distinct from that of other peptide-binding receptors, such as those for peptide hormones, which usually bind

**Fig. 3.22 MHC molecules bind peptides tightly within the cleft.** When MHC molecules are crystallized with a single synthetic peptide antigen, the details of peptide binding are revealed. In MHC class I molecules (panels a and c) the peptide is bound in an elongated conformation with both ends tightly bound at either end of the cleft. In the case of MHC class II molecules (panels b and d), the peptide is also bound in an elongated conformation but the ends of the peptide are not tightly bound and the peptide extends beyond the cleft. The upper surface of the peptide:MHC complex is recognized by T cells, and is composed of residues of the MHC molecule and the peptide. In representations c and d, the electrostatic potential of the MHC molecule surface is shown, with blue areas indicating a positive potential and red a negative potential.



only a single type of peptide. The crystal structures of peptide:MHC complexes have helped to show how a single binding site can bind peptides with high affinity while retaining the ability to bind a wide variety of different peptides.

An important feature of the binding of peptides to MHC molecules is that the peptide is bound as an integral part of the MHC molecule's structure, and MHC molecules are unstable when peptides are not bound. The stability of peptide binding is important, because otherwise, peptide exchanges occurring at the cell surface would prevent peptide:MHC complexes from being reliable indicators of infection or of uptake of specific antigen. As a result of this stability, when MHC molecules are purified from cells, their bound peptides co-purify with them, and this has enabled the peptides bound by specific MHC molecules to be analyzed. The peptides are released from the MHC molecules by denaturing the complex in acid, and can then be purified and sequenced. Pure synthetic peptides can also be incorporated into previously empty MHC molecules and the structure of the complex determined, revealing details of the contacts between the MHC molecule and the peptide. From the sequences of peptides bound to specific MHC molecules, combined with structural analysis of the peptide:MHC complex, a detailed picture of the binding interactions has been built up. We will first discuss the peptide-binding properties of MHC class I molecules.

### 3-16 MHC class I molecules bind short peptides of 8–10 amino acids by both ends.

The binding of a peptide in the peptide-binding cleft of an MHC class I molecule is stabilized at both ends by contacts between atoms in the free amino and carboxy termini of the peptide and invariant sites that are found at each end of the cleft of all MHC class I molecules (Fig. 3.23). These contacts are thought to be the main stabilizing contacts for peptide:MHC class I complexes because synthetic peptide analogues lacking terminal amino and carboxyl groups fail to bind stably to MHC class I molecules. Other residues in the peptide serve as additional anchors. Peptides that bind to MHC class I molecules are usually 8–10 amino acids long. The peptide lies in an elongated conformation along the groove; variations in peptide length appear to be

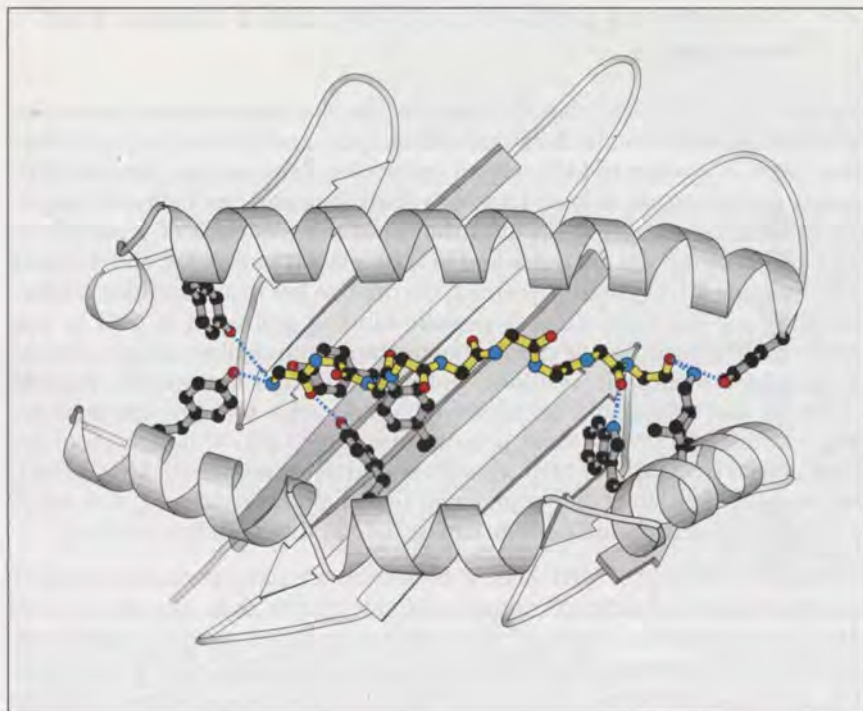


**Fig. 3.23 Peptides are bound to MHC class I molecules by their ends.** MHC class I molecules interact with the backbone of a bound peptide (shown in yellow) through a series of hydrogen bonds and ionic interactions (shown as dotted blue lines) at each end of the peptide. The amino terminus of the peptide is to the left; the carboxy terminus to the right. Black circles are carbon atoms; red are oxygen; blue are nitrogen. The amino acid residues in the MHC molecule that form these bonds are common to all MHC class I molecules and their side chains are shown in full (in gray) upon a ribbon diagram of the MHC class I groove. A cluster of tyrosine residues common to all MHC class I molecules forms hydrogen bonds to the amino terminus of the bound peptide, while a second cluster of residues forms hydrogen bonds and ionic interactions with the peptide backbone at the carboxy terminus and with the carboxy terminus itself.

accommodated, in most cases, by a kinking in the peptide backbone. However, two examples of MHC class I molecules where the peptide is able to extend out of the groove at the carboxy terminus suggest that some length variation may also be accommodated in this way.

These interactions give all MHC class I molecules their broad peptide-binding specificity. In addition, MHC molecules are highly polymorphic. There are hundreds of different versions, or alleles, of the MHC class I genes in the human population as a whole, and each individual carries only a small selection of them. The main differences between the allelic MHC variants are found at certain sites in the peptide-binding cleft, resulting in different amino acids in key peptide interaction sites in the different MHC variants. The consequence of this is that the different MHC variants preferentially bind different peptides. The peptides that can bind to a given MHC variant have the same or very similar amino acid residues at two or three particular positions along the peptide sequence. The amino acid side chains at these positions insert into pockets in the MHC molecule that are lined by the polymorphic amino acids. Because the binding of these side chains anchors the peptide to the MHC molecule, the peptide residues involved have been called **anchor residues**. Both the position and identity of these anchor residues can vary, depending on the particular MHC class I variant that is binding the peptide. However, most peptides that bind to MHC class I molecules have a hydrophobic (or sometimes basic) anchor residue at the carboxy terminus (Fig. 3.24). Changing an anchor residue can prevent the peptide from binding and, conversely, most synthetic peptides of suitable length that contain these anchor residues will bind the appropriate MHC class I molecule, in most cases irrespective of the amino acids at other positions in the peptide. These features of peptide binding enable an individual MHC class I molecule to bind a wide variety of different peptides, yet allow different MHC class I allelic variants to bind different sets of peptides.

|                |   |   |   |   |   |   |   |   |                  |
|----------------|---|---|---|---|---|---|---|---|------------------|
| H <sup>1</sup> | R | G | Y | V | Y | Q | Q | L | COO <sup>-</sup> |
| H <sup>2</sup> | S | I | I | N | F | E | K | L | COO <sup>-</sup> |
| H <sup>3</sup> | A | P | G | N | Y | P | A | L | COO <sup>-</sup> |

|                |   |   |   |   |   |   |   |   |   |                  |
|----------------|---|---|---|---|---|---|---|---|---|------------------|
| H <sup>1</sup> | T | Y | Q | R | T | R | A | L | V | COO <sup>-</sup> |
| H <sup>2</sup> | S | Y | F | P | E | I | T | H | I | COO <sup>-</sup> |
| H <sup>3</sup> | K | Y | Q | A | V | T | T | T | L | COO <sup>-</sup> |
| H <sup>4</sup> | S | Y | I | P | S | A | E | K | I | COO <sup>-</sup> |

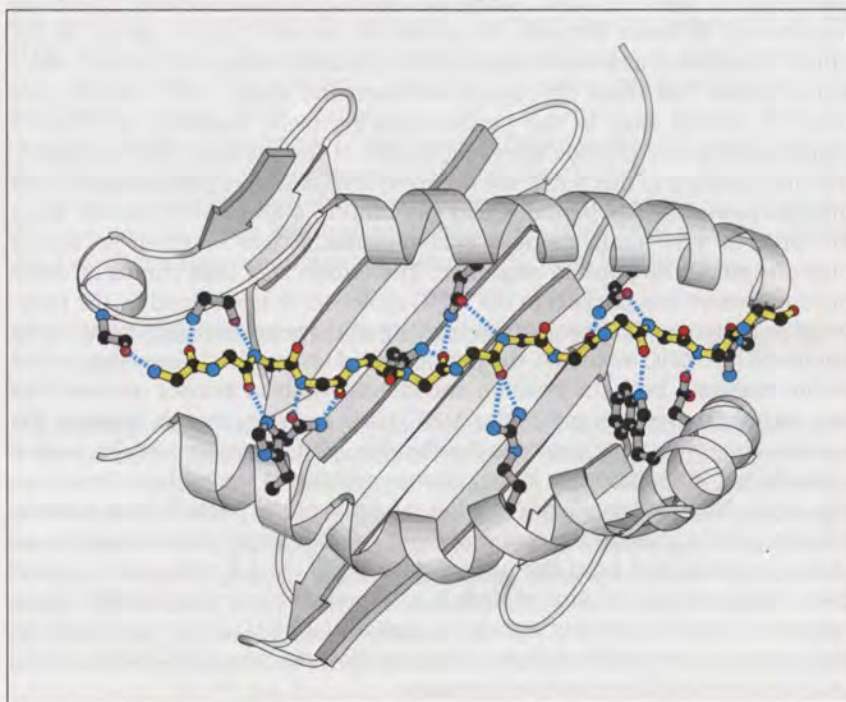
**Fig. 3.24 Peptides bind to MHC molecules through structurally related anchor residues.** Peptides eluted from two different MHC class I molecules are shown. The anchor residues (green) differ for peptides that bind different alleles of MHC class I molecules but are similar for all peptides that bind to the same MHC molecule. The upper and lower panels show peptides that bind to two different alleles of MHC class I molecules. The anchor residues that bind a particular MHC molecule need not be identical, but are always related (for example, phenylalanine (F) and tyrosine (Y) are both aromatic amino acids, whereas valine (V), leucine (L), and isoleucine (I) are all large hydrophobic amino acids). Peptides also bind to MHC class I molecules through their amino (blue) and carboxy (red) termini.

### 3-17 The length of the peptides bound by MHC class II molecules is not constrained.

Peptide binding to MHC class II molecules has also been analyzed by elution of bound peptides and by X-ray crystallography, and differs in several ways from peptide binding to MHC class I molecules. Peptides that bind to MHC class II molecules are at least 13 amino acids long and can be much longer. The clusters of conserved residues that bind the two ends of a peptide in MHC class I molecules are not found in MHC class II molecules, and the ends of the peptide are not bound. Instead, the peptide lies in an extended conformation along the MHC class II peptide-binding groove. It is held in this groove both by peptide side chains that protrude into shallow and deep pockets lined by polymorphic residues, and by interactions between the peptide backbone and side chains of conserved amino acids that line the peptide-binding cleft in all MHC class II molecules (Fig. 3.25). Although there are fewer crystal structures of MHC class II-bound peptides than of MHC class I, the available data show that amino acid side chains at residues 1, 4, 6, and 9 of an MHC class II-bound peptide can be held in these binding pockets.

The binding pockets of MHC class II molecules are more permissive in their accommodation of different amino acid side chains than are those of the MHC class I molecule, making it more difficult to define anchor residues and predict which peptides will be able to bind particular MHC class II molecules (Fig. 3.26). Nevertheless, by comparing the sequences of known binding peptides, it is usually possible to detect a pattern of permissive amino acids for each of the different alleles of MHC class II molecules, and to model how the amino acids of this peptide sequence motif will interact with the amino acids that make up the peptide-binding cleft in the MHC class II molecule. Because the peptide is bound by its backbone and allowed to emerge from both ends of the binding groove there is, in principle, no upper limit to the length of peptides that could bind to MHC class II molecules. However, it appears that longer peptides bound to MHC class II molecules are trimmed

**Fig. 3.25 Peptides bind to MHC class II molecules by interactions along the length of the binding groove.** A peptide (yellow; shown as the peptide backbone only, with the amino terminus to the left and the carboxy terminus to the right), is bound by an MHC class II molecule through a series of hydrogen bonds (dotted blue lines) that are distributed along the length of the peptide. The hydrogen bonds toward the amino terminus of the peptide are made with the backbone of the MHC class II polypeptide chain, whereas throughout the peptide's length bonds are made with residues that are highly conserved in MHC class II molecules. The side chains of these residues are shown in gray upon the ribbon diagram of the MHC class II groove.



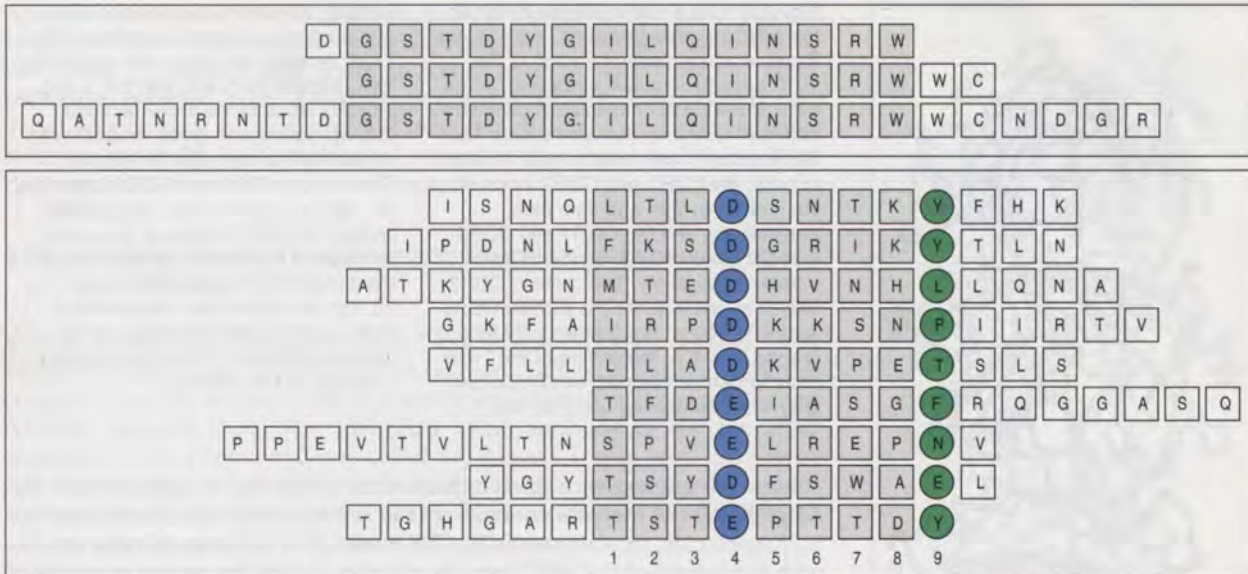


**Fig. 3.26 Peptides that bind MHC class II molecules are variable in length and their anchor residues lie at various distances from the ends of the peptide.** The sequences of a set of peptides that bind to the mouse MHC class II A<sup>k</sup> allele are shown in the upper panel. All contain the same core sequence but differ in length. In the lower panel, different peptides binding to the human MHC class II allele HLA-DR3 are shown. The

lengths of these peptides can vary, and so by convention the first anchor residue is denoted as residue 1. Note that all of the peptides share a negatively charged residue (aspartic acid (D) or glutamic acid (E)) in the P4 position (blue) and tend to have a hydrophobic residue (for example, tyrosine (Y), leucine (L), proline (P), phenylalanine (F)) in the P9 position (green).

by peptidases to a length of 13–17 amino acids in most cases. Like MHC class I molecules, MHC class II molecules that lack bound peptide are unstable, but the critical stabilizing interactions that the peptide makes with the MHC class II molecule are not yet known.

### 3-18 The crystal structures of several MHC:peptide:T-cell receptor complexes all show the same T-cell receptor orientation over the MHC:peptide complex.

At the time that the first X-ray crystallographic structure of a T-cell receptor was published, a structure of the same T-cell receptor bound to a peptide:MHC class I ligand was also produced. This structure (Fig. 3.27), which had been forecast by site-directed mutagenesis of the MHC class I molecule, showed the T-cell receptor aligned diagonally over the peptide and the peptide-binding groove, with the T-cell receptor  $\alpha$  chain lying over the  $\alpha_2$  domain and the amino-terminal end of the bound peptide, the T-cell receptor  $\beta$  chain lying over the  $\alpha_1$  domain and the carboxy-terminal end of the peptide, with the CDR3 loops of both T-cell receptor  $\alpha$  and T-cell receptor  $\beta$  meeting over the central amino acids of the peptide. The T-cell receptor is threaded through a valley between the two high points on the two surrounding  $\alpha$  helices that form the walls of the peptide-binding cleft.

Analysis of other MHC class I:peptide:T-cell receptor complexes and of the single example so far of an MHC class II:peptide:T-cell receptor complex (Fig. 3.28) shows that all of them have a very similar orientation, particularly for the  $V_\alpha$  domain, although some variability does occur in the location and orientation of the  $V_\beta$  domain. In this orientation, the  $V_\alpha$  domain makes contact primarily with the amino terminus of the bound peptide, whereas the  $V_\beta$  domain contacts primarily the carboxy terminus of the bound peptide. Both

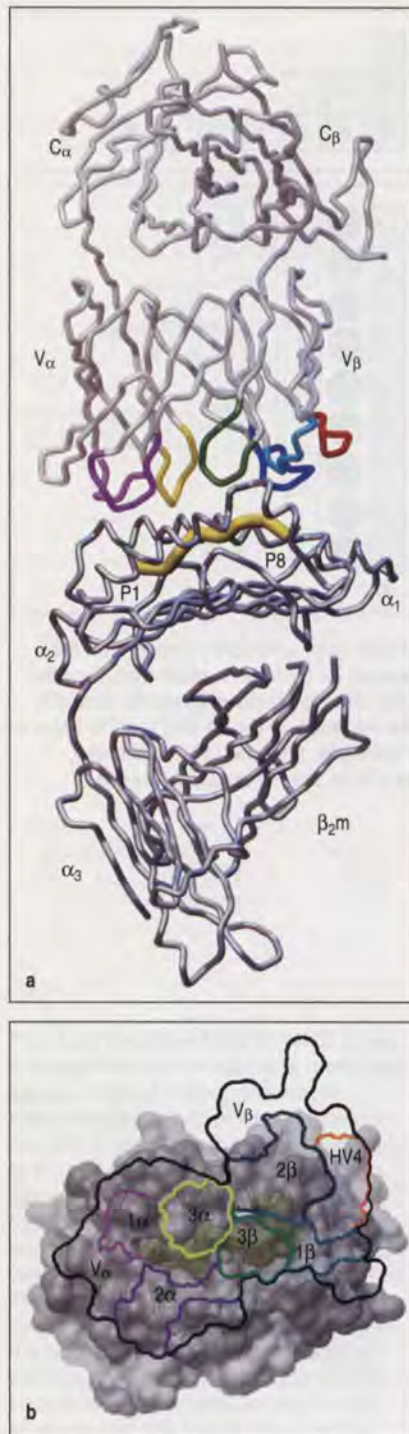


**Fig. 3.27 The T-cell receptor binds to the MHC:peptide complex.** Panel a: the T-cell receptor binds to the top of the MHC:peptide complex, straddling, in the case of the class I molecule shown here, both the  $\alpha_1$  and  $\alpha_2$  domain helices. The CDRs of the T-cell receptor are indicated in color; the CDR1 and CDR2 loops of the  $\beta$  chain in light and dark blue, respectively; and the CDR1 and CDR2 loops of the  $\alpha$  chain in light and dark purple, respectively. The  $\alpha$  chain CDR3 loop is in yellow while the  $\beta$  chain CDR3 loop is in green. The  $\beta$  chain HV4 loop is orange. Panel b: the outline of the T-cell receptor antigen-binding site (thick black line) is superimposed upon the top

surface of the MHC:peptide complex (the peptide is shaded dull yellow). The T-cell receptor lies diagonally across the MHC:peptide complex, with the  $\alpha$  and  $\beta$  CDR3 loops of the T-cell receptor ( $3\alpha$ ,  $3\beta$ , yellow and green, respectively) contacting the center of the peptide. The  $\alpha$  chain CDR1 and CDR2 loops ( $1\alpha$ ,  $2\alpha$ , light and dark purple, respectively) contact the MHC helices at the amino terminus of the bound peptide, whereas the  $\beta$  chain CDR1 and CDR2 loops ( $1\beta$ ,  $2\beta$ , light and dark blue, respectively) make contact with the helices at the carboxy terminus of the bound peptide. Courtesy of I.A. Wilson.

chains also interact with the  $\alpha$  helices of the MHC class I molecule (see Fig. 3.27). The T-cell receptor contacts are not symmetrically distributed over the MHC molecule, so whereas the  $V_\alpha$  CDR1 and CDR2 loops are in close contact with the helices of the MHC:peptide complex around the amino terminus of the bound peptide, the  $\beta$ -chain CDR1 and CDR2 loops, which interact with the complex at the carboxy terminus of the bound peptide, have variable contributions to the binding. This suggests that the  $V_\alpha$  contacts are responsible for the conserved orientation of the T-cell receptor on the MHC:peptide complex.

Comparison of the three-dimensional structure of the T-cell receptor to that of the same T-cell receptor complexed to its MHC-peptide ligand could address the question of whether the T-cell receptor, like some other receptors, undergoes a conformational change, or 'induced fit,' in its three-dimensional structure when it binds its specific ligand. To date, there is no certain answer, owing to the limitations of the available structures. For one thing, all the T-cell receptors analyzed to date have either been bound to ligands that do not produce activation, or are bound to ligands that can activate them, but the comparable unliganded receptor structures are not available. Also, the crystals of the T-cell receptor are all formed at 0 °C or below, which locks the receptor into a single conformation. However, subtly different peptides can have strikingly different effects when the same T cell recognizes either of the two peptides complexed with MHC. This could be due to differences in how T-cell receptor conformation is altered by binding the two related yet different ligands. Recent evidence also suggests that the temperature at which the T-cell receptor binds to a particular peptide:MHC complex makes a large difference in the extent of T-cell receptor aggregation; protein conformation is affected by temperature, and so these differences may well result from a conformational change.

From an examination of these structures it is hard to predict whether the main binding energy is contributed by T-cell receptor contacts with the bound peptide, or by T-cell receptor contacts with the MHC molecule. It is known that alterations as simple as changing a leucine to isoleucine in the peptide are sufficient to alter the T-cell response from strong killing to absolutely no response at all. Studies show that mutations of single residues in the presenting MHC molecules can have the same effect. Thus, the specificity of T-cell recognition involves both the peptide and its presenting MHC molecule. This dual specificity underlies the MHC restriction of T-cell responses, a phenomenon that was observed long before the peptide-binding properties of MHC molecules were known. We will recount the story of how MHC restriction was discovered when we return to the issue of how MHC polymorphism affects antigen recognition by T cells in Chapter 5.

Another consequence of this dual specificity is a need for T-cell receptors to be able to interact appropriately with the antigen-presenting surface of MHC molecules. It appears that there is some inherent specificity for MHC molecules encoded in the T-cell receptor genes, as well as selection during T-cell development for a repertoire of receptors able to interact appropriately with the particular MHC molecules present in that individual. We will be discussing the evidence for this in Chapter 7.

### 3-19 A distinct subset of T cells bears an alternative receptor made up of $\gamma$ and $\delta$ chains.

During the search for the gene for the T-cell receptor  $\alpha$  chain, another T-cell receptorlike gene was unexpectedly discovered. This gene was named T-cell receptor  $\gamma$ , and its discovery led to a search for further T-cell receptor genes. Another receptor chain was identified using antibody to the predicted sequence of the  $\gamma$  chain and was called the  $\delta$  chain. It was soon discovered that a minority population of T cells bore a distinct type of T-cell receptor made up of  $\gamma\delta$  heterodimers rather than  $\alpha\beta$  heterodimers. The development of these cells is described in Sections 7-13 and 7-14.

To date, there is no crystallographic structure of a  $\gamma\delta$  T-cell receptor, although it is expected to be similar in shape to  $\alpha\beta$  T-cell receptors.  $\gamma\delta$  T-cell receptors may be specialized to bind certain kinds of ligands, including heat-shock proteins and nonpeptide ligands such as mycobacterial lipid antigens. It seems likely that  $\gamma\delta$  T-cell receptors are not restricted by the 'classical' MHC class I and class II molecules. They may bind the free antigen, much as immunoglobulins do, and/or they may bind to peptides or other antigens presented by nonclassical MHC-like molecules. These are proteins that resemble MHC class I molecules but are relatively nonpolymorphic. We still know little about how  $\gamma\delta$  T-cell receptors bind antigen and thus how these cells function, and what their role is in immune responses. The structure and rearrangement of the genes for  $\gamma\delta$  T-cell receptors is covered in Sections 4-13 and 7-13 and the functions of  $\gamma\delta$  T cells are considered in Chapter 8.

### Summary.

The receptor for antigen on most T cells, the  $\alpha\beta$  T-cell receptor, is composed of two protein chains, T-cell receptor  $\alpha$  and T-cell receptor  $\beta$ , and resembles in many respects a single Fab fragment of immunoglobulin. T-cell receptors are always membrane-bound.  $\alpha\beta$  T-cell receptors do not recognize antigen in its native state, as do the immunoglobulin receptors of B cells, but recognize a composite ligand of a peptide antigen bound to an MHC molecule. MHC molecules are highly polymorphic glycoproteins encoded by genes in the major histocompatibility complex (MHC). Each MHC molecule binds a wide variety of different peptides, but the different variants each preferentially recognize sets of peptides with particular sequence and physical features. The peptide antigen is generated intracellularly, and bound stably in a peptide-binding cleft on the surface of the MHC molecule. There are two classes of MHC molecules and these are bound in their nonpolymorphic domains by CD8 and CD4 molecules that distinguish two different functional classes of  $\alpha\beta$  T cells. CD8 binds MHC class I molecules and can bind simultaneously to the same class I MHC:peptide complex being recognized by a T-cell receptor, thus acting as a co-receptor and enhancing the T-cell response; CD4 binds MHC class II molecules and acts as a co-receptor for T-cell receptors that recognise class II MHC:peptide ligands. T-cell receptors interact directly with both the antigenic peptide and polymorphic features of the MHC molecule that displays it, and this dual specificity underlies the

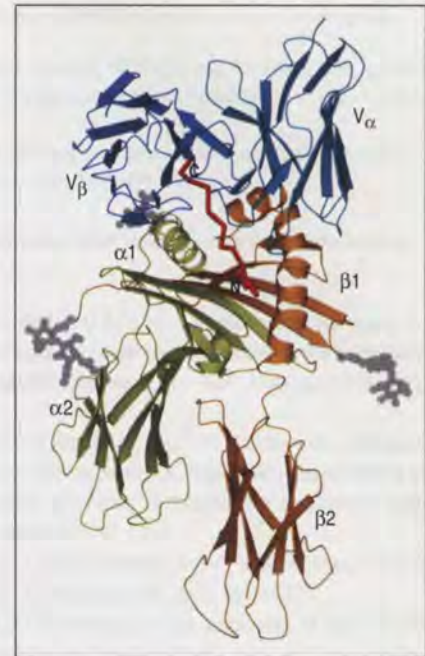


**Fig. 3.28 The T-cell receptor interacts with MHC class I and MHC class II molecules in a similar fashion.** The structure of a T-cell receptor binding to an MHC class II molecule has been determined, and shows the T-cell receptor binding to an equivalent site, and in an equivalent orientation, to the way that TCRs bind to MHC class I molecules (see Fig. 3.27). The structure of the molecules is shown in a cartoon form, with the MHC class II  $\alpha$  and  $\beta$  chains shown in light green and orange respectively. Only the  $V_\alpha$  and  $V_\beta$  domains of the T-cell receptor are shown, colored in blue. The peptide is colored red, while carbohydrate residues are indicated in gray. The TCR sits in a shallow saddle formed between the MHC class II  $\alpha$  and  $\beta$  chain  $\alpha$ -helical regions, at roughly 90° to the long axis of the MHC class II molecule and the bound peptide. Courtesy of E.L. Reinherz.

MHC restriction of T-cell responses. A second type of T-cell receptor, composed of a  $\gamma$  and a  $\delta$  chain, is structurally similar to the  $\alpha\beta$  T-cell receptor but appears to bind different ligands, including nonpeptide ligands. It is not thought to be MHC restricted. It is found on a minority population of T cells, the  $\gamma\delta$  T cells, whose biological function is still not clear.

### Summary to Chapter 3.

B cells and T cells use different, but structurally similar, molecules to recognize antigen. The antigen-recognition molecules of B cells are immunoglobulins, and are made both as a membrane-bound receptor for antigen, the B-cell receptor, and as secreted antibodies that bind antigens and elicit humoral effector functions. The antigen-recognition molecules of T cells, on the other hand, are made only as cell-surface receptors. Immunoglobulins and T-cell receptors are highly variable molecules, with the variability concentrated in that part of the molecule, the variable (V) region, that binds to antigen. Immunoglobulins bind a wide variety of chemically different antigens, whereas the major  $\alpha\beta$  form of T-cell receptors will recognize only peptide fragments of foreign proteins bound to the MHC molecules that are ubiquitous on cell surfaces.

Binding of antigen by immunoglobulins has chiefly been studied using antibodies. The binding of antibody to its corresponding antigen is highly specific, and this specificity is determined by the shape and physicochemical properties of the antigen-binding site. The part of the antibody that elicits effector functions, once the variable part has bound an antigen, is located at the other end of the molecule from the antigen-binding sites, and is termed the constant region. There are five main functional classes of antibody, each encoded by a different type of constant region. As we will see in Chapter 9, these in turn interact with different components of the immune system to incite an inflammatory response and eliminate the antigen.

The T-cell receptor differs in several respects from the B-cell immunoglobulins. Among the most important of these differences is the absence of a secreted form of the receptor. This reflects the functional differences between T cells and B cells. B cells deal with pathogens and their protein products circulating within the body; secretion of a soluble antigen-recognition molecule by the activated B cell after antigen has been encountered enables them to mop up antigen effectively throughout the extracellular spaces of the body. T cells, on the other hand, are specialized for cell-cell interactions. They either kill cells that are infected with intracellular pathogens and that bear foreign antigenic peptides on their surface, or interact with cells of the immune system that have taken up foreign antigen and are displaying it on the cell surface. They thus have no requirement for a soluble, secreted receptor.

An additional distinctive feature of the T-cell receptor compared with immunoglobulins is that it recognizes a composite ligand made up of the foreign peptide bound to a self MHC molecule. This forces T cells to interact with infected body cells to become activated. Each T-cell receptor is specific for a particular combination of peptide and a self MHC molecule.

MHC molecules are encoded by a family of highly polymorphic genes and, although each individual expresses several, this represents only a small selection of all possible variants. During T-cell development, the T-cell receptor repertoire is selected so that the T cells of each individual recognize antigen only in conjunction with their own MHC molecules. Expression of multiple variant MHC molecules each with a different peptide-binding repertoire helps ensure that T cells from an individual will be able to recognize at least some peptides generated from nearly every pathogen.

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