KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act 1956)

Coimbatore – 641 021.

LECTURE PLAN
DEPARTMENT OF BIOTECHNOLOGY

STAFF NAME: Dr. R S. SARANYA

SUBJECT NAME: IMMUNOLOGY SUB.CODE:17BTU303

SEMESTER: III CLASS: II B.Sc. (BT)

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
UNIT-I			
1	1	Immune Response: An overview, components of mammalian immune system	T1: 1-35
2	1	Antigens- Essential features of Ag, haptens, Carrier molecule, Immunological valence, Antigenic determinants	T1: 30-105
3	1	Adjuvants: Freund's complete and incomplete.	T2: 91-92
4	1	Antibodies - Molecular structure of Immunoglobulins or Antibodies	T1: 37
5	1	Humoral & Cellular immune responses	T2: 397-403
6	1	T-lymphocytes& immune response (cytotoxic T-cell, helper T-cell, suppressor T-cells), T-cell receptors	T2: 62-65
7	1	Genome rearrangements during B-lymphocyte differentiation	T1: 144-146
8	1	Antibody affinity maturation class switching	T2: 215-216
9	1	Assembly of T-cell receptor genes by somatic recombination	T3: 213-218
10	1	Unit test	
	Total No of Hours Planned for Unit I =10		
UNIT-II			
1	1	Regulation of immunoglobulin gene expression	T2: 165-178

2	1	Clonal selection theory, allotypes & idiotypes	T1: 26, 28, 42-45
3	1	Allelic exclusion	T1: 227, 238
4	1	Immunologic memory	T1: 28-30
5	1	Heavy chain gene transcription	T4: 120-121
6	1	Genetic basis of antibody diversity	T2: 178-184, 404
7	1	Hypotheses (germ line & somatic mutation)	T4: 131
8	1	Antibody diversity	T2: 178-184, 404
9	1	Unit test	
	Total No of Hours Planned for Unit II = 09		
UNIT-III			
1	1	Hypersensitivity Reactions (HS)	T1: 322-348
2	1	Type I: Allergies and anaphylaxis	T1: 322-332
3	1	Continuation of Type I: Allergies and anaphylaxis	T1: 322-332
4	1	Type II: Antibody mediated HS reactions, Mechanism and pathogencity	T1: 322-336
5	1	Continuation of Type II: Antibody mediated HS reactions, Mechanism and pathogencity	T1: 336 - 341
6	1	Type III: Immune complex mediated HS reactions: Mechanism & pathogenecity	T1: 336-341
7	1	Type IV: Delayed type (or) cell-mediated HS reactions; Mechanisms and pathogenecity	T1: 341-345
8	1	Type V: Stimulatory HS reactions. Mechanism and pathogenesis.	T2: 345
9	1	Unit test	
	Total No of Hours Planned for Unit III = 09		
UNIT-IV			
1	1	Major Histocompatibility complexes	T1: 31, 70-78
2	1	Class I MHC antigens, antigen processing	T1: 32, 70-73
3	1	Class II MHC antigens, antigen processing	T1: 31

4	1	Immunity to infection – immunity to different organisms	T4: 425
5	1	pathogen defense strategies	T4: 425
6	1	Avoidance of recognition	T4: 426
7	1	Autoimmune diseases	T1: 396-420, 421-449
8	1	Immunodeficiency	T1: 305-321
9	1	AIDS	T1: 313-319
10	1	Unit test	
	Total No of Hours Planned for Unit IV = 10		
UNIT-V			
1	1	Vaccines & Vaccination	T2: 443-457
2	1	Adjuvants, cytokines	T2: 313-333
3	1	DNA vaccines, recombinant vaccines	T2: 452-453, 446
4	1	Bacterial vaccines, viral vaccines, vaccines to other infectious agents	T2: 461
5	1	Passive & active immunization	T1: 282,283
6	1	Introduction to immunodiagnostics – RIA	T3: 130
7	1	ELISA	T1: 111, 112, 114
8	1	Previous year ESE Question papers discussion	
9	1	Previous year ESE Question papers discussion	
10	1	Unit test	
	Total No of Hours Planned for Unit V = 10		
Total Planned Hours	**48**		

TEXT BOOK

1. Abbas, A.K., Lichtman, A.H., & Pillai, S. (2007). *Cellular and Molecular Immunology* (6th ed.). Philadelphia: Saunders Publication.
2. Delves, P., Martin, S., Burton, D., & Roitt, I.M. (2006). *Roitt's Essential Immunology* (11th ed.). Wiley-lackwell Scientific Publication, Oxford.

3. Goldsby, R.A., Kindt, T.J., Osborne, B.A. (2007). *Kuby's Immunology* (6th ed.). New York: W.H. Freeman and Company.
4. Murphy, K., Travers, P., & Walport , M. (2008). *Janeway's Immunobiology* (7th ed.). New York : Garland Science Publishers.
5. Peakman, M., & Vergani, D. (2009). *Basic and Clinical Immunology* (2nd ed.). Edinberg: Churchill Livingstone Publishers.
6. Richard, C., & Geiffrey, S. (2009). *Immunology* (6th ed.). Wiley Blackwell Publication

SEMESTER III

17BTU303 IMMUNOLOGY 4H - 4C

Total hours/week: L:4 T:0 P:0 Marks: Internal: 40 External: 60 Total: 100

Scope: Immunology has its own jargon, and by the end of the course, one will be able to speak immunology as well as understand and use the basic concepts.

Objective: Immunity Rules is an overview of immunology: functions and the physical organization of the immune system.

UNIT-I

Immune Response: An overview, components of mammalian immune system, Antigens- Essential features of Ag, haptens, Carrier molecule, Immunological valence, Antigenic determinants. Adjuvants: Freund's complete and incomplete. Antibodies - Molecular structure of Immuno-globulins or Antibodies, Humoral & Cellular immune responses, Tlymphocytes& immune response (cytotoxic T-cell, helper T-cell, suppressor T-cells), T-cell receptors, genome rearrangements during B-lymphocyte differentiation, Antibody affinity maturation class switching, assembly of T-cell receptor genes by somatic recombination.

UNIT-II

Regulation of immunoglobulin gene expression: Clonal selection theory, allotypes & idiotypes, allelic exclusion, immunologic memory, heavy chain gene transcription, genetic basis of antibody diversity, hypotheses (germ line & somatic mutation), antibody diversity.

UNIT-III

Hypersensitivity Reactions (HS): Type I: Allergies and anaphylaxis; Type II: Antibody mediated HS reactions; Mechanism and pathogencity; Type III: Immune complex mediated HS reactions: Mechanism & pathogenecity; Type IV: Delayed type (or) cell-mediated HS reactions; Mechanisms and pathogenecity. Type V: Stimulatory HS reactions. Mechanism and pathogenesis.

UNIT-IV

Major Histocompatibility complexes: Class I & class II MHC antigens, antigen processing. Immunity to infection – immunity to different organisms, pathogen defense strategies, avoidance of recognition. Autoimmune diseases, Immunodeficiency-AIDS.

UNIT-V

Vaccines & Vaccination: Adjuvants, cytokines, DNA vaccines, recombinant vaccines, bacterial vaccines, viral vaccines, vaccines to other infectious agents, passive & active immunization Introduction to immunodiagnostics – RIA, ELISA.

References

1. Abbas, A.K., Lichtman, A.H., & Pillai, S. (2007). *Cellular and Molecular Immunology* (6th ed.). Philadelphia: Saunders Publication.
2. Delves, P., Martin, S., Burton, D., & Roitt, I.M. (2006). *Roitt's Essential Immunology* (11th ed.). Wiley-lackwell Scientific Publication, Oxford.
3. Goldsby, R.A., Kindt, T.J., Osborne, B.A. (2007). *Kuby's Immunology* (6th ed.). New York: W.H. Freeman and Company.
4. Murphy, K., Travers, P., & Walport , M. (2008). *Janeway's Immunobiology* (7th ed.). New York : Garland Science Publishers.
5. Peakman, M., & Vergani, D. (2009). *Basic and Clinical Immunology* (2nd ed.). Edinberg: Churchill Livingstone Publishers.
6. Richard, C., & Geiffrey, S. (2009). *Immunology* (6th ed.). Wiley Blackwell Publication.

UNIT-I

SYLLABUS

Immune Response: An overview, components of mammalian immune system, Antigens- Essential features of Ag, haptens, Carrier molecule, Immunological valence, Antigenic determinants. Adjuvants: Freund's complete and incomplete. Antibodies - Molecular structure of Immuno-globulins or Antibodies, Humoral & Cellular immune responses, Tlymphocytes& immune response (cytotoxic T-cell, helper T-cell, suppressor T-cells), T-cell receptors, genome rearrangements during B-lymphocyte differentiation, Antibody affinity maturation class switching, assembly of T-cell receptor genes by somatic recombination.

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IMMUNE RESPONSE

IMMUNE SYSTEM -- AN OVERVIEW

The immune system is composed of many interdependent cell types that collectively protect the body from bacterial, parasitic, fungal, viral infections and from the growth of tumor cells. Many of these cell types have specialized functions. The cells of the immune system can engulf bacteria, kill parasites or tumor cells, or kill viral-infected cells. Often, these cells depend on the T helper subset for activation signals in the form of secretions formally known as cytokines, lymphokines, or more specifically interleukins.

CELLS OF IMMUNE SYSTEM

Hematopoiesis

All blood cells arise from a type of cell called hematopoietic stem cell (HSC). Stem cells that can differentiate into other cell types. They are self renewing, they maintain their population level by cell division. In human, hematopoietic, the formation and development of red and white blood cells begins in the embryonic yolk sac during the first week of development.

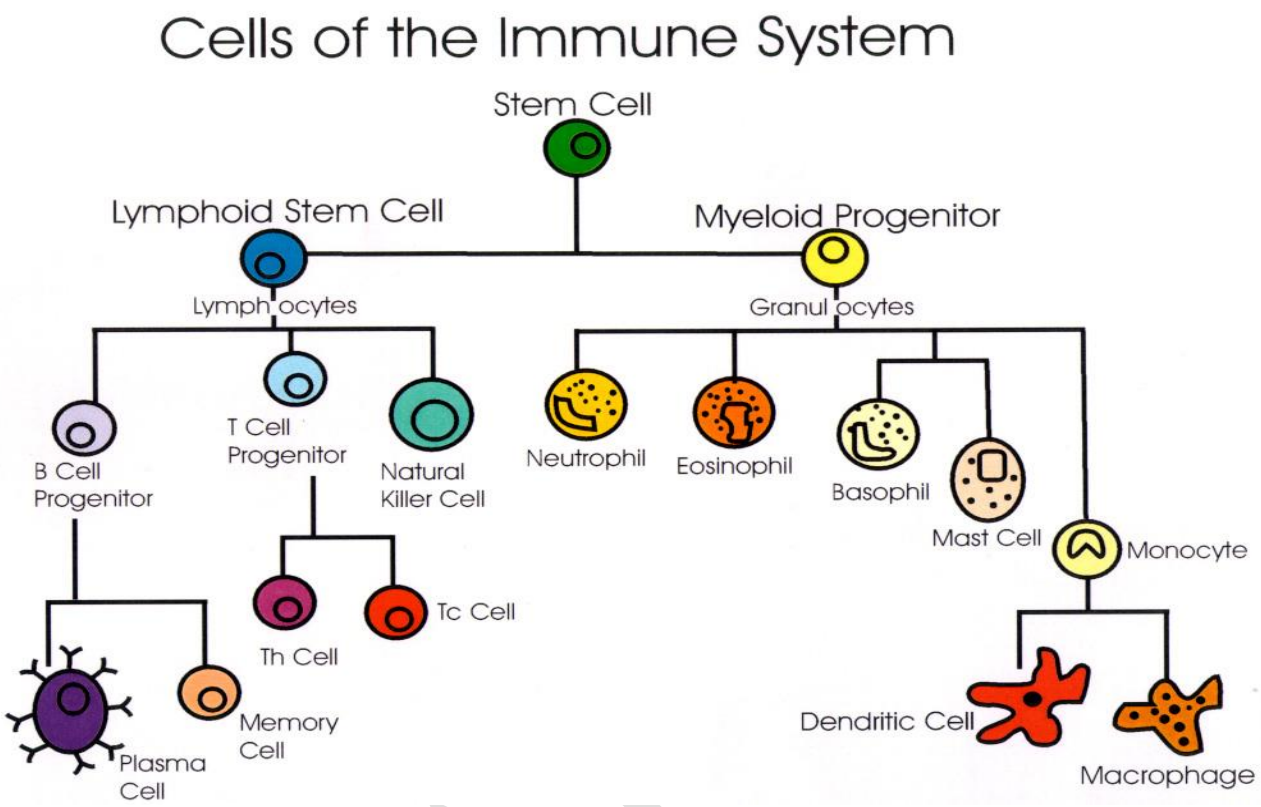

Fig: Cells of immune system

The Cells of the Immune System

Antigen-presenting cells	Cells which do not have antigen-specific receptors. Instead, they capture and process antigens, present them to T cell receptors. These cells include macrophages, dentritic cells and B cells.
B cells	Also known as B cell lymphocytes. B cells spend their entire early life in the bone marrow. Upon maturity, their job is to travel throughout the blood and lymph looking for antigens with which they can interlock. Once a B cell has identified an antigen, it starts replicating itself. These cloned cells mature into antibody-manufacturing *plasma cells*.
Basophils	Similar to mast cells, but distributed throughout the body. Like mast cells, basophils release histamine upon encountering certain antigens, thereby triggering an allergic reaction.
Cytotoxic T cells	Also called cytotoxic T lymphocytes or CTLs.

Dendritic cells	Mostly found in the skin and mucosal epithelium, where they are referred to as Langerhan's cells. Unlike macrophages, dendritic cells can also recognize viral particles as non-self. In addition, they can present antigens via both MHC I and MHC II, and can thus activate both CD8 and CD4 T cells, directly.
Granulocytes	Leukocytes (white blood cells) containing granules in the cytoplasm. Also known as a granular leukocyte. They seem to act as a first line of defense, as they rush toward an infected area and engulf the offending microbes. Granulocytes kill microbes by digesting them with killer enzymes contained in small units called lysosomes.
Helper T cells	These cells travel through the blood and lymph, looking for antigens (such as those captured by *antigen-presenting cells*). Upon locating an antigen, they notify other cells to assist in combating the invader. This is sometimes done through the use of cytokines (or specifically, lymphokines) which help destroy target cells and stimulate the production of healthy new tissue. Interferon is an example of such a cytokine.
Leukocytes	White blood cells. These are the cells which provide immunity, and they can be subdivided into three classes: lymphocytes, granulocytes and monocytes
Lymphocytes	Small white blood cells which are responsible for much of the work of the immune system. Lymphocytes can be divided into three classes: B cells, T cells and null cells.
Macrophages	Literally, "large eaters." These are large, long-lived phagocytes which capture foreign cells, digest them and present protein fragments (peptides) from these cells and manifest them on their exterior. In this manner, they present the antigens to the T cells. Macrophages are strategically located in lymphoid tissues, connective tissues and body cavities, where they are likely to encounter antigens. They also act as effector cells in cell-mediated immunity.
Mast cells	Cells concentrated within the respiratory and gastrointestinal tracts, and within the deep layers of the skin. These cells release histamine upon encountering certain antigens, thereby triggering an allergic reaction.
Memory cells	Specialized B cells which grant the body the ability to manufacture more of a particular antibody as needed, in case a particular antigen is ever encountered again.
Monocytes	Large, agranular leukocytes with relatively small, eccentric, oval or kidney-shaped nuclei.
Plasma cells	Specialized B cells which churn out antibodies—more than two thousand per second. Most of these die after four to five days; however, a few survive to become *memory cells*.

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T cells	Also known as T cell lymphocytes. Unlike B cells, these cells leave the marrow at an early age and travel to the thymus, where they mature. Here they are imprinted with critical information for recognizing "self" and "non-self" substances. Among the subclasses of T cells are helper T cells and cytotoxic (or killer) T cells.

ORGANS OF THE IMMUNE SYSTEM

A number of morphologically and functionally diverse organs and tissues have various functions in the development of immune responses. These can be distinguished by function as the primary and secondary lymphoid organs (Figure). The thymus and bone marrow are the primary (or central) lymphoid organs, where maturation of lymphocytes takes place. The lymph nodes, spleen, and various mucosal-associated lymphoid tissues (MALT) such as gut-associated lymphoid tissue (GALT) are the secondary (or peripheral) lymphoid organs, which trap antigen and provide sites for mature lymphocytes to interact with that antigen. In addition, tertiary lymphoid tissues, which normally contain fewer lymphoid cells than secondary lymphoid organs, can import lymphoid cells during an inflammatory response. Most prominent of these are cutaneous-associated lymphoid tissues.

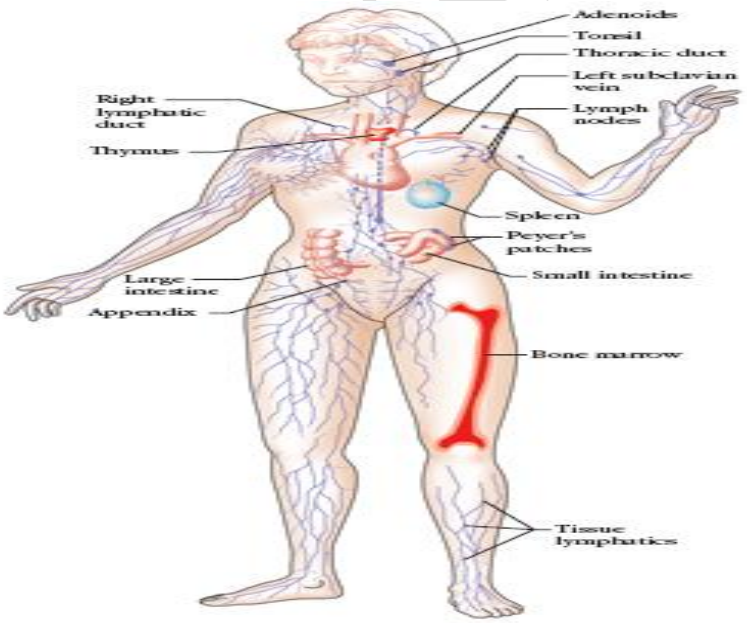


Figure: The human lymphoid system. The primary organs (bone marrow and thymus) are shown in red; secondary organs and tissues, in blue. These structurally and functionally diverse

lymphoid organs and tissues are interconnected by the blood vessels (not shown) and lymphatic vessels (purple) through which lymphocytes circulate. Only one bone is shown, but all major bones contain ma-row and thus are part of the lymphoid system.

PRIMARY LYMPHOID ORGANS:

Immature lymphocytes generated in hematopoiesis mature and become committed to a particular antigenic specificity within the primary lymphoid organs. Only after a lymphocyte has matured within a primary lymphoid organ is the cell immune competent (capable of mounting an immune response). T cells arise in the thymus, and in many mammals—humans and mice for example—B cells originate in bone marrow.

Also called central lymphoid organs, these are responsible for synthesis and maturation of immune competent cells. These include the bone marrow and the thymus.

(i) THYMUS:

The thymus is a gland located in the anterior mediastinum just above the heart, which reaches its greatest size just prior to birth, then atrophies with age.

The thymus is the site of T-cell development and maturation. It is a flat, bi-lobed organ situated above the heart. Each lobe is surrounded by a capsule and is divided into lobules, which are separated from each other by strands of connective tissue called trabeculae. Each lobule is organized into two compartments: the outer compartment, or *cortex,* is densely packed with immature T cells, called thymocytes, whereas the inner compartment, or *medulla,* is sparsely populated with thymocytes.

Both the cortex and medulla of the thymus are criss-crossed by a three-dimensional stromal-cell network com-posed of epithelial cells, dendritic cells, and macrophages, which make up the framework of the organ and contribute to the growth and maturation of thymocytes. Many of these stromal cells interact physically with the developing thymo-cytes (Figure). Some thymic epithelial cells in the outer cortex, called nurse cells, have long membrane extensions that surround as many as 50 thymocytes, forming large multicellular complexes. Other cortical epithelial cells have long interconnecting cytoplasmic extensions that form a network and have been shown to interact with numerous thymocytes as they traverse the cortex.

The function of the thymus is to generate and select a repertoire of T cells that will protect the body from infection. As thymocytes develop, an enormous diversity of T-cell re-ceptors is generated by a random process that produces some T cells with receptors capable of recognizing antigen-MHC complexes. However, most of the T-cell recep-tors produced by this random process are incapable of recognizing antigen-MHC complexes and a small portion react with combinations of self antigen-MHC complexes. Using mechanisms that are discussed in Chapter 10, the thymus induces the death of those T cells that cannot recognize anti-gen-MHC complexes and those that react with self-antigen– MHC and pose a danger of causing autoimmune disease. More than 95% of all thymocytes die by apoptosis in the thymus without ever reaching maturity.

Children with no development of thymus suffer from DiGeorge syndrome that is characterized by deficiency in T cell development but normal numbers of B cells.

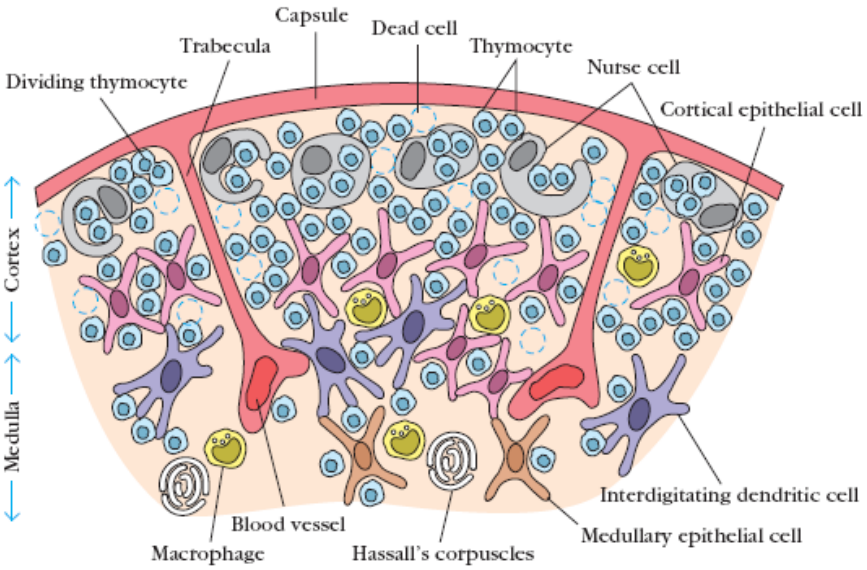


Fig: Diagrammatic cross section of a portion of the thymus, showing several lobules separated by connective tissue strands (trabeculae). The densely populated outer cortex is thought to contain many immature thymocytes (blue), which undergo rapid proliferation coupled with an enormous rate of cell death. Also present in the outer cortex are thymic nurse cells (gray), which are specialized epithelial cells with long membrane extensions that surround as many as 50 thymocytes. The medulla is sparsely populated and is thought to contain thymocytes that are more mature. During their stay within the thymus, thymocytes interact with various stromal cells, including cortical epithelial cells (light red), medullary epithelial cells (tan), interdigitating dendritic cells (purple), and macrophages (yellow). These cells produce thymic hormones and express high levels of class I and class II MHC molecules. Hassalls corpuscles, found in the medulla, contain concentric layers of degenerating epithelial cells.

(ii) BONE MARROW:

In humans and mice, bone marrow is the site of B-cell origin and development. Arising from lymphoid progenitors, immature B cells proliferate and differentiate within the bone marrow, and stromal cells within the bone marrow interact directly with the B cells and secrete various cytokines that are required for development. Like thymic selection during T-cell maturation, a selection process within the bone marrow eliminates B cells with self-reactive antibody receptors.

Bone marrow is not the site of B-cell development in all species. In birds, a lymphoid organ called the bursa of Fabricius, in cattle and sheep, the primary lymphoid tissue hosting the maturation, proliferation, and diversification of B cells early in gestation is the fetal spleen. Later in gestation, this function is assumed by a patch of tissue embedded in the wall of the intestine

called the ileal Peyer's patch The rabbit, too, uses gut-associated tissues such as the appendix as primary lymphoid tissue

Lymphatic System

As blood circulates under pressure, its fluid component (plasma) seems through the thin wall of the capillaries into the surrounding tissue. Much of this fluid, called interstitial fluid, returns to the blood through the capillary membranes. The remainder of the interstitial fluid, now called lymph, flows from the spaces in connective tissue into a network of tiny open lymphatic capillaries and then into a series of progressively larger collecting vessels called lymphatic vessels (Figure).

The largest lymphatic vessel, the thoracic duct, empties into the left sub-clavian vein near the heart. In this way, the lymphatic system captures fluid lost from the blood and returns it to the blood, thus ensuring steady-state levels of fluid within the circulatory system. The heart does not pump the lymph through the lymphatic system; instead the flow of lymph is achieved as the lymph vessels are squeezed by movements of the body's muscles. A series of one-way valves along the lymphatic vessels ensures that lymph flows only in one direction.

When a foreign antigen gains entrance to the tissues, it is picked up by the lymphatic system (which drains all the tissues of the body) and is carried to various organized lymphoid tissues such as lymph nodes, which trap the foreign antigen. As lymph passes from the tissues to lymphatic vessels, it becomes progressively enriched in lymphocytes. Thus, the lymphatic system also serves as a means of transporting lymphocytes and antigen from the connective tissues to organized lymphoid tissues where the lymphocytes may interact with the trapped antigen and undergo activation.

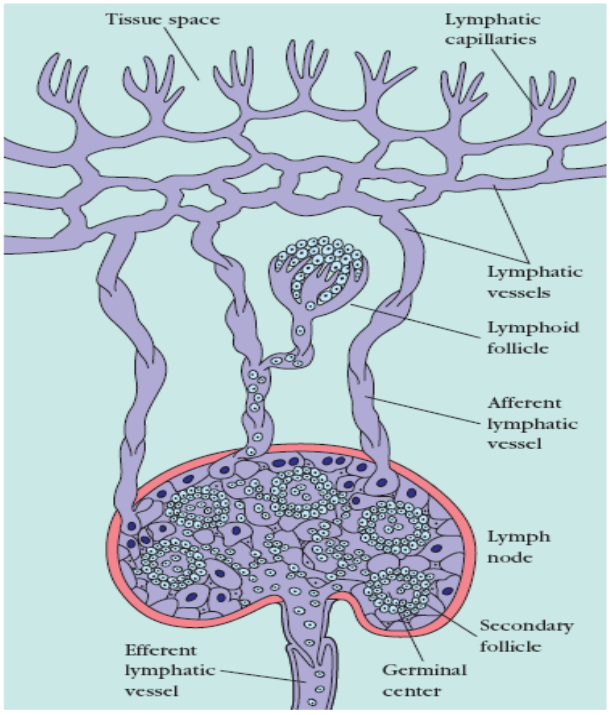


Fig: Lymphatic vessels. Small lymphatic capillaries opening into the tissue spaces pick up interstitial tissue fluid and carry it into progressively larger lymphatic vessels, which carry the fluid, now called lymph, into regional lymph nodes. As lymph leaves the nodes, it is carried through larger efferent lymphatic vessels, which eventually drain into the circulatory system at the thoracic duct or right lymph duct

PERIPHERAL LYMPHOID ORGANS (SECONDARY):

While primary lymphoid organs are concerned with production and maturation of lymphoid cells, the secondary or peripheral lymphoid organs are sites where the lymphocytes localize, recognize foreign antigen and mount response against it. These include the lymph nodes, spleen, tonsils, adenoids, appendix, and clumps of lymphoid tissue in the small intestine known as Peyer's patches. They trap and concentrate foreign substances, and they are the main sites of production of antibodies. Some lymphoid organs are capsulated such as lymph node and spleen while others are non-capsulated, which include mostly mucosa-associated lymphoid tissue (MALT).

(i) LYMPH NODE:

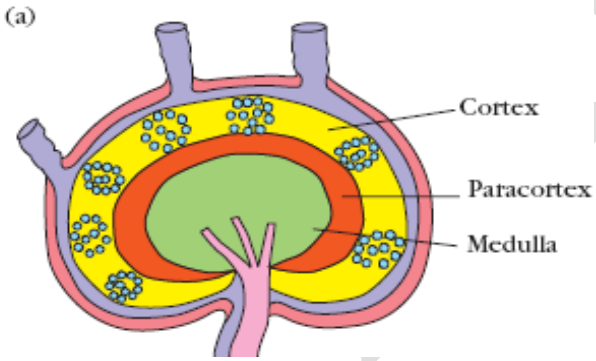
Clusters of lymph nodes are strategically placed in the neck, axillae, groin, mediastinum and abdominal cavity, where they filter antigens from the interstitial tissue fluid and the lymph during its passage from the periphery to the thoracic duct. The key lymph nodes are the axillary lymph nodes, the inguinal lymph nodes, the mesenteric lymph nodes and the cervical lymph nodes.

Lymph nodes are encapsulated bean-shaped structures containing a reticular network packed with lymphocytes, macrophages, and dendritic cells. Clustered at junctions of the lymphatic vessels, lymph nodes are the first organized lymphoid structure to encounter antigens that enter the tissue spaces. As lymph percolates through a node, any particulate antigen that is brought in with the lymph will be trapped by the cellular network of phagocytic cells and dendritic cells (follicular and inter digitating). The overall architecture of a lymph node supports an ideal mi-cro environment for lymphocytes to effectively encounter and respond to trapped antigens.

Morphologically, a lymph node can be divided into three roughly concentric regions: the cortex, the paracortex, and the medulla, each of which supports a distinct microenviron-ment (Figure 2-18). The outermost layer, the cortex, contains lymphocytes (mostly B cells), macro-phages, and follicular dendritic cells arranged in primary follicles. After antigenic challenge, the primary follicles enlarge into secondary follicles, each containing a germinal center. In children with B-cell deficiencies, the cortex lacks primary follicles and germinal centers. Beneath the cortex is the para cortex, which is popu-lated largely by T lymphocytes and also contains inter digitating dendritic cells thought to have migrated from tissues to the node. These inter digitating dendritic cells express high levels of class II MHC molecules, which are necessary for presenting antigen to T_H cells. Lymph nodes taken from neonatal thymectomized mice have unusually few cells in the paracortical region; the paracortex is therefore sometimes re-ferred to as a thymus-dependent area in contrast to the cortex, which is a thymus-independent area. The innermost layer of a lymph node, the medulla, is more sparsely populated with lymphoid-lineage cells; of those present, many are plasma cells actively secreting antibody molecules.

As antigen is carried into a regional node by the lymph, it is trapped, processed, and presented together with class II MHC molecules by inter digitating dendritic cells in the para-cortex, resulting in the activation of T_H cells. The initial activation of B cells is also thought to take place within the T-cell-rich paracortex. Once activated, T_H and B cells form small foci consisting largely of proliferating B cells at the edges of the paracortex. Some B cells within the foci differentiate into plasma cells secreting IgM and IgG. These foci reach maximum size within 4–6 days of antigen challenge. Within 4–7 days of antigen challenge, a few B cells and T_H cells migrate to the primary follicles of the cortex. It is not known what causes this migration. Within a primary follicle, cellular interactions between follicular dendritic cells, B cells, and T_H cells take place, leading to development of a secondary follicle with a central germinal center. Some of the plasma cells generated in the germinal center move to the medullary areas of the lymph node, and many migrate to bone marrow.

Afferent lymphatic vessels pierce the capsule of a lymph node at numerous sites and empty lymph into the sub-capsular sinus (Figure b). Lymph coming from the tissues percolates slowly inward through the cortex, paracortex, and medulla, allowing phagocytic cells and dendritic cells to trap any bacteria or particulate material (e.g., antigen-antibody complexes) carried by the lymph. After infection or the introduction of other antigens into the body, the lymph leaving a node through its single efferent lymphatic vessel is enriched with antibodies newly secreted by medullary plasma cells and also has a fiftyfold higher concentration of lymphocytes than the afferent lymph.



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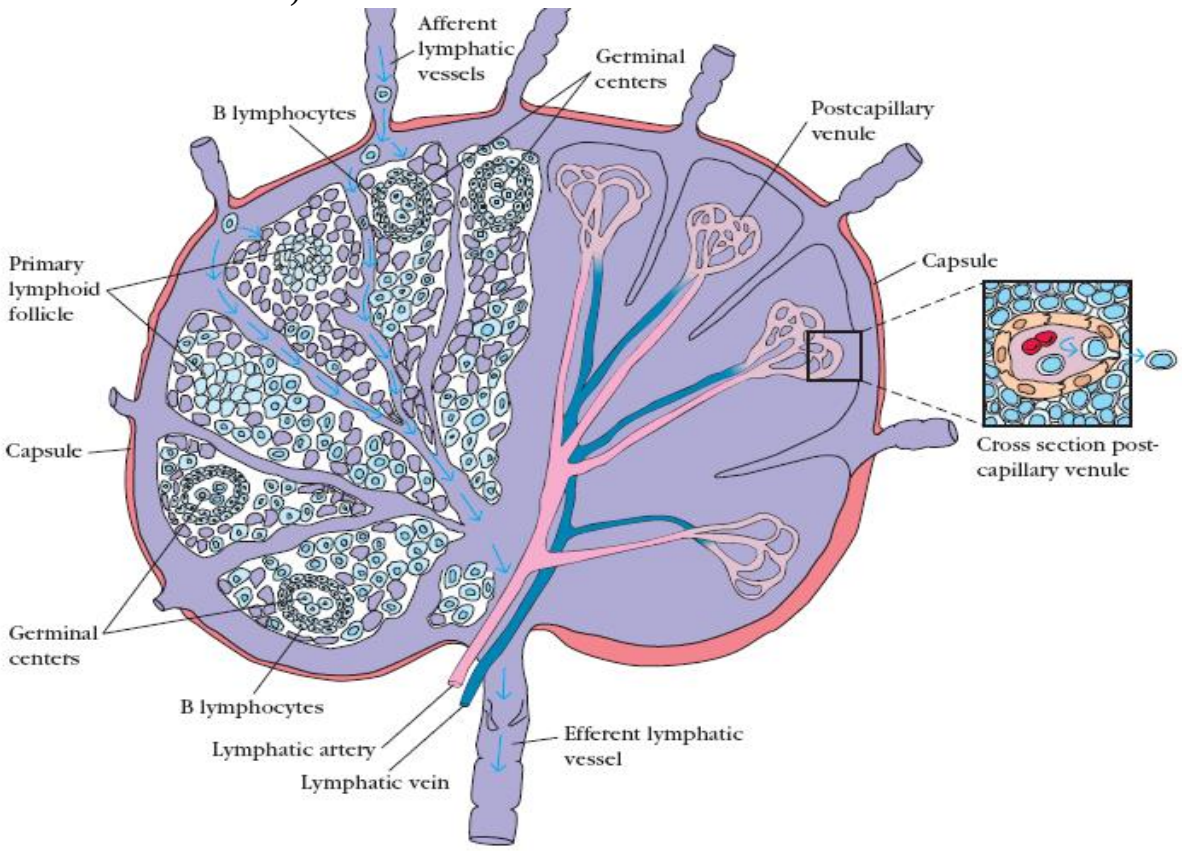


Fig: Structure of a lymph node. (a) The three layers of a lymph node support distinct microenvironments**. (b)** The left side depicts the arrangement of reticulum and lymphocytes within the various regions of a lymph node. Macrophages and dendritic cells, which trap antigen, are present in the cortex and paracortex. T_H cells are concentrated in the paracortex; B cells are located primarily in the cortex, within follicles and germinal centers. The medulla is populated largely by antibody-producing plasma cells. Lymphocytes circulating in the lymph are carried into the node by afferent lymphatic vessels; they either enter the reticular matrix of the node or pass through it and leave by the efferent lymphatic vessel. The right side of (b) depicts the lymphatic artery and vein and the post capillary venules. Lymphocytes in the circulation can pass into the node from the post capillary venues by a process called extravasations

SPLEEN

The spleen plays a major role in mounting immune responses to antigens in the blood stream. It is a large, ovoid secondary lymphoid organ situated high in the left abdominal cavity and weighing about 150 grams. It is the largest single lymphoid organ in the body. While lymph nodes are specialized for trapping antigen from local tissues, the spleen specializes in filtering blood and trapping blood-borne antigens; thus, it can respond to systemic infections. Unlike the lymph nodes, the spleen is not supplied by lymphatic vessels. Instead, blood-borne antigens and lymphocytes are carried into the spleen through the splenic artery. Experiments with

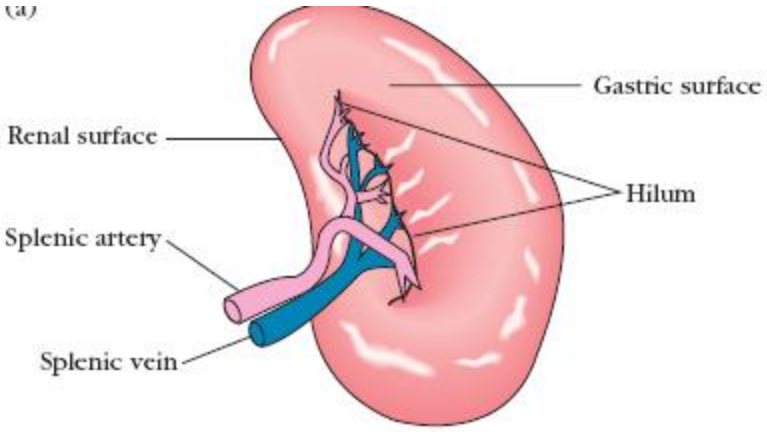
radioactively labeled lymphocytes show that more re-circulating lymphocytes pass daily through the spleen than through all the lymph nodes combined.

The spleen is surrounded by a capsule that extends a number of projections (trabeculae) into the interior to form a compartmentalized structure. The compartments are of two types, the red pulp and white pulp, which are separated by a diffuse marginal zone (Figure 2-19). The splenic red pulp consists of a network of sinusoids populated by macrophages and numerous red blood cells (erythrocytes) and few lymphocytes; it is the site where old and defective red blood cells are destroyed and removed. Many of the macrophages within the red pulp contain engulfed red blood cells or iron pigments from degraded hemoglobin. The splenic white pulp sur-rounds the branches of the splenic artery, forming a periarteriolar lymphoid sheath (PALS) populated mainly by T lymphocytes. Primary lymphoid follicles are attached to the
PALS. These follicles are rich in B cells and some of them contain germinal centers. The marginal zone, located peripheral to the PALS, is populated by lymphocytes and macrophages.

Blood-borne antigens and lymphocytes enter the spleen through the splenic artery, which empties into the marginal zone. In the marginal zone, antigen is trapped by interdigitating dendritic cells, which carry it to the PALS. Lymphocytes in the blood also enter sinuses in the marginal zone and migrate to the PALS.

The initial activation of B and T cells takes place in the T-cell-rich PALS. Here inter digitating dendritic cells capture antigen and present it combined with class II MHC molecules to T_H cells. Once activated, these T_H cells can then activate B cells. The activated B cells, together with some T_H cells, then migrate to primary follicles in the marginal zone. Upon antigenic challenge, these primary follicles develop into characteristic secondary follicles containing germinal centers (like those in the lymph nodes), where rapidly dividing B cells (centroblasts) and plasma cells are surrounded by dense clusters of concentrically arranged lymphocytes.

In children, splenectomy often leads to an increased incidence of bacterial sepsis caused primarily by *Streptococcus pneumoniae, Neisseria meningitidis,* and *Haemophilus influenzae.* Splenectomy in adults has less adverse effects, although it leads to some in-crease in blood-borne bacterial infections (bacteremia).



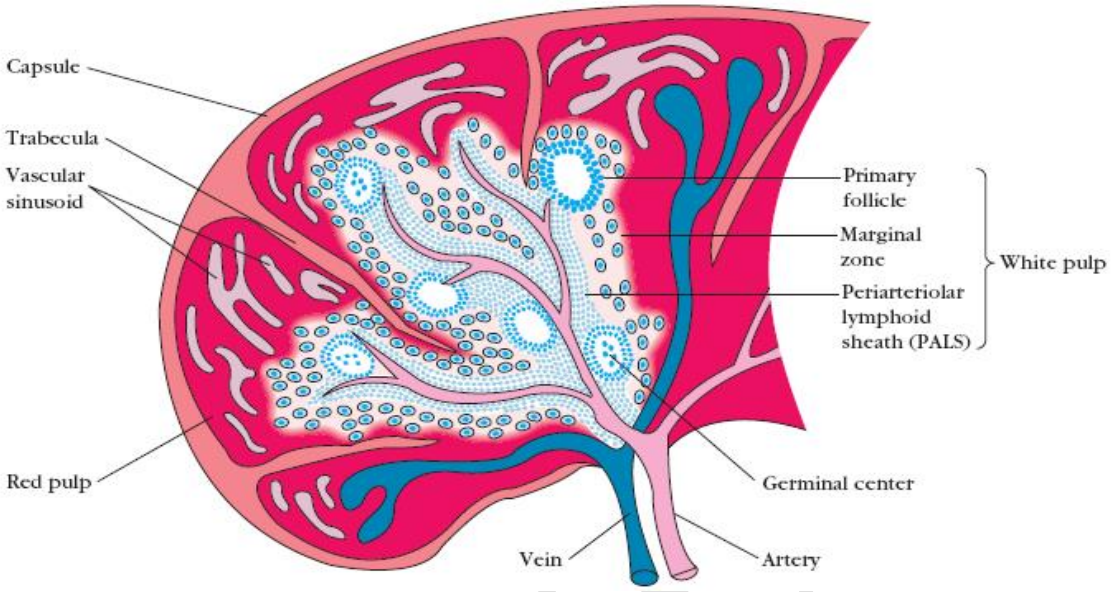


Fig: (a) The spleen, which is about 5 inches long in adults, is the largest secondary lymphoid organ. It is specialized for trapping blood-borne antigens**. (b) Diagrammatic cross section of the spleen**. The splenic artery pierces the capsule and divides into progressively smaller arterioles, ending in vascular sinusoids that drain back into the splenic vein. The erythrocyte-filled red pulp surrounds the sinusoids. The white pulp forms a sleeve, the periarteriolar lymphoid sheath (PALS), around the arteri-oles; this sheath contains numerous T cells. Closely associated with the PALS is the marginal zone, an area rich in B cells that contains lymphoid follicles that can develop into secondary follicles contain-ing germinal centers

MUCOSA ASSOCIATED LYMPHOID TISSUE (MALT):

Approximately >50% of lymphoid tissue in the body is found associated with the mucosal system. MALT is composed of gut-associated lymphoid tissues (GALT) lining the intestinal tract, bronchus-associated lymphoid tissue (BALT) lining the respiratory tract, and lymphoid tissue lining the genitourinary tract. The respiratory, alimentary and genitourinary tracts are guarded by sub epithelial accumulations of lymphoid tissue that are not covered by connective tissue capsule. They may occur as diffuse collections of lymphocytes, plasma cells and phagocytes throughout the lung and lamina propria of intestine or as clearly organized tissue with well-formed lymphoid follicles. The well-formed follicles include the tonsils (lingual, palatine and pharyngeal), Peyer's patches in the intestine and appendix. The major function of these organs is to provide local immunity by way of IgA (also IgE) production. Diffuse accumulations of lymphoid tissue are seen in the lamina propria of the intestinal wall. The intestinal epithelium overlying the Peyer's patches is specialized to allow the transport of antigens into the lymphoid tissue. This function is carried out by cuboidal absorptive epithelial cells termed "M" cells, so called because they have numerous microfolds on their luminal surface. M cells endocytosis, transport and present antigens to sub epithelial lymphoid cells. Majority of intra-epithelial lymphocytes are T cells, and most often CD8+ lymphocytes. The

intestinal lamina propria contains CD4+ lymphocytes, large number of B cells, plasma cells, macrophages, dendritic cells, eosinophils and mast cells. Peyer's patches contain both B cells and CD4+ T cells.

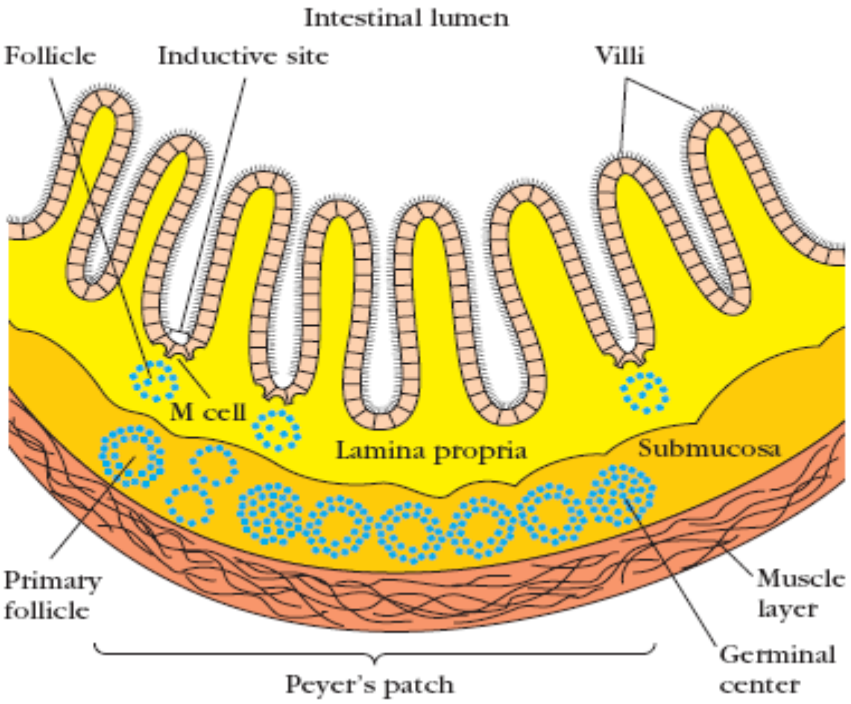


Fig: Cross-sectional diagram of the mucous membrane lining the intestine showing a nodule of lymphoid follicles that constitutes a Peyer's patch in the sub mucosa. The intestinal lamina propria contains loose clusters of lymphoid cells and diffuse follicles.

ANTIGEN

An antigen is any substance that causes immune system to produce antibodies against it. An antigen may be a foreign substance from the environment such as chemicals, bacteria, viruses, or pollen. An antigen may also be formed within the body, as with bacterial toxins or tissue cells.

Essential features of Antigen:

- Antigen, foreign substance that, when introduced into the body, is capable of stimulating an immune response, specifically activating lymphocytes
- Virtually any large foreign molecule can act as an antigen, including those contained in bacteria, viruses, protozoa, helminthes, foods, snake venoms, egg white, serum components, red blood cells, and other cells and tissues of various species including humans.
- An antigen that induces an immune response stimulates the lymphocytes to produce antibody

- On the surface of the antigens are regions, called antigenic determinants (epitope), that fit and bind to receptor molecules of complementary structure on the surface of the lymphocytes
- The binding of the lymphocytes' receptors to the antigens' surface molecules stimulates the lymphocytes to multiply and to initiate an immune response by the production of antibody, activation of cytotoxic cells, or both.
- The amount of antibody formed in response to stimulation depends on the kind and amount of antigen involved, the route of entry to the body, and individual characteristics of the host

Factors influencing antigenicity:

Molecular size: Large molecules are better antigen than small molecules Eg: hemocyanin- a large protein is a potent antigen

Structural stability: To recognize a molecule or part of a molecule as foreign, the cells of immune system must recognize its specific shape. Consequently, highly flexible molecules that have fixed shape are poor antigens. Eg: Gelatin-poor antigen

Degradability: The cells of immune system recognize small molecular fragments and soluble antigens. If a molecule cannot be broken up or solubilized, then it cannot acts as an antigen. Eg-Stainless steel pin

Foreignness: The cells whose function is it respond to antigen are selected in such a way that they do not usually respond to normal body components. They will respond, however, to foreign molecules differ even in minor respects from those usually found within the body. This property is **immunogenicity** and this depends on the degree of foreignness.

Specificity and Cross-Reactivity

Specificity measures the degree to which the immune system differentiates between different antigens. **Cross-reactivity** measures the extent to which different antigens appear similar to the immune system. The molecular determinants of specificity and cross-reactivity define the nature of antigenic variation and the selective processes that shape the distribution of variants in populations.

The surfaces of parasite molecules contain many overlapping antibody-binding sites (epitopes). An antibody bound to an epitope covers about 15 amino acids on the surface of a parasite molecule. However, only about 5 of the parasite's amino acids contribute to the binding energy. A change in any of those 5 key amino acids can greatly reduce the strength of antibody binding.

Antibodies have a variable region of about 50 amino acids that contains many overlapping paratopes. Each paratope has about 15 amino acids, of which about 5 contribute most of the binding energy for epitopes. Paratopes and epitopes define complementary regions of shape and charge rather than particular amino acid compositions. A single paratope can bind to unrelated epitopes, and a single epitope can bind to unrelated paratopes.

Naive B cells make IgM antibodies that typically bind with low affinity to epitopes. A particular epitope stimulates division of B cells with relatively higher-affinity IgM antibodies for the epitope. As the stimulated B cell clones divide rapidly, they also mutate their antibody-binding regions at a high rate. Mutant lineages that bind with higher affinity to the target antigen divide

more rapidly and out compete weaker-binding lineages. This mutation and selection produces high-affinity antibodies, typically of type IgA or IgG.

Each natural antibody can bind with low affinity to many different epitopes. Natural antibodies from different B cell lineages form a diverse set that binds with low affinity to almost any antigen. One in vitro study of HIV suggested that these background antibodies bind to the viruses with such low affinity that they do not interfere with infection. By contrast, in vivo inoculations with several different pathogens showed that the initial binding by natural antibodies lowered the concentrations of pathogens early in infection by one or two orders of magnitude.

Poor binding conditions cause low-affinity binding to be highly specific because detectable bonds form only between the strongest complementary partners. By contrast, favorable binding conditions cause low-affinity binding to develop a relatively broad set of complementary partners, causing relatively low specificity. The appropriate measure of affinity varies with the particular immune process. Early stimulation of B cells appears to depend on the equilibrium binding affinity for antigens. By contrast, competition between B cell clones for producing affinity-matured antibodies appears to depend on the dynamic rates of association between B cell receptors and antigens.

Polyclonal immune responses raise antibodies against many epitopes on the surface of an antigen. Cross-reactivity declines linearly with the number of amino acid substitutions between variant antigens because each exposed amino acid contributes only a small amount to the total binding between all antibodies and all epitopes. By contrast, a monoclonal antibody usually binds to a single epitope on the antigen surface. Cross-reactivity declines rapidly and nonlinearly with the number of amino acid substitutions in the target epitope because a small number of amino acids control most of the binding energy.

Immunogenicity

Immunogenicity is the ability of a particular substance, such as an antigen or epitope, to provoke an immune response in the body of a human or animal. In other words, immunogenicity is the ability to induce a humoral and/or cell mediated immune response.

Differentiation has to be made between wanted and unwanted immunogenicity.

- Wanted immunogenicity is typically related with vaccines, where the injection of an antigen (the vaccine) has to lead to an immune response against the pathogen (the virus, bacterium or substance).
- Unwanted immunogenicity is when the organism mounts an immune response against an antigen which is undesired. Unwanted immunogenicity is strongly linked with therapeutic proteins. A fraction of the patient treated with those drugs mount anti-drug-antibodies, which leads to inactivation of the drug and in rare cases to adverse effects .

Immunogenicity Vs Antigenicity

Immunogenicity and antigenicity are related but distinct immunologic properties that sometimes are confused. **Immunogenicity** is the ability to induce a humoral and/or cell-mediated immune response:

B cells + antigen → effector B cells + memory B cells
↓
(plasma cells)

T cells + antigen → effector T cells + memory T cells
↓
(e.g., CTLs, T_Hs)

Although a substance that induces a specific immune response is usually called an antigen, it is more appropriately called an **immunogen.**

Antigenicity is the ability to combine specifically with the final products of the above responses (i.e., antibodies and/or cell-surface receptors). Although all molecules that have the property of immunogenicity also have the property of antigenicity, the reverse is not true. Some small molecules, called haptens, are antigenic but incapable, by themselves, of inducing a specific immune response. In other words, they lack immunogenicity

Immunogenic potency of antigens

Proteins are significantly more immunogenic than polysaccharides. T cell response is required to drive immunogenicity.

Since lipids and nucleicacids arenon-immunogenic haptens,theyrequire conjugation with an epitope such as a protein or polysaccharide before they can evoke an immunologic response.

- Proteins or polysaccharides are used for studies of humoral immune response.
- Only proteins can serve as immunogens for cell-mediated immunity.

Immunogenicity is influenced by multiple characteristics of an antigen:

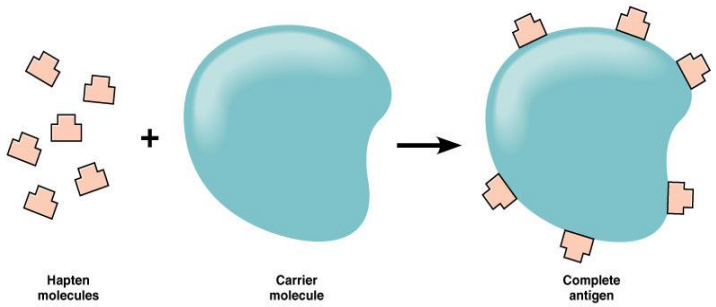
- Phylogenetic distance
- Molecular size
- Epitope density
- Chemical composition and heterogeneity
- Protein structure, aa-polymers, Glu-Lys, Tyr, Phe
- Degradability ability to be processed & presented to T cells
- D-amino acids

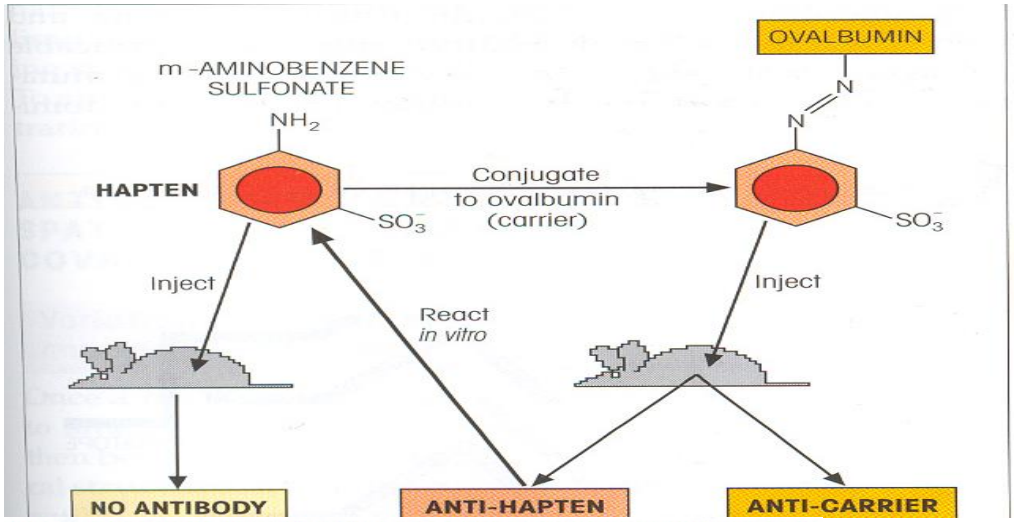
The Biological System is also contributes to Immunogenicity. They are

- Genotype of the recipient animal
- Immunogen dosage and route of administration

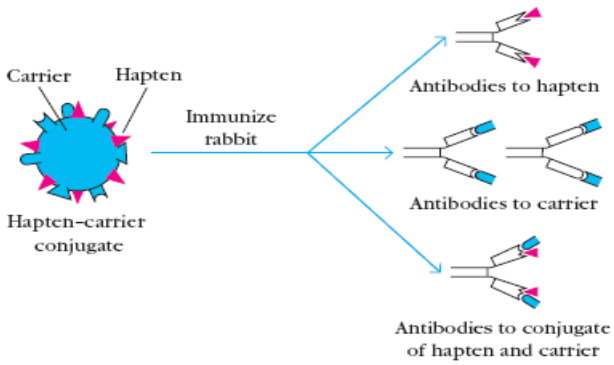
HAPTEN

- A **hapten** is a small molecule that can elicit an immune response only when attached to a large carrier such as a protein; the carrier may be one that also does not elicit an immune response by itself. (In general, only large molecules, infectious agents, or insoluble foreign matter can elicit an immune response in the body.) Once the body has generated antibodies to a hapten-carrier adduct, the small-molecule hapten may also be able to bind to the antibody, but it will usually not initiate an immune response; usually only the hapten-carrier adduct can do this. Sometimes the small-molecule hapten can even block immune response to the hapten-carrier adduct by preventing adduct from binding to the antibody, a process called hapten inhibition.
- **Haptens** are low-molecular weight molecules which contain an antigenic determinant but which are not itself antigenic unless complexed with an immunogenic carrier. Classical haptens include di- and trinitrophenol (DNP & TNP), dimethylaminonaphthalene sulfonate (dansyl), and a number of toxins, including urushiol, which is the toxin found in poison ivy.
- A substance that is capable of reacting with a specific antibody but cannot induce the formation of antibodies unless bound to a carrier protein or other molecule. Also called incomplete antigen, partial antigen.
- The first haptens used were aniline and its carboxyl derivatives (o-, m-, and p-aminobenzoic acid). A well-known example of a hapten is urushiol, which is the toxin found in poison ivy. When absorbed through the skin from a poison ivy plant, urushiol undergoes oxidation in the skin cells to generate the actual hapten, a reactive molecule called a quinone, which then reacts with skin proteins to form hapten adducts. Usually, the first exposure causes only sensitization, in which there is a proliferation of effector T-cells. After a second exposure later, the proliferated T cells can become activated, generating an immune reaction, producing the typical blisters of poison ivy exposure. Some haptens can induce autoimmune disease. An example is hydralazine, a blood pressure-lowering drug that occasionally can produce drug-induced lupus erythematosus in certain individuals. This also appears to be the mechanism by which the anaesthetic gas halothane can cause a life-threatening hepatitis, as well as the mechanism by which penicillin-class drugs cause autoimmune hemolytic anemia.Other haptens that are commonly used in molecular biology applications include fluorescein, biotin, digoxigenin, and dinitrophenol.
- Penicillin bound to a protein can result in Ab production to the penicillin (results in an allergy to penicillin





A hapten on its own will not induce antibodies.



Injection with:	Antibodies formed:
Hapten (DNP)	None
Protein carrier (BSA)	Anti-BSA
Hapten–carrier conjugate (DNP-BSA)	Anti-DNP (major) Anti-BSA (minor) Anti-DNP/BSA (minor)

Fig: A hapten-carrier conjugate contains multiple copies of the hapten—a small nonimmunogenic organic compound such as dinitrophenol (DNP)—chemically linked to a large protein carrier such as bovine serum albumin (BSA). Immunization with DNP alone elicits no anti-DNP antibodies, but immunization with DNP-BSA elicits three types of antibodies. Of these, anti-DNP antibody is predominant, indicating that in this case the hapten is the immunodominant epitope in a hapten-carrier conjugate, as it often is in such conjugates.

Many biologically important substances, including drugs, peptide hormones, and steroid hormones, can function as haptens.

Carrier molecule (Carrier Proteins)

Carrier Proteins for Immunogen Preparation

A carrier protein is any protein used for coupling with peptides or other haptens that are not sufficiently large or complex on their own to induce an immune response and produce antibodies. The carrier protein, because it is large and complex, confers immunogenicity to the conjugated hapten, resulting in antibodies being produced against epitopes on the hapten and carrier. Many proteins can be used as carriers and are chosen based on immunogenicity, solubility, and availability of useful functional groups through which conjugation with the hapten can be achieved. The two most commonly used carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA).

In a typical immune response, antibodies are produced by B-lymphocytes. In the majority of hapten-carrier systems, the B cells will produce antibodies that are specific for both the hapten and carrier. Because an antibody response will be directed against epitopes on both the carrier protein and hapten, it is important to plan carefully how hapten-specific antibodies will be identified and purified from the final immunized serum. To create the best immunogen, it may be beneficial to prepare the conjugates with several different carriers and with a range of hapten:carrier coupling ratios.

IMMUNOLOGICAL VALENCE

The number of epitopes on any antigen(s) to which antibody can bind is termed its valence. It is also defined as the number of antigen binding sites (ABS) antibodies possess.

Affinity

It is defined as the tightness of binding of an ABS of the antibody to the antigenic determinant (epitope) of the Ag. The tighter the binding, the less likely the antibody is to dissociate from Ag. Different antibodies to a single epitope of an antigen vary considerably in their affinity for that epitope. Clearly, the affinity of an antibody population is critical when the Ag is a toxin or virus, and must be neutralized by rapid and firm binding with the antibody. Antibodies produced by a memory response have higher affinity (about 1000 times higher) than those in a primary response (soon after the first exposure to the Ag).

Antibody Valence & Avidity

The valence of an antibody is the maximum number of antigenic determinants with which it can react. For example, IgG contains 2 Fab regions and can bind 2 molecules of antigens or 2 epitopes of the same antigenic particle, and thus is said to have a valence of 2. Having multiple binding sites for an antigen dramatically increases its binding to antigens on particles such as bacteria or virus. This synergistic and strengthened binding effect is termed avidity, which provides firmness of association between a multi-determinant antigen and the antibodies produced against it. In other words, avidity is the sum total of the strength of binding of these 2 molecules to each other at multiple sites (It is distinct from affinity, which is the strength of binding of at a single site). Determining the avidity of an antibody population is very difficult since it involves evaluating some function of the group interactions of a large number of different antibodies, with a large number of different antigenic determinants. But the importance of avidity is immense. For instance, 2 binding sites on IgG are 10-100 times more effective at neutralizing a virus than 2 unlinked binding sites. In case of IgM, whose valence may be up to 10, the avidity shoots up to a million times. This can be explained as follows.

Antibody with a single binding site to the antigen can bind it, but may also dissociate later. This results in the parting of the antibody from the antigen. However, if the valence is more than 1 for epitopes, even if dissociation occurs at one site, the association at other site(s) remains, and helps the reassociation at the first site. Hence, larger is the number of the bonds formed between the antigen and antibody, it is less likely that they will dissociate from each other.

Importance of Avidity: Despite relatively poor intrinsic affinity for an antigenic determinant but a high valence value, antibodies are able to clear their specific antigens (virus etc.) with great efficiency due to a derived high avidity feature.

ANTIGENIC DETERMINANT (EPITOPE)

An **epitope**, also known as **antigenic determinant**, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells. The part of an antibody that recognizes the epitope is called a paratope.

Adjuvants

Adjuvants (from Latin *adjuvare,* to help) are substances that, when mixed with an antigen and injected with it, enhance the immunogenicity of that antigen, without having any specific antigenic effect in it. Adjuvants are often used to boost the immune response when an antigen has low immunogenicity or when only small amounts of an antigen are available. For example, the antibody response of mice to immunization with BSA can be increased fivefold or more if the BSA is administered with an adjuvant. Precisely how adjuvants augment the immune response is not entirely known, but they appear to exert one or more of the following effects:

- Antigen persistence is prolonged.
- Co-stimulatory signals are enhanced.
- Local inflammation is increased.
- The nonspecific proliferation of lymphocytes is stimulated.

Adjuvants in immunology are often used to modify or augment the effects of a vaccine by stimulating the immune system to respond to the vaccine more vigorously, and thus providing increased immunity to a particular disease. Adjuvants accomplish this task by mimicking specific sets of evolutionarily conserved molecules, so called PAMPs, which include liposomes, lipopolysaccharide (LPS), molecular cages for antigen, components of bacterial cell walls, and endocytosed nucleic acids such as double-stranded RNA (dsRNA), single-stranded DNA (ssDNA), and unmethylated CpG dinucleotide-containing DNA. Because immune systems have evolved to recognize these specific antigenic moieties, the presence of an adjuvant in conjunction with the vaccine can greatly increase the innate immune response to the antigen by augmenting the activities of dendritic cells (DCs), lymphocytes, and macrophages by mimicking a natural infection. Furthermore, because adjuvants are attenuated beyond any function of virulence, they pose little or no independent threat to a host organism.

Inorganic adjuvants

Aluminium salts

There are many adjuvants, some of which are inorganic (such as alum), that also carry the potential to augment immunogenicity. Two common salts include aluminium phosphate and aluminium hydroxide. These are the most common adjuvants in human vaccines.

Aluminum potassium sulfate (alum) prolongs the persistence of antigen. When an antigen is mixed with alum, the salt precipitates the antigen. Injection of this alum precipitate results in a slower release of antigen from the injection site, so that the effective time of exposure to the antigen increases from a few days without adjuvant to several weeks with the adjuvant. The alum precipitate also increases the size of the antigen, thus increasing the likelihood of phagocytosis.

Organic adjuvants

While Aluminium salts are popularly used in human vaccines, the organic compound Squalene is also used. However, organic adjuvants are more commonly used in animal vaccines.

Oil-based

Oil-based adjuvants are commonly used in some veterinary vaccines.

Water-in-oil adjuvants- also prolong the persistence of antigen. A preparation known as **Freund's incomplete adjuvant** contains antigen in aqueous solution, mineral oil, and an emulsifying agent such as mannide monooleate, which disperses the oil into small droplets surrounding the antigen; the antigen is then released very slowly from the site of injection. This preparation is based on **Freund's complete adjuvant,** the first deliberately formulated highly effective adjuvant, developed by Jules Freund many years ago and containing heat-killed Mycobacteria as an additional ingredient. Muramyl dipeptide, a component of the mycobacterial cell wall, activates macrophages, making Freund's complete adjuvant far more potent than the

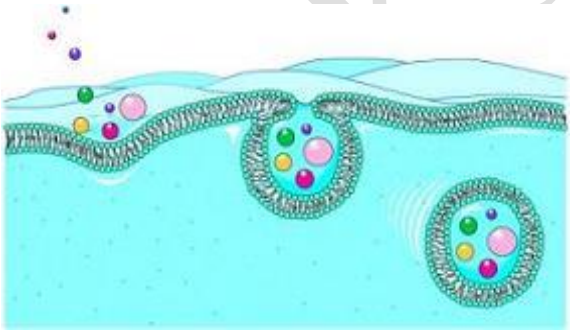
in-complete form. Activated macrophages are more phagocytic than unactivated macrophages and express higher levels of class II MHC molecules and the membrane molecules of the B7 family. The increased expression of class II MHC increases the ability of the antigen-presenting cell to present antigen to T_H cells. B7 molecules on the antigen-presenting cell bind to CD28, a cell-surface protein on T_H cells, triggering co-stimulation, an enhancement of the T-cell immune response. Thus, antigen presentation and the requisite co-stimulatory signal usually are increased in the presence of adjuvant.

Alum and Freund's adjuvants also stimulate a local, chronic inflammatory response that attracts both phagocytes and lymphocytes. This infiltration of cells at the site of the adjuvant injection often results in formation of a dense, macrophage-rich mass of cells called a **granuloma.** Because the macrophages in a granuloma are activated, this mechanism also enhances the activation of T_H cells.

Other adjuvants (e.g., synthetic polyribonucleotides and bacterial lipopolysaccharides) stimulate the nonspecific pro-liferation of lymphocytes and thus increase the likelihood of antigen-induced clonal selection of lymphocytes

Virosomes

Another market-approved adjuvant and carrier system are virosomes. During the last two decades, a variety of technologies have been investigated to improve the widely-used adjuvants based on aluminium salts. These salts are unfavorable, since they develop their effect by inducing local inflammation, which is also the basis for the extended side-effect pattern of this adjuvant. In contrast, the adjuvant capabilities of virosomes are independent of any inflammatory reaction. Virosomes contain a membrane-bound hemagglutinin and neuraminidase derived from the influenza virus, and serve to amplify fusogenic activity. Therefore facilitate the uptake into antigen presenting cells (APC) and induce a natural antigen-processing pathway. The delivery of the antigen by virosomes to the immune system in a way that mimics a natural path may be a reason why virosome-based vaccines stand out due to their excellent safety profile



Today, adjuvants play an important role in the efficacy of vaccines. Stimulating the correct immune response is a must when selecting an adjuvant to use for a new vaccine. Since one adjuvant alone is rarely optimal for all antigens, it is critical to have a selection of different types of adjuvants for evaluation with one antigen.

ANTIBODIES

Antibodies are glycoprotein belonging to the immunoglobulin super family; the terms antibody and immunoglobulin are often used interchangeably. They present on the B-cell membrane and secreted by plasma cells. Membrane-bound antibody confers antigenic specificity on B cells;

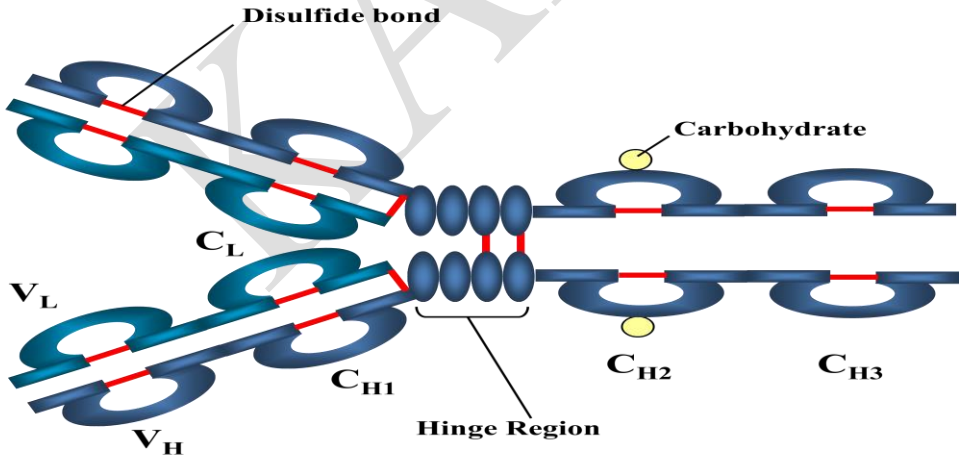
antigen-specific proliferation of B-cell clones is elicted by the interaction of membrane antibody with antigen. Secreted antibodies circulate in the blood, where they serve as the effectors of humoral immunity by searching out and neutralizing antigens or marking them for elimination. All antibodies share structural features, bind to antigen, and participate in a limited number of effect or functions.

The antibodies produced in response to a particular anti-gen are heterogeneous. Most antigens are complex and contain many different antigenic determinants, and the immune system usually responds by producing antibodies to several epitopes on the antigen. This response requires the recruitment of several clones of B cells. Their outputs are monoclonal antibodies, each of which specifically binds a single antigenic determinant. Together, these monoclonal antibodies make up the polyclonal and heterogeneous serum anti-body response to an immunizing antigen.

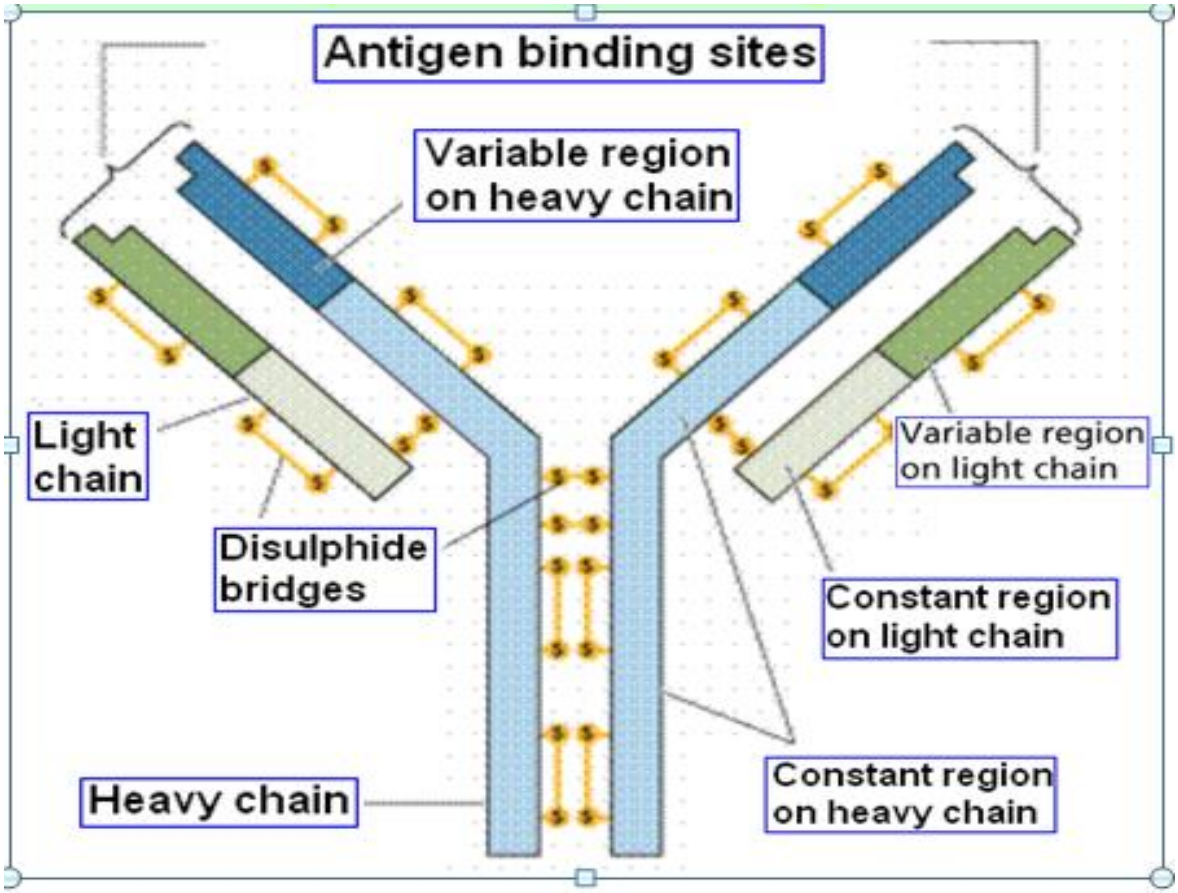
Immunoglobulins generally assume one of two roles: immunoglobulins may act as i) plasma membrane bound antigen receptors on the surface of a B-cell or ii) as antibodies free in cellular fluids functioning to intercept and eliminate antigenic determinants.

ANTIBODY-Structure

- Antibodies are typically made of basic structural units—each with two large heavy chains and two small light chains.



Structure



- Antibodies are heavy (~150 kDa) globular plasma proteins
- They have sugar chains added to some of their amino acid residues, so antibodies are glycoproteins.
- Immunoglobulins are composed of four polypeptide chains: two "light" chains (lambda or kappa), and two "heavy" chains (alpha, delta, gamma, epsilon or mu).
- The type of heavy chain determines the immunoglobulin isotype (IgA, IgD, IgG, IgE, IgM, respectively).
- Light chains are composed of 220 amino acid residues while heavy chains are composed of 440-550 amino acids. Each chain has "constant" and "variable" regions as shown in the figure.
- Variable regions (V region) are contained within the amino (NH_2) terminal end of the polypeptide chain (amino acids 1-110). When comparing one antibody to another, these amino acid sequences are quite distinct.
- Constant regions (C region), comprising amino acids 111-220 (or 440-550), are rather uniform, in comparison, from one antibody to another, within the same isotype.
- "Hypervariable" regions or "Complementarity Determining Regions" (CDRs) are found within the variable regions of both the heavy and light chains. These regions serve to recognize and bind specifically to antigen.

- The four polypeptide chains are held together by covalent disulfide (-S-S-) bonds

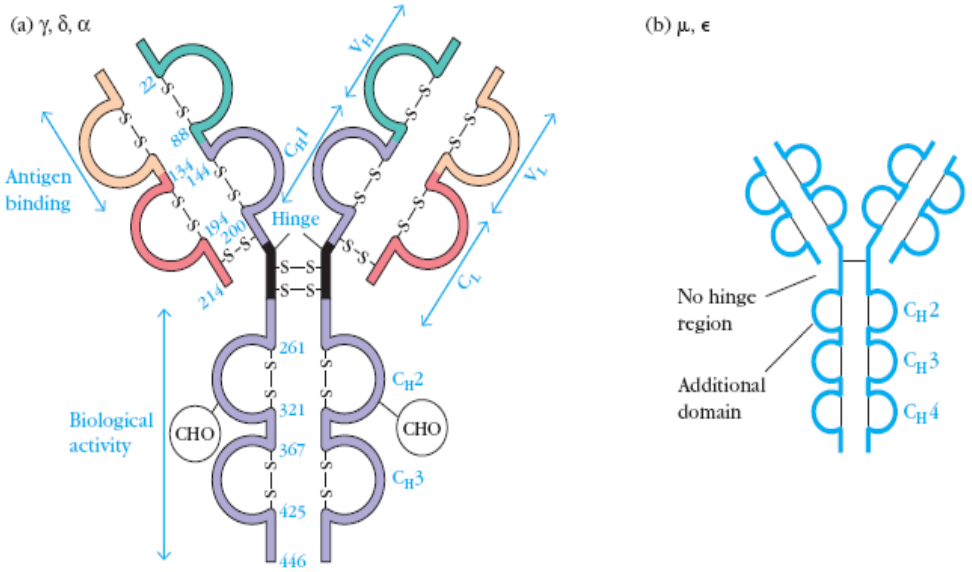


Fig:Heavy and light chains are folded into domains, each containing about 110 amino acid residues and an intrachain disulfide bond that forms a loop of 60 amino acids. The amino-terminal domains, corresponding to the V regions, bind to antigen effector functions are mediated by the other domains. (b) the and heavy chains contain an additional domain that replaces the hinge region.

Immunoglobulin domains

The Ig monomer is a "Y"-shaped molecule that consists of four polypeptide chains; two identical *heavy chains* and two identical *light chains* connected by disulfide bonds. Each chain is composed of structural domains called immunoglobulin domains. These domains contain about 70-110 amino acids and are classified into different categories (for example, variable or IgV, and constant or IgC) according to their size and function. They have a characteristic immunoglobulin fold in which two beta sheets create a “sandwich” shape, held together by interactions between conserved cysteines and other charged amino acids.

Heavy chain

There are five types of mammalian Ig heavy chain denoted by the Greek letters: α, δ, ε, γ, and μ. The type of heavy chain present defines the *class* of antibody; these chains are found in IgA, IgD, IgE, IgG, and IgM antibodies, respectively. Distinct heavy chains differ in size and composition; α and γ contain approximately 450 amino acids, while μ and ε have approximately 550 amino acids.

1. Fab region
2. Fc region

3. Heavy chain (blue) with one variable (V_H) domain followed by a constant domain (C_H1), a hinge region, and two more constant (C_H2 and C_H3) domains.
4. Light chain (green) with one variable (V_L) and one constant (C_L) domain
5. Antigen binding site (paratope)
6. Hinge regions.

Each heavy chain has two regions, the *constant region* and the *variable region*. The constant region is identical in all antibodies of the same isotype, but differs in antibodies of different isotypes. Heavy chains γ, α and δ have a constant region composed of *three* tandem (in a line) Ig domains, and a hinge region for added flexibility; heavy chains μ and ε have a constant region composed of *four* immunoglobulin domains. The variable region of the heavy chain differs in antibodies produced by different B cells, but is the same for all antibodies produced by a single B cell or B cell clone. The variable region of each heavy chain is approximately 110 amino acids long and is composed of a single Ig domain.

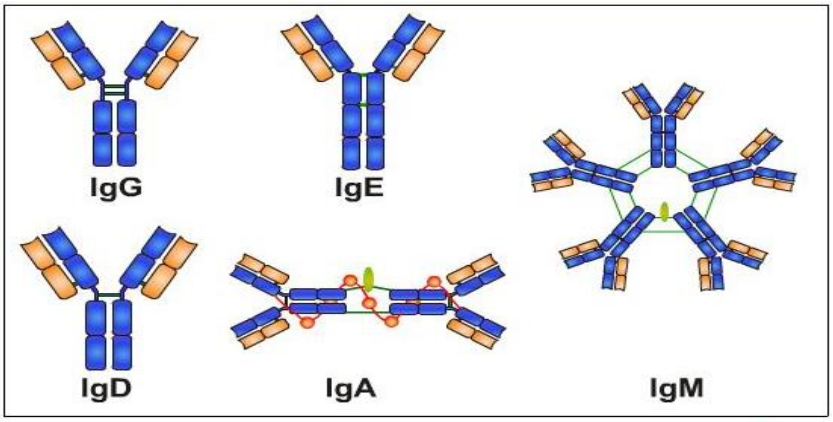
Light chain

In mammals there are two types of immunoglobulin light chain, which are called lambda (λ) and kappa (κ). A light chain has two successive domains: one constant domain and one variable domain. The approximate length of a light chain is 211 to 217 amino acids. Each antibody contains two light chains that are always identical; only one type of light chain, κ or λ, is present per antibody in mammals. Other types of light chains, such as the iota (ι) chain, are found in lower vertebrates like sharks (Chondrichthyes) and bony fishes (Teleostei).

Immunoglobulin Classes and Subclasses

- Immunglobulin molecules are divided into distinct classes and subclasses in terms of the differences in amino acid sequence of constant region of heavy chain, i.e.γ,α,μ,δ,and εchains
- Immunoglobulin Classes of Mammals
  - IgG - Gamma (γ) heavy chains
  - IgM - Mu (μ) heavy chains
  - IgA - Alpha (α) heavy chains
  - IgD - Delta (δ) heavy chains
  - IgE - Epsilon (ε) heavy chains
- IgG has a family of subclass, IgG1, IgG2, IgG3, IgG4(cattle has no)
- IgA is divided into two subclasses, IgA1 and IgA2(sheep)
- Mammalian antibodies can be divided into five classes: IgG, IgM, IgA, IgD and IgE, based on the number of Y units and the type of heavy chain
- The light chains of any antibody can be classified as either a kappa or Antibody Structure lambda type based on small polypeptide structural differences
- The heavy chain determines the subclass of each antibody
- The subclasses of antibodies differ in the number of disulfide bonds and the length of the hinge region

Fig: Five Classes of Immunoglobulin



- The most commonly used antibody in immunochemical procedures is of the IgG class because they are the major immunoglobulin (Ig) released in serum

IgG Immunoglobulins

IgG, a monomer, is the predominant Ig class present in human serum. Produced as part of the secondary immune response to an antigen, this class of immunoglobulin constitutes approximately 80% of total serum Ig. IgG is the only class of Ig that can cross the placenta in humans, and it is largely responsible for protection of the newborn during the first months of life. Because of its relative abundance and excellent specificity toward antigens, IgG is the principle antibody used in immunological research and clinical diagnostics.

- IgG, the most abundant class in serum, constitutes about 80% of the total serum immunoglobulin. The IgG molecule consists of two heavy chains and two or two light chain.s There are four human IgG subclasses, distinguished by differences in -chain sequence and numbered according to their decreasing average serum concentrations: IgG1, IgG2, IgG3, and IgG4.
- IgG1, IgG3, and IgG4 readily cross the placenta and play an important role in protecting the developing fetus.
- IgG3 is the most effective complement activator, followed by IgG1; IgG2 is less efficient, and IgG4 is not able to activate complement at all.
- IgG1 and IgG3 bind with high affinity to Fc receptors on phagocytic cells and thus mediate opsonization. IgG4 has an intermediate affinity for Fc receptors, and IgG2 has an extremely low affinity.

Properties of IgG:

- Molecular weight: 150,000
- H-chain type (MW): gamma (53,000)
- Serum concentration: 10 to 16mg/mL
- Percent of total immunoglobulin: 80%
- Glycosylation (by weight): 3%
- Distribution: intra- and extravascular

- Function: secondary response

IgM Immunoglobulins

Serum IgM exists as a pentamer in mammals, predominates in primary immune responses to most antigens, is the most efficient complement fixing immunoglobulin and comprises approximately 10% of normal human serum Ig content. IgM is also expressed on the plasma membrane of the B lymphocytes as a monomer. It is the B cell antigen receptor and the H chains each contain an additional hydrophobic domain for anchoring in the membrane. Monomers of serum IgM are bound together by disulfide bonds and a joining (J) chain.

Each of the five monomers is composed of two light chains (either kappa or lambda) and two heavy chains. Unlike in IgG (and the generalized structure shown above), the heavy chain in IgM monomers is composed of one variable and four constant regions, the additional constant domain replacing the hinge region. IgM can cause cell agglutination as a result of recognition of epitopes on invading microorganisms. This antibody-antigen immune complex is then destroyed by complement fixation or receptor mediated endocytosis by macrophages. IgM is the first immunoglobulin class to be synthesized by the neonate and plays a role in the pathogenesis of some autoimmune diseases.

IgM accounts for 5%–10% of the total serum immunoglob-ulin, with an average serum concentration of 1.5 mg/ml. Monomeric IgM, with a molecular weight of 180,000, is ex-pressed as membrane-bound antibody on B cells.

IgM is secreted by plasma cells as a pentamer in which five monomer units are held together. The five monomer subunits are arranged with their Fc regions in the center of the pentamer and the ten antigen-binding sites on the periphery of the molecule. Each pentamer contains an additional Fc-linked polypeptide called the **J (joining) chain,** which is disulfide-bonded to the carboxyl-terminal cysteine residue of two of the ten chains. The J chain ap-pears to be required for polymerization of the monomers to form pentameric IgM; it is added just before secretion of the pentamer.

IgM is the first immunoglobulin class produced in a primary response to an antigen, and it is also the first im-munoglobulin to be synthesized by the neonate. Because of its pentameric structure with 10 antigen-binding sites, serum IgM has a higher valency than the other isotypes. An IgM molecule can bind 10 small hapten molecules.

Because of its large size, IgM does not diffuse well and therefore is found in very low concentrations in the intercellular tissue fluids. The presence of the J chain allows IgM to bind to receptors on secretory cells, which transport it across epithelial linings to enter the external secretions that bathe mucosal surfaces.

Properties of IgM:

- Molecular weight: 900,000
- H-chain type (MW): mu (65,000)
- Serum concentration: 0.5 to 2mg/mL
- Percent of total immunoglobulin: 10%
- Glycosylation (by weight): 12%

- Distribution: mostly intravascular
- Function: primary response

IgA Immunoglobulins

Although IgA constitutes only 10%–15% of the total immunoglobulin in serum, it is the predominant immunoglobulin class in external secretions such as breast milk, saliva, tears, and mucus of the bronchial, genitourinary, and digestive tracts. In serum, IgA exists primarily as a monomer, but polymeric forms (dimers, trimers, and some tetramers) are sometimes seen, all containing a J-chain polypeptide. The IgA of external secretions, called **secretory IgA,** consists of a dimer or tetramer, a J-chain polypeptide, and a polypeptide chain called **secretory component.**

IgA exists in serum in both monomeric and dimeric forms, comprising approximately 15% of the total serum Ig. Secretory IgA, a dimer, provides the primary defense mechanism against some local infections because of its abundance in mucosal secretions (e.g., saliva, tears). The principal function of secretory IgA may not be to destroy antigen but to prevent passage of foreign substances into the circulatory system.

Properties of IgA:

- Molecular weight: 320,000 (secretory)
- H-chain type (MW): alpha (55,000)
- Serum concentration: 1 to 4mg/mL
- Percent of total immunoglobulin: 15%
- Glycosylation (by weight): 10%
- Distribution: intravascular and secretions
- Function: protect mucus membranes.IgA-secreting plasma cells are concentrated along mucous membrane surfaces. Breast milk contains secretory IgA and many other molecules that help protect the newborn against infection during the first month of life

IgD and IgE Immunoglobulins

IgD and IgE are found in serum in much smaller quantities than other Igs. Membrane IgD is a receptor for antigen found mostly on mature B-lymphocytes. IgE primarily defends against parasitic invasion and is responsible for allergic reactions.

Properties of IgD:

- Molecular weight: 180,000
- H-chain type (MW): delta (70,000)
- Serum concentration: 0 to 0.4mg/mL
- Percent of total immunoglobulin: 0.2%
- Glycosylation (by weight): 13%
- Distribution: lymphocyte surface
- Function: unknown

- IgD was first discovered when a patient developed a multiple myeloma whose myeloma protein failed to react with anti-isotype antisera against the then-known isotypes: IgA, IgM, and IgG. When rabbits were immunized with this myeloma protein, the resulting antisera were used to identify the same class of antibody at low levels in normal human serum. The new class, called IgD, has a serum concentration of 30 g/ml and constitutes about 0.2% of the total immunoglobulin in serum. IgD, together with IgM, is the major membrane-bound immunoglobulin expressed by mature B cells, and its role in the physiology of B cells is under investigation. No biological effector function has been identified for IgD.

Properties of IgE:

- Molecular weight: 200,000
- H-chain type (MW): epsilon (73,000)
- Serum concentration: 10 to 400ng/mL
- Percent of total immunoglobulin: 0.002%
- Glycosylation (by weight): 12%
- Distribution: basophils and mast cells in salive and nasal secretions
- Function: protect against parasites
- IgE binds to Fc receptors on the membranes of blood basophils and tissue mast cells. Cross-linkage of receptor-bound IgE molecules by antigen (allergen) induces basophils and mast cells to translocate their granules to the plasma membrane and release their contents to the extracellular environment, a process known as degranulation. As a result, a variety of pharmacologically active mediators are released and give rise to allergic manifestations

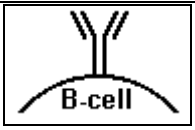
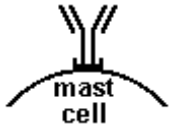
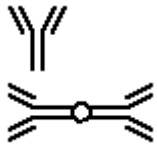
TABLE Chain composition of the five immunoglobulin classes in humans

Class	Heavy chain	Subclasses	Light chain	Molecular formula
IgG	γ	γ1, γ2, γ3, γ4	κ or λ	$\gamma_2\kappa_2$ $\gamma_2\lambda_2$
IgM	μ	None	κ or λ	$(\mu_2\kappa_2)_n$ $(\mu_2\lambda_2)_n$ $n = 1$ or 5
IgA	α	α1, α2	κ or λ	$(\alpha_2\kappa_2)_n$ $(\alpha_2\lambda_2)_n$ $n = 1, 2, 3,$ or 4
IgE	ϵ	None	κ or λ	$\epsilon_2\kappa_2$ $\epsilon_2\lambda_2$
IgD	δ	None	κ or λ	$\delta_2\kappa_2$ $\delta_2\lambda_2$

BASIC IMMUNOGLOBULIN FUNCTION

Antibodies function in a variety of ways designed to eliminate the antigen that elicited their production. Some of these functions are independent of the particular class (isotype) of immunoglobulin. These functions reflect the antigen binding capacity of the molecule as defined by the variable and hypervariable (idiotypic) regions. For example, an antibody might bind to a toxin and prevent that toxin from entering host cells where its biological effects would be activated. Similarly, a different antibody might bind to the surface of a virus and prevent that virus from entering its host cell. In contrast, other antibody functions are dependent upon the immunoglobulin class (isotype). These functions are contained within the constant regions of the molecule. For example, only IgG and IgM antibodies have the ability to interact with and initiate the complement cascade. Likewise, only IgG molecules can bind to the surface of macrophages via Fc receptors to promote and enhance phagocytosis. The following table summarizes some immunoglobulin properties.

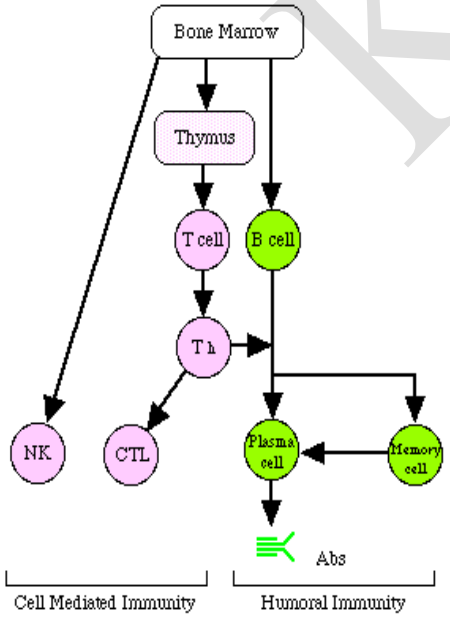
Isotype	Structure	Placental transfer	Binds mast cell surfaces	Binds phagocytic cell surfaces	Activates complement	Additional features
IgM		-	-	-	+	First Ab in development and response.

IgD		-	-	-	-	B-cell receptor.
IgG		+	-	+	+	Involved in opsonization and ADCC. Four subclasses; IgG1, IgG2, IgG3, IgG4.
IgE		-	+	-	-	Involved in allergic responses.
IgA		-	-	-	-	Two subclasses; IgA1, IgA2. Also found as dimer (sIgA) in secretions.

IMMUNE RESPONSE

The immune response is how the body recognizes and defends itself against bacteria, viruses, and substances that appear foreign and harmful. There are two types of immune response

1. Cell mediated immune response
2. Humoral mediated immune response



KAHE

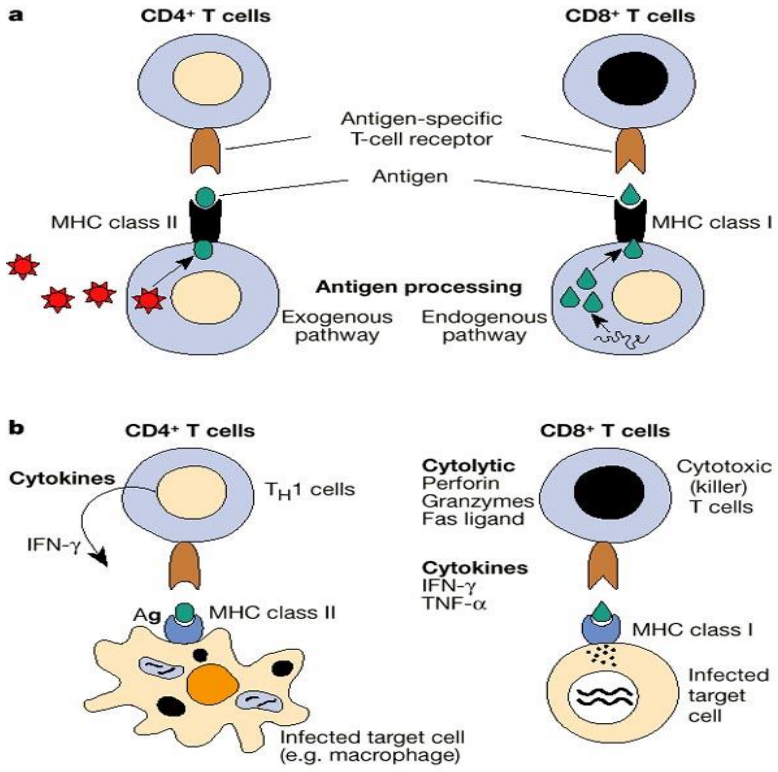
1. CELL MEDIATED IMMUNITY

Cell-mediated immunity

These $CD4^+$ cells bind to antigen presented by antigen-presenting cells (APCs) like phagocytic macrophages and dendritic cells. The T cells then release lymphokines that attract other cells to the area. The result is inflammation: the accumulation of cells and molecules that attempt to wall off and destroy the antigenic material (the rash following exposure to poison ivy is an example).

CM is adaptive immune response against intracellular microbes is mediated by T cells and can be transferred from immunized to naïve individuals to T cells and not by antibodies.

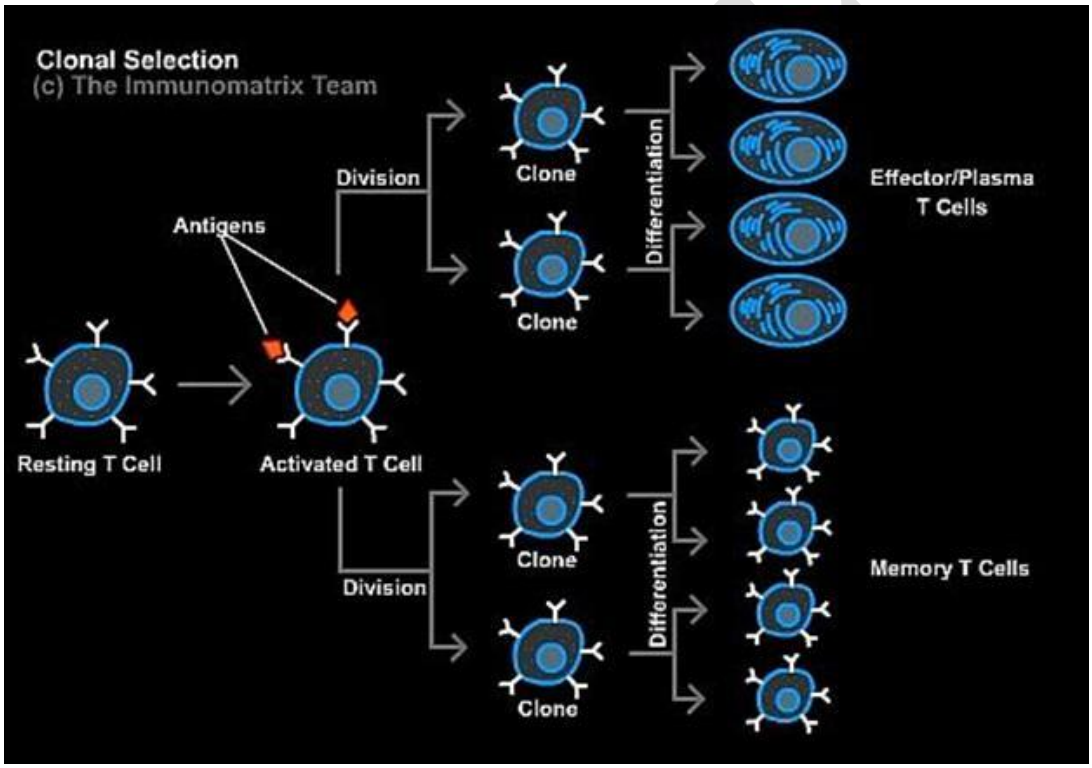
There are two types (main) cell mediated immunity. In one, which is exemplified by DTH reactions, CD4+ Th1 Cells, as well as CD8 Th2 cells, recognize antigens of microbes that have been ingested by phagocyte and activate the phagocytes to kill the microbes. In the second type of CM, CD8+ CTLs kill any nucleated cell that contains foreign antigens (microbial /tumor antigens) in cytosol.



Cell mediated immune response consists of several steps;

- naive cell recognition of cell- Associated antigens in peripheral lymphoid organs,
- clonal expansion of T cells and their differentiation into effector cells.
- Migration of affecter cell to the site of infection or antigen challenge and elimination of microbes or antigen.

CD4 helper T lymphocytes may differentiate into specialized effector cells Th1 cells that secrete IFN-g which favors phagocyte mediated immunity, or into Th2 cells that secrete Il-1and Il4 and Il5, which favor IgE and eosinophil/ mast cell mediated immune reaction. The differentiation of naïve CD4+Tcells into Th1 and Th2 population is controlled by cytokines produced by the T cells themselves.



CD8+ T cells differentiate in to effector CTLs acquiring the capacity to kill targets, under the influence of co stimulators and help from CD4+T c ells.

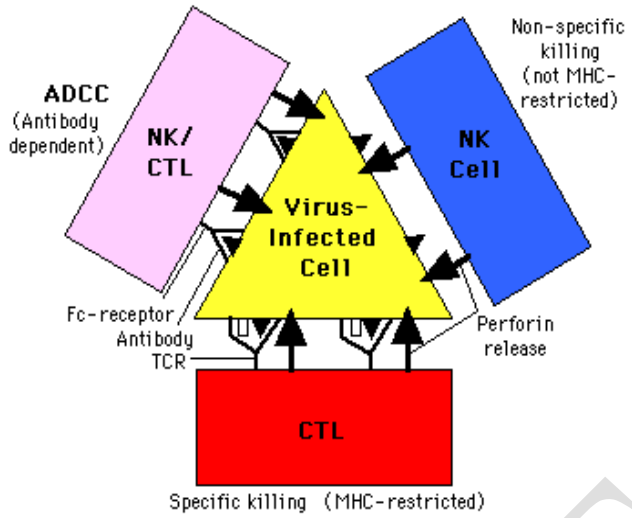


Fig: Activated NK and CTL act on a tumor cells and kill it

The migration of T cells to sites of infection is mediated by chemokines and the binding of adhesion molecules to their ligands on activated endothelium.

The activation of macrophages by TH1 cells is mediated by IFN-g and CD40L-CD40 interactions. Activated macrophages kill phogocytosed microbes, stimulate inflammation, and repair damaged tissues. If the infection is not fully resolved activated macrophages cause tissue damage and fibrosis.

HUMORAL IMMUNITY

Humoral immunity is mediated by secreted antibodies and its physiological function is defense against extracellular microbes (including viruses) and microbial exotoxins. Humoral immunity can be transferred to other individuals by the transfer of serum (antibodies). Defect in humoral immunity leads to enhanced infections by bacteria and fungi. Antibodies also participate in autoimmune disorders and hypersensitivity.

When an antigen with multiple epitopes gains entry into the body, different clones of B cells recognize and produce antibodies against different epitopes, thus the natural response is said to by polyclonal. However, by using hybridoma technology it is possible to develop a clone of B cells directed against a single epitope, and produce monoclonal antibodies.

Antibodies are produced by plasma cells in the secondary lymphoid organs, but antibodies can perform their effector functions at any site in the body. Once the antibodies enter the circulation or mucosa, they can easily reach sites of infection. Circulating antibodies can recognize antigen present in blood or can pass through the endothelium into tissue spaces and render their effector functions.

The first exposure to a microbe or an antigen, either by infection or by vaccination, leads to the activation of naive B lymphocytes. These B cells differentiate into antibody producing plasma cells and memory cells. Some of the antibody producing cells migrate to the bone marrow and live in this site for several years, where they continue to produce antibodies even when antigen has been eliminated. It is estimated that over half the IgG found in serum of normal individuals is derived from these long-lived antibody producing cells, which were induced by exposure to various antigens throughout the life of the individual. When the same antigen enters the body again, the circulating antibodies provide immediate protection against infection. At the same time, memory cells too are activated by the antigen and the resulting secondary response provides high level of protection.

Antibody production by activated B cells is the core the humoral response: antibody effects, such as complement activation by IgM and certain IgGs, opsonization via F(c)Rs and pathogen/toxin neutralization by high-affinity IgG and IgA; and processes related to B cell activation, such as T_H2 activation and cytokine production, germinal center formation, isotype switching, affinity maturation and memory cell production. The F(c) region of IgG binds to F(c) receptors, playing a critical role (along with receptors for complement byproducts) in clearing extracellular bacteria. Intracellular bacteria are cleared by cell-mediated immunity.

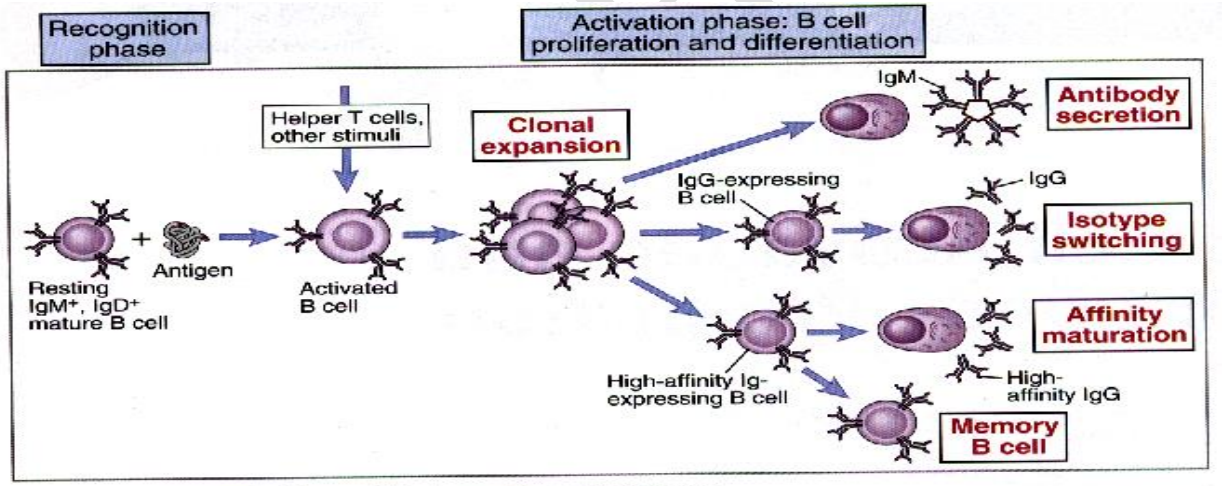
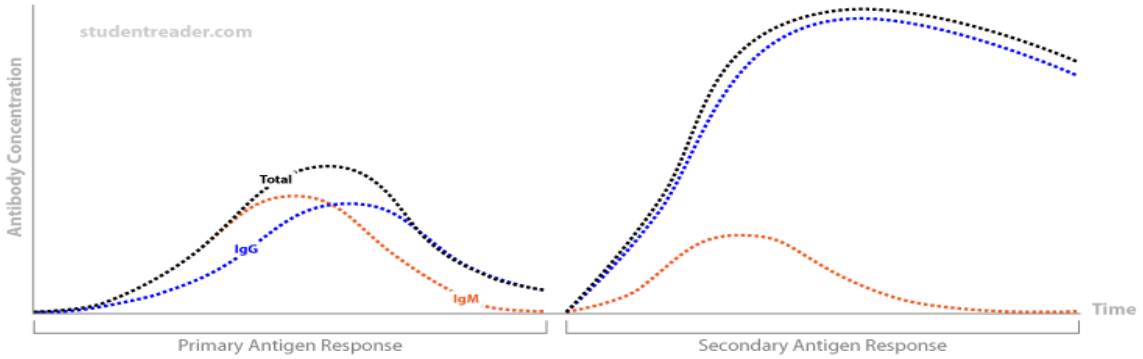


Figure 9–1 Phases of the humoral immune response.
The activation of B cells is initiated by specific recognition of antigens by the surface Ig receptors of the cells. Antigen and other stimuli, including helper T cells, stimulate the proliferation and differentiation of the specific B cell clone. Progeny of the clone may produce IgM or other Ig isotypes (e.g., IgG), may undergo affinity maturation, or may persist as memory cells.

Antigens are grouped into thymus-dependent antigens and thymus-independent antigens.
Activation by thymus-dependent antigens requires two signals: first, binding of the antigen itself to the B cell; second, binding of a thymocyte to the B cell. Thymus-independent antigens, conversely, activate B cells on their own; in some cases, however, T_H cytokine secretion (but not binding) is needed for maximum B cell activity.



Activation of naïve B cells by thymus-dependent and -independent antigens leads to the primary humoral response. The primary responses is characterized by a lag phase — during which naïve B cells undergo clonal selection, clonal expansion and differentiation into memory or antibody-secreting cells — followed by an exponential increase in circulating antibodies that peaks, plateaus and declines. The lag ranges from 4-10 days and the peak antibody titer can occur as late as 14 days later. IgM is secreted initially, but the B cell population usually undergoes *class switching* to secrete increasing amounts of IgG. Memory B cells formed during the primary response enter the G_0 phase and can live through the patient's entire life.

Activation of memory cells (both B and T type) by thymus-dependent antigens leads to the secondary humoral response. The secondary humoral response lasts longer and is highly effective due to *class-switching* (secretion of non-IgM antibodies), *affinity maturation* (antibodies with higher affinity), a shorter lag of 1-4 days and a 100-1000x greater magnitude. Memory B cells are responsible for secretion of high levels of high-affinity antibodies, and for *class-switching* to antibody isotypes best suited for clearing the pathogens. *Original antigenic sin* results in an apparent secondary response to a primary infection — if the primary infection has any epitopes encountered before, then those epitopes will elicit a secondary response.

Primary and Secondary Response

Property	**Primary Response**	**Secondary Response**
Responding B Cell :	Naïve B cell	Memory B cell
Lag Period:	4-7 days	1-3 days
Length of Response:	7-10 days	3-5 days
Magnitude:	Varies based on antigen	100-1000x greater than 1° response
Isotype Produced:	Initially IgM, then IgG	Mostly IgG
Antigens:	Thymus-dependent and -independent	Thys-dependent
Antibody Affinity:	Lower	Higher

Consequences after antibody production

Antibodies have three ways of attacking and eliminating an invading pathogen

- neutralization – Antibody prevents bacterial adherence to target cells by binding to antigen ligand
- opsonization – Antibody promotes phagocytosis by coating the outside of the pathogen and making it look extra "tasty" to macrophages and T cells.
- complement cascade activation – Antibody activates complement cascade *(see innate immunity)*, which enhances opsonization and facilitates the lysis of certain bacteria

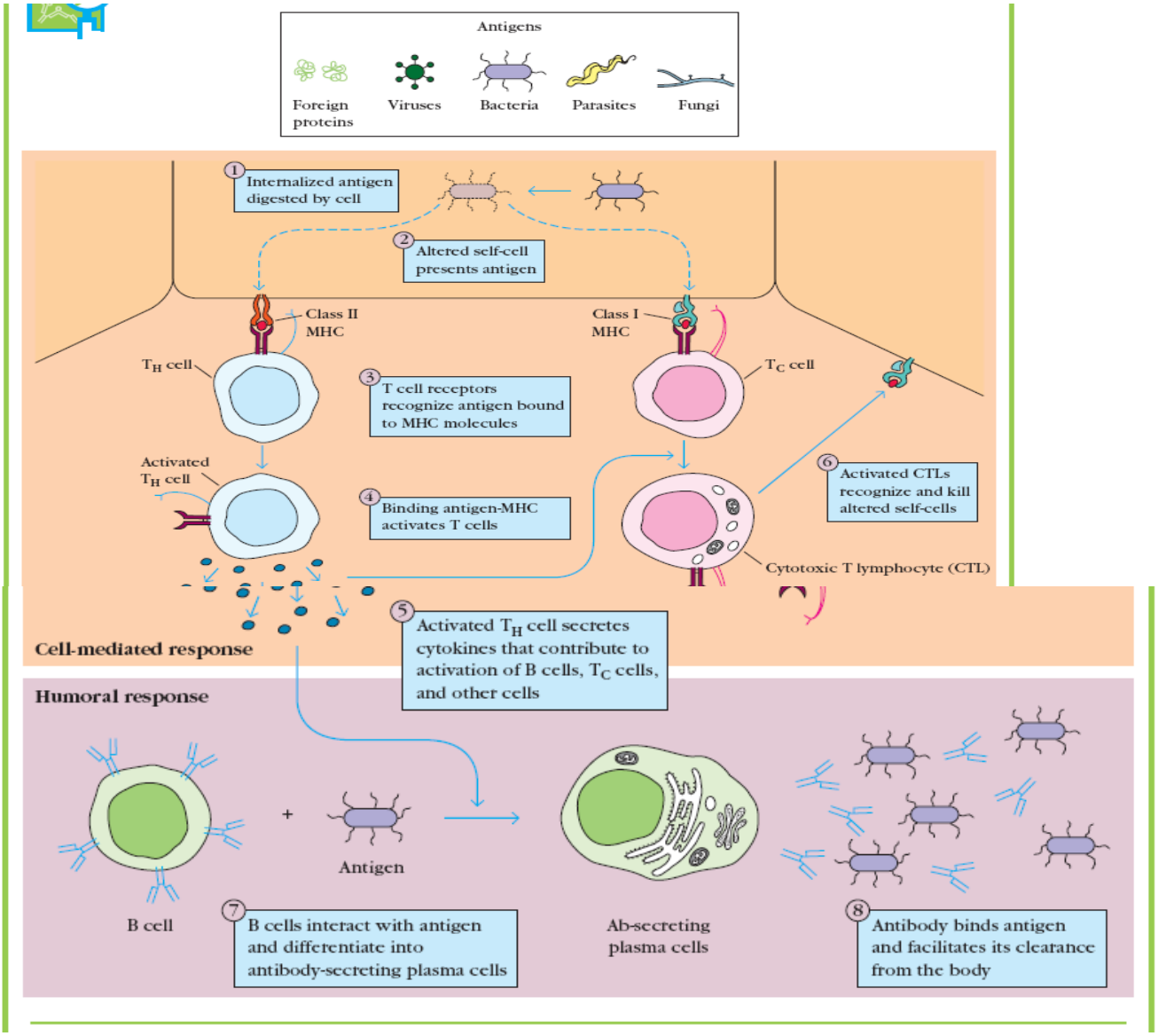
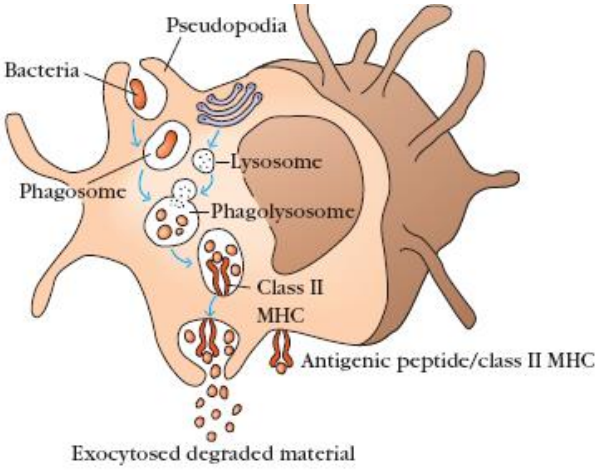


Fig: Overview of the humoral and cell-mediated branches of the immune system. In the humoral response, B cells interact with antigen and then differentiate into antibody-secreting plasma cells. The secreted antibody binds to the antigen and facilitates its clearance from the body. In the cell-mediated response, various subpopulations of T cells recognize antigen presented on self-cells. T_H cells respond to antigen by producing cytokines. T_C cells respond to antigen by developing into cytotoxic T lymphocytes (CTLs), which mediate killing of altered self-cells (e.g., virus infected cells).

Phagocytosis

Macrophages are capable of ingesting and digesting exogenous antigens, such as whole microorganisms and insoluble particles, and endogenous matter, such as injured or dead host cells, cellular debris, and activated clotting factors. In the first step in phagocytosis, macrophages are attracted by and move toward a variety of substances generated in an immune response; this process is called chemotaxis. The next step in phagocytosis is adherence of the antigen to the macrophage cell membrane. Complex antigens, such as whole bacterial cells or viral particles, tend to adhere well and are readily phagocytosed; isolated proteins and encapsulated bacteria tend to adhere poorly and are less readily phagocytosed. Adherence induces membrane protrusions, called pseudopodia, to extend around the attached material. Fusion of the pseudopodia encloses the material within a membrane-bounded structure called a phagosome, which then enters the endocytic processing pathway. In this pathway, a phagosome moves toward the cell interior, where it fuses with a lysosome to form a phagolysosome. Lysosomes contain lysozyme and a variety of other hydrolytic enzymes that digest the ingested material. The digested contents of the phagolysosome are then eliminated in a process called exocytosis. The macrophage membrane has receptors for certain classes of antibody. If an antigen (e.g., a bacterium) is coated with the appropriate antibody, the complex of antigen and antibody binds to antibody receptors on the macrophage membrane more readily than antigen alone and phagocytosis is enhanced. In one study, for example, the rate of phagocytosis of an antigen was 4000-fold higher in the presence of specific antibody to the antigen than in its absence. Thus, antibody functions as an opsonin, a molecule that binds to both antigen and macrophage and enhances phagocytosis. The process by which particulate antigens are rendered more susceptible to phagocytosis is called opsonization.



Antimicrobial And Cytotoxic Activities

A number of antimicrobial and cytotoxic substances produced by activated macrophages can destroy phagocytosed microorganisms.

Oxygen-Dependent Killing Mechanisms

Activated phagocytes produce a number of reactive oxygen intermediates (ROIs) and reactive nitrogen intermediates that have potent antimicrobial activity. During phagocytosis, a metabolic process known as the respiratory burst occurs in activated macrophages. This process results in the activation of a membrane-bound oxidase that catalyzes the reduction of oxygen to superoxide anion, a reactive oxygen intermediate that is extremely toxic to ingested microorganisms. The superoxide anion also generates other powerful oxidizing agents, including hydroxyl radicals and hydrogen peroxide. As the lysosome fuses with the phagosome, the activity of myeloperoxidase produces hypochlorite from hydrogen per oxide and chloride ions. Hypochlorite, the active agent of household bleach, is toxic to ingested microbes. When macrophages are activated with bacterial cell-wall components such as lipopolysaccharide (LPS) or, in the case of mycobacteria, muramyl dipeptide (MDP), together with a T-cell–derived cytokine (IFN-γ), they begin to express high levels of nitric oxide synthetase (NOS), an enzyme that oxidizes L-arginine to yield L-citrulline and nitric oxide (NO), a gas:

L-arginine + O_2 + NADPH $\rightarrow$NO + L-citrulline + NADP

Nitric oxide has potent antimicrobial activity; it also can combine with the superoxide anion to yield even more potent antimicrobial substances. Recent evidence suggests that much of the antimicrobial activity of macrophages against bacteria, fungi, parasitic worms, and protozoa is due to nitric oxide and substances derived from it.

Oxygen-Independent Killing Mechanisms

Activated macrophages also synthesize lysozyme and various hydrolytic enzymes whose degradative activities do not require oxygen. In addition, activated macrophages produce a group of antimicrobial and cytotoxic peptides, commonly known as defensins. These molecules are

cysteine-rich cationic peptides containing 29–35 amino-acid residues. Each peptide, which contains six invariant cysteines, forms a circular molecule that is stabilized by intramolecular disulfide bonds. These circularized defensin peptides have been shown to form ion-permeable channels in bacterial cell membranes. Defensins can kill a variety of bacteria, including Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Pseudomonas aeruginosa, and Haemophilus influenzae. Activated macrophages also secrete tumor necrosis factor α (TNF-α), a cytokine that has a variety of effects and is cytotoxic for some tumor cells.

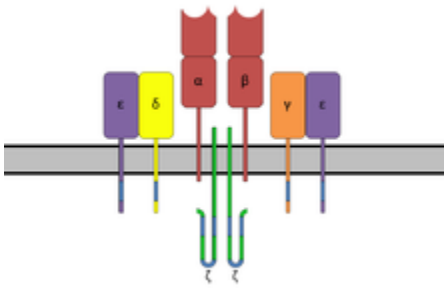
T LYMPHOCYTES AND IMMUNE RESPONSE

T lymphocytes also arise in the bone marrow. Unlike B cells, which mature within the bone marrow, T cells migrate to the thymus gland to mature. During its maturation within the thymus, the T cell comes to express a unique antigen-binding molecule, called the T-cell receptor, on its membrane.Unlike membrane-bound antibodies on B cells, which can recognize antigen alone, T-cell receptors can recognize only antigen that is bound to cell-membrane proteins called major histocompatibility complex (MHC) molecules. MHC molecules that function in this recognition event, which is termed "antigen presentation," are polymorphic (genetically diverse) glycoproteins found on cell membranes. There are two major types of MHC molecules: Class I MHC molecules, which are expressed by nearly all nucleated cells of vertebrate species, consist of a heavy chain linked to a small invariant protein called _2-microglobulin. Class II MHC molecules, which consist of an alpha and a beta glycoprotein chain, are expressed only by antigen-presenting cells. When a naive T cell encounters antigen combined with a MHC molecule on a cell, the T cell proliferates and differentiates into memory T cells and various effector T cells. There are two well-defined subpopulations of T cells: T helper (TH) and T cytotoxic (TC) cells. Although a third type of T cell, called a T suppressor (TS) cell, has been postulated, recent evidence suggests that it may not be distinct from TH and TC subpopulations. T helper and T cytotoxic cells can be distinguished from one another by the presence of either CD4 or CD8 membrane glycoproteins on their surfaces. T cells displaying CD4 generally function as TH cells, whereas those displaying CD8 generally function as TC cells.

After a TH cell recognizes and interacts with an antigen– MHC class II molecule complex, the cell is activated—it becomes an effector cell that secretes various growth factors known collectively as cytokines. The secreted cytokines play an important role in activating B cells, TC cells, macrophages, and various other cells that participate in the immune response. Differences in the pattern of cytokines produced by activated TH cells result in different types of immune response. Under the influence of TH-derived cytokines, a TC cell that recognizes an antigen–MHC class I molecule complex proliferates and differentiates into an effector cell called a cytotoxic T lymphocyte (CTL). In contrast to the TC cell, the CTL generally does not secrete many cytokines and instead exhibits cell-killing or cytotoxic activity. The CTL has a vital function in monitoring the cells of the body and eliminating any that display antigen, such as virus-infected cells, tumor cells, and cells of a foreign tissue graft. Cells that display foreign antigen complexed with a class I MHC molecule is called altered self-cells; these are targets of CTLs.

T CELL RECEPTOR

The **T-cell receptor**, or TCR, is a molecule found on the surface of **T cells**, or **T** lymphocytes, which is responsible for recognizing fragments of antigen as peptides bound to major histocompatibility complex (MHC) molecules.



GENOME REARRANGEMENTS DURING B-LYMPHOCYTES DIFFERENTIATION

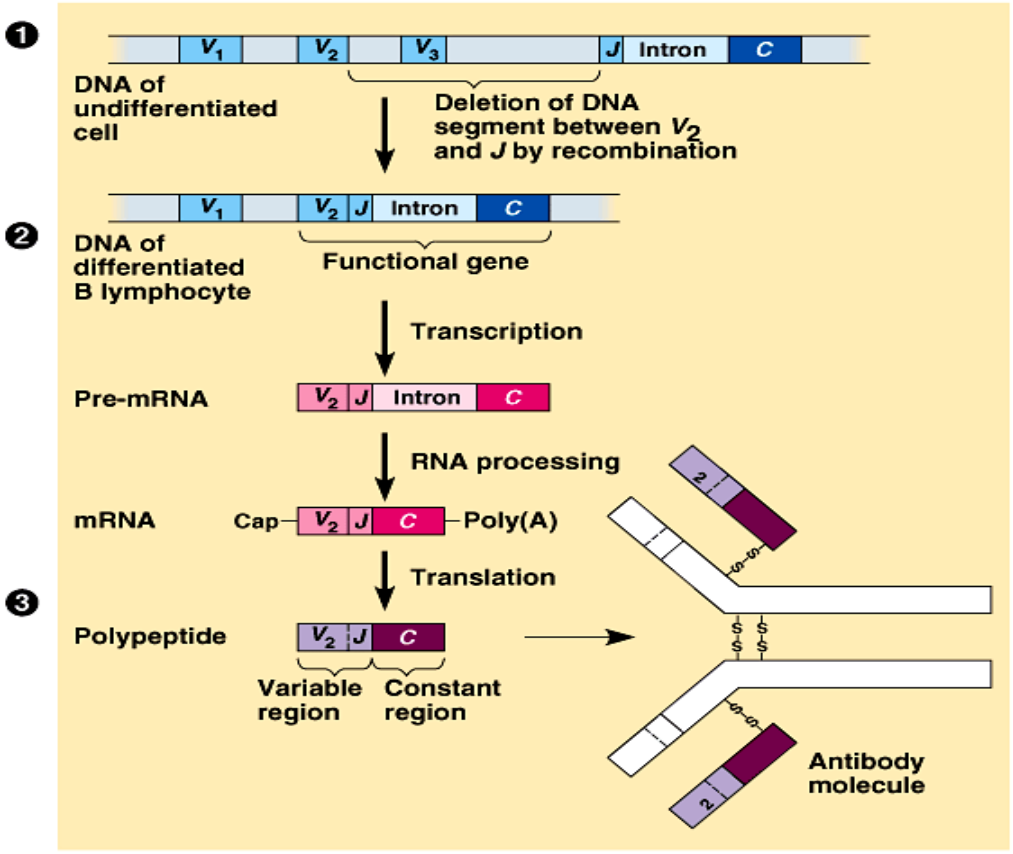
B-Cell Maturation The generation of mature B cells first occurs in the embryo and continues throughout life. Before birth, the yolk sac, fetal liver, and fetal bone marrow are the major sites of B-cell maturation; after birth, generation of mature B cells occurs in the bone marrow.

Progenitor B Cells Proliferate in Bone Marrow

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

At the earliest developmental stage, pro-B cells require direct contact with stromal cells in the bone marrow. This interaction is mediated by several cell-adhesion molecules, including VLA-4 on the pro-B cell and its ligand, VCAM-1, on the stromal cell. After initial contact is made, a receptor on the pro-B cell called c-Kit interacts with a stromal-cell surface molecule known as stem-cell factor (SCF). This interaction activates c-Kit, which is a tyrosine kinase, and the pro-B cell begins to divide and differentiate into a pre-B cell and begins expressing a receptor for IL-7. The IL-7 secreted by the stromal cells drives the maturation process, eventually inducing down-regulation of the adhesion molecules on the pre-B cells, so that the proliferating

cells can detach from the stromal cells. At this stage, pre-B cells no longer require direct contact with stromal cells but continue to require IL-7 for growth and maturation.



Ig-Gene Rearrangment Produces Immature B Cells

B-cell maturation depends on rearrangement of the immunoglobulin DNA in the lymphoid stem cells. The mechanisms of Ig-gene rearrangement occur in the pro-B cell stage is a heavy-chain DH-to-JH gene rearrangement; this is followed by a VH-to-DHJH rearrangement. If the first heavy-chain rearrangement is not productive, then VH-DH-JH rearrangement continues on the other chromosome. Upon completion of heavy-chain rearrangement, the cell is classified as a pre-B cell. Continued development of a pre-B cell into an immature B cell requires a productive light-chain gene rearrangement. Because of allelic exclusion, only one light-chain isotope is expressed on the membrane of a B cell. Completion of a productive light-chain rearrangement commits the now immature B cell to a particular antigenic specificity determined by the cell's heavy-chain VDJ sequence and light-chain VJ sequence. Immature B cells express mIgM (membrane IgM) on the cell surface. As would be expected, the recombinase enzymes RAG-1 and RAG-2, which are required for both heavy-chain and light-chain gene rearrangements, are expressed during the pro-B and pre-B cell stages. The enzyme terminal deoxyribonucleotidyl transferase (TdT), which catalyzes insertion of N-nucleotides at the DH-JH and VH-DHJH coding joints, is active during the pro-B cell stage and ceases to be active early in

the pre–B-cell stage. Because TdT expression is turned off during the part of the pre–B-cell stage when light-chain rearrangement occurs, N-nucleotides are not usually found in the VL-JL coding joints.

The bone-marrow phase of B-cell development culminates in the production of an IgM-bearing immature B cell. At this stage of development the B cell is not fully functional, and antigen induces death or unresponsiveness (anergy) rather than division and differentiation. Full maturation is signaled by the co-expression of IgD and IgM on the membrane. This progression involves a change in RNA processing of the heavy-chain primary transcript to permit production of two mRNAs, one encoding the membrane form of the μ chain and the other encoding the membrane form of the ϒchain.

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

During one stage in T-cell development,
the β chain of the T-cell receptor associates with pre-Tα to form the pre–T-cell receptor A parallel situation occurs during B-cell development. In the pre-B cell, the membrane μchain is associated with the **surrogate light chain,** a complex consisting of two proteins: a V-like sequence called **Vpre-B** and a C-like sequence called λ5, which associate non covalently to form a light chain–like structure.

The membrane-bound complex of heavy chain and surrogate light chain appears on the pre-B cell associated with the Ig-α/Ig-β heterodimer to form the pre–B-cell receptor. Only pre-B cells that are able to express membrane bound heavy chains in association with surrogate light chains are able to proceed along the maturation pathway. There is speculation that the pre–B-cell receptor recognizes a not-yet-identified ligand on the stromal-cell membrane, thereby transmitting a signal to the pre-B cell that prevents VH to DHJH rearrangement of the other heavy-chain allele, thus leading to allelic exclusion. Following the establishment of an effective pre–B-cell receptor, each pre-B cell undergoes multiple cell divisions, producing 32 to 64 descendants. Each of these progeny pre-B cells may then rearrange different light-chain gene segments, thereby increasing the overall diversity of the antibody repertoire. The critical role of the pre–B-cell receptor was demonstrated with knockout mice in which the gene encoding the

5 protein of the receptor was disrupted. B-cell development in these mice was shown to be blocked at the pre-B stage, which suggests that a signal generated through the receptor is necessary for pre-B cells to proceed to the immature B-cell stage.

B-Cell Activation and Proliferation

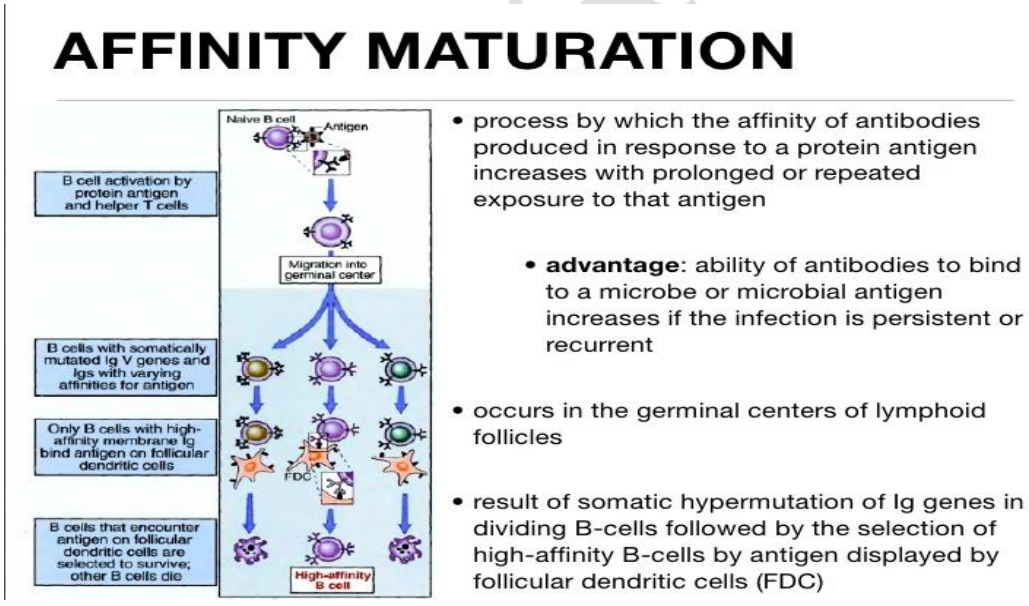
After export of B cells from the bone marrow, activation, proliferation, and differentiation occur in the periphery and require antigen. Antigen-driven activation and clonal selection of naive B cells leads to generation of plasma cells and memory B cells. In the absence of antigen-induced activation,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

Thymus-Dependent and Thymus- Independent Antigen Have Different Requirements for Response Depending on the nature of the antigen, B-cell activation proceeds by two different routes, one dependent upon TH cells, the other not. The B-cell response to thymus-dependent

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

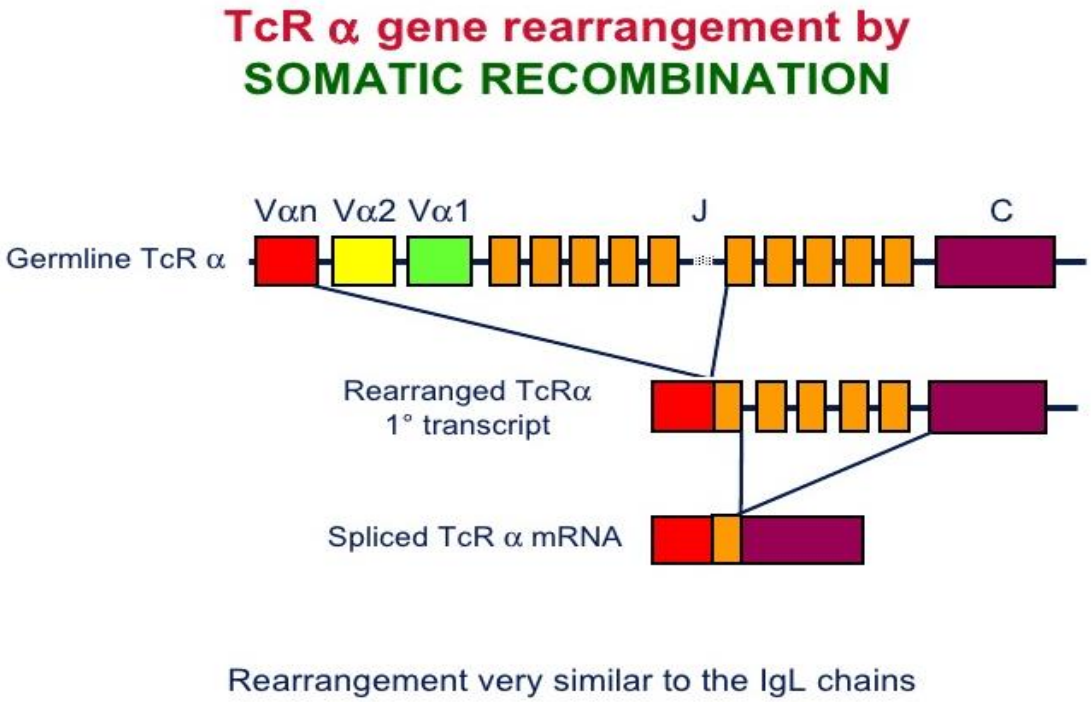
Antibody affinity maturation class switching

Affinity maturation and class switching of antibodies are temporally, but not mechanistically, related processes. The basis of affinity maturation is the selection, in the germinal centers, of antibodies that bind the antigen better. Early in an immune response, the selection is from the primary repertoire; later, it is from mutants generated by hypermutation at the immunoglobulin loci. Recently, the door has been opened for the study of the molecular mechanism of hypermutation, which is expected to make a major contribution to general biology. Class switching has been studied in the past for its obvious clinical importance, but also at the basic level of DNA recombination. Progress in understanding class switching has been trailing the progress made in V(D)J recombination, but new in vitro systems and gene-targeted mice are closing the gap.



Assembly of T cell Receptor Genes by Somatic Recombination

T-cell receptor genes are assembled by somatic recombination from sets of gene segments in the same way as are the immunoglobulin genes. This leads to a T-cell receptor in which the highest diversity is in the central part of the receptor, which contacts the bound peptide fragment of the ligand



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Possible Questions

Two mark questions

1. What is immunology?
2. Write any three unique features of specific immunity.
3. Define immunity.
4. What are types of immunity?
5. State the functions of spleen.
6. Name the secondary lymphoid organs.
7. What is phagocytosis?
8. Name the primary lymphoid organs.
9. What is Hematopoiesis?
10. Expand MALT.
11. Expand GALT.
12. Define adaptive immunity.
13. What is antigen?
14. Define antigenicity.
15. Define immunogenicity.
16. What is epitope?
17. What is B-Cell?
18. Define hapten.
19. Comment on adjuvants.

Eight mark questions

1. Write any three unique features of specific immunity.
2. Describe the structure of lymph node with the help of diagram.
3. What is Hematopoiesis? Explain in detail.
4. State the cell of the immune system.
5. Explain the details about memory of immune system.
6. Describe the structure of bone marrow with the help of diagram.
7. Describe the anatomical and physiological barriers in the first line of defence against disease?
8. Explain in detailed note on essential features of antigens.

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9. Explain in detailed note on features of T cell receptors.
10. Describe the structure of antibody with the help of diagram.
11. Explain the types of immunoglobulin
12. Comment on the structure of IgM and IgG

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UNIT-II

SYLLABUS

Regulation of immunoglobulin gene expression: Clonal selection theory, allotypes & idiotypes, allelic exclusion, immunologic memory, heavy chain gene transcription, genetic basis of antibody diversity, hypotheses (germ line & somatic mutation), antibody diversity.

REGULATION OF IMMUNOGLOBULIN GENE EXPRESSION

CLONAL SELECTION THEORY

Instructive hypothesis

There is only one common receptor encoded in the germ line and that different receptors are generated using the antigen as a template. Each antigen would cause the one common receptor to be folded to fit the antigen. It could not explain why the one common receptor did not fold around self antigens.

Selective theory

Instructional theories postulated that antigens play a central role in determining antibody specificity. Conversely, selective theories stated that an antigen reacts with an already-existing antibody.

Clonal selection hypothesis

The germ line encodes many different clones of immunologically competent cells(ICC) bearing antigen receptors(Abs) against all possible antigens. Antigen selects those clones of cells that have the appropriate receptor (Ig). Any cell or clones of cells bearing receptors for self molecules are destroyed during embryonic life.

ALLOTYPE

Although all members of a species inherit the same set of isotype genes, multiple alleles exist for some of the genes. These alleles encode subtle amino acid differences, called allotypic determinants that occur in some, but not all, members of a species. The sum of the individual

allotypic determinants displayed by an antibody determines its allotype**.** In humans, allotypes have been characterized forall four IgG subclasses, for one IgA subclass, and for the light chain. The chain allotypes are referred to as Gm markers. At least 25 different Gm allotypes have been identified; they are designated by the class and subclass followed by the allele number, for example, G1m (1), G2m (23), G3m (11), G4m (4a). Of the two IgA subclasses, only the IgA2 subclass has allotypes, as A2m (1) and A2m (2). The light chain has three allotypes, designated m (1), m (2), and m (3). Each of these allotypic determinants represents differences in one to four amino acids that are encoded by different alleles. Antibody to allotypic determinants can be produced by injecting antibodies from one member of a species into another member of the same species who carries different allotypic determinants. Antibody to allotypic determinants sometimes is produced by a mother during pregnancy in response to paternal allotypic determinants on the fetal immunoglobulins. Antibodies to allotypic determinants can also arise from a blood transfusion.

IDIOTYPE

The unique amino acid sequence of the VH and VL domains of a given antibody can function not only as an antigen-binding site but also as a set of antigenic determinants. The idiotypic determinants arise from the sequence of the heavy- and light-chain variable regions. Each individual antigenic determinant of the variable region is referred to as an idiotope**.** In some cases an idiotope may be the actual antigen-binding site, and in some cases an idiotope may comprise variable region sequences outside of the antigen binding site. Each antibody will present multiple idiotopes; the sum of the individual idiotopes is called the idiotype of the antibody. Because the antibodies produced by individual B cells derived from the same clone have identical variable-region sequences, they all have the same idiotype. Anti-idiotype antibody is produced by injecting antibodies that have minimal variation in their isotypes and allotypes, so that the idiotypic difference can be recognized.

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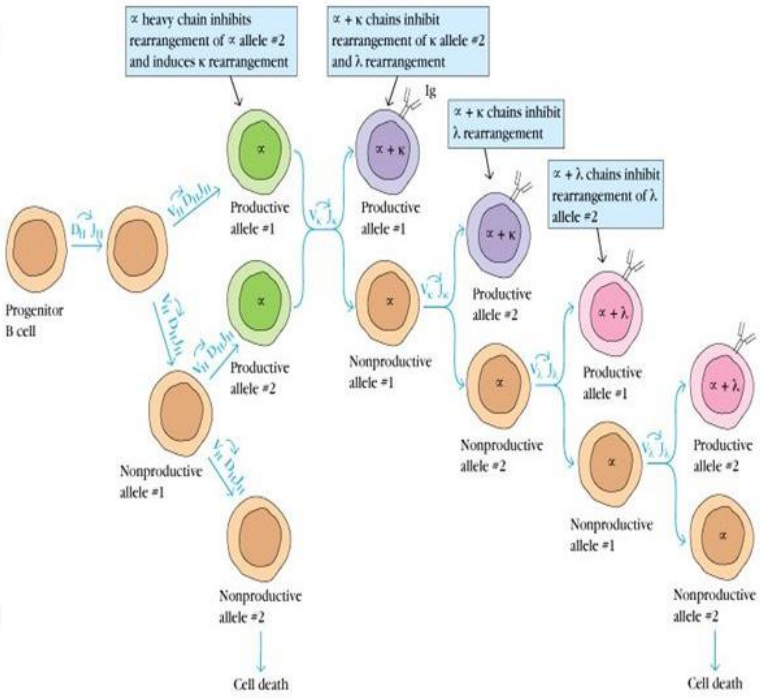
ALLELIC EXCLUSION

Allelic exclusion is a process by which only one allele of a gene is expressed while the other allele is silenced. For autosomal genes, diploid organisms inherit one copy from each parent.

Allelic Exclusion

- These diversity mechanisms often generate non-functional Ig genes: genes that contain stop codons or don't stay in the proper reading frame. The developing B cells use a mechanism called "**allelic exclusion**", in which each B cell makes only 1 active L chain and 1 active H chain. The cell tries each copy of the L genes and each copy of the H genes in turn:
  - If an active chain is made, no further DNA splicing occurs.
  - However, if a non-functional Ig is made, the cell then tries the next L or H gene.
  - This process continues until an active product is made from both H and L, or until all genes have been tried (in which case the cell dies).



IMMUNOLOGICAL MEMORY

Memory B cells are a B cell sub-type that are formed within germinal centers following primary infection and are important in generating an accelerated and more robust antibody-mediated immune response in the case of re-infection (also known as a *secondary immune response*).

Primary response

During an initial infection (or primary immune response) involving a T-dependent antigen, naive follicular B cells are activated in the presence of T_{FH} cells within the follicles of secondary lymphoid organs (i.e. spleen and lymph nodes) and undergo clonal expansion to produce a foci of B cells that are specific for the antigen. Most of these clones differentiate into the plasma cells, also called effector B cells which produce a first wave of protective antibodies and help clear the infection, but a fraction persist as dormant memory cells that survive in the body on a long-term basis after having gone through a highly mutative and selective germinal center reaction. Activated B cells that fail to undergo germinal center differentiation do not persist as effective memory B cells and are rapidly negatively selected against.

Within germinal centers, B cells proliferate and mutate the genetic region coding for their surface antibody (also known as immunoglobulin). The process is called somatic hyper mutation and is responsible for introducing spontaneous mutations with a frequency of about 1 in every 1600 cell division (a relatively high frequency considering the low mutation frequency of other cells of the body being 1 in 10^6 cell divisions). Then after gaining a set number of mutations, germinal center B cells are subjected to a round of selection by T_{FH} cells. B cell clones that have mutated and gained higher affinity surface immunoglobulin that better recognize antigen receive cellular contact-dependent survival signals from interacting with their cognate T_{FH} cells[4] and go on to one of three fates: (i) differentiate into plasma cells that have improved affinity towards antigen (therefore more efficient than their earlier the generation of plasma cells in clearing the infection), (ii) affinity matured memory B cells, or (iii) retained in the germinal center to re-enter another round of mutative replication and T_{FH} cell-dependent selection. Therefore, as an infection proceeds, memory B cells selected in the later stages of a germinal center response are found to have accumulated the highest numbers of immunoglobulin mutation events with superior affinity towards their targeted antigen. Conversely, during the course of a germinal center reaction, low affinity or potentially auto-reactive germinal center B cell clones, or those that have gained non-functional mutations are out-competed by higher affinity clones and eventually undergo cellular apoptosis.

Secondary response and memory

With each such subsequent exposure to the same antigen, the number of different responding B cell clones increases to generate a polyclonal response and effectively a greater number of memory B cells persist. Thus, a stronger antibody response (i.e. higher titres of more diverse antibody molecules) having improved affinity towards antigen is typically observed in the secondary immune response. It is unclear at what stage such a model reaches saturation to provide an optimal level of antibody-mediated immune protection against the same antigen. However, the fact that all the accumulation of cells of a single clone population express many of the one same type of antibody and that these memory B cells survive for long periods of time in a body underscores their functional significance during vaccination and the administration of booster shots.

HEAVY CHAIN GENE TRANSCRIPTION

Organization and Expression of Immunoglobulin Genes

As immunoglobulin (Ig) sequence data accumulated virtually every antibody molecule studied was found to contain a unique amino acid sequence in its variable region but only one of a limited number of invariant sequences in its constant region. The genetic basis for this combination of constancy and tremendous variation in a single protein molecule lies in the organization of the immunoglobulin genes. In germ-line DNA, multiple gene segments encode portions of a single immunoglobulin heavy or light chain. These gene segments are carried in the germ cells but cannot be transcribed and translated into complete chains until they are rearranged into functional genes. During B-cell maturation in the bone marrow, certain of these gene segments are randomly shuffled by a dynamic genetic system capable of generating more than 106 combinations. Subsequent processes increase the diversity of the repertoire of antibody binding sites to a very large number that exceeds 106 by at least two or three orders of magnitude. The processes of Bcell development are carefully regulated: the maturation of a progenitor B cell progresses through an ordered sequence of Ig-gene rearrangements, coupled with modifications to the gene that contribute to the diversity of the final product. By the end of this process, a mature, immune competent B cell will contain coding sequences for one functional heavy chain variable-region and one light-chain variable-region. The individual B cell

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is thus antigenically committed to a specific epitope. After antigenic stimulation of a mature B cell in peripheral lymphoid organs, further rearrangement of constant-region gene segments can generate changes in the isotype expressed, which produce changes in the biological effector functions of the immunoglobulin molecule without changing its specificity. Thus, mature B cells contain chromosomal DNA that is no longer identical to germ-line DNA. While we think of genomic DNA as a stable genetic blueprint, the lymphocyte cell lineage does not retain an intact copy of this blueprint. Genomic rearrangement is an essential feature of lymphocyte differentiation, and no other vertebrate cell type has been shown to undergo this process. This chapter first describes the detailed organization of the immunoglobulin genes, the process of Ig-gene rearrangement, and the mechanisms by which the dynamic immunoglobulin genetic system generates more than 108 different antigenic specificities. Then it describes the mechanism of class switching, the role of differential RNA processing in the expression of immunoglobulin genes, and the regulation of Ig-gene transcription. The chapter concludes with the application of our knowledge of the molecular biology of immunoglobulin genes to the engineering of antibody molecules for therapeutic and research applications.

GENETIC BASIS OF ANTIBODY DIVERSITY

For several decades, immunologists sought to imagine a genetic mechanism that could explain the tremendous diversity of antibody structure. Two different sets of theories emerged. The germ-line theories maintained that the genome contributed by the germ cells, egg and sperm, contains a large repertoire of immunoglobulin genes; thus, these theories invoked no special genetic mechanisms to account for antibody diversity. They argued that the immense survival valueof the immune system justified the dedication of a significant fraction of the genome to the coding of antibodies. In contrast, the somatic-variation theories maintained that the genome contains a relatively small number of immunoglobulin genes, from which a large number of antibody specificities are generated in the somatic cells by mutation ore combination. As the amino acid sequences of more and more immunoglobulin's were determined, it became clear that there must be mechanisms not only for generating antibody diversity but also for maintaining constancy. Whether diversity was generated by germ-line or by somatic mechanisms, a paradox remained: How could stability be maintained in the constant (C) region while some kind of

diversifying mechanism generated the variable (V) region? Neither the germ-line nor the somatic-variation proponents could offer a reasonable explanation for this central feature of immunoglobulin structure. Germ-line proponents found it difficult to account for an evolutionary mechanism that could generate diversity in the variable part of the many heavy- and light-chain genes while preserving the constant region of each unchanged. Somatic-variation proponents found it difficult to conceive of a mechanism that could diversify the variable region of a single heavy- or light-chain gene in the somatic cells without allowing alteration in the amino acid sequence encoded by the constant region. A third structural feature requiring an explanation emerged when amino acid sequencing of the human myeloma protein called Ti1 revealed that identical variable region sequences were associated heavy chain constant regions. A similar phenomenon was observed in rabbits by C. Considerable additional evidence has confirmed that a single variable-region sequence, defining a particular antigenic specificity, can be associated with multiple heavy-chain constant-region sequences; in other words, different classes, or isotypes, of antibody (e.g., IgG, IgM) can be expressed with identical variable-region sequences.

Variable-Region Gene Rearrangements

The preceding sections have shown that functional genes that encode immunoglobulin light and heavy chains are assembled by recombinational events at the DNA level. These events and the parallel events involving T-receptor genes are the only known site-specific DNA rearrangements in vertebrates. Variable-region gene rearrangements occur in an ordered sequence during B-cell maturation in the bone marrow. The heavy-chain variable-region genes rearrange first, then the light-chain variable-region genes. At the end of this process, each B cell contains a single functional variable region DNA sequence for its heavy chain and another for its light chain. The process of variable-region gene rearrangement produces mature, immune competent B cells; each such cell is committed to produce antibody with a binding site encoded by the particular sequence of its rearranged V genes. As described later in this chapter, rearrangements of the heavy chain constant-region genes will generate further changes in the immunoglobulin class (isotype) expressed by a B cell, but those changes will not affect the cell's antigenic specificity. The steps in variable-region gene rearrangement occur in an ordered sequence, but they are

random events that result in the random determination of B-cell specificity. The order, mechanism, and consequences of these rearrangements are described in this section.

Light-Chain DNA Undergoes-J Rearrangements

Expression of both _ and _ light chains requires rearrangement of the variable-region V and J gene segments. In humans, any of the functional V_ genes can combine with any of the four functional J-C combinations. In the mouse, things are slightly more complicated. DNA rearrangement can join the V-1 gene segment with either the J-1 or the J-3 gene segment, or the V-2 gene segment can be joined with the J-2 gene segment. In human or mouse light-chain DNA, any one of the V-gene segments can be joined with any one of the functional J-gene segments Rearranged and genes contain the following regions in order from the 5' to 3' end: a short leader (L) exon, a noncoding sequence (intron), a joined VJ gene segment, a second intron, and the constant region. Upstream from each leader gene segment is a promoter sequence. The rearranged light chain sequence is transcribed by RNA polymerase from the exon through the C segment to the stop signal, generating a light-chain primary RNA transcript. The introns in the primary transcript are removed by RNA processing enzymes, and the resulting light-chain messenger RNA then exits from the nucleus. The light chain mRNA binds to ribosomes and is translated into the light-chain protein. The leader sequence at the amino terminus pulls the growing polypeptide chain into the lumen of the rough endoplasmic reticulum and is then cleaved, so it is not present in the finished light-chain protein product.

Heavy-Chain DNA Undergoes V-D-J Rearrangement

Generation of a functional immunoglobulin heavy-chai gene requires two separate rearrangement events within the variable region. As illustrated in Figure 5-5, a DH gene segment first joins to a JH segment; the resulting DHJH segment then moves next to and joins a VH segment to generate a VHDHJH unit that encodes the entire variable region. In heavy-chain DNA, variable-region rearrangement produces a rearranged gene consisting of the following sequences, starting from the 5'end: a short L exon, an intron, a joined VDJ segment, another intron, and a series of C gene segments. As with the light-chain genes, a promoter sequence is

located a short distance upstream from each heavy-chain leader sequence. Once heavy-chain gene rearrangement is accomplished, RNA polymerase can bind to the promoter sequence and transcribe the entire heavy-chain gene, including the introns. Initially, both C_ and C_ gene segments are transcribed. Differential polyadenylation and RNA splicing remove the introns and process the primary transcript to generate mRNA including either the C_ or the C_ transcript. These two mRNAs are then translated, and the leader peptide of the resulting nascent polypeptide is cleaved, generating finished. The production of two different heavy-chain mRNAs allows a mature, immune competent B cell to express both IgM and IgD with identical antigenic specificity on its surface.

Recombination Signal Sequences Direct Recombination

The discovery of two closely related conserved sequences in variable-region germ-line DNA paved the way to fuller understanding of the mechanism of gene rearrangements. DNA sequencing studies revealed the presence of unique recombination signal sequences (RSSs) flanking each germ-line V, D, and J gene segment. One RSS is located 3' to each V gene segment, 5'to each J gene segment, and on both sides of each D gene segment. These sequences function as signals for the recombination process that rearranges the genes. Each RSS contains a conserved palindromic heptamer and a conserved AT-rich monomer sequence separated by an intervening sequence of 12 or 23 base pairs. The intervening 12 and 23-bp sequences correspond, respectively, to one and two turns of the DNA helix; for this reason the sequences are called one-turn recombination signal sequences and two turn signal sequences. The V signal sequence has a one-turn spacer, and the J-signal sequence has a two-turn spacer. In light-chain DNA, this order is reversed; that is, the V- signal sequence has a two-turn spacer, and the J-signal sequence has a one-turn spacer. In heavy-chain DNA, the signal sequences of the VH and JH gene segments have two-turn spacers; the signals on either sides of the DH gene segment have one-turn spacers. Signal sequences having a one-turn spacer can join only with sequences having a two-turn spacer (the so called one-turn/two-turn joining rule). This joining rule ensures, for example, that a VL segment joins only to a JL segment and not to another VL segment; the rule likewise ensures that VH, DH, and JH segments join in proper order and that segments of the same type do not join each other.

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Gene Segments are joined by Recombinases

V-(D)-J recombination, which takes place at the junctions between RSSs and coding sequences, is catalyzed by enzymes collectively called V (D) J recombinase**.** Identification of the enzymes that catalyze recombination of V, D, and J gene segments began in the late 1980s and is still ongoing. In 1990 David Schatz, Marjorie Oettinger, and David Baltimore first reported the identification of two recombination-activating genes, designated *RAG-1* and*RAG-2,* whose encoded proteins act synergistically and are required to mediate V-(D)-J joining. The RAG-1 and RAG-2 proteins and the enzyme terminal deoxynucleotidyl transferase (TdT) are the only lymphoid-specific gene products that have been shown to be involved in V-(D)-J rearrangement. The recombination of variable-region gene segments consists of the following steps, catalyzed by a system of recombinase enzymes. Cleavage of one strand of DNA by RAG-1 and RAG-2 at the junctures of the signal sequences and coding sequences _ A reaction catalyzed by RAG-1 and RAG-2 in which the free 3'-OH group on the cut DNA strand attacks the phosphodiester bond linking the opposite strand to the signal sequence, simultaneously producing a hairpin structure at the cut end of the coding sequence and a flush, 5'phosphorylated, double-strand break at the signal sequence _ Cutting of the hairpin to generate sites for the addition of P-region nucleotides, followed by the trimming of a few nucleotides from the coding sequence by a single strand endonuclease. Addition of up to 15 nucleotides, called N-region nucleotides, at the cut ends of the V, D, and J coding sequences of the heavy chain by the enzyme terminal deoxynucleotidyl transferase. Repair and ligation to join the coding sequences and to join the signal sequences, catalyzed by normal double strand break repair (DSBR) enzymes Recombination results in the formation of a coding joint, falling between the coding sequences, and a signal joint, between the RSSs. The transcriptional orientation of the gene segments to be joined determines the fate of the signal joint and intervening DNA. Less frequently, the two gene segments have opposite orientations. In this case joining occurs by inversion of the DNA, resulting in the retention of both the coding joint and the signal joint (and intervening DNA) on the chromosome. In the human locus, about half of the V- gene segments are inverted with respect to J and their joining is thus by inversion.

HYPOTHESES

Somatic mutation, genetic alteration acquired by a cell that can be passed to the progeny of the mutated cell in the course of cell division. Somatic mutations differ from germ line mutations, which are inherited genetic alterations that occur in the germ cells (i.e., sperm and eggs). Somatic mutations are frequently caused by environmental factors, such as exposure to ultraviolet radiation or to certain chemicals.

A **germ line mutation** or **germinal mutation** is any detectable and heritable variation in the lineage of germ cells. Mutations in these cells are transmitted to offspring, while, on the other hand, those in somatic cells are not. A germ line mutation gives rise to a **constitutional mutation** in the offspring, that is, a mutation that is present in virtually every cell. A constitutional mutation can also occur very soon after fertilization, or continue from a previous constitutional mutation in a parent

ANTIBODY DIVERSITY

Generation of Antibody Diversity

As the organization of the immunoglobulin genes was deciphered,the sources of the vast diversity in the variable region began to emerge. The germ-line theory, mentioned earlier, argued that the entire variable-region repertoire is encoded in the germ line of the organism and is transmitted from parent to offspring through the germ cells (egg and sperm). The somatic-variation theory held that the germ line contains a limited number of variable genes, which are diversified in the somatic cells by mutational or recombinational events during development of the immune system. With the cloning and sequencing of the immunoglobulin genes, both models were partly vindicated. To date, seven means of antibody diversification have been identified in mice and humans: Multiple germ-line gene segments Combinatorial V-(D)-J joining Junctional flexibility P-region nucleotide addition (P-addition) N-region nucleotide addition (N-addition) Somatic hypermutation. Combinatorial association of light and heavy chains Although the exact contribution of each of these avenues of diversification to total antibody diversity is not known, they each contribute significantly to the immense number of distinct antibodies that the mammalian immune system is capable of generating.

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There Are Numerous Germ-Line V, D, and J Gene Segments

An inventory of functional V, D, and J gene segments in the germ-line DNA of one human reveals 51 VH, 25 D, 6 JH,40 V, 5 J, 31 V, and 4 J gene segments. In addition to these functional segments, there are many pseudo genes. It should be borne in mind that these numbers were largely derived from a landmark study that sequenced the DNA of the immunoglobulin loci of a single individual. The immunoglobulin loci of other individuals might contain slightly different numbers of particular types of gene segments. In the mouse, although the numbers are known with less precision than in the human, there appear to be about 85 V gene segments and 134 VH gene segments, 4 functional JH, 4 functional J, 3 functional J, and an estimated 13 DH gene segments, but only three V gene segments. Although the number of germ-line genes found in either humans or mice is far fewer than predicted by early proponents of the germ line model, multiple germ-line V, D, and J gene segments clearly do contribute to the diversity of the antigen-binding sites in antibodies.

Combinatorial V-J and V-D-J Joining Generates Diversity

The contribution of multiple germ-line gene segments to antibody diversity is magnified by the random rearrangement of these segments in somatic cells. It is possible to calculate how much diversity can be achieved by gene rearrangements). In humans, the ability of any of the 51 VH gene segments to combine with any of the 27 DH segments and any of the 6 JH segments allows a considerable amount of heavy-chain gene diversity to be. Similarly, 40 V gene segments randomly combining with 5 J segments has the potential of generating 200 possible combinations at the locus, while 30 V and 4 J gene segments allow up to 120 possible combinations at the human locus. It is important to realize that these are minimal calculations of potential diversity. Junctional flexibility and P- and N-nucleotide addition, as mentioned above, and, especially, somatic hypermutation, which will be described shortly, together make an enormous contribution to antibody diversity. Although it is not possible to make an exact calculation of their contribution, most workers in this field agree that they raise the potential for antibody combining-site diversity in humans to well over 1010. This does not mean that, at any given time, a single individual has a repertoire of 1010 different antibody combining sites. These

very large numbers describe the set of possible variations, of which any individual carries a subset that is smaller by several orders of magnitude.

Junctional Flexibility Adds Diversity

The enormous diversity generated by means of V, D, and J combinations is further augmented by a phenomenon called junctional flexibility. As described above, recombination involves both the joining of recombination signal sequences to form a signal joint and the joining of coding sequences to form a coding joint. Although the signal sequences are always joined precisely, joining of the coding sequences is often imprecise. In one study, for example, joining of the V-21 and J-1 coding sequences was analyzed in several pre-B cell lines. Sequence analysis of the signal and coding joints revealed the contrast in junctional precision. As illustrated previously, junctional flexibility leads to many nonproductive rearrangements, but it also generates productive combinations that encode alternative amino acids at each coding joint, thereby increasing antibody diversity. The amino acid sequence variation generated by junctional flexibility in the coding joints has been shown to fall within the third hyper variable region (CDR3) in immunoglobulin heavy-chain and light-chain DNA. Since CDR3 often makes a major contribution to antigen binding by the antibody molecule, amino acid changes generated by junctional flexibility are important in the generation of antibody diversity.

P-Addition Adds Diversity at Palindromic Sequences

As described earlier, after the initial single-strand DNA cleavage at the junction of a variable-region gene segment and attached signal sequence, the nucleotides at the end of the coding sequence turn back to form a hairpin structure. The subsequent addition of complementary nucleotides to this strand (P-addition) by repair enzymes generates a palindromic sequence in the coding joint, and so these nucleotides are called P-nucleotides. Variation in the position at which the hairpin is cut thus leads to variation in the sequence of the coding joint.

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N-Addition Adds Considerable Diversity by Addition of Nucleotides

Variable-region coding joints in rearranged heavy-chain genes have been shown to contain short amino acid sequences that are not encoded by the germ-line V, D, or J gene segments. These amino acids are encoded by nucleotides added during the D-J and V to D-J joining process by a terminal deoxynucleotidyl transferase (TdT) catalyzed reaction Evidence that TdT is responsible for the addition of these N-nucleotides has come from transfection studies in fibroblasts. When fibroblasts were transfected with the *RAG-1* and *RAG-2* genes, V-D-J rearrangement occurred but no N-nucleotides were present in the coding joints. However, when the fibroblasts were also transfected with the gene encoding TdT, then V-D-J rearrangement was accompanied by addition of N-nucleotides at the coding joints. Up to 15 N-nucleotides can be added to both the DH-JH and VH-DHJH joints. Thus, a complete heavy-chain variable region is encoded by a VHNDHNJH unit. The additional heavy chain diversity generated by N-region nucleotide addition is quite large because N regions appear to consist of wholly random sequences. Since this diversity occurs at V-D-J coding joints, it is localized in CDR3 of the heavy-chain genes.

Somatic Hypermutation Adds Diversity in Already-Rearranged Gene Segments

All the antibody diversity described so far stems from mechanisms that operate during formation of specific variable regions by gene rearrangement. Additional antibody diversity is generated in rearranged variable-region gene units by a process called somatic hypermutation**.** As a result of somatic hypermutation, individual nucleotides in VJ or VDJ units are replaced with alternatives, thus potentially altering the specificity of the encoded immunoglobulins. Normally, somatic hypermutation occurs only within germinal centers (see Chapter 11), structures that form in secondary lymphoid organs within a week or so of immunization with an antigen that activates a T-cell-dependent B-cell response. Somatic hyper mutation is targeted to rearranged Vregions located within a DNA sequence containing about 1500 nucleotides, which includes the whole of the VJ or VDJ segment. Somatic hyper mutation occurs at a frequency approaching 10_3 per base pair per generation. This rate is at least a hundred thousand-fold higher (hence the name hypermutation) than the spontaneous mutation rate, about 10_8/ bp/generation, in other genes. Since the combined length of the H-chain and L-chain variable-region genes is about 600 bp, one

expects that somatic hypermutation will introduce at least one mutation per every two cell divisions in the pair of VH and VL genes that encode an antibody. The mechanism of somatic hypermutation has not yet been determined.Most of the mutations is nucleotide substitutions rather than deletions or insertions. Somatic hypermutation introduces these substitutions in a largely, but not completely, random fashion. Recent evidence suggests that certain nucleotide motifs and palindromic sequences within VH and VL may be especially susceptible to somatic hypermutation. Somatic hypermutations occur throughout the VJ or VDJ segment, but in mature B cells they are clustered within the CDRs of the VH and VL sequences, where they are most likelyto influence the overall affinity for antigen. Following exposure to antigen, those B cells with higher-affinity receptors will be preferentially selected for survival. Since CDR3 often makes a major contribution to antigen binding by the antibody molecule, amino acid changes generated by junctional flexibility are important in the generation of antibody diversity.

P-Addition Adds Diversity at Palindromic Sequences

As described earlier, after the initial single-strand DNA cleavage at the junction of a variable-region gene segment and attached signal sequence, the nucleotides at the end of the coding sequence turn back to form a hairpin structure. This hairpin is later cleared by an endonuclease. This second cleavage sometimes occurs at a position that leaves a short single strand at the end of the coding sequence. The subsequent addition of complementary nucleotides to this strand (P-addition) by repair enzymes generates a palindromic sequence in the coding joint, and so these nucleotides are called P-nucleotides (Figure 5-13a). Variation in the position at which the hairpin is cut thus leads to variation in the sequence of the coding joint.

N-Addition Adds Considerable Diversity by Addition of Nucleotides

Variable-region coding joints in rearranged heavy-chain genes have been shown to contain short amino acid sequences that are not encoded by the germ-line V,D, or J gene segments. These amino acids are encoded by nucleotides added during the D-J and V to D-J joining process by a terminal deoxynucleotidyl transferase (TdT) catalyzed reaction differential selection is an increase in the antigen affinity of a population of B cells. The overall process, called affinity

maturation, takes place within germinal centers, and is described more fully in Chapter 11. Claudia Berek and Cesar Milstein obtained experimental evidence demonstrating somatic hypermutation during the course of an immune response to a hapten-carrier conjugate. These researchers were able to sequence mRNA that encoded antibodies raised against a hapten in response to primary, secondary, or tertiary immunization (first, second, or third exposure) with a hapten-carrier conjugate. The hapten they chose was 2-phenyl-5-oxazolone (phOx), coupled to a protein carrier. They chose this hapten because it had previously been shown that the majority of antibodies it induced were encoded by a single germ-line VH and V gene segment. By day 14 after primary immunization, analysis of eight hybridomas revealed that six continued to use the germ-line VH Ox-1 gene segment and all continued to use the V Ox-1 gene segment. Now, however, all of these hybridomas included one or more mutations from the germ-line sequence. Hybridomas analyzed from the secondary and tertiary responses showed a larger percentage utilizing germ-line VH gene segments other than the VH Ox-1 gene. In those hybridoma clones that utilized the VH Ox-1 and V-Ox-1 gene segments, most of the mutations were clustered in the CDR1 and CDR2 hypervariable regions. The number of mutations in the anti-phOx hybridomas progressively increased following primary, secondary, and tertiary immunizations, as did the overall affinity of the antibodies for phOx.

Synthesis, Assembly, and Secretion of Immunoglobulins

Immunoglobulin heavy- and light-chain mRNAs are translated on separate polyribosomes of the rough endoplasmic reticulum (RER). Newly synthesized chains contain an amino-terminal leader sequence, which serves to guide the chains into the lumen of the RER, where the signal sequence is then cleaved. The assembly of light (L) and heavy (H) chains into the disulfide-linked and glycosylated immunoglobulin molecule occurs as the chains pass through the cisternae of the RER. The complete molecules are transported to the Golgi apparatus and then into secretory vesicles, which fuse with the plasma membrane. The order of chain assembly varies among the immunoglobulin classes. In the case of IgM, the H and L chains assemble within the RER to form half-molecules, and then two half-molecules assemble to form the complete molecule. In the case of Ig G, two H chains assemble, then an H2L intermediate is assembled, and finally the complete H2L2 molecule is formed. Inter chain disulfide bonds are formed, and the polypeptides

are glycosylated as they move through the Golgi apparatus. If the molecule contains the trans membrane sequence of the membrane form, it becomes anchored in the membrane of a secretory vesicle and is inserted into the plasma membrane as the vesicle fuses with the plasma membrane. If the molecule contains the hydrophilic sequence of secreted immune globulins, it is transported as a free molecule in a secretory vesicle and is released from the cell when the vesicle fuses with the plasma membrane.

Regulation of Ig-Gene Transcription

The immunoglobulin genes are expressed only in B-lineage cells, and even within this lineage, the genes are expressed at different rates during different developmental stages. As withother eukaryotic genes, three major classes of cis regulatory sequences in DNA regulate transcription of immunoglobulin genes: Promoters: relatively short nucleotide sequences, extending about 200 bp upstream from the transcription initiation site, that promote initiation of RNA transcription in a specific direction Enhancers: nucleotide sequences situated some distance upstream or downstream from a gene that activate transcription from the promoter sequence in an orientation-independent manner Silencers: nucleotide sequences that down-regulate transcription, operating in both directions over a distance. The locations of the three types of regulatory elements in germ-line immunoglobulin DNA are shown in Figure 5-19.All of these regulatory elements have clusters of sequence motifs that can bind specifically to one or more nuclear proteins. Each VH and VL gene segment has a promoter located just upstream from the leader sequence. In addition, the J cluster and each of the DH genes of the heavy-chain locus are preceded by promoters. Like other promoters, the immunoglobulin promoters contain a highly conserved ATrich sequence called the TATA box, which serves as a site for the binding of a number of proteins that are necessary for the initiation of RNA transcription. The actual process of transcription is performed by RNA polymerase II, which starts transcribing DNA from the initiation site, located about 25 bp downstream of the TATA box. Ig promoters also contain an essential and conserved octamer that confers B-cell specificity on the promoter. The octamer binds two transcription factors, oct-1, found in many cell types, and oct-2, found only in B cells.

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Antibody Genes and Antibody Engineering

There are many clinical applications in which the exquisite specificity of a mouse monoclonal antibody would be useful. However, when mouse monoclonal antibodies are introduced into humans they are recognized as foreign and evoke an antibody response that quickly clears the mouse monoclonal antibody from the bloodstream. In addition, circulating complexes of mouse and human antibodies can cause allergic reactions. In some cases, the buildup of these complexes in organs such as the kidney can cause serious and even life-threatening reactions. Clearly, one way to avoid these undesirable reactions is to use human monoclonal antibodies for clinical applications. However, the preparation of human monoclonal antibodies has been hampered by numerous technical problems. In response to the difficulty of producing human monoclonal antibodies and the complications resulting from the use of mouse monoclonal antibodies in humans, there is now a major effort to engineer monoclonal antibodies and antibody binding sites with recombinant DNA technology. The growing knowledge of antibody gene structure and regulation has made possible what Cesar Milstein, one of the inventors of monoclonal antibody technology, has called "man-made antibodies." It is now possible to design and construct genes that encode immunoglobulin molecules in which the variable regions come from one species and the constant regions come from another. New genes have been created that link nucleotide sequences coding non antibody proteins with sequences that encode antibody variable regions specific for particular antigens. These molecular hybrids or chimeras may be able to deliver powerful toxins to particular antigenic targets, such as tumor cells. Finally, by replacement of the immunoglobulin loci of one species with that of another, animals of one species have been endowed with the capacity to respond to immunization by producing antibodies encoded by the donor's genetically transplanted Ig genes. By capturing a significant sample of all of the immunoglobulin heavy- and light-chain variable-region genes via incorporation into libraries of bacteriophage, it has been possible to achieve significant an useful reconstructions of the entire antibody repertoires of individuals. The next few sections describe each of these types of antibody genetic engineering.

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Chimeric and Hybrid Monoclonal Antibodies Have Potent Clinical Potential

One approach to engineering an antibody is to clone recombinant DNA containing the promoter, leader, and variable region sequences from a mouse antibody gene and the constant-region exons from a human antibody gene. The antibody encoded by such a recombinant gene is a mouse-human chimera, commonly known as a humanized antibody. Its antigenic specificity, which is determined by the variable region, is derived from the mouse DNA; its isotype, which is determined by the constant region, is derived from the human DNA. Because the constant regions of these chimeric antibodies are encoded by human genes, the anti- bodies have fewer mouse antigenic determinants and are far less immunogenic when administered to humans than mouse monoclonal antibodies. The ability of the mouse variable regions remaining in these humanized antibodies to provide the appropriate binding site to allow specific recognition of the target antigen has encouraged further re exploration of this approach. It is possible to produce chimeric human-mouse antibodies in which only the sequences of the CDRs are of mouse origin. Another advantage of humanized chimeric antibodies is that they retain the biological effector functions of human antibody and are more likely to trigger human complement activation or Fc receptor binding. One such chimeric human mouse antibody has been used to treat patients with B-cell varieties of non-Hodgkin's lymphoma. Chimeric monoclonal antibodies that function as immunotoxins can also be prepared. In this case, the terminal constant-region domain in a tumor specific monoclonal antibody is replaced with toxin chains. Because these immunotoxins lack the terminal Fc domain, they are not able to bind to cells bearing Fc receptors. These immunotoxins can bind only to tumor cells, making them highly specific as therapeutic reagents. Hetero conjugates, or bispecific antibodies, are hybrids of two different antibody molecules. They can be constructed by chemically cross linking two different antibodies or by synthesizing them in hybridomas consisting of two different monoclonal-antibody-producing cell lines that have been fused. Both of these methods generate mixtures of monospecific and bispecific antibodies from which the desired bi specific molecule must be purified. Using genetic engineering to construct genes that will encode molecules only with the two desired specificities is a much simpler and more elegant approach. Several bi specific molecules have been designed in which one half of the antibody has specificity for a tumor and the other half has specificity for a surface molecule on an immune effector cell, such as an NK cell, an activated macrophage, or a

cytotoxic T lymphocyte(CTL). Such hetero conjugates have been designed to activate the immune effector cell when it is cross linked to the tumor cell so that it begins to mediate destruction of the tumor cell.

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Possible Questions

Two marks

1. Number of domains in light chain
2. What is an idiotype
3. Allotypes are used for?
4. Which type is involved in hypersenstivity
5. Differentiate autoimmunity and alloimmunity
6. Define anti idiotypic antibodies
7. Define germ line theory

Eight mark questions

1. Explain clonal selection theory
2. What are memory cells? How they are produced?
3. Brief note on Immunologic memory
4. What is antibody diversity? What are the three theories which account for antibody diversity?
5. Explain the instructive and selective theories of antibody formation

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UNIT-III

SYLLABUS

Introduction: Hypersensitivity Reactions (HS): Type I: Allergies and anaphylaxis; Type II: Antibody mediated HS reactions; Mechanism and pathogencity; Type III: Immune complex mediated HS reactions: Mechanism & pathogenecity; Type IV: Delayed type (or) cell-mediated HS reactions; Mechanisms and pathogenecity. Type V: Stimulatory HS reactions. Mechanism and pathogenesis.

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HYPERSENSITIVITY REACTIONS

Hypersensitive Reaction

An immune response mobilizers a battery of effector molecules that act to remove antigen by various mechanisms, these effector molecules induce a localized inflammatory response that eliminates antigen without extensively damaging the host's tissue. Under certain circumstances, however, this inflammatory response can have deleterious effects, resulting in significant tissue damage or even death. This inappropriate immune response is termed hypersensitivity or allergy**.** Although the word *hypersensitivity* implies an increased response, the response is not always heightened but may, instead, be an inappropriate immune response to an antigen. Hypersensitive reactions may develop in the course of either humoral or cell-mediated responses.

The ability of the immune system to respond inappropriately to antigenic challenge was recognized early in this century. Two French scientists, Paul Portier and Charles Richet, investigated the problem of bathers in the Mediterranean reacting violently to the stings of Portuguese Man of War jellyfish. Portier and Richet concluded that the localized reaction of the bathers was the result of toxins. To counteract this reaction, the scientists experimented with the use of isolated jellyfish toxins as vaccines. Their first attempts met with disastrous results. Portier and Richet injected dogs with the

purified toxins, followed later by a booster of toxins. Instead of reacting to the booster by producing antibodies against the toxins, the dogs immediately reacted with vomiting, diarrhea, asphyxia, and, in some instances, death. Clearly this was an instance where the animals "over reacted" to the antigen. Portier and Richet coined the term *anaphylaxis*, loosely translated from Greek to mean the opposite of *prophylaxis*, to describe this overreaction. Richet was subsequently awarded the Nobel Prize in Physiology or Medicine in 1913 for his work on anaphylaxis.

We currently refer to anaphylactic reactions within the humoral branch initiated by antibody or antigen-antibody complexes as immediate hypersensitivity**,** because the symptoms are manifest within minutes or hours after a sensitized recipient encounters antigen. Delayed-type hypersensitivity **(**DTH**)** is so named in recognition of the delay of symptoms until days after exposure. This chapter examines the mechanisms and consequences of the four primary types of hypersensitive reactions.

Gell and Coombs Classification

Several forms of hypersensitive reaction can be distinguished, reflecting differences in the effector molecules generated in the course of the reaction. In immediate hypersensitive reactions, different antibody isotypes induce different immune effector molecules. IgE antibodies, for example, induce mast-cell degranulation with release of histamine and other biologically active molecules. IgG and IgM antibodies, on the other hand, induce hypersensitive reactions by activating complement. The effector molecules in the complement reactions are the membrane-attack complex and such complement split products as C3a, C4a, and C5a.In delayed-type hypersensitivity reactions, the effect or molecules are various cytokines secreted by activated TH or TC cells. As it became clear that several different immune mechanisms give rise to hypersensitive reactions, P. G. H. Gell and R. R. A. Coombs proposed a classification scheme in which hypersensitive reactions are divided into four types. Three types of hypersensitivity occur within the humoral branch and are mediated by antibody or antigen-antibody complexes: IgE-mediated (type I), antibody-mediated (type II), and immune complex–mediated (type III). A fourth type of hypersensitivity depends on reactions within the cell-mediated branch, and is termed delayed-type hypersensitivity, or DTH (type IV). Each type involves distinct mechanisms, cells, and mediator molecules (Figure 16-1).

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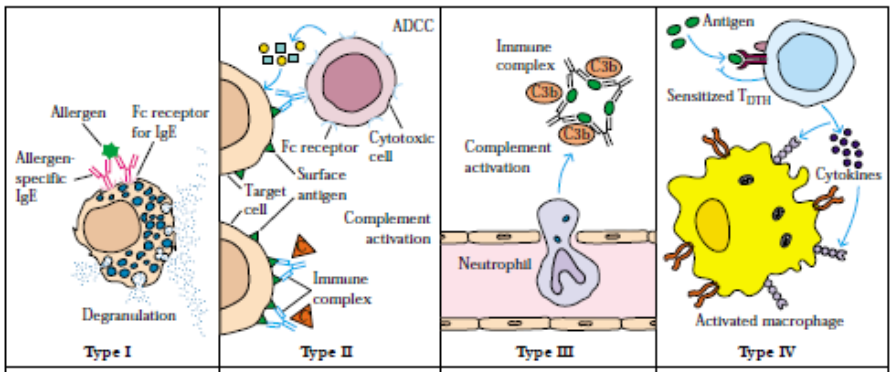

Type I	Type II	Type III	Type IV
IgE-Mediated Hypersensitivity	IgG-Mediated Cytotoxic Hypersensitivity	Immune Complex-Mediated Hypersensitivity	Cell-Mediated Hypersensitivity
Ag induces crosslinking of IgE bound to mast cells and basophils with release of vasoactive mediators	Ab directed against cell surface antigens meditates cell destruction via complement activation or ADCC	Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils	Sensitized T_H1 cells release cytokines that activate macrophages or T_C cells which mediate direct cellular damage
Typical manifestations include systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema	Typical manifestations include blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia	Typical manifestations include localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulnephritis, rheumatoid arthritis, and systemic lupus erythematosus	Typical manifestations include contact dermatitis, tubercular lesions and graft rejection

Fig: The four types of hypersensitive responses.

This classification scheme has served an important function in identifying the mechanistic differences among various hypersensitive reactions, but it is important to point out that secondary effects blur the boundaries between the four categories.

IgE-Mediated (Type I) Hypersensitivity

A type I hypersensitive reaction is induced by certain types of antigens referred to as allergens**,** and has all the hallmarks of a normal humoral response. That is, an allergen induces a humoral antibody response by the same mechanisms for other soluble antigens, resulting in the generation of antibody-secreting plasma cells and memory cells. What distinguishes a type I hypersensitive response from a normal humoral response is that the plasma cells secrete IgE. This class of antibody binds with high affinity to Fc receptors on the surface of tissue mast cells and blood basophils. Mast cells and basophils coated by IgE aresaid to be sensitized. A later exposure to the same allergen cross-links the membrane-bound IgE on sensitized mast cellsand basophils, causing degranulation of these cells (Figure16-2). The pharmacologically active mediators released from the granules act on the surrounding tissues. The principal effects—vasodilation and smooth-muscle contraction—maybe either systemic or localized, depending on the extent of mediator release.

Type I Reactions

As depicted in figure, several components are critical to development of type I hypersensitive reactions. This section will consider these components first and then describe the mechanism of degranulation.

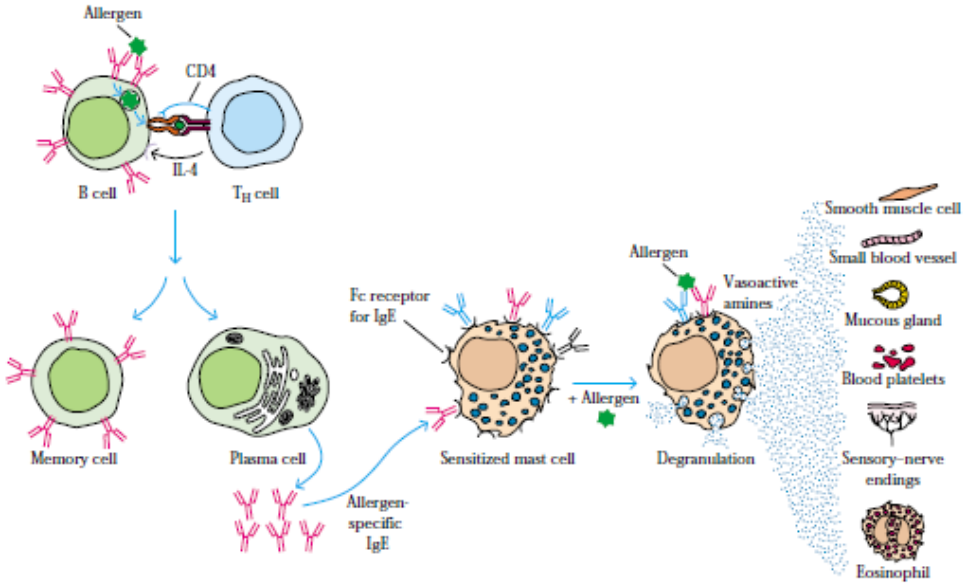

Fig: General mechanism underlying a type I hypersensitive reaction. Exposure to an allergen activates B cells to form IgE secreting plasma cells. The secreted IgE molecules bind to Ig E specific Fc receptors on mast cells and blood basophils. (Many molecules of IgE with various specificities can bind to the IgE-Fc receptor.) Second exposure to the allergen leads to cross linking of the bound IgE, triggering the release of pharmacologically active mediators, vasoactive amines, from mast cells and basophils. The mediators cause smooth-muscle contraction, increased vascular permeability, and vasodilation.

Allergens

The majority of humans mount significant IgE responses only as a defense against parasitic infections. After an individualhas been exposed to a parasite, serum IgE levels increase and remain high until the parasite is successfully cleared from the body. Some persons, however, may have an abnormality called atopy**,** a hereditary predisposition to the development of immediate hypersensitivity reactions against common environmental antigens. The IgE regulatory defects suffered by atopic individuals allow no parasitic antigens to stimulate inappropriate IgE production, leading to tissue damaging type I hypersensitivity. The term *allergen* refers specifically to no parasitic antigens capable

of stimulating type I hypersensitive responses in allergic individuals. The abnormal IgE response of atopic individuals is at least partly genetic—it often runs in families. Atopic individuals have abnormally high levels of circulating IgE and also more than normal numbers of circulating eosinophils.

These individuals are more susceptible to allergies such as hay fever, eczema, and asthma. The genetic propensity to atopic responses has been mapped to several candidate loci. One locus, on chromosome5q, is linked to a region that encodes a variety of cytokines, including IL-3, IL-4, IL-5, IL-9, IL-13, and GM-CSF. A second locus, on chromosome 11q, is linked to a region that encodes the_ chain of the high-affinity IgE receptor. It is known that inherited atopy is multigenic and that other loci probably also are involved. Indeed, as information from the Human Genome Project is analyzed, other candidate genes may be revealed. Most allergic IgE responses occur on mucous membrane surfaces in response to allergens that enter the body by either inhalation or ingestion.

Those that have include the allergens from rye grass pollen, ragweed pollen, codfish, birch pollen, timothy grass pollen, and bee venom. Each of these allergens has been shown to be a multiantigenic system that contains a number of allergenic components. Ragweed pollen, a major allergen in the United States, is a case in point. It has been reported that a square mile of ragweed yields 16 tons of pollen in a single season. Indeed, all regions of the United States are plagued by ragweed pollen as well as pollen from trees indigenous to the region. The pollen particles are inhaled, and their tough outer wall is dissolved by enzymes in the mucous secretions, releasing the allergenic substances.

Chemical fractionation of ragweed has revealed a variety of substances, most of which are not allergenic but are capable of eliciting an IgM or IgG response. Of the five fractions that are allergenic (i.e., able to induce an IgE response), two evoke allergenic reactions in about 95% of ragweed-sensitive individuals and are called major allergens; these are designated the E and K fractions. The other three, called Ra3, Ra4, and Ra5, are minor allergens that induce an allergic response in only 20% to 30% of sensitive subjects. Why are some pollens (e.g., ragweed) highly allergenic, whereas other equally abundant pollens (e.g., nettle) are rarely allergenic? No single physicochemical property seems to distinguish the highly allergenic E and K fractions of ragweed from the less allergenic Ra3, Ra4, and Ra5 fractions and from the non allergenic fractions. Rather, allergens as a group appear to possess diverse properties. Some allergens, including foreign serum and egg albumin, are potent antigens; others, such as plant pollens, are weak antigens. Although most allergens are small proteins or protein-

bound substances having a molecular weight between 15,000 and 40,000, attempts to identify some common chemical property of these antigens have failed. It appears that allergenicity is a consequence of a complex series of interactions involving not only the allergen but also the dose, the sensitizing route, sometimes an adjuvant, and—most important, as noted above—the genetic constitution of the recipient.

TYPE I HYPERSENSITIVITY

Type I hypersensitivity is also known as immediate or anaphylactic hypersensitivity. The reaction may involve skin, eyes (conjunctivitis), nasopharynx , rhinitis), bronchopulmonary tissues (asthma) and gastrointestinal tract (gastroenteritis). The reaction may cause a range of symptoms from minor inconvenience to death. The reaction usually takes 15 - 30 minutes from the time of exposure to the antigen, although sometimes it may have a delayed onset (10 - 12 hours).

Immediate hypersensitivity is mediated by IgE. The primary cellular component in this hypersensitivity is the mast cell or basophil. The reaction is amplified and/or modified by platelets, neutrophils and eosinophils. A biopsy of the reaction site demonstrates mainly mast cells and eosinophils.

The mechanism of reaction involves preferential production of IgE, in response to certain antigens (often called allergens). The precise mechanism as to why some individuals are more prone to type-I hypersensitivity is not clear. However, it has been shown that such individuals preferentially produce more of TH2 cells that secrete IL-4, IL-5 and IL-13 which in turn favor IgE class switch. IgE has very high affinity for its receptor (Fcε; CD23) on mast cells and basophils.

A subsequent exposure to the same allergen cross links the cell-bound IgE and triggers the release of various pharmacologically active substances. Cross-linking of IgE Fc-receptor is important in mast cell triggering. Mast cell degranulation is preceded by increased Ca++ influx, which is a crucial process; ionophores which increase cytoplasmic Ca^{++} also promote degranulation, whereas, agents which deplete cytoplasmic Ca^{++} suppress degranulation.

The agents released from mast cells and their effects are listed. Mast cells may be triggered by other stimuli such as exercise, emotional stress, chemicals (*e.g.*, photographic developing medium, calcium ionospheres, codeine, *etc.*). These reactions, mediated by agents without IgE-allergen interaction, are not hypersensitivity reactions, although they produce the same symptoms type 1 hypersensitivity.

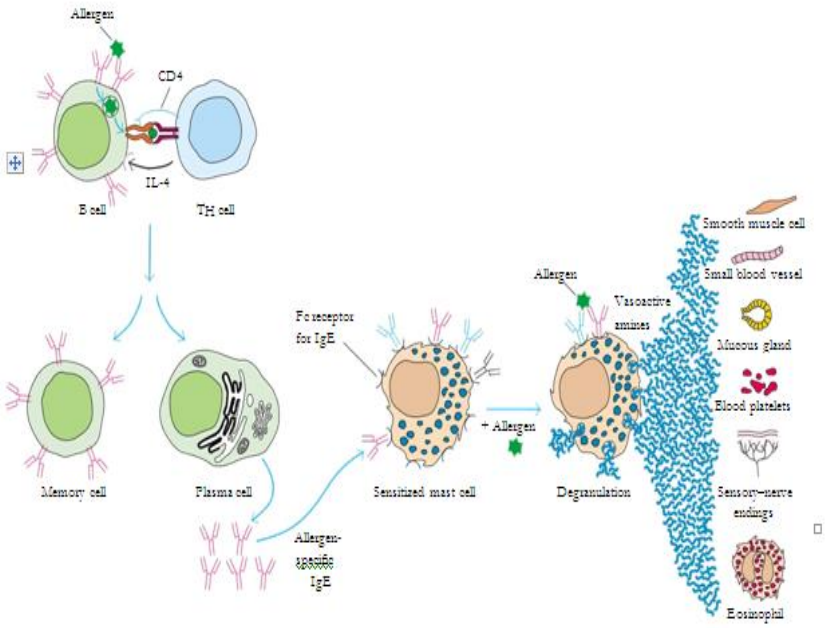

Fig:Induction and effector mechanism in Type I hypersensitivity

The reaction is amplified by PAF (platelet activation factor) which causes platelet aggregation and release of histamine, heparin and vasoactive amines. Eosinophil chemotactic factor of anaphylaxis (ECF-A) and neutrophil chemotactic factors attract eosinophils and neutrophils, respectively, which release various hydrolytic enzymes that cause necrosis.

Diagnostic tests for immediate hypersensitivity include skin (prick and intradermal) tests (fig. 1A), measurement of total IgE and specific IgE antibodies against the suspected allergens. Total IgE and specific IgE antibodies are measured by a modification of enzyme immunoassay (ELISA).

Symptomatic treatment is achieved with anti-histamines which block histamine receptors. Chromolyn sodium inhibits mast cell degranulation, probably, by inhibiting Ca^{++} influx. Late onset allergic

symptoms, particularly bronchoconstriction which is mediated by leukotrienes, are treated with leukotriene receptor blockers (Singulair, Accolate) or inhibitors of the cyclooxygenase pathway (Zileutoin). Symptomatic, although short term, relief from bronchoconstriction is provided by bronchodilators (inhalants) such as isoproterenol derivatives (Terbutaline, Albuterol). Thophylline elevates cAMP by inhibiting cAMP-phosphodiesterase and inhibits intracellular Ca^{++} release is also used to relieve bronchopulmonary symptoms. Hyposensitization (immunotherapy or desensitization) is another treatment modality which is successful in a number of allergies, particularly to insect venoms and, to some extent, pollens. Suppressor T cells that specifically inhibit IgE antibodies may play a role.

The use of IgG antibodies against the Fc portions of IgE that binds to mast cells has been approved for treatment of certain allergies, as it can block mast cell sensitization.

EG-Asthma, atopic dermatitis, Food allegy

TYPE II HYPERSENSITIVITY

Type II hypersensitivity is also known as cytotoxic hypersensitivity and may affect a variety of organs and tissues. The antigens are normally endogenous, although exogenous chemicals (haptens) which can attach to cell membranes can also lead to type II hypersensitivity. Drug-induced hemolytic anemia, granulocytopenia and thrombocytopenia are such examples. The reaction time is minutes to hours.

Type II hypersensitivity is primarily mediated by antibodies of the IgM or IgG classes and complement. Type II hypersensitive reactions involve antibody-mediated destruction of cells. Antibody can activate the complement system, creating pores in the membrane of a foreign cell, or it can mediate cell destruction by antibody-dependent cell-mediated cytotoxicity (ADCC). In this process, cytotoxic cells with Fc receptors bind to the Fc region of antibodies on target cells and promote killing of the cells. Antibody bound to a foreign cell also can serve as an opsonin, enabling phagocytic cells with Fc or C3b re-ceptors to bind and phagocytose the antibody-coated cell.

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Eg: **i)Blood transfusion reaction**

The clinical manifestations of transfusion reactions result from massive intravascular hemolysis of the transfused red blood cells by antibody plus complement.

ii) Hemolytic Disease of the Newborn

Hemolytic disease of the newborn develops when maternal IgG antibodies specific for fetal blood-group antigens cross the placenta and destroy fetal red blood cells. The conse-quences of such transfer can be minor, serious, or lethal. Severe hemolytic disease of the newborn, called erythroblastosis fetalis**,** most commonly develops when an Rh^+ fetus ex-presses an Rh antigen on its blood cells that the Rh^- mother does not express.

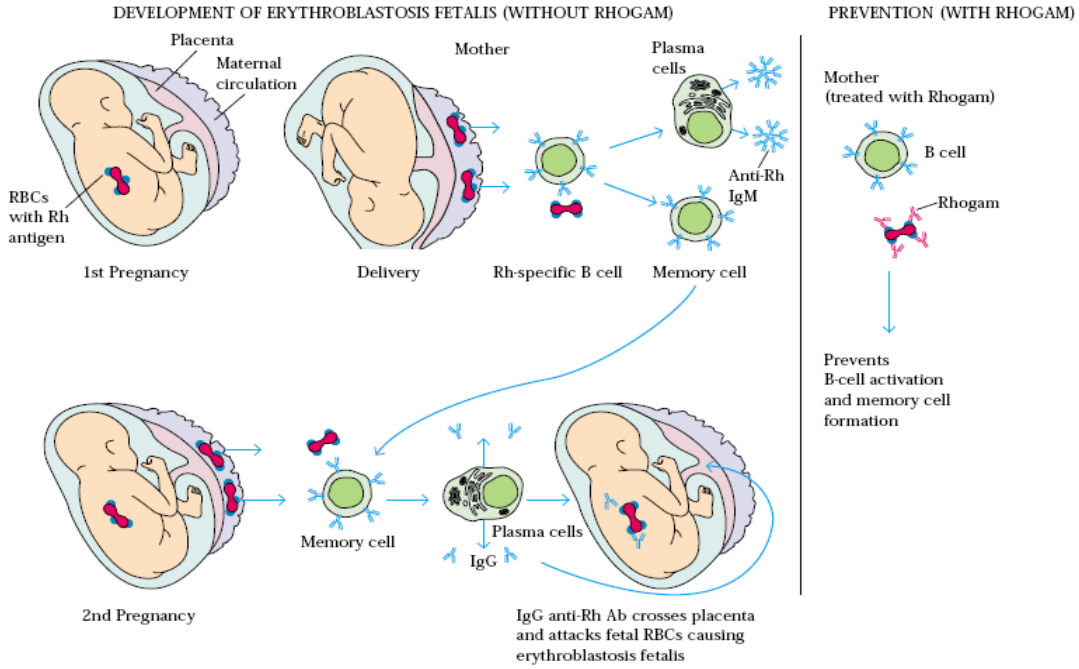


Figure: Type II hypersensitivity mechanisms

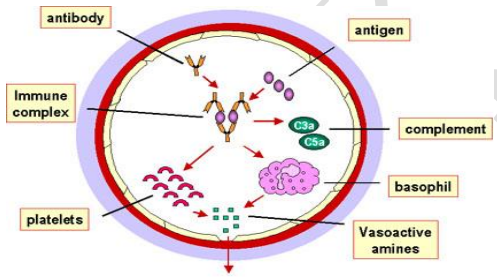
Diagnostic tests include detection of circulating antibody against the tissues involved and the presence of antibody and complement in the lesion (biopsy) by immunofluorescence. The staining pattern is normally smooth and linear, such as that seen in Goodpasture's nephritis (renal and lung basement membrane) and pemphigus (skin intercellular protein, desmosome).

Treatment involves anti-inflammatory and immunosuppressive agents.

TYPE III HYPERSENSITIVITY

Type III hypersensitivity is also known as immune complex hypersensitivity. The reaction may be general (*e.g.*, serum sickness) or may involve individual organs including skin (*e.g.*, systemic lupus erythematosus, Arthus reaction), kidneys (*e.g.*, lupus nephritis), lungs (*e.g.*, aspergillosis), blood vessels (*e.g.*, polyarteritis), joints (*e.g.*, rheumatoid arthritis) or other organs. This reaction may be the pathogenic mechanism of diseases caused by many microorganisms.

The reaction may take 3 - 10 hours after exposure to the antigen (as in Arthus reaction). It is mediated by soluble immune complexes. They are mostly of the IgG class, although IgM may also be involved. The antigen may be exogenous (chronic bacterial, viral or parasitic infections), or endogenous (non-organ specific autoimmunity: *e.g.*, systemic lupus erythematosus, SLE). The antigen is soluble and not attached to the organ involved. Primary components are soluble immune complexes and complement (C3a, 4a and 5a). The damage is caused by platelets and neutrophils.



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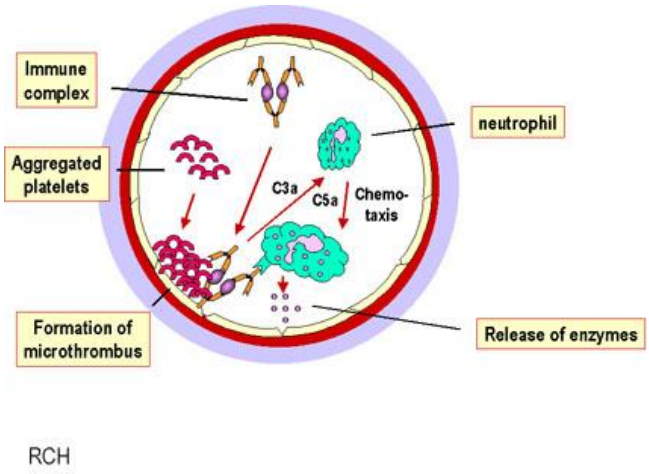

Figure :Mechanism of damage in type-III hypersensitivity

The lesion contains primarily neutrophils and deposits of immune complexes and complement. Macrophages infiltrating in later stages may be involved in the healing process.

The affinity of antibody and size of immune complexes are important in production of disease and determining the tissue involved.

Mechanism:

The reaction of antibody with antigen generates immune complexes. Generally this complexing of antigen with anti-body facilitates the clearance of antigen by phagocytic cells. In some cases, however, large amounts of immune complexes can lead to tissue-damaging type III hypersensitive reactions. The magnitude of the reaction depends on the quantity of immune complexes as well as their distribution within the body. When the complexes are deposited in tissue very near the site of antigen entry, a localized reaction develops. When the complexes are formed in the blood, a reaction can de-velop wherever the complexes are deposited. In particular, complex deposition is frequently observed on blood-vessel walls, in the synovial membrane of joints, on the glomerular basement membrane of the kidney, and on the choroid plexus of the brain. The deposition of these complexes initiates a reaction that results in the recruitment of neutrophils to the site. The tissue there is injured as a consequence of granular release from the neutrophil.

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Type III hypersensitive reactions develop when immune complexes activate the complement system's array of immune effector molecules (see Figure). The C3a, C4a, and C5a complement split products are anaphylatoxins that cause localized mast-cell de-granulation and consequent increase in local vascular permeability. C3a, C5a, and C5b67 are also chemotactic factors for neutrophils, which can accumulate in large numbers at the site of immune-complex deposition. Larger immune complexes are deposited on the basement membrane of blood-vessel walls or kidney glomeruli, whereas smaller complexes may pass through the basement membrane and be deposited in the subepithelium. The type of lesion that results depends on the site of deposition of the complexes.

Much of the tissue damage in type III reactions stems from release of lytic enzymes by neutrophils as they attempt to phagocytes immune complexes. The C3b complement component acts as an opsonin, coating immune complexes. A neutrophil binds to a C3b-coated immune complex by means of the type I complement receptor, which is specific for C3b. Because the complex is deposited on the basement-membrane surface, phagocytosis is impeded, so that lytic enzymes are released during the unsuccessful attempts of the neutrophil to ingest the adhering immune complex. Further activation of the membrane-attack mechanism of the complement system can also contribute to the destruction of tis-sue. In addition, the activation of complement can induce aggregation of platelets, and the resulting release of clotting factors can lead to formation of microthrombi.

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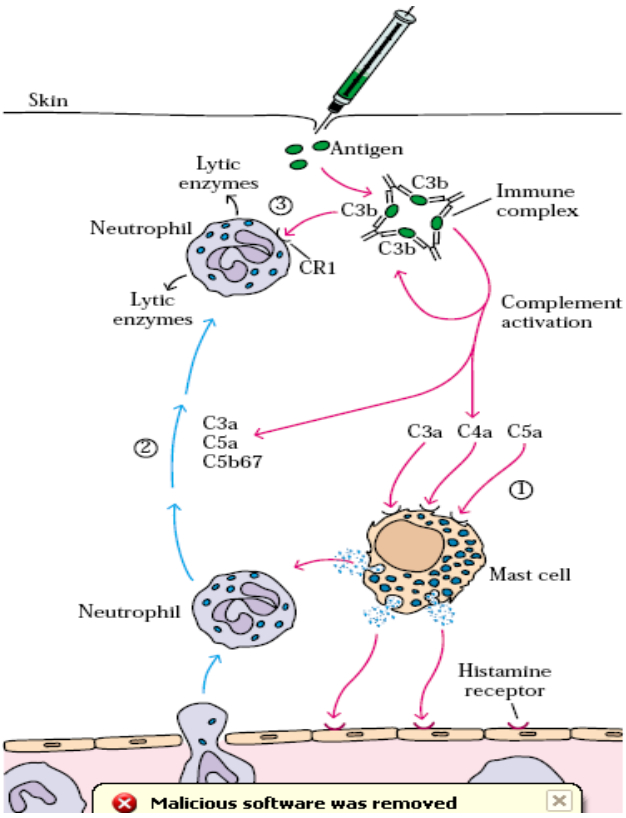

Fig:Development of a localized Arthus reaction (type III hypersensitive reaction).

Diagnosis involves examination of tissue biopsies for deposits of immunoglobulin and complement by immunofluorescence microscopy. The immunofluorescent staining in type III hypersensitivity is granular (as opposed to linear in type II such as seen in Goodpasture's syndrome). The presence of immune complexes in serum and depletion in the level of complement are also diagnostic. Polyethylene glycol-mediated turbidity (nephelometry) are also utilized to detect immune complexes.

Treatment includes anti-inflammatory agents.

TYPE IV HYPERSENSITIVITY

Type IV hypersensitivity is also known as cell mediated or delayed type hypersensitivity. The classical example of this hypersensitivity is tuberculin (Montoux) reaction which peaks 48 hours

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after the injection of antigen (PPD or old tuberculin). The lesion is characterized by induration and erythema.

Type IV hypersensitivity is involved in the pathogenesis of many autoimmune and infectious diseases (tuberculosis, leprosy, blastomycosis, histoplasmosis, toxoplasmosis, leishmaniasis, *etc.*) and granulomas due to infections and foreign antigens. Another form of delayed hypersensitivity is contact dermatitis (poison ivy , chemicals, heavy metals, *etc.*) in which the lesions are more papular. Type IV hypersensitivity can be classified into three categories depending on the time of onset and clinical and histological presentation.

Table 3 - Delayed hypersensitivity reactions

Type	Reaction time	Clinical appearance	Histology	Antigen and site
contact	48-72 hr	eczema	lymphocytes, followed by macrophages; edema of epidermis	Epidermal (organic chemicals, poison ivy, heavy metals, *etc.*)
tuberculin	48-72 hr	local induration	lymphocytes, monocytes, macrophages	intradermal (tuberculin, lepromin, *etc.*)
granuloma	21-28 days	hardening	macrophages, epitheloid and giant cells, fibrosis	Persistent antigen or foreign body presence (tuberculosis, leprosy, *etc.*)

Mechanisms of damage in delayed hypersensitivity include T lymphocytes and monocytes and/or macrophages. Cytotoxic T cells (Tc) cause direct damage whereas helper T (TH1) cells secrete cytokines which activate cytotoxic T cells and recruit and activate monocytes and macrophages, which cause the bulk of the damage. The delayed hypersensitivity lesions mainly contain monocytes and a few T cells.

Major lymphokines involved in delayed hypersensitivity reaction include monocyte chemotactic factor, interleukin-2, interferon-gamma, TNF alpha/beta, *etc.*

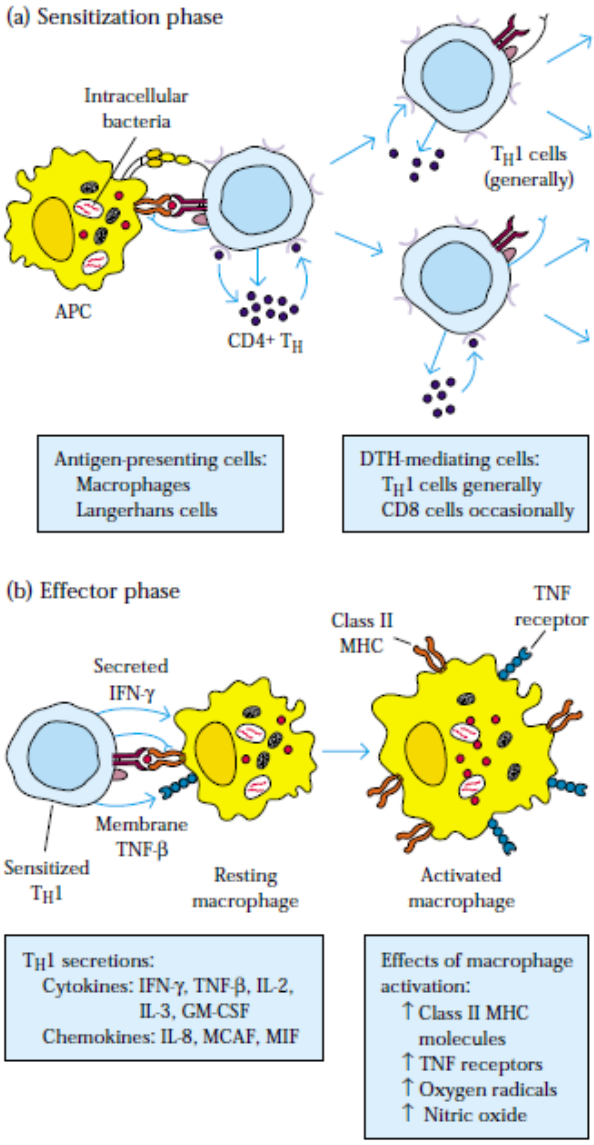
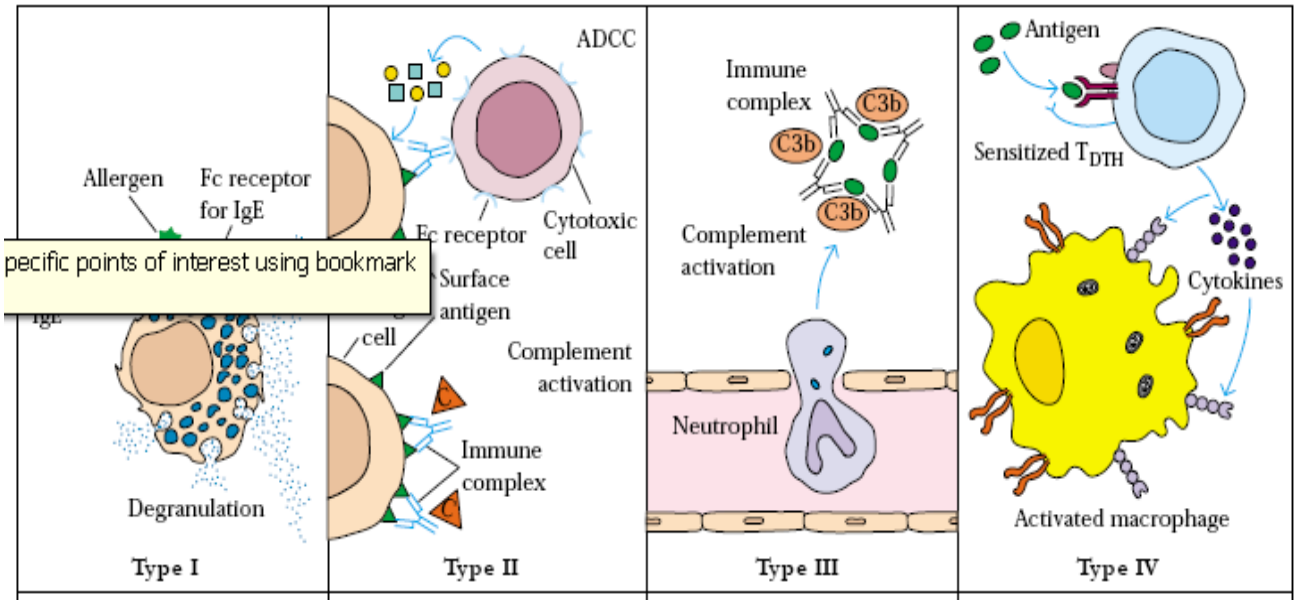


Figure 4. Mechanisms of damage in delayed hypersensitivity

Diagnostic tests *in vivo* include delayed cutaneous reaction (*e.g.* Montoux test (figure 5)) and patch test (for contact dermatitis). In vitro tests for delayed hypersensitivity include mitogenic response, lympho-cytotoxicity and IL-2 production.

Corticosteroids and other immunosuppressive agents are used in treatment.

Table 5 - Comparison of Different Types of hypersensitivity				
characteristics	**type-I (anaphylactic)**	**type-II (cytotoxic)**	**type-III (immune complex)**	**type-IV (delayed type)**
antibody	IgE	IgG, IgM	IgG, IgM	None
antigen	exogenous	cell surface	soluble	tissues & organs
response time	15-30 minutes	minutes-hours	3-8 hours	48-72 hours
appearance	weal & flare	lysis and necrosis	erythema and edema, necrosis	erythema and induration
histology	basophils and eosinophil	antibody and complement	complement and neutrophils	monocytes and lymphocytes
transferred with	antibody	antibody	antibody	T-cells
examples	allergic asthma, hay fever	Erythroblastosis fetalis, Good pasture's nephritis	SLE, farmer's lung disease	tuberculin test, poisonivy, granuloma



Type I	Type II	Type III	Type IV
IgE-Mediated Hypersensitivity	IgG-Mediated Cytotoxic Hypersensitivity	Immune Complex-Mediated Hypersensitivity	Cell-Mediated Hypersensitivity
Ag induces crosslinking of IgE bound to mast cells and basophils with release of vasoactive mediators	Ab directed against cell surface antigens meditates cell destruction via complement activation or ADCC	Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils	Sensitized T_H1 cells release cytokines that activate macrophages or T_C cells which mediate direct cellular damage
Typical manifestations include systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema	Typical manifestations include blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia	Typical manifestations include localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulnephritis, rheumatoid arthritis, and systemic lupus erythematosus	Typical manifestations include contact dermatitis, tubercular lesions and graft rejection

Type V hypersensitivity

Type V hypersensitivity is the final type of hypersensitivity in which antibodies are produced with the property of stimulating specific cell targets. The clearest example is Grave's disease caused by antibodies that stimulate the thyroid-stimulating hormone receptor, leading to overactivity of the thyroid gland.

Instead of binding to cell surfaces, the antibodies recognise and bind to the cell surface receptor, which either prevents the intended ligand binding with the receptor or mimics the effects of the ligand, thus impairing cell signaling.

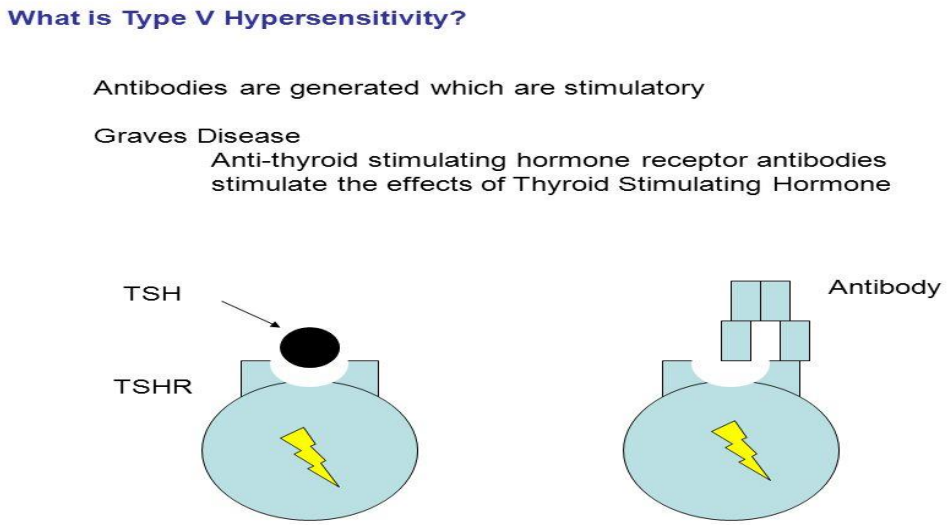
Some clinical examples:

- Graves' disease
- Myasthenia gravis

The use of Type 5 is rare. These conditions are more frequently classified as Type 2, though sometimes they are specifically segregated into their own subcategory of Type 2.

Graves disease: There is a moderate diffuse goitre, evidence of weight loss due to thyrotoxicosis, and changes of thyroid-associated ophthalmopathy (eyelid retraction, proptosis due to the eyeball being pushed forward by swollen extraocular muscles, and periorbital oedema).

Myasthenia gravis (MG) is a long term neuromuscular disease that leads to varying degrees of skeletal muscle weakness. The most commonly affected muscles are those of the eyes, face, and swallowing. It can result in double vision, drooping eyelids, trouble talking, and trouble walking. Onset can be sudden. Those affected often have a large thymus gland or develop a thymoma. Myasthenia gravis is an autoimmune synaptopathy. The disorder occurs when the immune system malfunctions and generates antibodies that attack the body's tissues. The antibody in myasthenia gravis attacks a normal human protein, targeting a protein called the nicotinic acetylcholine receptor, or a related protein called a muscle-specific kinase.



Possible Questions

Two mark questions

1. Define Hypersensitivity.
2. What is allergy?
3. What is Allergens?
4. What is haemolytic disease?
5. Comment on cell mediated hypersensitivity?
6. Draw the diagram for Hypersensitivity reaction I process.
7. Define Inflammation

Eight mark questions

1. Explain in detail about Hypersensitivity reaction I.
2. Explain Hypersensitivity reaction II in detail.
3. Comment on IgE mediated Hypersensitivity.
4. Describe the diagnostic tests for Hypersensitivity reaction II.
5. Give a brief account on Hypersensitivity reaction III.
6. Elaborate the treatment details for Hypersensitivity reactions.
7. Explain the different types of hypersensitivity reactions.
8. Describe in detail about auto immune diseases.
9. Compare the different types of hypersensitivity.
10. Give an account on IgG mediated cytotoxic hypersensitivity.
11. Briefly explain about immune complex mediated hypersensitivity.

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UNIT-IV

SYLLABUS

Introduction: Major Histocompatibility complexes: Class I & class II MHC antigens, antigen processing. Immunity to infection – immunity to different organisms, pathogen defense strategies, avoidance of recognition. Autoimmune diseases, Immunodeficiency-AIDS.

MAJOR HISTOCOMPATIBILITY COMPLEX

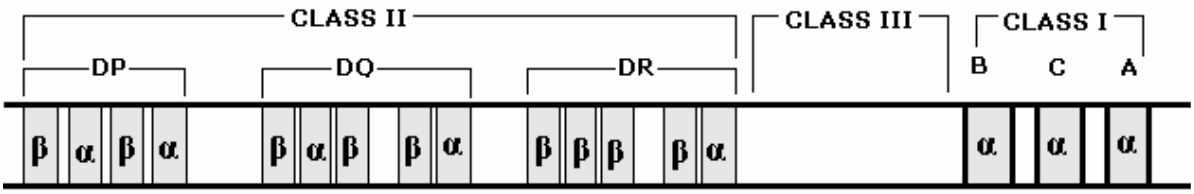
Major Histocompatibility Complex

- MHC complex is group of genes on a single chromosome that codes the MHC antigens.
- Major as well as minor histocompatibility antigens (also called transplantation antigens) mediate rejection of grafts between two genetically different individuals.
- However, the role played by the major histocompatibility antigens supersedes the minor histocompatibility antigens.
- HLA (human leukocyte antigens) are the MHC antigens of humans, and called so because they were first detected on leukocytes. H-2 antigens are their equivalent MHC antigens of mouse.
- A set of MHC alleles present on each chromosome is called an MHC haplotype. Monozygotic human twins have the same histocompatibility molecules on their cells, and they can accept transplants of tissue from each other.
- Histocompatibility molecules of one individual act as antigens when introduced into a different individual.
- George Snell, Jean Dausset and Baruj Benacerraf received the Nobel Prize in 1980 for their contributions to the discovery and understanding of the MHC in mice and humans.
- MHC gene products were identified as responsible for graft rejection.

- MHC gene products that control immune responses are called the immune response (Ir) genes. Immune response genes influence responses to infections.
- The essential role of the HLA antigens lies in the induction and regulation of the immune response and defence against microorganisms.
- The physiologic function of MHC molecules is the presentation of peptide antigen to T lymphocytes.
- These antigens and their genes can be divided into three major classes: class I, class II and class III.

Structure

- The MHC complex resides in the short arm of chromosome 6 and overall size of the MHC is approximately 3.5 million base pairs.
- The complete three-dimensional structure for both class I and class II MHC molecules has been determined by x-ray crystallography.
- The class I gene complex contains three loci A, B and C, each of which codes of α chain polypeptides.
- The class II gene complex also contains at least three loci, DP, DQ and DR; each of these loci codes for one α and a variable number of β chain polypeptides.
- Class III region is not actually a part of the HLA complex, but is located within the HLA region, because its components are either related to the functions of HLA antigens or are under similar control mechanisms to the HLA genes.
- Class III antigens are associated with proteins in serum and other body fluids (e.g.C4, C2, factor B, TNF) and have no role in graft rejection.



Nomenclature

- HLA specificities are identified by a letter for locus and a number (A1, B5, etc.), and the haplotypes are identified by individual specificities (e.g., A1, B7, Cw4, DP5, DQ10, DR8).

- Specificities which are defined by genomic analysis (PCR), are named with a letter for the locus and a four digit number (e.g. A0101, B0701, C0401, etc.).

Inheritance

- Histocompatibility genes are inherited as a group (haplotype), one from each parent. Thus, MHC genes are co dominantly expressed in each individual.
- A heterozygous human inherits one paternal and one maternal haplotype, each containing three Class-I (B, C and A) and three Class II (DP, DQ and DR) loci. Each individual inherits a maximum of two alleles for each locus.
- The maximum number of class I MHC gene products expressed in an individual is six; that for class II MHC products can exceed six but is also limited.
- Thus, as each chromosome is found twice (diploid) in each individual, a normal tissue type of an individual will involve 12 HLA antigens.
- Haplotypes, normally, are inherited intact and hence antigens encoded by different loci are inherited together.
- However, on occasions, there is crossing over between two parental chromosomes, thereby resulting in new recombinant haplotypes.
- There is no somatic DNA recombination that occurs for antibodies and for the TCR, so the MHC genes lack recombinational mechanisms for generating diversity.
- Many alleles of each locus permit thousands of possible assortments. There are at least 1000 officially recognized HLA alleles.

Expression

- Class I antigens are expressed on all nucleated cells (except those of the central nervous system) and platelets.
- The class II antigens are expressed on antigen presenting cells such as B lymphocytes, dendritic cells, macrophages, monocytes, Langerhans cells, endothelial cells and thymic epithelial cells.
- Cytokines, especially interferon gamma (IFN-γ), increase the level of expression of class I and class II MHC molecules.

MHC Class I Molecule

- Class I MHC molecules contain two separate polypeptide chains, the heavier (44-47 KDa) alpha chain and the lighter (12 KDa) beta chain.

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- The carboxyl end of α chain resides inside the cell while the amino end projects on the surface of cell with a short intervening hydrophobic segment traverses the membrane.
- The α chain is coded by the MHC genes and has three globular domains α1, α2 and α3. β2-microglobulin is encoded by a gene on another chromosome.
- The α3 domain is non-covalently associated with the β2 microglobulin. Both α chain and β2-microglobulin are members of the Ig superfamily. Without the β2 microglobulin, the class I antigen will not be expressed on the cells surface.
- Individuals with defective β2 microglobulin gene do not express any class I antigen and hence they have a deficiency of cytotoxic T cells.
- A peptide-binding groove is formed between α1 and α2 helices with beta-pleated sheet as its floor.
- A peptide of 8- 10 amino acids long can be presented in this groove. The alloantigenic sites that carry determinants specific to each individual are found in the α1 and α2 domains.
- The greatest variability in amino acids (or polymorphism) occurs in the α1 and α2 sequences that line the wall and floor of the groove that binds the peptides.
- The polymorphism among class I MHC gene products creates variation in the chemical surface of the peptide-binding groove so that various peptide molecules can be accommodated.
- The specific binding of a peptide molecule in the peptide-binding groove of MHC requires the peptide to have one or more specific amino acid at a fixed position.
- Such sites are termed anchor sites. The other amino acids can be variable so that each MHC molecule can bind many different peptides.
- The α1 and α2 domains also bind T cell receptor (TCR) of CD8 T lymphocytes. The parts of these domains that are in contact with TCR also show polymorphism.
- The immunoglobulin-like region of α3 domain is constant (shows no variation) and is non-covalently bound β2 microglobulin.
- The importance of the highly conserved region of α3 is that CD8 molecules present on CD8 T lymphocytes binds to this region.

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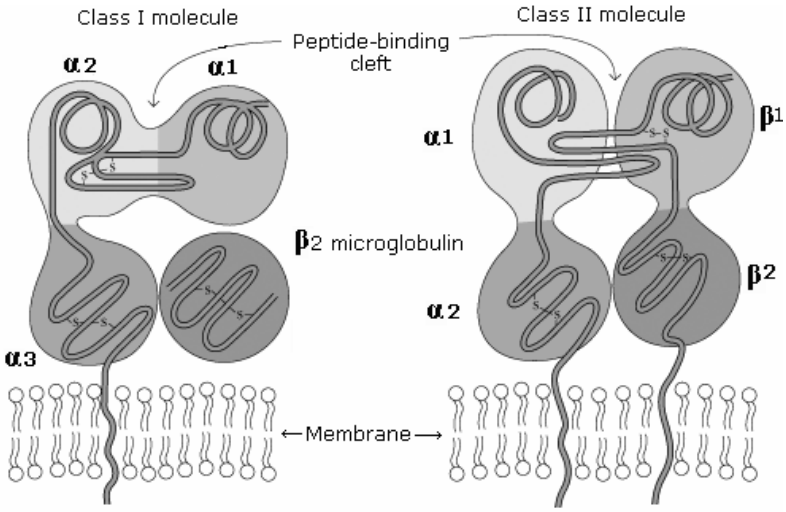
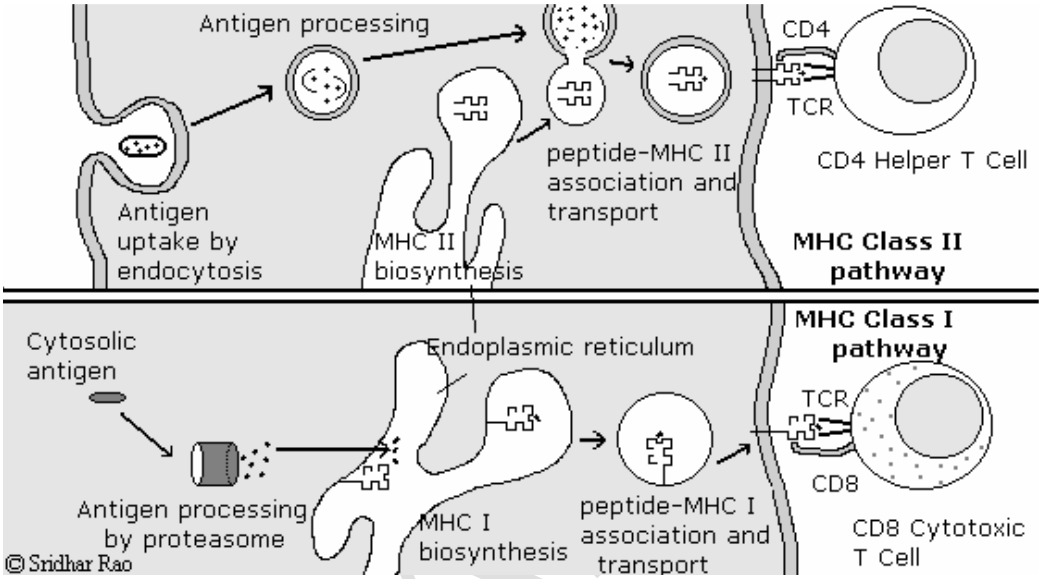


- CD8 T lymphocytes recognizes peptide antigen only when it is presented by the antigen presenting cell in the peptide binding groove of MHC I molecules.
- Class I molecules present peptide fragments in the cytosol (endogenous antigen, which could be fragments of viral or tumour proteins) to the CD8 lymphocytes.

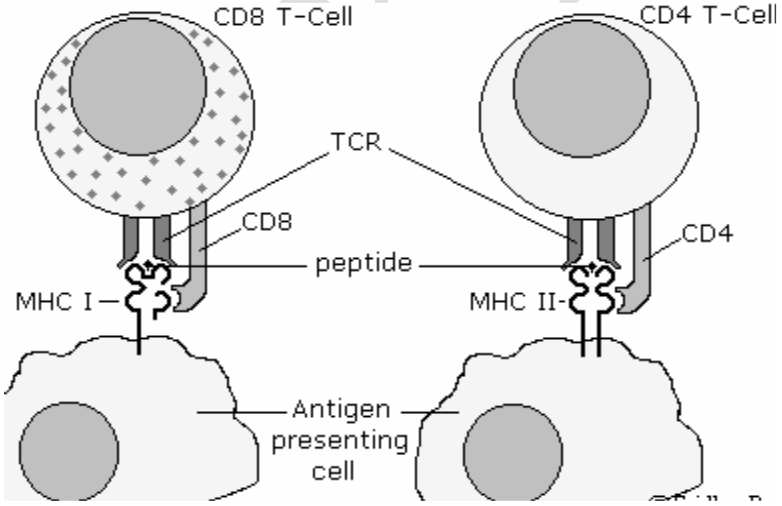
MHC Class II Molecule

- MHC class II molecules comprise two non-identical and non-covalently associated polypeptide chains (αand β).
- These two chains have amino ends on the surface, a short transmembrane stretch and intracytoplasmic carboxyl ends. Both α chain (34 kDa) and β chain (28 kDa) are MHC-encoded and polymorphic.
- The domains closest to the membrane in each chain are structurally related to immunoglobulins. With the exception of the α1 domain, all domains are stabilized by disulfide bridges.
- The β chain is shorter than the α chain and contains the alloantigenic sites. A peptide binding groove is formed in between α1 and β1 domains with a beta pleated floor.
- As in the case for class I MHC, the greatest polymorphic variability in the amino acids is in those facing the groove.
- This in turn determines the chemical structure of the groove and influences the specificity and affinity of peptide binding. Peptides associated with class II MHC are 13-25 amino acids long.

- As with class I MHC, anchor sites for one or more amino acids also exist in the groove of the class II MHC molecule. α2 and β2 are largely non-polymorphic.
- During antigen presentation, CD4 molecule of Helper T lymphocyte binds to β2 domain of the class II MHC molecules.
- Exogenous antigens (fragments of bacterial cells or viruses that are engulfed and processed by antigen presenting cell) are presented to helper T-cells along with MHC II molecules.



Because each MHC molecule (I and II) can bind many different peptides, the binding is said to be degenerate.



CD 4 Helper Tlymphocytes canrecognize peptideantigen only whenpresented along MHCII molecules.

CD8Cytotoxic T lymphocytes can recognize peptide antigen only when presented along MHC I molecules.

- Class I MHC molecules
- Class II MHC molecules

Each of these molecules plays a unique role in antigen recognition, ensuring that the immune system can recognize and respond to the different types of antigen that it encounters.

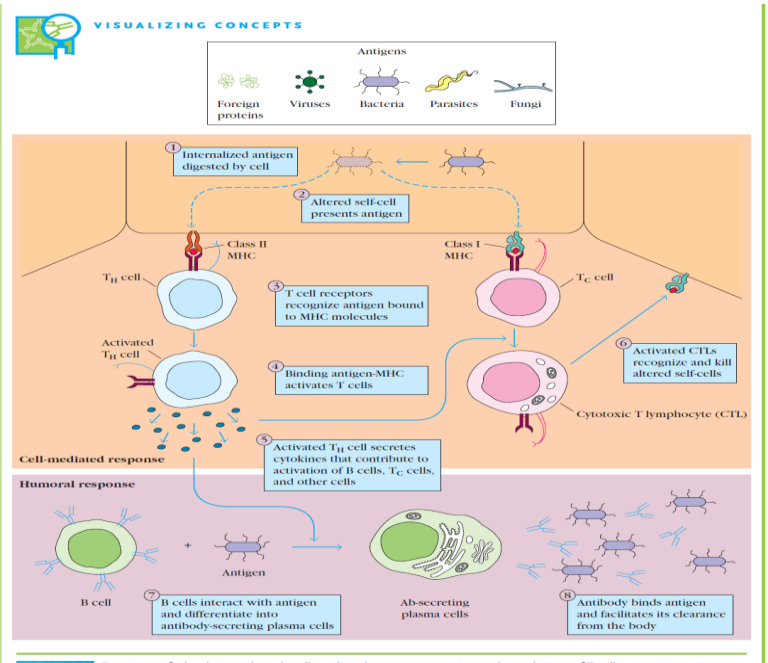


FIGURE 1-7 Overview of the humoral and cell-mediated branches of the immune system. In the humoral response, B cells interact with antigen and then differentiate into antibody-secreting plasma cells. The secreted antibody binds to the antigen and facilitates its clearance from the body. In the cell-mediated response, various subpopulations of T cells recognize antigen presented on self-cells. T_H cells respond to antigen by producing cytokines. T_C cells respond to antigen by developing into cytotoxic T lymphocytes (CTLs), which mediate killing of altered self-cells (e.g., virus-infected cells).

B and T Lymphocytes Utilize Similar Mechanisms To Generate Diversity in Antigen Receptors

The antigenic specificity of each B cell is determined by the membrane-bound antigen-binding receptor (i.e., antibody) expressed by the cell. As a B cell matures in the bone marrow, its specificity is created by random rearrangements of a series of gene segments that encode the antibody molecule. As a result of this process, each mature B cell possesses a single functional gene encoding the antibody heavychain and a single functional gene encoding the antibody light chain; the cell therefore synthesizes and displays antibody with one specificity on its membrane. All antibody molecules on a given B lymphocyte have identical specificity, giving each B lymphocyte, and the clone of daughter cells to which it gives rise, a distinct specificity for a single epitope on an antigen. The mature B lymphocyte is therefore said to be antigenically committed**.**

The random gene rearrangements during B-cell maturation in the bone marrow generate an enormous number of different antigenic specificities. The resulting B-cell population, which consists of individual B cells each expressing a unique antibody, is estimated to exhibit collectively more than 1010 different antigenic specificities. The enormous diversity in the mature B-cell population is later reduced by a selection process in the bone marrow that eliminates any B cells with membrane-bound

antibody that recognizes self components. The selection process helps to ensure that selfreactive antibodies (auto-antibodies) are not produced.

The attributes of specificity and diversity also characterize the antigen-binding T-cell receptor (TCR) on T cells. As in B cell maturation; the process of T-cell maturation includes random rearrangements of a series of gene segments that encode the cell's antigen-binding receptor. Each T lymphocyte cell expresses about 105 receptors, and all of the receptors on the cell and its clonal progeny have identical specificity for antigen. The random rearrangement of the TCR genes is capable of generating on the order of 109 unique antigenic specificities. This enormous potential diversity is later diminished through a selection process in the thymus that eliminates any T cell with self-reactive receptors and ensures that only T cells with receptors capable of recognizing antigen associated with MHC molecules will be able to mature.

The Major Histocompatibility Molecules Bind Antigenic Peptides

The major histocompatibility complex (MHC) is a large genetic complex with multiple loci. The MHC loci encode two major classes of membrane-bound glycoproteins: **class I** and **class II MHC molecules.** As noted above, TH cells generally recognize antigen combined with class II molecules, whereas TC cells generally recognize antigen combined with class I molecules (Figure 1-8).

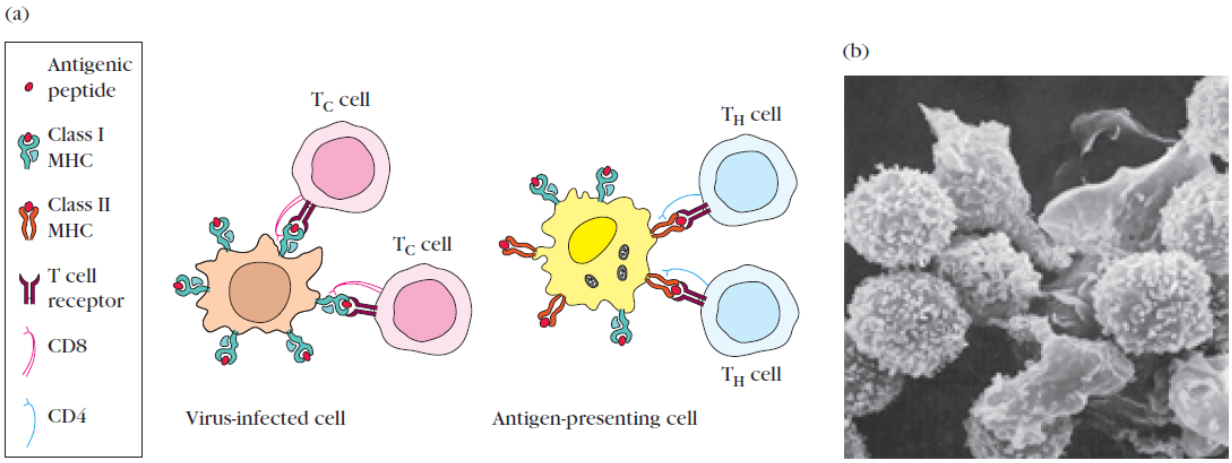


FIGURE 1-8 The role of MHC molecules in antigen recognition by T cells. (a) Class I MHC molecules are expressed on nearly all nucleated cells. Class II MHC molecules are expressed only on antigen-presenting cells. T cells that recognize only antigenic peptides displayed with a class II MHC molecule generally function as T helper (T_H) cells. T cells that recognize only antigenic peptides displayed with a class I MHC molecule generally function as T cytotoxic (T_C) cells. (b) This scanning electron micrograph reveals numerous T lymphocytes interacting with a single macrophage. The macrophage presents processed antigen combined with class II MHC molecules to the T cells. [*Photograph from W. E. Paul (ed.), 1991,* Immunology: Recognition and Response, *W. H. Freeman and Company, New York; micrograph courtesy of M. H. Nielsen and O. Werdelin.*]

MHC molecules function as antigen-recognition molecules, but they do not possess the fine specificity for antigen characteristic of antibodies and T-cell receptors. Rather, each MHC molecule can bind to a spectrum of antigenic peptides derived from the intracellular degradation of antigen molecules. In both class I and class II MHC molecules the distal regions (farthest from the membrane) of different alleles display wide variation in their amino acid sequences. These variable regions form a cleft within which the antigenic peptide sits and is presented to T lymphocytes. Different allelic forms of the genes encoding class I and class II molecules confer different structures on the antigen-binding cleft with different specificity. Thus the ability to present an antigen to T lymphocytes is influenced by the particular set of alleles that an individual inherits.

ANTIGEN PROCESSING

In order for a foreign protein antigen to be recognized by a T cell, it must be degraded into small antigenic peptides that form complexes with class I or class II MHC molecules. This conversion of proteins into MHC-associated peptide fragments is called *antigen processing and presentation.* Whether a particular antigen will be processed and presented together with class I MHC or class II MHC molecules appears to be determined by the route that the antigen takes to enter a cell.

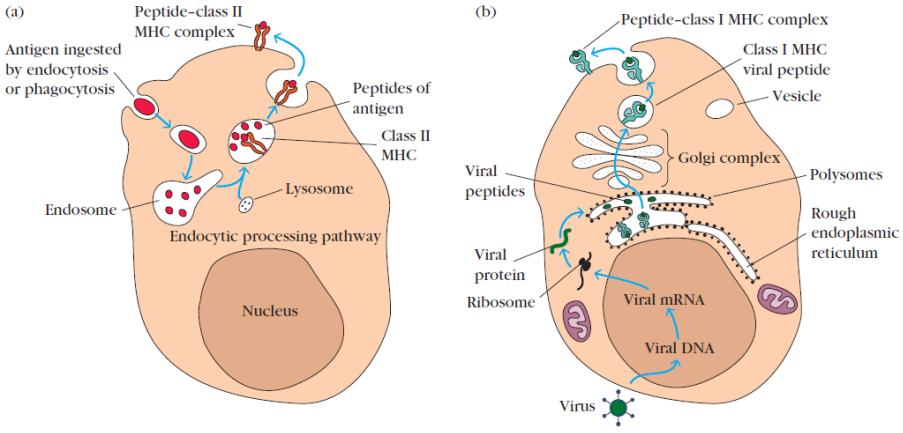


FIGURE 1-9 Processing and presentation of exogenous and endogenous antigens. (a) Exogenous antigen is ingested by endocytosis or phagocytosis and then enters the endocytic processing pathway. Here, within an acidic environment, the antigen is degraded into small peptides, which then are presented with class II MHC molecules on the membrane of the antigen-presenting cell. (b) Endogenous antigen, which is produced within the cell itself (e.g., in a virus-infected cell), is degraded within the cytoplasm into peptides, which move into the endoplasmic reticulum, where they bind to class I MHC molecules. The peptide–class I MHC complexes then move through the Golgi complex to the cell surface.

Exogenous antigen is produced outside of the host cell and enters the cell by endocytosis or phagocytosis. Antigen presenting cells (macrophages, dendritic cells, and B cells) degrade ingested exogenous antigen into peptide fragments within the endocytic processing pathway. Experiments

suggest that class II MHC molecules are expressed within the endocytic processing pathway and that peptides produced by degradation of antigen in this pathway bind to the cleft within the class II MHC molecules. The MHC molecules bearing the peptide are then exported to the cell surface. Since expression of class II MHC molecules is limited to antigen- presenting cells, presentation of exogenous peptide– class II MHC complexes is limited to these cells. T cells displaying CD4 recognize antigen combined with class II MHC molecules and thus are said to be *class II MHC restricted.* These cells generally function as T helper cells.

Endogenous antigen is produced within the host cell itself. Two common examples are viral proteins synthesized within virus-infected host cells and unique proteins synthesized by cancerous cells. Endogenous antigens are degraded into peptide fragments that bind to class I MHC molecules within the endoplasmic reticulum. The peptide–class I MHC complex is then transported to the cell membrane. Since all nucleated cells express class I MHC molecules, all cells producing endogenous antigen use this route to process the antigen. T cells displaying CD8 recognize antigen associated with class I MHC molecules and thus are said to be *class I MHC restricted.* These cytotoxic T cells attack and kill cells displaying the antigen–MHC class I complexes for which their receptors are specific.

IMMUNITY TO INFECTIONS

Immune Response to Infectious Diseases

One of the first and most important features of host innate immunity is the barrier provided by the epithelial surfaces of the skin and the lining of the gut. The difficulty of penetrating these epithelial barriers ensures that most pathogens never gain productive entry into the host. In addition to providing a physical barrier to infection, the epithelia also produce chemicals that are useful in preventing infection. The secretion of gastric enzymes by specialized epithelial cells lowers the pH of the stomach and upper gastrointestinal tract, and other specialized cells in the gut produce antibacterial peptides .A major feature of innate immunity is the presence of the normal gut flora, which can competitively inhibit the binding of pathogens to gut epithelial cells. Innate responses can also block the establishment of infection. For example, the cell walls of some gram-positive bacteria contain a peptidoglycan that activates the alternative complement pathway, resulting in the generation of C3b, which opsonizes bacteria and enhances phagocytosis. Some bacteria produce endotoxins such as LPS, which stimulate the production of cytokines such as TNF-_, IL-1, and IL-6 by macrophages or endothelial cells. These cytokines can activate macrophages. Phagocytosis of bacteria by macrophages and other phagocytic cells is another highly effective line of innate defense. However, some types of bacteria that commonly grow intracellularly have developed mechanisms that allow them to resist degradation within the

phagocyte. Viruses are well known for the stimulation of innate responses. In particular, many viruses induce the production of interferons, which can inhibit viral replication by inducing an antiviral response. Viruses are also controlled by NK cells. As described, NK cells frequently form the first line of defense against viral infections. Generally, pathogens use a variety of strategies to escaped estruction by the adaptive immune system. Many pathogens reduce their own antigenicity either by growing within host cells, where they are sequestered from immune attack, or by shedding their membrane antigens. Other pathogens camouflage themselves by mimicking the surfaces of host cells, either by expressing molecules with amino acid sequences similar to those of host cell-membrane molecules or by acquiring a covering of host membrane molecules. Some pathogens are able to suppress the immune response selectively or to regulate it so that a branch of the immune system is activated that is ineffective against the pathogen. Continual variation in surface antigens is another strategy that enables a pathogen to elude the immune system. This antigenic variation may be due to the gradual accumulation of mutations, or it may involve an abrupt change in surface antigens. Both innate and adaptive immune responses to pathogens provide critical defense, but infectious diseases, which have plagued human populations throughout history, still cause the death of millions each year. Although widespreaduse of vaccines and drug therapy has drastically reduced mortality from infectious diseases in developed countries, such diseases continue to be the leading cause of death in theThird World. It is estimated that over 1 billion people are infected worldwide, resulting in more than 11 million deaths every year. Despite these alarming numbers, estimated expenditures for research on infectious diseases prevalent in the Third World are less than 5% of total health research expenditures worldwide. Not only is this a tragedy for these countries, but some of these diseases are beginning and a new drug-resistant strain of *Mycobacterium tuberculosis*is spreading at an alarming rate in the United States. In this chapter, the concepts described in earlier chapters,antigenicity and immune effector mechanisms, as well as vaccine development are applied to selected infectious diseases caused by viruses, bacteria, protozoa, andhelminths—the four main types of pathogens.

IMMUNITY TO DIFFERENT ORGANISMS

Viral Infections

A number of specific immune effector mechanisms, together with nonspecific defense mechanisms, are called into play to eliminate an infecting virus (Table 17-1). At the same time,

the virus acts to subvert one or more of these mechanisms to prolong its own survival. The outcome of the infection depends on how effectively the host's defensive mechanisms resist the offensive tactics of the virus. The innate immune response to viral infection is primarily through the induction of type I and the activation of NK cells. Double stranded RNA (dsRNA) produced during the viral life cycle can induce the expression of IFN-_ and IFN-_ by the infected cell. Macrophages, monocytes, and fibroblasts also are capable of synthesizing these cytokines, but the mechanisms that induce the production of type I interferons in these cells are not completely understood. Once bound, IFN-_ and IFN- activate the JAK-STAT pathway, which in turn induces the transcription of several genes. One of these genes encodes an enzyme known as 2_-5_-oligo-adenylate synthetase [2-5(A) synthetase], which activates a ribonuclease (RNAse L) that degrades viral RNA.

Many Viruses are neutralized by Antibodies

Antibodies specific for viral surface antigens are often crucial in containing the spread of a virus during acute infection and in protecting against reinjection. Antibodies are particularly effective in protecting against infection if they are localized at the site of viral entry into the body.Most viruses express surface receptor molecules that enable them to initiate infection by binding to specific host-cell membrane molecules. For example, influenza virus binds to sialic acid residues in cellmembrane glycoproteins and glycolipids; rhinovirus binds to intercellular adhesion molecules (ICAMs); and Epstein-Barrvirus binds to type 2 complement receptors on B cells. If antibody to the viral receptor is produced, it can block infection altogether by preventing the binding of viral particles to host cells. Secretory IgA in mucous secretions plays an important role in host defense against viruses by blocking viral attachment of mucosal epithelial cells. The advantage of the attenuated oral polio vaccine, considered in Chapter 18, is that it induces production of secretory IgA, which effectively blocks attachment of poliovirus along the gastrointestinal tract. Viral neutralization by antibody sometimes involves mechanisms that operate after viral attachment to host cells.In some cases, antibodies may block viral penetration by binding to epitopes that are necessary to mediate fusion ofthe viral envelope with the plasma membrane. If the induced antibody is of a complement-activating isotype, lysis of enveloped virions can ensue. Antibody or complement can also agglutinate viral particles and function as an opsonizingagent to facilitate Fc- or C3b-receptor–mediated phagocytosis of the viral particles.

Cell-Mediated Immunity is Important for Viral Control and Clearance

Although antibodies have an important role in containing the spread of a virus in the acute phases of infection, they are not usually able to eliminate the virus once infection has occurred—particularly if the virus is capable of entering a latent state in which its DNA is integrated into host chromosomal DNA. Once an infection is established, cell-mediated immune mechanisms are most important in host defense. In general, CD8+ TC cells and CD4+ TH1 cells are the main

components of cell-mediated antiviral defense, although in some cases CD4+ TC cells have also been implicated. Activated TH1 cells produce a number of cytokines, including IL-2, IFN-, and TNF that defend against viruses either directly or indirectly. IFN-_ acts directly by inducing an antiviral state in cells. IL-2 acts indirectly by assisting in the recruitment of CTL precursors into an effector population. Both IL-2 and IFN activate NK cells, which play an important role in host defense during the first days of many viral infections until a specific CTL response develops. In most viral infections, specific CTL activity arises within 3–4 days after infection, peaks by 7–10 days, and then declines. Within 7–10 days of primary infection, most virions have been eliminated, paralleling the development of CTLs. CTLs specific for the virus eliminate virus-infected self-cells and thus eliminate potential sources of new virus. The role of CTLs in defense against viruses is demonstrated by the ability of virus-specific CTLs to confer protection for the specific virus on non immune recipients by adoptive transfer. The viral specificity of the CTL as well can be demonstrated with. Adoptive transfer: adoptive transfer of a CTL clone specific for influenza virus strain X protects mice against influenza virus X but not against influenza virus strain Y.

Viruses Can Evade Host Defense Mechanisms

Despite their restricted genome size, a number of viruses have been found to encode proteins that interfere at various levels with specific or non specific host defenses. Presumably, the advantage of such proteins is that they enable viruses to replicate more effectively amidst host antiviral defenses. As described above, the induction of IFN-α and IFN-γ is a major innate defense against viral infection, but some viruses have developed strategies to evade the action of IFN-_/_. These include hepatitis C virus, which has been shown to overcome the antiviral effect of the interferons by blocking or inhibiting the action of PKR. Another mechanism for evading host responses, utilized in particular by herpes simplex viruses (HSV) is inhibition of antigen presentation by infected host cells. HSV-1 and HSV-2 both express an immediate-early protein (a protein synthesized shortly after viral replication) called ICP47, which very effectively inhibits the human transporter molecule needed for antigen processing (TAP). Inhibition of TAP blocks antigen delivery to class I MHC receptors on HSV-infected cells, thus preventing presentation of viral antigen to CD8+ T cells. This results in the trapping of empty class I MHC molecules in the endoplasmic reticulum and effectively shuts down a CD8+ T-cell response to HSV-infected cells. The targeting of MHC molecules is not unique to HSV. Other viruses have been shown to down-regulate class I MHC expression shortly after infection. Two of the best characterized examples, the adenoviruses and cytomegalovirus (CMV), use distinct molecular mechanisms to reduce the surface expression of class I MHC molecules, again inhibiting antigen presentation to CD8+ T cells. Some viruses CMV, measles virus, and HIV—have been shown to reduce levels of class II MHC molecules on the cell surface, thus blocking the function of antigen-specific antiviral helper T cells. Antibody-mediated destruction of viruses requires

complement activation, resulting either in direct lysis of the viral particle or opsonization and elimination of the virus by phagocytic cells. A number of viruses have strategies for evading complement-mediated destruction. Vaccinia virus, for example, secretes a protein that binds to the C4b component, inhibiting the classical complement pathway; and herpes simplex viruses have a glycoprotein component that binds to the C3b complement component, inhibiting both the classical and alternative pathways. A number of viruses escape immune attack by constantly changing their antigens. In the influenza virus, continual antigenic variation results in the frequent emergence of new infectious strains. The absence of protective immunity to these newly emerging strains leads to repeated epidemics of influenza. Antigenic variation among rhinoviruses, the causative agent of the common cold, is responsible for our inability to produce an effective vaccine for colds. Nowhere is antigenic variation greater than in the human immunodeficiency virus (HIV), the causative agent of AIDS. Estimates suggest that HIV accumulates mutations at a rate 65 times faster than does influenza virus. Because of the importance of AIDS, a section of Chapter 19 addresses this disease. A large number of viruses evade the immune response by causing generalized immune suppression. Among these are the paramyxoviruses that cause mumps, the measles virus, Epstein-Barr virus (EBV), cytomegalovirus, and HIV. In some cases, immunosuppression is caused by direct viral infection of lymphocytes or macrophages. The virus can then either directly destroy the immune cells by cytolytic mechanisms or alter their function. In other cases, immune suppression is the result of a cytokine imbalance. For example, EBV produces a protein, called BCRF1 that is homologous to IL-10; like IL-10, BCRF1 suppresses cytokine production by the TH1 subset, resulting in decreased levels of IL-2, TNF, and IFN

Bacterial Infections

Immunity to bacterial infections is achieved by means of antibody unless the bacterium is capable of intracellular growth, in which case delayed-type hypersensitivity has an important role. Bacteria enter the body either through a number of natural routes (e.g., the respiratory tract, the gastrointestinal tract, and the genitourinary tract) or through normally inaccessible routes opened up by breaks in mucous membranes or skin. Depending on the number of organisms entering and their virulence, different levels of host defense are enlisted. If the inoculum size and the virulence are both low, then localized tissue phagocytes may be able to eliminate the bacteria with an innate, nonspecific defense. Larger inoculums or organisms with greater virulence tend to induce an adaptive, specific immune response.

Immune Responses to Extracellular and Intracellular Bacteria Can Differ

Infection by extracellular bacteria induces production of humoral antibodies, which are ordinarily secreted by plasma cells in regional lymph nodes and the submucosa of the respiratory and gastrointestinal tracts. The humoral immune response is the main protective response against

extracellular bacteria. The antibodies act in several ways to protect the host from the invading organisms, including removal of the bacteria and inactivation of bacterial toxins (Figure 17-8). Extracellular bacteria can be pathogenic because they induce a localized inflammatory response or because they produce toxins. The toxins, endotoxin or exotoxin, can be cytotoxic but also may cause pathogenesis in other ways. An excellent example of this is the toxin produced by diphtheria, which exerts a toxic effect on the cell by blocking protein synthesis. Endotoxins, such as lipopolysaccharides (LPS), are generally components of bacterial cell walls, while exotoxins, such as diphtheria toxin, are secreted by the bacteria. Antibody that binds to accessible antigens on the surface of a bacterium can, together with the C3b component of complement, act as an opsonin that increases phagocytosis and thus clearance of the bacterium. In the case of some bacteria—notably, the gram-negative organisms— complement activation can lead directly to lysis of the organism. Antibody-mediated activation of the complement system can also induce localized production of immune effector molecules that help to develop an amplified and more effective inflammatory response. For example, the complement split products C3a, C4a, and C5a act as anaphylatoxins, inducing local mast-cell degranulation and thus vasodilation and the extravasation of lymphocytes and neutrophils from the blood into tissue space. Other complement split products serve as chemotactic factors for neutrophils and macrophages, thereby contributing to the buildup of phagocytic cells at the site of infection. Antibody to a bacteria toxin may bind to the toxin and neutralize it; the antibody-toxin complexes are then cleared byphagocytic cells in the same manner as any other antigen antibody complex. While innate immunity is not very effective against intracellular bacterial pathogens, intracellular bacteria can activate NK cells, which, in turn, provide an early defense againstthese bacterium. Intracellular bacterial infections tend to induce a cell-mediated immune response, specifically, delayedtypehypersensitivity. In this response, cytokines secreted byCD4+ T cells are important—notably IFN-γ, which activates macrophages to kill ingested pathogens more effectively

KAHE

PATHOGEN DEFENSE STRATEGIES

Bacteria Can Effectively Evade Host Defense Mechanisms

There are four primary steps in bacterial infection: Attachment to host cells Proliferation Invasion of host tissue, Toxin-induced damage to host cells Host-defense mechanisms act at each of these steps, and many bacteria have evolved ways to circumvent some of these host defenses. Some bacteria have surface structures or molecules that enhance their ability to attach to host cells. A number of gram-negative bacteria, for instance, have pili (long hair like projections), which enable them to attach to the membrane of the intestinal or genitourinary tract. Other

bacteria, such as *Bordetella pertussis,* secrete adhesion molecules that attach to both the bacterium and the ciliatedepithelial cells of the upper respiratory tract. Secretory IgA antibodies specific for such bacterial structures can block bacterial attachment to mucosal epithelialcells and are the main host defense against bacterial attachment. However, some bacteria (e.g., *Neisseria gonorrhoeae,Haemophilus influenzae,* and *Neisseria meningitidis*) evade the IgA response by secreting proteases that cleave secretory IgA at the hinge region; the resulting Fab and Fc fragments have a shortened half-life in mucous secretions and are not able to agglutinate microorganisms. Some bacteria evade the IgA response of the host by changing these surface antigens. In *N. gonorrhoeae,* for example, pilin, the protein component of the pili, has a highly variable structure.Variation in the pilin amino acid sequence is generated by gene rearrangements of its coding sequence. The pilin locus consists of one or two expressed genes and 10–20 silent genes. Each gene is arranged into six regions called *mini cassettes.* Pilin variation is generated by a process of gene conversion, in which one or more mini cassettes from the silent genes replace a minicassette of the expression gene. This process generates enormous antigenic variation, which may contribute to the pathogenicity of *N. gonorrhoeae* by increasing the likelihood that expressed pili will bind firmly to epithelial cells. In addition, the continual changes in the pilin sequence allow the organism to evade neutralization by IgA. Some bacteria possess surface structures that serve to inhibit phagocytosis. A classic example is *Streptococcus pneumoniae,* whose polysaccharide capsule prevents phagocytes is very effectively. There are 84 serotypes of *S. pneumoniae* that differ from one another by distinct capsular polysaccharides. During infection, the host produce antibody against the infecting serotype. This antibody protects against reinjection with the same serotype but will not protect against infection by a different serotype. In this way, *S. pneumoniae* can cause disease many times in the same individual. On other bacteria, such as *Streptococcus pyogenes,* a surface protein projection called the M protein inhibits phagocytosis. Some pathogenic staphylococci are able to assemble a protective coat from host proteins. These bacteria secrete a coagulase enzyme that precipitates a fibrin coat around them, shielding them from phagocytic cells. Mechanisms for interfering with the complement system help other bacteria survive. In some gram-negative bacteria, for example, long side chains on the lipid A moiety of the cell-wall core polysaccharide help to resist complement mediated lysis. *Pseudomonas* secretes an enzyme, elastase that inactivates both the C3a and C5a anaphylatoxins, thereby diminishing the localized inflammatory reaction. A number of bacteria escape host defense mechanisms by their ability to survive within phagocytic cells. Some, such as*Listeria monocytogenes,* do this by escaping from the phagolysosome to the cytoplasm, which is a more favorable environment for their growth. Other bacteria, such as *Mycobacterium avium,* block lysosomal fusion with the phagolysosome; and some mycobacteria are resistant to the oxidative attack that takes place within the phagolysosome.

Immune Responses Can Contribute to Bacterial Pathogenesis

In some cases, disease is caused not by the bacterial pathogen itself but by the immune response to the pathogen. As described in Chapter 12, pathogen-stimulated overproduction of cytokines leads to the symptoms of bacterial septic shock, food poisoning, and toxic-shock syndrome. For instance, cell-wall endotoxins of some gram-negative bacteria activate macrophages, resulting in release of high levels of IL-1 and TNF, which can cause septic shock. In staphylococcal food poisoning and toxic-shock syndrome, exotoxins produced by the pathogens function as super antigens, which can activate all T cells that express T-cell receptors with a particular_ domain. The resulting overproduction of cytokines by activated TH cells causes many of the\ symptoms of these diseases.

Protozoan Diseases

Protozoans are unicellular eukaryotic organisms. They are responsible for several serious diseases in humans, including amoebiasis, Chagas' disease, African sleeping sickness, malaria, leishmaniasis, and toxoplasmosis. The type of immune response that develops to protozoan infection and the effectiveness of the response depend in part on the location of the parasite within the host. Many protozoans have life-cycle stages in which they are free within the bloodstream, and it is during these stages that humoral antibody is most effective. Many of these same pathogens are also capable of intracellular growth; during these stages, cell-mediated immune reactions are effective in host defense. In the development of vaccines for protozoan diseases, the branch of the immune system that is most likely to confer protection must be carefully considered.

Malaria (*Plasmodium* Species) Infects 600 Million People Worldwide

Malaria is one of the most devastating diseases in the world today, infecting nearly 10% of the world population and causing 1–2 million deaths every year. Malaria is caused by various species of the genus *Plasmodium,* of which *P. falciparum* is the most virulent and prevalent. The alarming development of multiple-drug resistance in *Plasmodium* and the increased resistance of its vector, the *Anopheles* mosquito, to DDT underscore the importance of developing new strategies to hinder the spread of malaria.

PLASMODIUM LIFE CYCLE AND PATHOGENESIS OF MALARIA

Plasmodium progresses through a remarkable series of developmental and maturational stages in its extremely complex life cycle. Female *Anopheles* mosquitoes, which feed on blood meals, serve as the vector for *Plasmodium,* and part of the parasite's life cycle takes place within the mosquito. (Because male *Anopheles* mosquitoes feed on plant juices, they do not transmit *Plasmodium.* Human infection begins when sporozoites, one of the *Plasmodium* stages, are

introduced into an individual's bloodstream as an infected mosquito takes a blood meal. Within 30 min, the sporozoites disappear from the blood as they migrate to the liver, where they infect hepatocytes. Sporozoites are long, slender cells that are covered b a 45-kDa protein called circumsporozoite (CS) antigen, which appears to mediate their adhesion to hepatocytes. The binding site on the CS antigen is a conserved region in the carboxyl-terminal end (called region II) that has a high degree of sequence homology with known cell-adhesion molecules. Within the liver, the sporozoites multiply extensively and undergo a complex series of transformations that culminate in the formation and release of merozoites in about a week. It has been estimated that a liver hepatocyte infected with a single sporozoite can release 5,000–10,000 merozoites. The released merozoites infect red blood cells, initiating the symptoms and pathology of malaria. Within a red blood cell, merozoites replicate and undergo successive differentiations; eventually the cell ruptures and releases new merozoites, which go on to infect more red blood cells. Eventually some of the merozoites differentiate into male and female gametocytes, which may be ingested by a female *Anopheles* mosquito during a blood meal. Within the mosquito's gut, the male and female gametocytes differentiate into gametes that fuse to form a zygote, which multiplies and differentiates into sporozoites with in the salivary gland. The infected mosquito is now set to initiate the cycle once again. The symptoms of malaria are recurrent chills, fever, and sweating. The symptoms peak roughly every 48 h, when successive generations of merozoites are released from infected red blood cells. An infected individual eventually becomes weak and anemic and shows splenomegaly. The large numbers of merozoites formed can block capillaries, causing intense headaches, renal failure, heart failure, or cerebral damage—often with fatal consequences. There is speculation that some of the symptoms of malaria may be caused not by *Plasmodium* itself but instead by excessive production of cytokines. This hypothesis stemmed from the observation that cancer patients treated in clinical trials with recombinant tumor necrosis factor (TNF) developed symptoms that mimicked malaria. The relation between TNF and malaria symptoms was studied by infecting mice with a mouse specific strain of *Plasmodium,* which causes rapid death by cerebral malaria. Injection of these mice with antibodies to TNF was shown to prevent the rapid death.

HOST RESPONSE TO PLASMODIUM INFECTION

In regions where malaria is endemic, the immune response to *Plasmodium* infection is poor. Children less than 14 years old mount the lowest immune response and consequently are most likely to develop malaria. In some regions, the childhood mortality rate for malaria reaches 50%, and worldwide the disease kills about a million children a year. The low immune response to *Plasmodium* among children can be demonstrated by measuring serum antibody levels to the sporozoite stage. Only 22% of the children living in endemic areas have detectable antibodies to the sporozoite stage, whereas 84% of the adults have such antibodies. Even in adults, the degree of immunity is far from complete, however, and most people living in endemic regions have lifelong low-level *Plasmodium* infections. A number of factors may contribute to the low levels

of immune responsiveness to *Plasmodium.* The intracellular phases of the life cycle in liver cells and erythrocytes also reduce the degree of immune activation generated by the pathogen and allow the organism to multiply while it is shielded from attack. Furthermore, the most accessible stage, the sporozoite, circulates in the blood for only about 30 min before it infects liver hepatocytes; it is unlikely that much immune activation can occur in such a short period of time. And even when an antibody response does develop to sporozoites, *Plasmodium* has evolved a way of overcoming that response by sloughing off the surface CS antigen coat, thus rendering the antibodies ineffective.

Diseases Caused by Parasitic Worms (Helminths)

Unlike protozoans, which are unicellular and often grow within human cells, helminths are large, multicellular organisms that reside in humans but do not ordinarily multiply there and are not intracellular pathogens. Although helminthes are more accessible to the immune system than protozoans, most infected individuals carry few of these parasites; for this reason, the immune system is not strongly engaged and the level of immunity generated to helminths is often very poor. Parasitic worms are responsible for a wide variety of diseases in both humans and animals.More than a billion people are infected with *Ascaris,* a parasitic roundworm that infects the small intestine, and more than 300 million people are infected with *Schistosoma,* a trematode worm that causes a chronic debilitating infection. Several helminths are important pathogens of domestic animals and invade humans who ingest contaminated food. These helminths include *Taenia,* a tapeworm of cattle and pigs, and *Trichinella,* the roundworm of pigs that causes trichinosis. Several *Schistosoma* species are responsible for the chronic, debilitating, and sometimes fatal disease schistosomiasis(formerly known as *bilharzia*). Three species, *S. mansoni, S. japonicum,* and *S. haematobium,* are the major pathogens in humans, infecting individuals in Africa, the Middle East, South America, the Caribbean, China, Southeast Asia, and the Philippines.A rise in the incidence of schistosomiasis in recent years has paralleled the increasing worldwide use of irrigation, which has expanded the habitat of the freshwater snail that serves as the intermediate host for schistosomes. Infection occurs through contact with free-swimming infectious larvae, called cer cariae, which are released from an infected snail at the rate of 300–3000 per day. When cercariae contact human skin, they secrete digestive enzymes that help them to bore into the skin, where they shed their tails and are transformed into schistosomules. The schistosomules enter the capillaries and migrate to the lungs, then to the liver, and finally to the primary site of infection, which varies with the species. *S. mansoni* and *S. japonicum* infect the intestinalmesenteric veins; *S. haematobium* infects the veins of the urinary bladder. Once established in their final tissue site, schistosomules mature into male and female adult worms. The worms mate and the females produce at least 300 spiny eggs a day. Unlike protozoan parasites, schistosomes and otherhelminths do not multiply within their hosts. The eggs produced by the female worm do not mature into adult worms in humans; instead, some of them pass into the feces or urine and are excreted to infect more snails. As many as half of the

eggs produced remain in the host, where they invade the intestinal wall, liver, or bladder and cause hemorrhage. A chronic state can then develop in which the adult worms persist and the unexcreted eggs induce cell-mediated delayed-type hypersensitive reactions, resulting in large granulomas that are gradually walled off by fibrous tissue. Although the eggs are contained by the formation of the granuloma, often the granuloma itself obstructs the venous blood flow to the liver or bladder. Although an immune response does develop to the schistosomes, in most individuals it is not sufficient to eliminate the adult worms, even though the intravascular sites of schistosome infestation should make the worm an easy target forimmune attack. Instead, the worms survive for up to 20 years. The schistosomules would appear to be the forms most susceptible to attack, but because they are motile, they can evade the localized cellular buildup of immune and inflammatory cells. Adult schistosome worms also have several unique mechanisms that protect them from immune defenses. The adult worm has been shown to decrease the expression of antigens on its outer membrane and also to enclose itself in a glycolipid and glycoprotein coat derived from the host, masking the presence of its own antigens. Among the antigens observed on the adult worm are the host's own ABO blood-group antigens and histocompatibility antigens.

The immune response is, of course, diminished by this covering made of the host's self-antigens, which must contribute to the lifelong persistence of these organisms. The relative importance of the humoral and cellmediatedresponses in protective immunity to schistosomiasis is controversial. These manifestations suggest that cytokines produced by a TH2-like subset are important for the immune response: IL-4, which induces B cells to classswitch to IgE production; IL-5, which induces bone-marrow precursors to differentiate into eosinophils; and IL-3, which (along with IL-4) stimulates growth of mast cells. Degranulation of mast cells releases mediators that increase the infiltration of such inflammatory cells as macrophages and eosinophils. The eosinophils express Fc receptors for IgE and IgG and bind to the antibody-coated parasite. Once bound to the parasite, an eosinophil can participate in antibodydependent cell-mediated cytotoxicity (ADCC), releasing mediators from its granules that damage the parasite. One eosinophil mediator, called basic protein, is particularly toxic to helminths. Immunization studies with mice, however, suggest that this humoral IgE response may not provide protective immunity. When mice are immunized with *S. mansoni* vaccine, the protective immune response that develops is not an IgE response, but rather a TH1 response characterized by IFN-γ production and macrophage accumulation. Furthermore, inbred strains of mice with deficiencies in mast cells or IgE develop protective immunity from vaccination, whereas inbred strains with deficiencies in cellmediated CD4+ T-cell responses fail to develop protective immunity in response to the vaccine. These studies suggest that the CD4+ T-cell response may be the most important in immunity to schistosomiasis. It has been suggested that the ability to induce an ineffective TH2-like response may have evolved in schistosomes as a clever defense mechanism to ensure that TH2 cells produced sufficient levels of IL-10 to inhibit protective immunity mediated by the TH1-like subset in the CD4+ T response. Antigens present on the

membrane of cercariae and young schistosomules are promising vaccine components because these stages appear to be most susceptible to immune attack. Injecting mice and rats with monoclonal antibodies to cercariae and young schistosomules passively transferred resistance to infection with live cercariae.When these protective antibodies were used in affinity columns to purify schistosome membrane antigens from crude membrane extracts, it was found that mice immunized and boosted with these purified antigens exhibited increased resistance to a later challenge with live cercariae. Schistosome cDNA libraries were then established and screened with the protective monoclonal antibodies to identify those cDNAs encoding surface antigens. Experiments using cloned cercariae or schistosomule antigens are presently under way to assess their ability to induce protective immunity in animal models. However, in developing an effective vaccine for schistosomiasis, a fine line separates a beneficial immune response, which at best limits the parasite load, from a detrimental response, which in itself may become pathologic.

AVOIDANCE OF RECOGNITION

These cells present receptors contained on the surface or within the cell, named pattern recognition receptors (PRRs), which recognize molecules that are broadly shared by pathogens but distinguishable from host molecules, collectively referred to as pathogen-associated molecular patterns (PAMPs). The cells of the innate system recognize and respond to pathogens in a generic way, but, unlike the adaptive immune system, the system does not provide long-lasting immunity to the host.

KAHE

AUTOIMMUNE DISEASES

Auto immune diseases

Early in the last century, Paul Ehrlich realized that the immune system could go awry and, instead of reacting against foreign antigens, could focus its attack on self-antigens. He termed this condition "horror autotoxicus."We now understand that, while mechanisms of self-tolerance normally protect an individual from potentially self-reactive lymphocytes, there are failures. They result in an inappropriate response of the immune system against self-components termed autoimmunity**.**

In the 1960s, it was believed that all self-reactive lymphocytes were eliminated during their development in the bone marrow and thymus and that a failure to eliminate these lymphocytes led to autoimmune consequences. Since the late 1970s, a broad body of experimental evidence has countered that belief, revealing that not all self-reactive lymphocytes are deleted during T-cell and B-cell

maturation. Instead, normal healthy individuals have been shown to possess mature, recirculating, self-reactive lymphocytes. Since the presence of these self-reactive lymphocytes in the periphery does not inevitably result in autoimmune reactions, their activity must be regulated in normal individuals through clonal anergy or clonal suppression. A breakdown in this regulation can lead to activation of self-reactive clones of T or B cells, generating humoral or cell-mediated responses against selfantigens. These reactions can cause serious damage to cells and organs, sometimes with fatal consequences. Sometimes the damage to self-cells or organs is caused by antibodies; in other cases, T cells are the culprit.

Auto recognition

For example, a common form of autoimmunity is tissue injury by mechanisms similar to type II hypersensitivity reactions. Type II hypersensitivity reactions involve antibody-mediated destruction of cells. Autoimmune hemolytic anemia is an excellent example of such an autoimmune disease. In this disease, antigens on red blood cells are recognized by auto-antibodies, which results in the destruction of the blood cells, which in turn results in anemia. Autoantibodies are also the major offender in Hashimoto's thyroiditis, in which antibodies reactive with tissue-specific antigens such as thyroid peroxidase and thyroglobulin cause severe tissue destruction. Other autoimmune diseases that involve auto-antibodies are listed in Table 20-1. Many autoimmune diseases are characterized by tissue destruction mediated directly by T cells. A well-known example is rheumatoid arthritis, in which self-reactive T cells attack the tissue in joints, causing an inflammatory response that results in swelling and tissue destruction. Other examples include insulin-dependent diabetes mellitus and multiple sclerosisThis chapter describes some common human autoimmune diseases. These can be divided into two broad categories: organ-specific and systemic autoimmune disease. Such diseases affect 5%–7% of the human population, often causing chronic debilitating illnesses. Several experimental animal models used to study autoimmunity and various mechanisms that may contribute to induction of autoimmune reactions also are described. Finally, current and experimental therapies for treating autoimmune diseases are described.

Classes of Auto Immuno Diseases

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TABLE 20-1 Some autoimmune diseases in humans

Disease	Self-antigen	Immune response
	ORGAN-SPECIFIC AUTOIMMUNE DISEASES	
Addison's disease	Adrenal cells	Auto-antibodies
Autoimmune hemolytic anemia	RBC membrane proteins	Auto-antibodies
Goodpasture's syndrome	Renal and lung basement membranes	Auto-antibodies
Graves' disease	Thyroid-stimulating hormone receptor	Auto-antibody (stimulating)
Hashimoto's thyroiditis	Thyroid proteins and cells	T_{DTH} cells, auto-antibodies
Idiopathic thrombocyopenia purpura	Platelet membrane proteins	Auto-antibodies
Insulin-dependent diabetes mellitus	Pancreatic beta cells	T_{DTH} cells, auto-antibodies
Myasthenia gravis	Acetylcholine receptors	Auto-antibody (blocking)
Myocardial infarction	Heart	Auto-antibodies
Pernicious anemia	Gastric parietal cells; intrinsic factor	Auto-antibody
Poststreptococcal glomerulonephritis	Kidney	Antigen-antibody complexes
Spontaneous infertility	Sperm	Auto-antibodies
	SYSTEMIC AUTOIMMUNE DISEASES	
Ankylosing sponkylitis	Vertebrae	Immune complexes
Multiple sclerosis	Brain or white matter	T_H1 cells and T_C cells, auto-antibodies
Rheumatoid arthritis	Connective tissue, IgG	Auto-antibodies, immune complexes
Scleroderma	Nuclei, heart, lungs, gastrointestinal tract, kidney	Auto-antibodies
Sjogren's syndrome	Salivary gland, liver, kidney, thyroid	Auto-antibodies
Systemic lupus erythematosus (SLE)	DNA, nuclear protein, RBC and platelet membranes	Auto-antibodies, immune complexes

Organ-Specific Autoimmune Diseases

In an organ-specific autoimmune disease, the immune response is directed to a target antigen unique to a single organ or gland, so that the manifestations are largely limited to that organ. The cells of the target organs may be damaged directly by humoral or cell-mediated effect or mechanisms. Alternatively, the antibodies may over stimulate or block the normal function of the target organ.

Some Autoimmune Diseases Are Mediated by Direct Cellular Damage

Autoimmune diseases involving direct cellular damage occur when lymphocytes or antibodies bind to cell-membrane antigens, causing cellular lysis and/or an inflammatory response in the affected organ. Gradually, the damaged cellular structure is replaced by connective tissue (scar tissue), and the function of the organ declines. This section briefly describes a few examples of this type of autoimmune disease.

Hashimoto's Thyroiditis

In Hashimoto's thyroiditis, which is most frequently seen in middle-aged women, an individual produces auto-antibodiesand sensitized TH1 cells specific for thyroid antigens. The DTH response is characterized by an intense infiltration of the thyroid gland by lymphocytes, macrophages, and plasma cells, which form lymphocytic follicles and germinal centers (Figure 20-1). The ensuing inflammatory response causes a goiter, or visible enlargement of the thyroid gland, a physiological response to hypothyroidism. Antibodies are formed to a number of thyroid proteins, including thyroglobulin and thyroid peroxidase, both of which are involved in the uptake of iodine. Binding of the auto-antibodies to these proteins interferes with iodine uptake and leads to decreased production of thyroid hormones (hypothyroidism).

Thyrotoxicosis

Hyperthyroidism, also known as over active thyroid and hyperthyreosis, is the condition that occurs due to excessive production of thyroid hormone by the thyroid gland. Thyrotoxicosis is the condition that occurs due to excessive thyroid hormone of any cause and therefore includes hyperthyroidism.

Systemic Autoimmune Diseases

In systemic autoimmune diseases, the response is directed toward a broad range of target antigens and involves a number of organs and tissues. These diseases reflect a general defect in immune regulation that results in hyperactive T cells and B cells. Tissue damage is widespread, both from cell mediated immune responses and from direct cellular damage caused by auto-antibodies or by accumulation of immune complexes.

Systemic Lupus Erythematosus Attacks Many Tissues

One of the best examples of a systemic autoimmune disease is **systemic lupus erythematosus (SLE),** which typically appears in women between 20 and 40 years of age; the ratio of female to male patients is 10:1. SLE is characterized by fever, weakness, arthritis, skin rashes, pleurisy, and kidney dysfunction. Lupus is more frequent in African-American and Hispanic women than in Caucasians, although it is not known why this is so. Affected individuals may produce autoantibodies to a vast array of tissue antigens, such as DNA, histones, RBCs, platelets, leukocytes, and clotting factors; interaction of these auto-antibodies with their specific antigens produces various symptoms. Auto-antibody specific for RBCs and platelets, for example, can lead to complement-mediated lysis, resulting in hemolytic anemia and thrombocytopenia, respectively. When immune complexes of auto-antibodies with various nuclear antigens are deposited along the walls of small blood vessels, a type III

hypersensitive reaction develops. The complexes activate the complement system and generate membrane-attack complexes and complement split products that damage the wall of the blood vessel, resulting in vasculitis and glomerulo nephritis.

Excessive complement activation in patients with severe SLE produces elevated serum levels of the complement split products C3a and C5a, which may be three to four times higher than normal. C5a induces increased expression of the type 3 complement receptor (CR3) on neutrophils, facilitating neutrophil aggregation and attachment to the vascular endothelium. As neutrophils attach to small blood vessels, the number of circulating neutrophils declines (neutropenia) and various occlusions of the small blood vessels develop (vasculitis). These occlusions can lead to widespread tissue damage. Laboratory diagnosis of SLE focuses on the characteristic antinuclear antibodies, which are directed against double stranded or single-stranded DNA, nucleoprotein, histones, and nucleolar RNA. Indirect immune fluorescent staining with serum from SLE patients produces various characteristic nucleus-staining patterns.

Autoimmune haemolytic anaemia

Autoimmune anemias include pernicious anemia, autoimmune hemolytic anemia, and drug-induced hemolytic anemia Pernicious anemia is caused by auto-antibodies to intrinsic factor, a membrane-bound intestinal protein on gastric parietal cells. Intrinsic factor facilitates uptake of vitamin B12from the small intestine. Binding of the auto-antibody to intrinsic factor blocks the intrinsic factor–mediated absorption of vitamin B12. In the absence of sufficient vitamin B12, which is necessary for proper hematopoiesis, the number of functional mature red blood cells decreases below normal. Pernicious anemia is treated with injections of vitamin B12, thus circumventing the defect in its absorption. An individual with autoimmune hemolytic anemia makes auto-antibody to RBC antigens, triggering complement mediated lysis or antibody-mediated opsonization and phagocytosis of the red blood cells. One form of autoimmune anemia is drug-induced: when certain drugs such as penicillin or the anti-hypertensive agent methyldopa interact with red blood cells, the cells become antigenic. The immunodiagnostic test for autoimmune hemolytic anemias generally involves a Coombs test, in which the red cells are incubated with an anti–human IgG antiserum. If IgG auto-antibodies are present on the red cells, the cells are agglutinated by the antiserum.

Rheumatoid Arthritis

Rheumatoid arthritisis a common autoimmune disorder, most often affecting women from 40 to 60 years old. The major symptom is chronic inflammation of the joints, although the hematologic, cardiovascular, and respiratory systems are also frequently affected. Many individuals with rheumatoid arthritis produce a group of auto-antibodies called rheumatoid factors that are reactive with determinants in the Fc region of IgG. The classic rheumatoid factor is an IgM antibody with that reactivity. Such auto-antibodies bind to normal circulating IgG, forming IgM-IgG complexes that are deposited in the joints. These immune complexes can activate the complement cascade, resulting in a type III hypersensitive reaction,which leads to chronic inflammation of the joints.

Goodpasture's Syndrome

In **Goodpasture's syndrome,** auto-antibodies specific for certain basement-membrane antigens bind to the basement membranes of the kidney glomeruli and the alveoli of the lungs. Subsequent complement activation leads to direct cellular damage and an ensuing inflammatory response mediated by a buildup of complement split products. Damage to the glomerular and alveolar basement membranes leads to progressive kidney damage and pulmonary hemorrhage. Death may ensue within several months of the onset of symptoms. Biopsies from patients with Goodpasture's syndrome stained with fluorescent-labeled anti-IgG and anti-C3b reveal linear deposits of IgG and C3b along the basement membranes.

Insulin-Dependent Diabetes Mellitus

A disease afflicting 0.2% of the population, **insulin-dependent diabetes mellitus (IDDM)** is caused by an autoimmune attack on the pancreas. The attack is directed against specialized insulin-producing cells (beta cells) that are located in spherical clusters, called the islets of Langerhans, scattered throughout the pancreas. The autoimmune attack destroys beta cells, resulting in decreased production of insulin and consequently increased levels of blood glucose. Several factors are important in the destruction of beta cells. First, activated CTLs migrate into an islet and begin to attack the insulinproducing cells. Local cytokine production during this response includes IFN-γ, TNF-α, and IL-1. Auto-antibody production can also be a contributing factor in IDDM. The first CTL infiltration and activation of macrophages, frequently referred to as insulitis (Figure 20-3), is followed by cytokine release and the presence of auto-antibodies, which leads to a cell-mediated DTH response. The subsequent beta-cell destruction is thought to be mediated by cytokines released during the DTH response and by lytic enzymes released from the activated macrophages. Auto-antibodies to beta cells

may contribute to cell destruction by facilitating either antibody-plus-complement lysis or antibody-dependent cell-mediated cytotoxicity (ADCC).

IMMUNODEFICIENCY –AIDS

Human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS) is a spectrum of conditions caused by infection with the human immunodeficiency virus (HIV). Following initial infection, a person may not notice any symptoms or may experience a brief period of influenza-like illness. Typically, this is followed by a prolonged period with no symptoms. As the infection progresses, it interferes more with the immune system, increasing the risk of common infections like tuberculosis, as well as other opportunistic infections, and tumors that rarely affect people who have working immune systems. These late symptoms of infection are referred to as acquired immunodeficiency syndrome (AIDS). This stage is often also associated with weight loss.

HIV is spread primarily by unprotected sex (including anal and oral sex), contaminated blood transfusions, hypodermic needles, and from mother to child during pregnancy, delivery, or breastfeeding. Some bodily fluids, such as saliva and tears, do not transmit HIV. Methods of prevention include safe sex, needle exchange programs, treating those who are infected, and male circumcision. Disease in a baby can often be prevented by giving both the mother and child antiretroviral medication. There is no cure or vaccine; however, antiretroviral treatment can slow the course of the disease and may lead to a near-normal life expectancy. Treatment is recommended as soon as the diagnosis is made. Without treatment, the average survival time after infection is 11 years.

Acquired Immune Deficiency Syndrome (AIDS)

It is now recognized that AIDS (acquired immune deficiency syndrome) is the first great pandemic of the second half of the twentieth century. First described in 1981 AIDS is the result of an infection by the human immunodeficiency virus (HIV), a lentivirus within the family Retroviridae. The disease appears to have begun in central Africa as early as the 1950s; HIV may have developed in the human population in the 1930s, or even earlier. Simian immunodeficiency viruses (SIVs) related to HIV-1 and HIV-2, the strains primarily responsible for AIDS, have been isolated from African primates. The SIV from chimpanzees seems to have infected humans

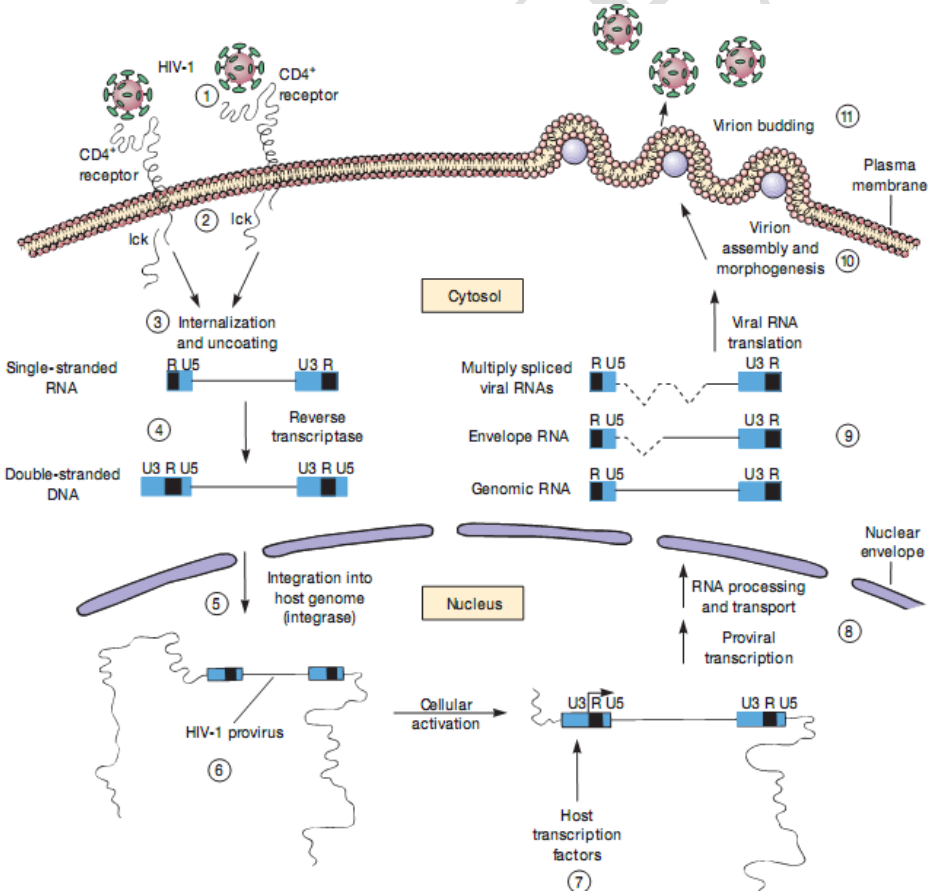
and developed into HIV-1; HIV-2 may have arisen from the SIV that infects sooty mangabeys. Once established, HIV-1 spread to the Caribbean and then to the United States and Europe. Epidemiologically AIDS occurs worldwide. The groups most at risk in acquiring AIDS are (in descending order of risk) homosexual/bisexual men; intravenous (IV) drug users; heterosexuals who have intercourse with drug users, prostitutes (sex trade workers), and bisexuals; transfusion patients or hemophiliacs who must receive clotting factor preparations made from donated blood; and children born of infected mothers. The mortality rate from AIDS is extremely high.

HIV-1 is an enveloped lentivirus and a member of the family Retroviridae with a cylindrical core inside its capsid. The core contains two copies of its plus single-stranded RNA genome and several enzymes. Thus far 10 virus-specific proteins have been discovered. One of them, the gp120 envelope protein, participates in HIV-1 attachment to CD4 cells.

The AIDS virus is acquired by direct exposure of a person's bloodstream to body fluids (blood, semen, vaginal secretions) containing the virus, through sexual contact, or perinatally from an infected mother to her fetus. It also is possible that a newborn can be infected through breast-feeding. Once inside the body, the virus gp120 envelope protein binds to the CD4 glycoprotein plasma membrane receptor on CD4 T cells, macrophages, dendritic cells, and monocytes. (Dendritic cells are present throughout the body's mucosal surfaces and bear the CD4 protein. Thus it is possible that these are the first cells infected by HIV in sexual transmission.) Recent evidence shows that the virus requires a coreceptor in addition to the CD4 receptor. Macrophage-tropic strains, which seem to predominate early in the disease and infect both macrophages and T cells, require the CCR5 (CC-CKR-5) chemokine receptor protein as well as CD4. A second chemokine coreceptor, called CXCR-4 or fusin, is T cell–tropic and used by an HIV strain that is active at later stages of the infection. This strain induces the formation of syncytia, as described later. Individuals with two defective copies of the CCR5 gene do not seem to get AIDS; apparently the virus cannot infect their T cells. People with one good copy of the CCR5 gene do get AIDS but survive several years longer than those with no mutation. Host cellnreceptors and virion adsorption (pp. 399–403); Replication and transcription inn retroviruses.

Entry into the host cell begins when the envelope fuses with the plasma membrane, and the virus releases its core and two RNA strands into the cytoplasm. Inside the infected cell, the

core protein remains associated with the RNA as it is copied into a single strand of DNA by the RNA/DNA-dependent DNA polymerase activity of the reverse transcriptase enzyme. The RNA is next degraded by another reverse transcriptase component, ribonuclease H, and the DNA strand is duplicated to form a double-stranded DNA copy of the original RNA genome. A complex of the double-stranded DNA (the provirus) and the integrase enzyme moves into the nucleus. Then the proviral DNA is integrated into the cell's DNA through a complex sequence of reactions catalyzed by the integrase. The integrated provirus can remain latent, giving no sign of its presence. Alternatively the provirus can force the cell to synthesize viral mRNA. Some of the RNA is translated to produce viral proteins by the cell's own ribosomes. Viral proteins and the complete HIV-1 RNA genome are then assembled into new virions that bud from the infected host cell. Eventually the host cell lyses.



Life Cycle of HIV-1. (1) After interaction of gp120 with the CD4 cell plasma membrane receptor, gp41-mediated membrane fusion occurs. (2) This leads to the entry of HIV-1 into the

cell. The lck denotes a lymphoid-specific tyrosine kinase that binds to CD4. (3) After internalization and uncoating, reverse transcription of viral RNA begins. (4) The double-stranded DNA form of the virus genome is produced in the presence of appropriate host factors. (5) The HIV-1 integrase promotes the insertion of this viral DNA duplex into the CD4 cell's genome after the DNA has entered the nucleus. (6) This gives rise to the HIV-1 provirus. (7) The expression of the HIV-1 gene is stimulated initially by the action of specific inducible and constitutive host transcription factors with binding sites in the long terminal repeat. Their binding leads to the sequential production of various viral mRNAs. (8) The first mRNAs produced correspond to the multiply spliced species of approximately 2.0 kilobases encoding tat, rev, and nef regulatory proteins. (9) Subsequently the viral structural proteins are produced, allowing the (10) assembly and morphogenesis of the virions. (11) The new HIV-1 virions that are produced by viral budding from the host CD4 cell can then reinitiate the retroviral life cycle by infecting other CD4 target cells.

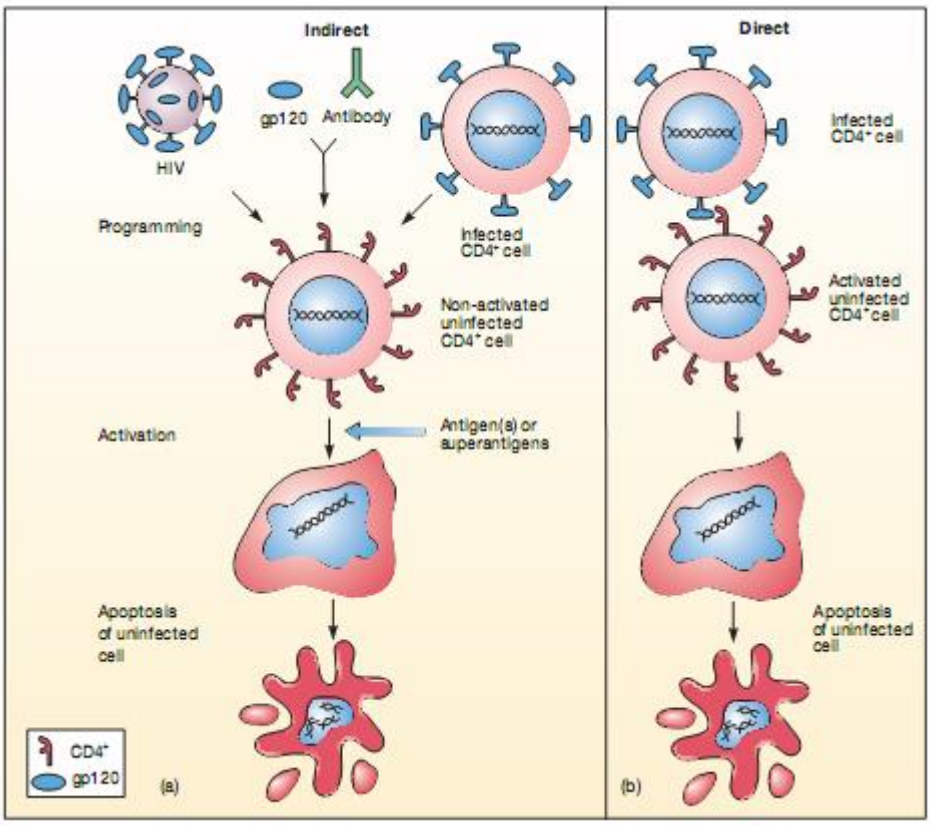
Second, a true case of AIDS can develop directly upon infection. The mean interval between HIV infection and the onset of AIDS appears to be about 8 to 10 years, although it varies considerably with each individual. At first, a person's immune system responds to the HIV-1 infection by manufacturing HIV-1 antibodies, but not in sufficient quantities to stop the viral attack. The virus becomes established within primarily CD4 T-helper cells, and HIV accumulates in lymphoid organs in large quantities even before symptoms appear. Initially, CD4 T-helper cells proliferate abnormally in the lymph nodes. Thereafter the lymph nodes' internal structure collapses due to viral replication. This leads to a decline in

the number of lymphocytes within the lymph nodes and results in a selective depletion of the CD4 T-cell subset that is critical to the propagation of the entire T-cell pool. When this CD4 population declines, interleukin-2 (IL-2) production also decreases. Because IL-2 stimulates the production of T cells in general, the whole T-cell population may decline. This leaves the infected person open to opportunistic infections: invasion by pathogens that proliferate widely only because the immune system is defective.

It should be noted that factors other than direct T-cell destruction also may be involved in AIDS pathogenesis. HIV may reduce the immune response by destroying or disabling dendritic cells,

which present foreign antigens to T cells. HIV also mutates exceptionally rapidly and thus could evade and eventually overwhelm the immune system. HIV may disrupt the balance between different types of T-helper cells and consequently decrease the killer T-cell population. It is possible that several different mechanisms contribute to T-cell destruction.

New findings suggest still another potential mechanism for the depletion of CD4 cells. In HIV-infected individuals the loss of CD4 cells is associated with lymphocyte activation; however, this activation does not result in cell proliferation, as it does normally, but rather in cell death by a mechanism known as programmed cell death.



Apoptosis is a homeostatic physiological suicide mechanism in which cell death occurs naturally during normal tissue turnover. Usually apoptosis occurs after activation of a calciumdependent endogenous endonuclease. Cells undergoing apoptosis display profound structural changes such as a decrease in cell volume, blebbing of the plasma membrane, and nuclear fragmentation. The nuclear DNA is cleaved into short oligo nucleosomal length DNA

fragments. The dying cell sheds small membrane-bound apoptotic bodies, which are phagocytosed and digested. (a) There may be several ways in which an HIV infection can indirectly trigger apoptosis. In all cases an initial event would program or prime the target cell so that apoptosis would be triggered by the binding of antigens or super antigens to the cell's T-cell receptors. Possibly the external gp120 envelope glycoprotein of the HIV virion binds to the CD4 protein on lymphocytes and programs the lymphocyte. A combination of free gp120 and antibodies to gp120 also could stimulate programmed cell death. First, the gp120 would bind to CD4 receptors. Then antibodies would attach to the gp120 and cause clustering of the receptors, thus priming the uninfected CD4 cell. It also is possible that binding of the infected cell's surface gp120 proteins to the CD4 receptors on an uninfected cell will program the uninfected cell for apoptosis in response to antigens. (b) Apoptosis may be directly triggered in an uninfected cell. The gp120 envelope proteins on the surface of an infected cell may combine with the CD4 proteins of an uninfected cell and directly stimulate programmed cell death without activation by antigens.

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Possible Questions

Two mark questions

1. Give a brief account on organ-specific autoimmune diseases.
2. Write short notes on classes of auto immune diseases.
3. Describe in detail about systemic autoimmune diseases.
4. Comment on Rheumatoid arthritis.
5. What is MHC?
6. State the role of MHC in antigen processing.
7. Make a note on MHC II complex.
8. Draw the diagram for MHC.
9. What is the role of MHC?
10. Define AIDS.
11. What are the types of MHC?
12. What is immunodeficiency?
13. Define immunity and explain immunity to infections.
14. Describe in detail the immunity against bacteria.
15. Give a brief account on immunology aspects of AIDS.
16. What is HIV? Explain in detail.
17. Define Pathogen.
18. Draw the neat diagram for HIV.
19. Describe in detail about the immunity to different organisms.
20. Give a brief account on immune response for viral infections.

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21. Define MHC I.
22. Explain about endogenous pathway in detail.
23. Make a note on immune response.
24. Describe in detail about antigen processing.
25. Give a brief account on exogenous pathway for antigen presentation on major histocompatibility complex.

Eight mark questions

1. Give a brief account on organ-specific autoimmune diseases.
2. Write short notes on classes of auto immune diseases.
3. Describe in detail about systemic autoimmune diseases.
4. Comment on Rheumatoid arthritis.
5. What is MHC?
6. State the role of MHC in antigen processing.
7. Make a note on MHC II complex.
8. Draw the diagram for MHC.
9. What is the role of MHC?
10. Define AIDS.
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24. Describe in detail about antigen processing.
25. Give a brief account on exogenous pathway for antigen presentation on major histocompatibility complex.

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UNIT-V

SYLLABUS

Vaccines & Vaccination: Adjuvants, cytokines, DNA vaccines, recombinant vaccines, bacterial vaccines, viral vaccines, vaccines to other infectious agents, passive & active immunization Introduction to immunodiagnostics – RIA, ELISA.

Vaccines and Vaccination

A vaccine is a product that produces immunity from a disease and can be administered through needle injections, by mouth, or by aerosol. A vaccination is the injection of a killed or weakened organism that produces immunity in the body against that organism. T-cell memory is very important for long-lasting immunity, because T-cells control both humoral and cell mediated immunity. When the immune system recognizes a foreign antigen for the first time, an immune response is produced. When T cells are involved, immunological T-cell memory is produced. When the body encounters same antigen subsequently, a stronger immune response is produced. This is because of existing immunological memory against that antigen. Further antigenic stimulus increases the immune response. First antigenic stimulus is "priming" whereas subsequent stimuli are "booster". This is the principle of active immunization. The term "vaccine" was coined by Louis Pasteur to commemorate first successful immunization against small pox by Edward Jenner.

The term vaccine was derived from "vacca", meaning cow, since Edward Jenner used cowpox virus (Vaccinia) to prevent smallpox infection. Vaccination involves deliberate exposure to antigen under conditions where disease should not result. Vaccination is aimed at inducing active immunity in an individual; so that subsequent contact with the microorganism following natural infection induces strong protective immune response. The protective immunity may involve secretion of neutralizing antibodies or production of memory CTL or Th1 cells. The use of vaccines is now being extended to immunize against tumours or to block fertilization (contraceptive vaccines). A vaccine is a suspension of whole (live or inactivated) or fractionated bacteria or viruses that have been rendered nonpathogenic,

and is given to induce an immune response and prevent disease. Even though no vaccine is entirely safe or completely effective, their use is strongly supported by their benefit-to-risk ratio.

Adjuvants

An adjuvant is a pharmacological or immunological agent that modifies the effect of other agents. Adjuvants may be added to a vaccine to modify the immune response by boosting it such as to give a higher amount of antibodies and a longer-lasting protection, thus minimizing the amount of injected foreign material.

Cytokines

Cytokines are produced by a broad range of cells, including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells; a given cytokine may be produced by more than one type of cell.

Properties of ideal vaccine

1. Provide long lasting immunity.
2. Should induce both humoral and cellular immunity.
3. Should not induce autoimmunity or hypersensitivity.
4. Should be inexpensive to produce, easy to store and administer.
5. Vaccines must also be perceived to be safe.

The vaccine vial may contain relevant antigen, adjuvant (usually alum), preservatives and/or traces of protein derived from the cells in which the vaccine agent was cultured e.g. egg protein

Types of vaccines

DNA Vaccines

These vaccines are still in experimental stage. Like recombinant vaccines, genes for the desired antigens arelocated and cloned. The DNA is injected into the muscle of the animal being vaccinated, usually with a "gene gun" that uses compressed gas to blow the DNA into the muscle

cells. DNA can be introduced into tissues bybombarding the skin with DNA-coated gold particles. It is also possible to introduce DNA into nasal tissue in nosedrops. Some muscle cells express the pathogen DNA to stimulate the immune system. DNA vaccines have inducedboth humoral and cellular immunity.

Advantages

1. DNA is very stable, it resists extreme temperature and hence storage and transport are easy.
2. A DNA sequence can be changed easily in the laboratory.
3. The inserted DNA does not replicate and encodes only the proteins of interest.
4. There is no protein component and so there will be no immune response against the vector itself.
5. Because of the way the antigen is presented, there is a cell-mediated response that may be directed against any antigen in the pathogen.

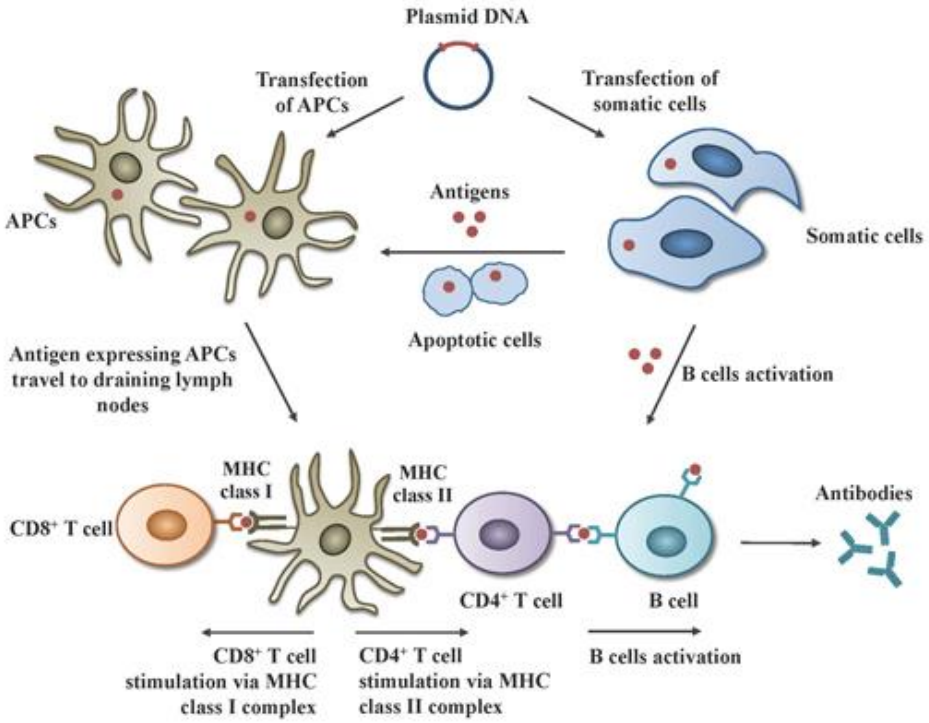

Fig: DNA vaccine

Disadvantages

1. Potential integration of DNA into host genome leading to insertional mutagenesis.
2. Induction of autoimmune responses: anti-DNA antibodies may be produced against introduced DNA.
3. Induction of immunologic tolerance: The expression of the antigen in the host may lead to specific non responsiveness to that antigen.

Recombinant vaccines

The vaccines are produced using recombinant DNA technology or genetic engineering. Recombinant vaccines arethose in which genes for desired antigens of a microbe are inserted into a vector. Different strategies are:

1. Using the engineered vector (e.g., Vaccinia virus) that is expressing desired antigen as a vaccine
2. The engineered vector (e.g., yeast) is made to express the antigen, such is vector is grown and the antigen is purified and injected as a subunit vaccine. Other expression vectors include the bacteria *Escherichia coli*, mutant Salmonella spp., and BCG.
3. Introduction of a mutation by deleting a portion of DNA such that they are unlikely to revert can create an attenuated live vaccine.
4. Live attenuated vaccines can also be produced by reassortment of genomes of virulent and a virulent strains.
5. Genes coding for significant antigens are introduced into plants, such that the fruits produced bear foreign antigens. This is edible vaccine and is still in experimental stage.

Examples

1. Hepatitis B Virus (HBV) vaccine is a recombinant subunit vaccine. Hepatitis B surface antigen is producedfrom a gene transfected into yeast (*Saccharomyces cerevisiae*) cells and purified for injection.
2. Vaccinia virus may be engineered to express protein antigens of HIV, rabies etc. Foreign genes cloned intothe viral genome is expressed on the surface of infected cells in association with class I MHC molecules. The antigen-MHC complex induces a Tc cell response.

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3. B subunit of cholera toxin, the B subunit of heat-labile *E. coli* enterotoxin (LT), and one of the glycoprotein membrane antigens of the malaria parasite are being developed using this technique.
4. *Salmonella typhimurium* engineered to express antigens of *Vibrio cholerae*.
5. Bacille Calmette vaccine strain engineered to express genes of HIV-1.
6. Reassortment of genomes between human and avian strains to create Influenza vaccine. Human and swine strains to create Rotavirus vaccine.

Advantages

1. Those vectors that are not only safe but also easy to grow and store can be chosen.
2. Antigens which do not elicit protective immunity or which elicit damaging responses can be eliminated from the vaccine. Example Cholera toxin A can be safely removed from cholera toxin.

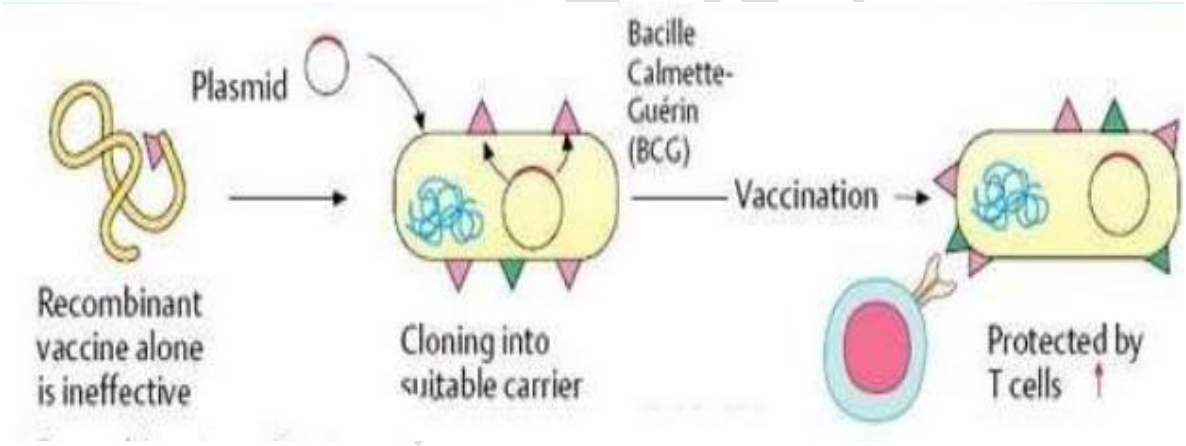

Fig: Recombinant vaccine

Disadvantages

1. Since the genes for the desired antigens must be located, cloned, and expressed efficiently in the newvector, the cost of production is high.
2. When engineered vaccinia virus is used to vaccinate, care must be taken to spare immunodeficientindividuals.

Bacterial Vaccines

When it is unsafe to use live microorganisms to prepare vaccines, they are killed or inactivated. These are preparations of the normal (wild type) infectious, pathogenic microorganisms that have been rendered non pathogenic, usually by treatment with using heat, formaldehyde or gamma irradiation so that they cannot replicate at all. Such killed vaccines vary greatly in their efficacy.

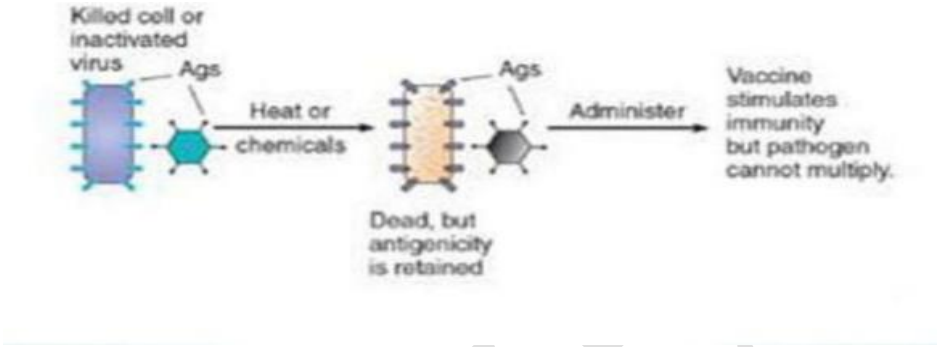

Fig: Killed vaccine

Table : Killed vaccines, source and the route of administration

Microorganism	Vaccine	Method	Route
Salmonella typhi, SC	TAB	Heat, Phenol ,Acetone	SC
Vibrio cholerae		Phenol	SC or ID
Yersinia pestis	Haffkine	Formalin	SC
Bordetella pertussis		Merthiolate	IM
Poliomyelitis	Salk	Formalin	IM
JE virus	Nakayama Strain	Formalin	IM
Rabies virus	Semple	Phenol	SC
	BPL	BPL	SC
	HDCV	BPL	IM or SC
	DEV	BPL	IM or SC
Influenza virus		Formalin	IM
Hepatitis A	HM175	Formalin	IM

Advantages

1. Safe to use and can be given to immune deficient and pregnant individuals.
2. Cheaper than live attenuated vaccine
3. Storage not as critical as live vaccine

Disadvantages

1. Since the microorganisms cannot multiply, a large number are required to stimulate immunity.
2. Periodic boosters must be given to maintain immunity.
3. Only humoral immunity can be induced.
4. Most killed vaccines have to be injected.
5. Some vaccines such as *Bordetella pertussis* induce ill effects like postvaccinial encephalomyelitis.
6. Anaphylactic reaction to neomycin or streptomycin may occur in (Inactivated Polio Vaccine) recipients.
7. Anaphylactic hypersensitivity to eggs may occur in recipients of influenza vaccine.
8. Inactivation, such as by formaldehyde in the case of the Salk vaccine, may alter antigenicity.
9. Presence of some un-inactivated microbes can lead to vaccine-associated disease.

Viral Vaccine

These vaccines are composed of live, attenuated microorganisms that cause a limited infection in their host ssufficient to induce an immune response, but insufficient to cause disease. To make an attenuated vaccine, the pathogen is grown in foreign host such as animals, embryonated eggs or tissue culture, under conditions that make it less virulent. The strains are altered to a non-pathogenic form; for example, its tropism has been altered so that it no longer grows at a site that can cause disease. Some mutants will be selected that have a better ability to grow in the foreign host. These tend to be less virulent for the original host. These vaccines may be given by injection or by the oral route. A major advantage of live virus vaccines is that because they cause infection, the vaccine very closely reproduces the natural stimulus to the immune system.

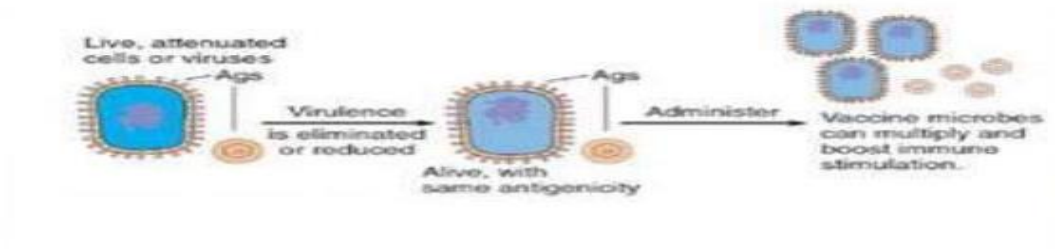

Fig: Live Attenuated Vaccine

Table : Live attenuated vaccine, source and route of administration.

Bacteria/virus	**Vaccine**	**Method**	**Route**
Vibrio	CVD103Hgr	Genetically modified	Oral
Salmonella	Ty21a	Genetically modified	Oral
Mycobacterium	BCG	Prolonged subculture	ID
Polio	Sabin	Passage in MK cells	Oral
JE	SA 14-14-2	Passage in weanling mice	IM
Yellow Fever	17D	Passage in chick embryo cells	SC
Influenza	-	Temperature sensitive mutant	IN
Mesales, Mumps, Rubella	MMR Rubella (Wistar RA 27/3)	Passage in fibroblasts cells	SC
Chicken pox	Oka/Merck	Human diploid cell cultures	SC
Small pox	Vaccinia virus	Naturally avirulent	ID

The influenza vaccine contains cold-adapted vaccine strains of the influenza virus that have been grown in tissue culture at progressively lower temperatures. After a dozen or more of these passages, the virus grows well only at around 25° C and in vivo growth is restricted to the upper respiratory tract.

Advantages

1. Infectious microbes can stimulate generation of memory cellular as well as humoral immune responses.
2. Since these can multiply in the host, fewer quantities must be injected to induce protection.
3. A single administration of vaccine often has a high efficacy in producing long-lived immunity. Multiple booster doses may not be required.
4. Whole microbes stimulate response to antigens in their natural conformation. They raise immune response to all protective antigens.
5. Some live vaccines can be given orally; such vaccines induce mucosal immunity and IgA synthesis, which gives more protection at the normal site of entry.
6. Oral preparations are less expensive than giving injections.
7. They can lead to elimination of wild type virus from the community.

Disadvantages

1. May very rarely revert to its virulent form and cause disease.
2. Live vaccines cannot be given safely to immune suppressed individuals. Administration of live attenuated vaccines to people with impaired immune function can cause serious illness or death in the vaccine recipient.
3. Since they are live and because their activity depends on their viability, proper storage is critical.
4. Spread to contacts of vaccine who have not consented to be vaccinated. In some cases, it turns out be an advantage.

Vaccines to other infections agents

Subunit Vaccines

Subunit vaccines contain purified antigens instead of whole organisms. Such a preparation consists of only thoseantigens that elicit protective immunity. Subunit vaccines are composed of toxoids, sub cellular fragments, or surfaceantigens. Administration of whole organism, as in case of pertussis was found unfavorable immune reactionsresulting in severe side effects.

Advantages

1. They can safely be given to immunosuppressed people
2. They are less likely to induce side effects.

Disadvantages

1. Antigens may not retain their native conformation, so that antibodies produced against the subunit may notrecognize the same protein on the pathogen surface.
2. Isolated protein does not stimulate the immune system as well as a whole organism vaccine.

Toxoids

Some bacterial diseases are not directly caused by a bacterium itself, but by a toxin produced by the bacterium. One example is tetanus: its symptoms are not caused by the *Clostridium tetani* bacterium, but by a neurotoxin it produces (tetanospasmin). Immunizations for this type of pathogen can be made by inactivating the toxin that causes disease symptoms. As with organisms or viruses used in killed or inactivated vaccines, this can be done via treatment with a chemical such as formalin, or by using heat or other methods. Immunizations created using inactivated toxins are called ***toxoids***. Toxoids can actually be considered killed or inactivated vaccines, but are sometimes given their own category to highlight the fact that they contain an inactivated toxin, and not an inactivated form of bacteria.

Passive and active immunization

Passive immunity can occur naturally, when maternal antibodies are transferred to the fetus through the placenta, and it can also be induced artificially, when high levels of antibodies specific to a pathogen or toxin (obtained from humans, horses, or other animals) are transferred to non-immune persons through blood.

Artificially acquired active immunity can be induced by a vaccine, a substance that contains antigen. A vaccine stimulates a primary response against the antigen without causing symptoms of the disease.

Radioimmunoassay

One of the most sensitive techniques for detecting antigen or antibody is radioimmunoassay (RIA). The technique was first developed in 1960 by two endocrinologists, S. A. Berson and Rosalyn Yalow, to determine levels of insulin–anti-in- sulin complexes in diabetics. Although their technique en- countered some skepticism, it soon proved its value for measuring hormones, serum proteins, drugs, and vitamins at concentrations of 0.001 micrograms per milliliter or less. In 1977, some years after Berson's death, the significance of the technique was acknowledged by the award of a Nobel Prize to Yalow. The principle of RIA involves competitive binding of ra- diolabeled antigen and unlabeled antigen to a high-affinity antibody. The labeled antigen is mixed with antibody at a concentration that saturates the antigen-binding sites of the antibody. Then test samples of unlabeled antigen of un- known concentration are added in progressively larger amounts. The antibody does not distinguish labeled from unlabeled antigen, so the two kinds of antigen compete for available binding sites on the antibody. As the concentration of unlabeled antigen increases, more labeled antigen will be displaced from the binding sites. The decrease in the amount of radio labeled antigen bound to specific antibody in the presence of the test sample is measured in order to determine the amount of antigen present in the test sample. The antigen is generally labeled with a gamma-emitting isotope such as 125I, but beta-emitting isotopes such as tritium (3H) are also routinely used as labels. The radiola- beled antigen is part of the assay mixture; the test sample may be a complex mixture, such as serum or other body fluids, that contains the unlabeled antigen. The first step in setting up an RIA is to determine the amount of antibody needed to bind 50%–70% of a fixed quantity of radioactive antigen (Ag∗) in the assay mixture. This ratio of antibody to Ag∗ is chosen to ensure that the number of epitopes presented by the labeled antigen always exceeds the total number of antibody binding sites. Consequently, unlabeled antigen added to the sample mixture will compete with ra- diolabeled antigen for the limited supply of antibody. Even a small amount of unlabeled antigen added to the assay mixture of labeled antigen and antibody will cause a de- crease in the amount of radioactive antigen bound, and this decrease will be proportional to the amount of unlabeled antigen added. To determine the amount of labeled antigen bound, the Ag-Ab complex is precipitated to separate it from free antigen (antigen not bound to Ab), and the ra- dioactivity in

the precipitate is measured. A standard curve can be generated using unlabeled antigen samples of known concentration (in place of the test sample), and from this plot the amount of antigen in the test mixture may be precisely determined.

Several methods have been developed for separating the bound antigen from the free antigen in RIA. One method in- volves precipitating the Ag-Ab complex with a secondary anti-isotype antiserum. For example, if the Ag-Ab complex contains rabbit IgG antibody, then goat anti-rabbit IgG will bind to the rabbit IgG and precipitate the complex. Another method makes use of the fact that protein A of Staphylococcus aureus has high affinity for IgG. If the Ag-Ab complex contains an IgG antibody, the complex can be precipitated by mixing with formalin-killed S. aureus. After removal of the complex by either of these methods, the amount of free la-beled antigen remaining in the supernatant can be measured in a radiation counter; subtracting this value from the total amount of labeled antigen added yields the amount of la- beled antigen bound.

Various solid-phase RIAs have been developed that make it easier to separate the Ag-Ab complex from the unbound antigen. In some cases, the antibody is covalently cross- linked to Sepharose beads. The amount of radiolabeled anti- gen bound to the beads can be measured after the beads have been centrifuged and washed. Alternatively, the antibody can be immobilized on polystyrene or polyvinylchloride wells and the amount of free labeled antigen in the supernatant can be determined in a radiation counter. In another approach, the antibody is immobilized on the walls of microtiter wells and the amount of bound antigen determined. Because the procedure requires only small amounts of sample and can be conducted in small 96-well microtiter plates (slightly larger than a 3 5 card), this procedure is well suited for determining the concentration of a particular anti- gen in large numbers of samples. For example, a microtiter RIA has been widely used to screen for the presence of the hepatitis B virus. RIA screening of donor blood has sharply reduced the incidence of hepatitis B infections in recipients of blood transfusions

Overview of ELISA

This chapter examines what areas of science are needed to allow optimal use of ELISA and notes their relationships. This information is useful for students and those instructing students. Diagrams, with brief descriptions of key points, are used to illustrate such relationships. Inherent in this exercise are considerations of the exact requirements by the operators in using the ELISA. Attention to increasing knowledge in those areas highlighted is essential both in developmental work to produce a working ELISA and in the ultimate

value of any test devised. A good deal of attention should be directed at defining, as clearly as possible, the objectives for the ELISA. The development of a diagnostic test for a specific disease requires that all other data pertaining to the biology of that disease, e.g., antigenicity and structure of the agent, antibody production in different animals following infection, qualitative assessment of antibodies by different assays, and availability of standard or control sera, are known. Some attention must be paid to the laboratory facilities available, e.g., equipment, reagents already developed, small laboratory animals, experimental large animals, cash to buy commercial products, and trained personnel. In this way, the chances of producing a sustainable test to solve the defined problem are significantly greater than when a test is developed by a dabbling technique with poor or no forward planning.

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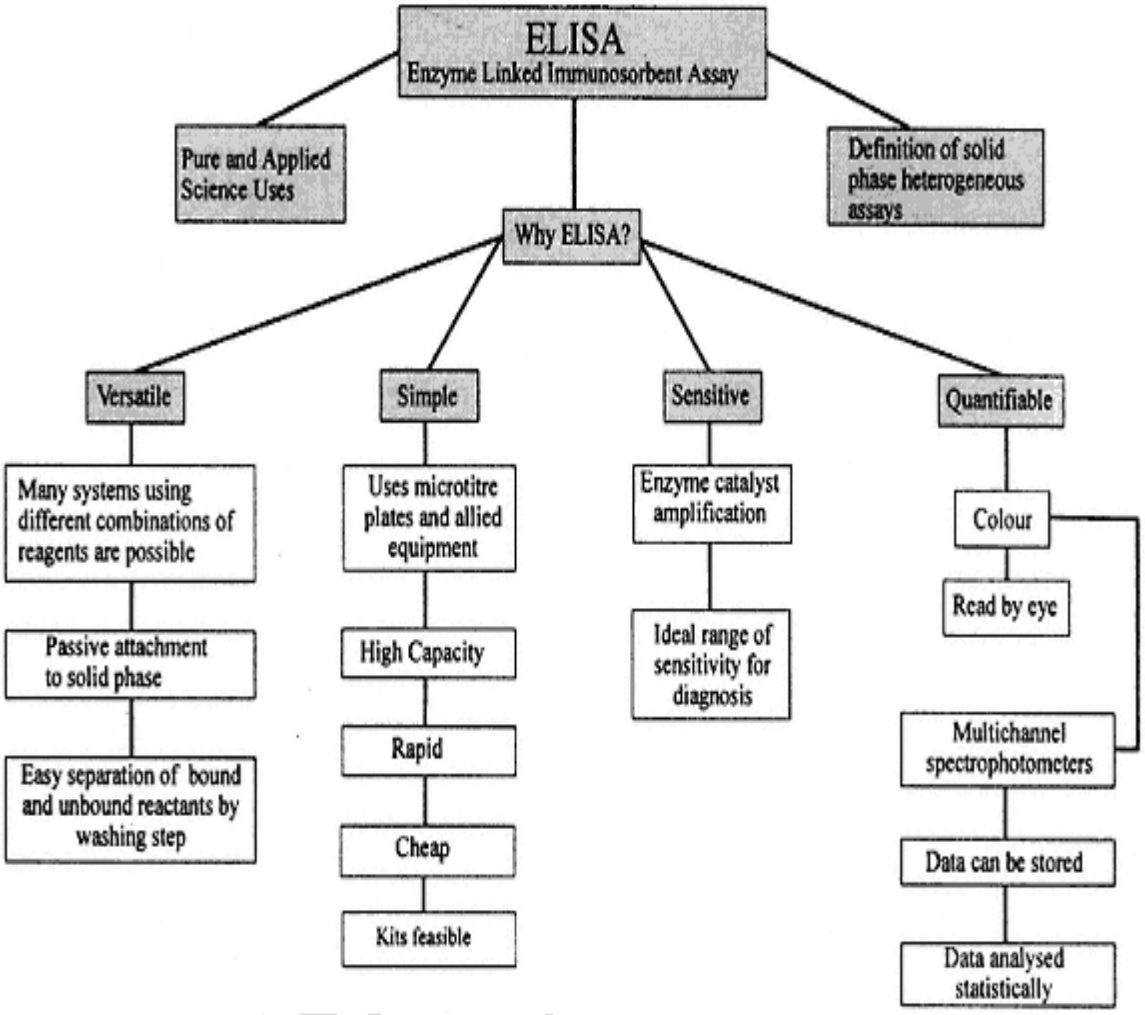

Fig. 1.

Scheme showing features of ELISA that makes it advantageous for a wide range of applications.

Figure 4 deals with some of the enzymatic systems in the ELISA, and illustrates areas that need to be understood in order to allow optimal performance to be maintained. Understanding enzyme kinetics, catalysis reactions, hazards, and buffer formulation (pH control) are all essential.

Figure 5 illustrates the use of ELISA's in binding and inhibition/competition interactions to allow an understanding of a problem. It is essential that the chemical and physical nature of antibodies and antigens are understood, particularly in cases of developmental work. As full an

understanding of the antigenic properties of agents being examined is needed to allow maximum exploitation of ELISA, particularly if the results are ever to be understood.

Figure 6 deals with data processing and analysis. Various essential statistical parameters must be elucidated, if data are to be interpreted. This is true in understanding how to calculate the variance in a result, and also for examining populations. Such studies actually define any ELISA's performance, allowing confidence in results to be measured, thereby allowing a meaning to be placed on results. The concepts of controlling assays with
references to standards is also needed.

Figure 7 extends the use of statistical understanding into epidemiological needs. A common use of ELISA is to provide data on populations studied. The areas of sampling (size, number, and so forth) are vital when planning disease control strategies. These simplified overviews should be used as reference points when considering the development and specific use of any ELISA. They should help

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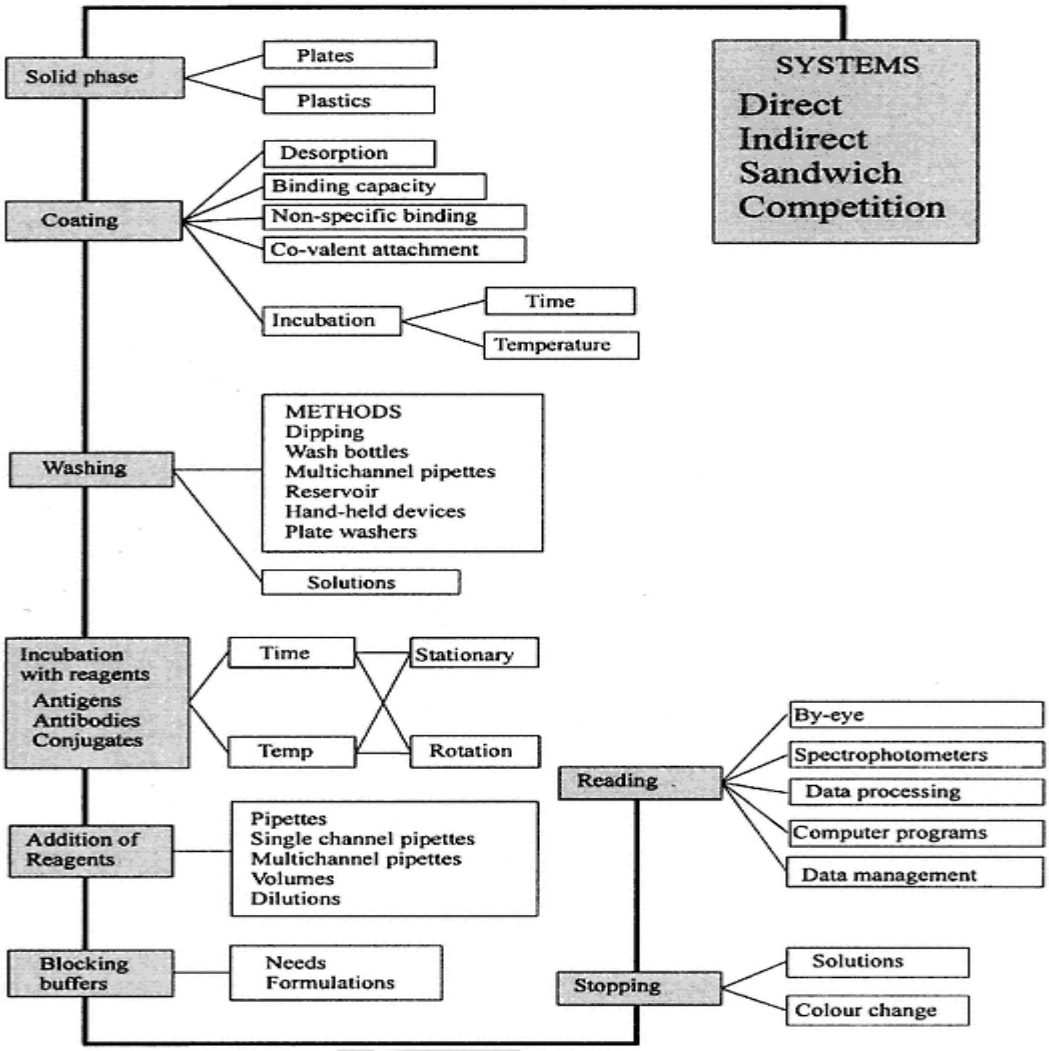


Fig. 2.

Scheme relating stages in ELISA. Specific stages vary according to the system utilized.

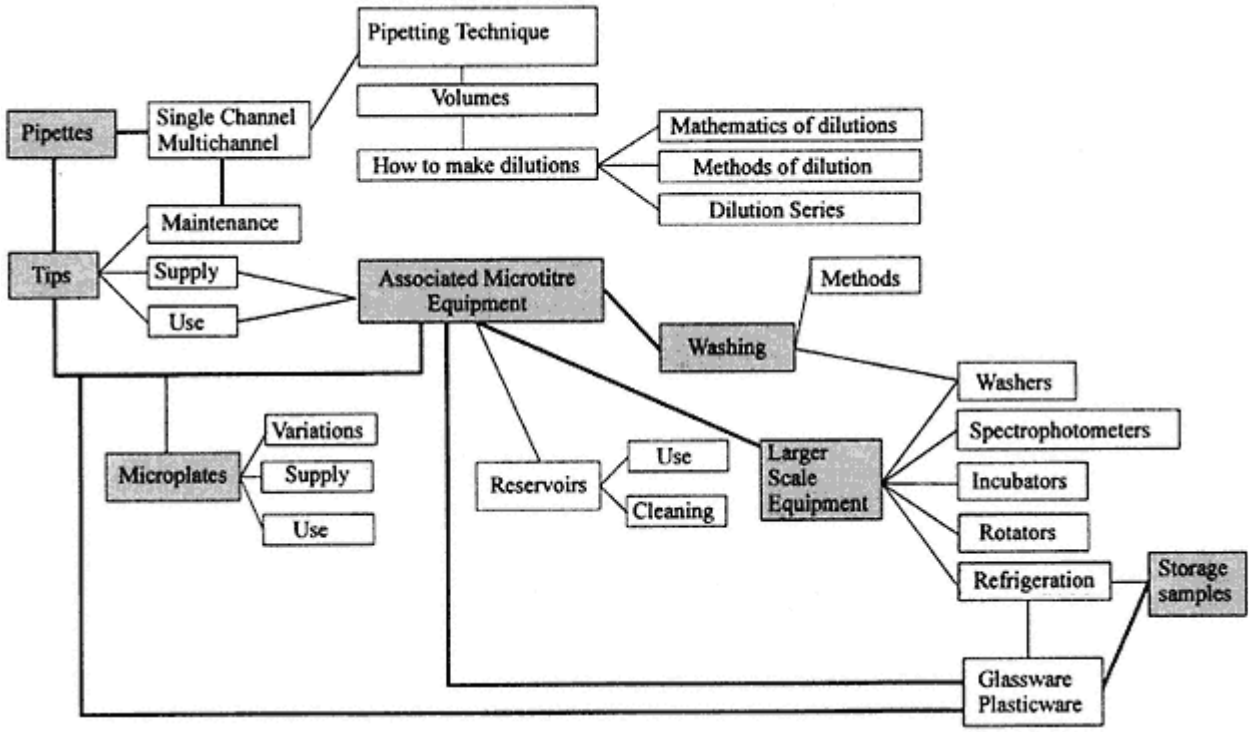


Fig. 3.

Scheme relating equipment needs and skills for ELISA.

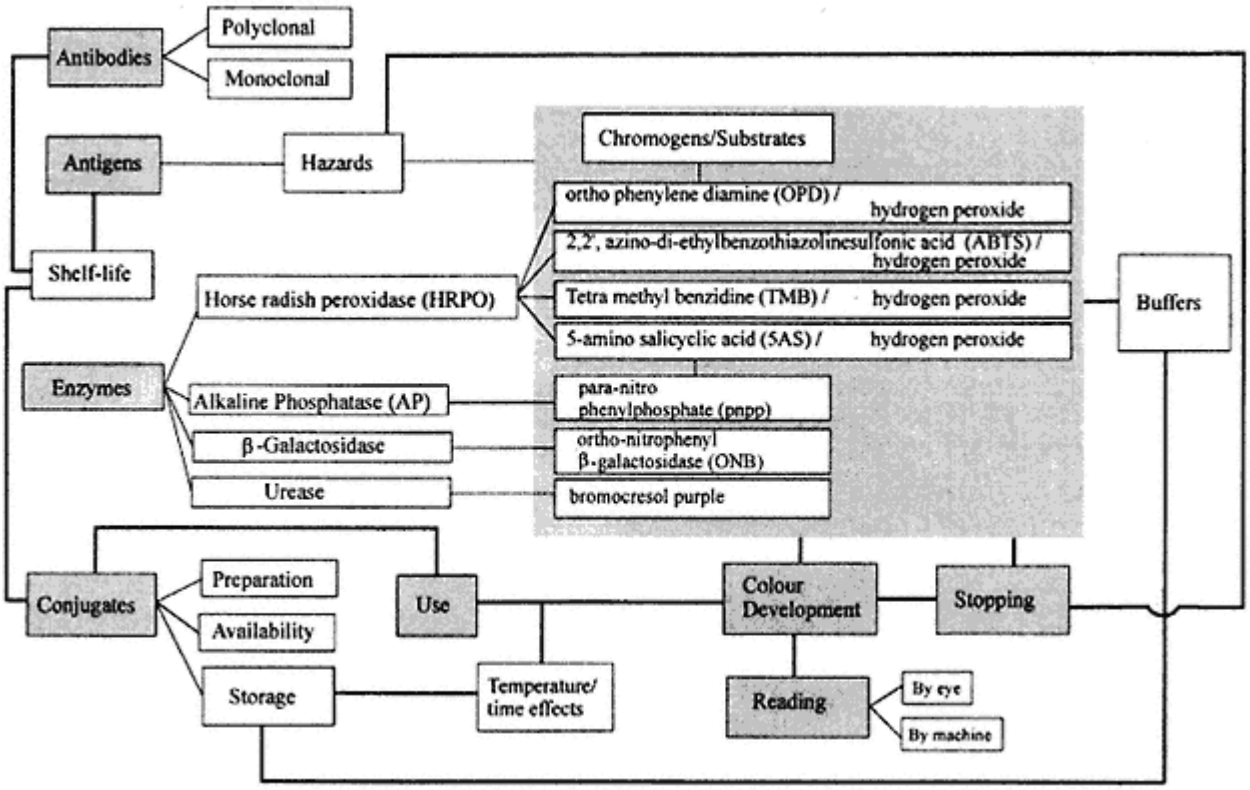


Fig. 4.

Relationships of enzyme systems to components of ELISA.

readers with limited exposure to ELISA, particularly after studying the details in later chapters. They are also useful for trainers in establishing areas of competence in students.

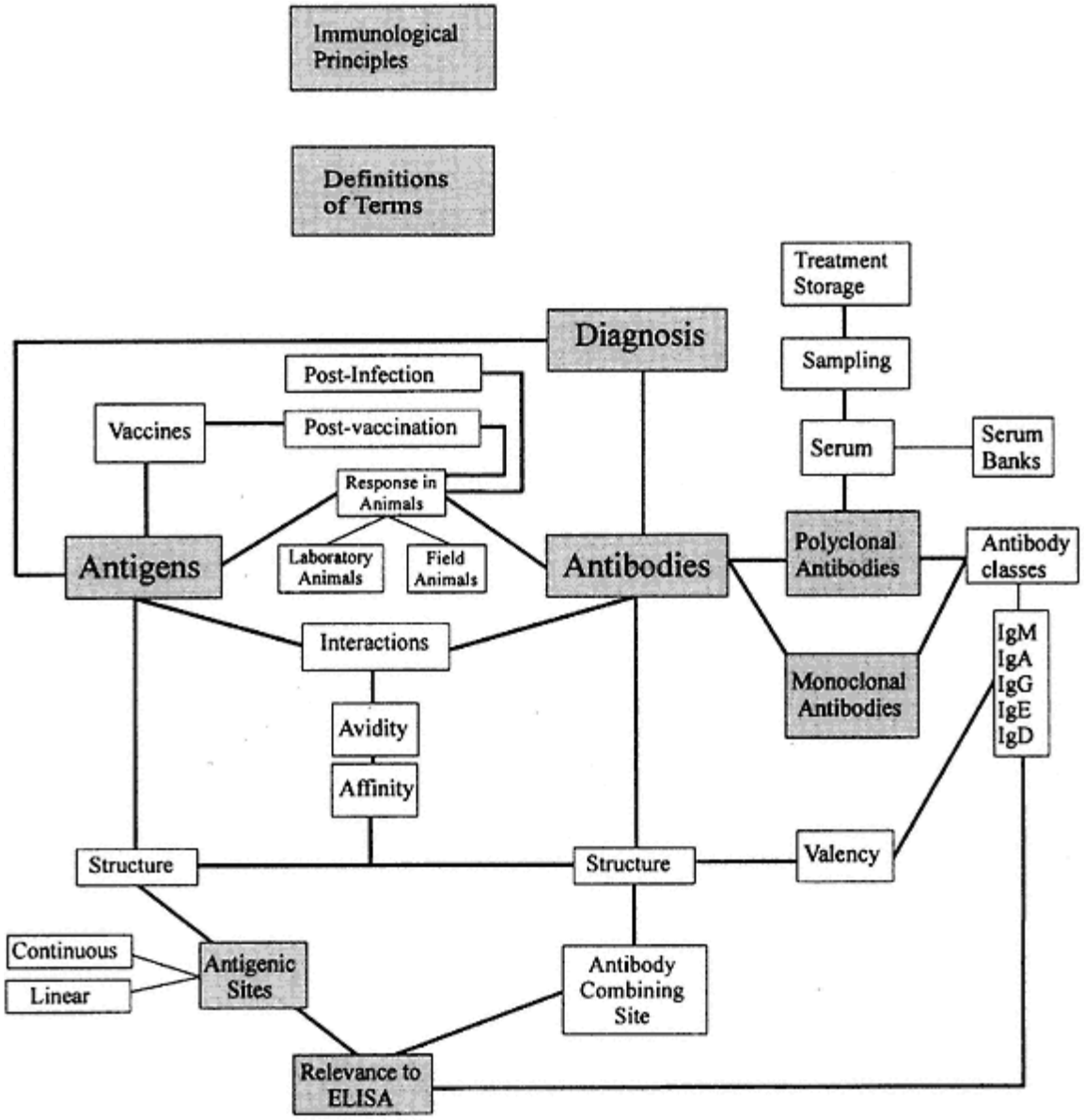


Fig. 5.

Requires features in immunological understanding in order to establish ELISA.

These are the key points to keep in mind at this early stage when considering then use of ELISA:

1. The ELISA is a tool to solve a problem.

2. Any problem should be defined, as clearly as possible, with reference to all previous work defining the specific agent involved and related agents.

3. Other methods for analyzing the problem should be reviewed, particularly when tests are already established. This has implications if the ELISA is to replace existing tests.

4. The capacity for testing has to be addressed. For example, when an ELISA may be used on a large scale (kit), then sufficient reagents, standard sera, conjugates (batches), and antigen preparations must be available. Research leading to successful assays in which reagents are difficult to prepare on a large scale, require extensive expertise to formulate, or are reliant on a specific limited batch of a commercial reagent are not sustainable.

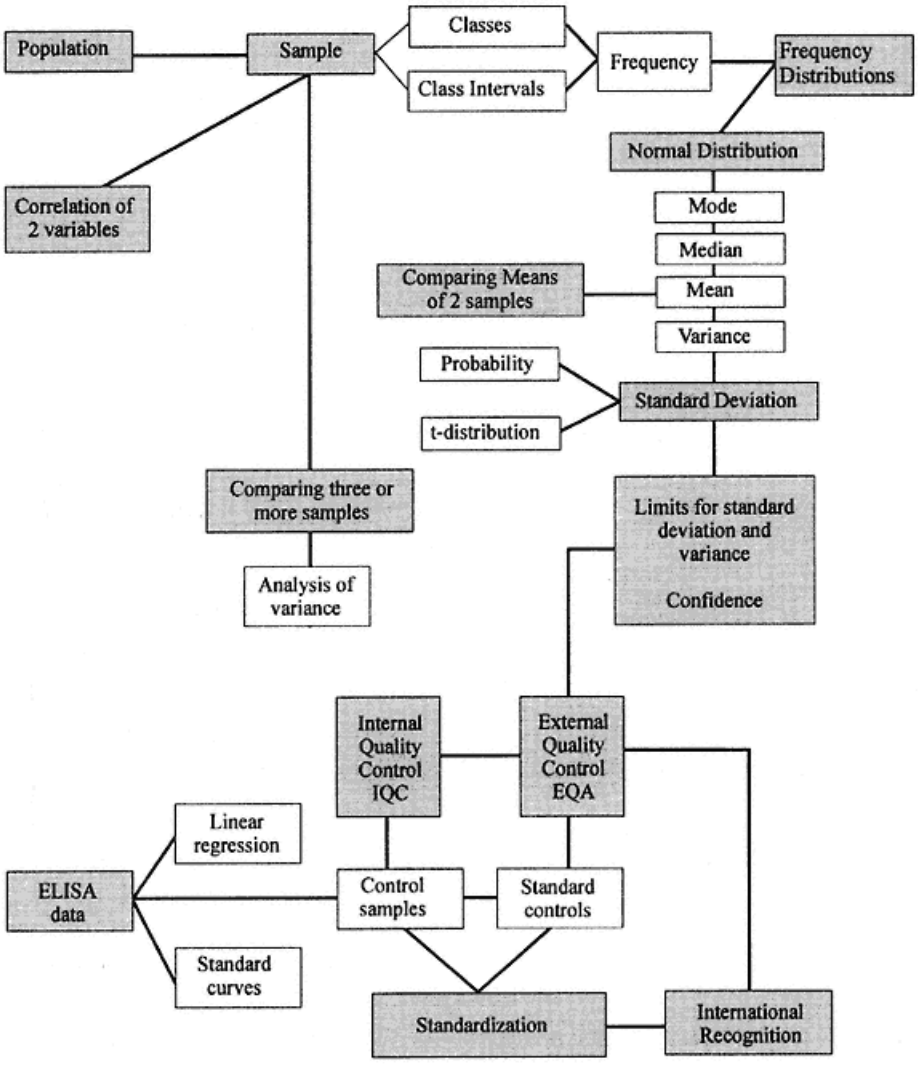
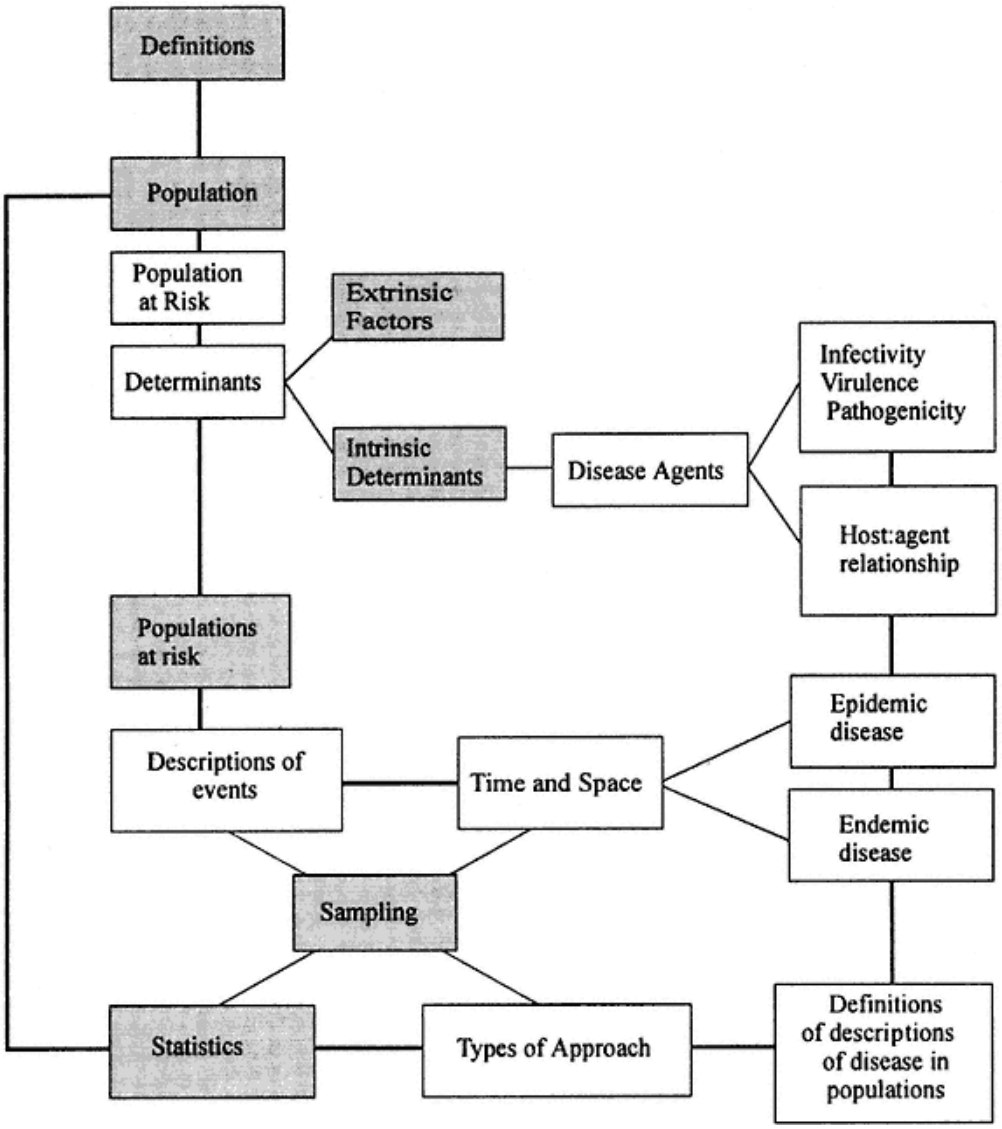


Fig. 6.

Important statistical factors needed to make use of ELISA. Note the links to quality control (internal) and the establishment of confidence in test results. Increasingly, assays need international recognition.

5. When a test may be of use to a wider group of scientists, the possible conditions (laboratory facilities, expertise) should be considered when developing assays. Such technology transfer factors are relevant, particularly in laboratories in developing countries.



Scheme relating basic areas in epidemiology that need to be understood in the context of data obtained from ELISA. Note the strong link with statistics/sampling, which is inherent in the test design.

The knowledge and skills required to both perform ELISA and make use of the data have to be gained through a variety of sources, including textbooks. As with all other techniques, the ultimate benefit is not the technique in itself, but the meaningful gathering and analysis of the data. One factor not included in all these examples is that of common sense: the ability to really consider what one is doing, and why, and not to overlook the simplicity of what is needed by being blinded by the technology for its own sake. Most problems are relatively
simple to examine after some clear thought. Thus, the good ELISA person will consider the problem first, obtain the necessary technical skills and equipment to perform a test,
and then obtain data that is from a planned perspective. As much data from all other tests and the scientific literature should also be sought. This is true for an assay developer, as well as a person using a supplied, predetermined kit. The skills required by the use of a kit are no less than those of the developer; indeed, a kit in the hands of an unskilled worker is often useless. The majority (90%) of problems observed in the practice of ELISA are operator faults caused by lack of common sense, failure to appreciate the need to stick to instructions, sloppy technique, or poorly maintained equipment. Most of the remaining percentage is caused by poor-quality water.

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Possible Questions

Two mark questions

1. General properties of Cytokines
2. What is an adjuvant
3. Principle Of ELISA
4. Type of test used in blood typing
5. List out the agents useful for passive immunization
6. Give the principle of indirect ELISA
7. What is the principle of RIA
8. Explain vaccine

Eight mark questions

1. Explain the mode of action of adjuvants
2. Laboratory diagnostics of the HIV-infection
3. Explain about Agar gel double immunodiffusion precipitation tests and Indirect hemmagglutination tests
4. Enumerate the advantages of using monoclonal antibodies compared to polyclonal antisera
5. What are the different types of ELISA?
6. Explain the technique of RIA and Immunodiffusion
7. Describe the role of cytokines in immunogenic reactions
8. Write a note on preparation of vaccines

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