TOLERANCE AND AUTO IMMUNITY

TEACHING OBJECTIVES:

1. Understand the concept and significance of tolerance
2. Know the factors that determine induction of tolerance
3. Understand the mechanism of tolerance induction
4. Understand the concepts of autoimmunity and disease
5. Know the features of major autoimmune diseases
6. Know the theories on etiology of autoimmune disease

TOLERANCE

Introduction:

Tolerance refers to the specific immunological non-reactivity to an antigen resulting from a previous exposure to the same antigen. While the most important form of tolerance is non-reactivity to self antigens, it is possible to induce tolerance to non-self (foreign) antigens. When an antigen induces tolerance, it is termed tolerogen.

Tolerance to self antigens: We normally do not mount a strong immune response against our own (self) antigens, a phenomenon called self-tolerance. When the immune system recognizes a self antigen and mounts a strong response against it, autoimmune disease develops. Nonetheless, the immune system has to recognize self-MHC to mount a response against a foreign antigen. Thus, the immune system is constantly challenged to discriminate self vs non-self and mediate the right response.

Induction of tolerance to non-self : Tolerance can also be induced to non-self (foreign) antigens by modifying the antigen, by injecting the antigen through specific routes such as oral, administering the antigen when the immune system is developing, etc. Certain bacteria and viruses have devised clever ways to induce tolerance so that the host does not kill these microbes. Ex: Patients with lepromatous type of leprosy do not mount an immune response against *Mycobacterium leprae*.

Tolerance to tissues and cells:

Tolerance to tissue and cell antigens can be induced by injection of hemopoietic (stem) cells in neonatal or severely immunocompromised (by lethal irradiation or drug treatment) animals. Also, grafting of allogeneic bone marrow (or thymus) in early life results in tolerance to the donor type cells and tissues. Such animals are known as chimeras. These findings are of significant importance in bone marrow grafting.

Immunologic features of tolerance:

Tolerance is different from non-specific immunosuppression, and immunodeficiency. It is an active antigen dependent process in response to the antigen. Like immune response, tolerance is specific and like immunological memory, it can exist in T-cell, B cells or both and like immunological memory, tolerance at the T cell level is longer lasting than tolerance at the B cell level.

Induction of tolerance in T cells is easier and requires relatively smaller amounts of tolerogen than tolerance in B cells. Maintenance of immunological tolerance requires persistence of antigen. Tolerance can be broken naturally (as in autoimmune diseases) or artificially (as shown in experimental animals, by x-irradiation, certain drug treatments and by exposure to cross reactive antigens).

Tolerance may be induced to all epitopes or only some epitopes on an antigen and tolerance to a single antigen may exist at B cell level or T cells level or at both levels.

Mechanisms of tolerance induction:

The exact mechanism of induction and maintenance of tolerance is not fully understood. Experimental data, however, point to several possibilities.

Clonal deletion: T and B lymphocytes during development come across self antigens and such cells undergo clonal deletion through a process known as apoptosis or programmed cell death. For example, T cells that develop in the thymus first express neither CD4 nor CD8. Such cells next acquire both CD4 and CD8 called double-positive cells and express low levels of αβ TCR. Such cells undergo positive selection after interacting with class I or class II MHC molecules expressed on cortical epithelium. During this process, cells with low affinity for MHC are positively selected. Unselected cells die by apoptosis, a process called "death by neglect". Next, the cells loose either CD4 or CD8. Such T cells then encounter self-peptides presented by self MHC molecules expressed on dendritic cells. Those T cells with high affinity receptors for MHC + self-peptide undergo clonal deletion also called negative selection through induction of apoptosis. Any disturbance in this process can lead to escape of auto-reactive T-cells that can trigger autoimmune disease. Likewise, differentiating early B cells when they encounter self-antigen, cell associated or soluble, undergo deletion. Thus, clonal deletion plays a key role in ensuring tolerance to self antigen.

Peripheral tolerance: The clonal deletion is not a fool proof system and often T and B cells fail to undergo deletion and therefore such cells can potentially cause autoimmune disease once they reach the peripheral lymphoid organs. Thus, the immune system has devised several additional check points so that tolerance can be maintained.

Activation-induced cell death: T cells upon activation not only produce cytokines or carryout their effector functions but also die through programmed cell death or apoptosis. In this process, the death receptor (Fas) and its ligand (FasL) play a crucial role. Thus, normal T cells express Fas but not FasL. Upon activation, T cells express FasL which binds to Fas and triggers apoptosis by activation of caspase-8. The importance of Fas and FasL is clearly demonstrated by the observation that mice with mutations in Fas (*lpr* mutation) or FasL (*gld* mutation) develop severe lymphoproliferative and autoimmune disease and die within 6 months while normal mice live up to 2 years. Similar mutations in these apoptotic genes in humans leads to a lymphoproliferative disease called autoimmune lymphoproliferative syndrome (ALPS).

Clonal anergy: Auto-reactive T cells when exposed to antigenic peptides on antigen presenting cells (APC) that do not possess the co-stimulatory molecules CD80 (B7-1) or CD86 (B7-2) become anergic

(nonresponsive) to the antigen. Also, while activation of T cells through CD28 triggers IL-2 production, activation of CTLA4 leads to inhibition of IL-2 production and anergy. Also, B cells when exposed to large amounts of soluble antigen down-regulate their surface IgM and become anergic. These cells also up-regulate the Fas molecules on their surface. An interaction of these B cells with Fas-ligand bearing T cells results in their death via apoptosis.

Clonal ignorance: T cells reactive to self-antigen not represented in the thymus will mature and migrate to the periphery, but they may never encounter the appropriate antigen because it is sequestered in inaccessible tissues. Such cells may die out for lack of stimulus. Auto-reactive B cells, that escape deletion, may not find the antigen or the specific T-cell help and thus not be activated and die out.

Anti-idiotype antibody: These are antibodies that are produced against the specific idiotypes of other antibodies. Anti-idiotypic antibodies are produced during the process of tolerization and have been demonstrated in tolerant animals. These antibodies may prevent the B cell receptor from interacting with the antigen.

Regulatory T cells (Formerly called suppressor cells): Recently, a distinct population of T cells has been discovered called regulatory T cells. Regulatory T cells come in many flavors, but the most well characterized include those that express CD4+ and CD25+. Because activated normal CD4 T cells also express CD25, it was difficult to distinguish regulatory T cells and activated T cells. The latest research suggests that regulatory T cells are defined by expression of the forkhead family transcription factor Foxp3. Expression of Foxp3 is required for regulatory T cell development and function. The precise mechanism/s through which regulatory T cells suppress other T cell function is not clear. One of the mechanisms include the production of immunosuppressive cytokines such as TGF-β and IL-10. Genetic mutations in Foxp3 in humans leads to development of a severe and rapidly fatal autoimmune disorder known as **I**mmune dysregulation, **P**olyendocrinopathy, **E**nteropathy, **X**-linked (**IPEX**)syndrome. This disease provides the most striking evidence that regulatory T cells play a critical role in preventing autoimmune disease.

Table 1. Factors which determine induction of immune response or tolerance following challenge with antigen.

determinant	favor immune response	favor tolerance
physical form of antigen	large, aggregated, complex molecules;	soluble, aggregate-free, relatively smaller, less complex molecules, Ag not processed by APC or processed inappropriately
route of Ag administration	sub-cutaneous or intramuscular	oral or sometimes intravenous
dose of antigen	optimal dose	very large (or sometime very small) dose
age of responding animal	older and immunologically mature	Newborn (mice), immunologically immature
differentiation state of cells	fully differentiated cells; memory T and memory B cells	relatively undifferentiated: B cells with only IgM (no IgD), T cells (*e.g.* cells in thymic cortex)

AUTOIMMUNITY

Definition:

Autoimmunity can be defined as breakdown of mechanisms responsible for self-tolerance and induction of an immune response against components of the self. Such an immune response may not always be harmful (e.g., anti-idiotype antibodies or recognition of self-MHC molecules). However, in numerous (autoimmune) diseases it is well recognized that products of the immune system cause severe damage to the self.

Effector mechanisms in autoimmune diseases:

Both antibodies and effector T cells and their products can be involved in the damage in autoimmune diseases.

General classification:

Autoimmune diseases are generally classified on the basis of the organ or tissue involved. These diseases may fall in an organ-specific category in which the immune response is directed against antigen(s) associated with the target organ being damaged or a non-organ-specific (also sometimes referred to as systemic) in which the antibody is directed against an antigen or many antigens not associated with the target organ and the disease is seen through out the body (Table 1). The antigen involved, in most autoimmune diseases is evident from the name of the disease (Table 1).

Genetic predisposition for autoimmunity:

Studies in mice and observations in humans suggest a genetic predisposition for autoimmune diseases. Association between certain HLA types and autoimmune diseases has been noted (HLA: B8, B27, DR2, DR3, DR4, DR5 etc.).

Etiology of autoimmunity disease:

The exact etiology of autoimmune diseases is not known. However, various theories have been offered. These include sequestered antigen, escape of auto-reactive clones, loss of Regulatory T cells, cross-reactive antigens including exogenous antigens (pathogens) and altered self antigens (chemical and viral infections).

Sequestered antigen: Lymphoid cells may not be exposed to some self-antigens during their differentiation, because they may be late-developing antigens or may be confined to specialized organs (e.g., testes, brain, eye, etc.). A release of antigens from these organs, resulting from accidental traumatic injury or surgery, can result in the stimulation of an immune response and initiation of an autoimmune disease.

Escape of auto-reactive clones: The negative selection in the thymus may not be fully functional to eliminate self reactive cells. Not all self antigens may be represented in the thymus or certain antigens may not be properly processed and presented.

Lack of regulatory T cells: There are fewer regulatory T-cells in many autoimmune diseases.

Table 2. Spectrum of autoimmune diseases, target organs and diagnostic tests.

Organ Specific	Disease	Target	Antibody to	Diagnostic Test
	Hashimoto's Thyroiditis	Thyroid	Thyroglobulin, thyroid peroxidase (microsomal)	RIA, Passive, CF, hemagglutination
	Primary Myxedema	Thyroid	Cytoplasmic	Immunofluorescence (IF)
	Graves' disease	Thyroid	TSH receptor	Bioassay, Competition for TSH receptor
	Pernicious anemia	Red cells	Intrinsic factor (IF), Gastric parietal cell	B-12 binding to IF immunofluorescence
	Addison's disease	Adrenal	Adrenal cells	Immunofluorescence
	Premature onset menopause	Ovary	Steroid producing cells	Immunofluorescence
	Male infertility	Sperms	Spermatozoa	Agglutination,
	Insulin dependent juvenile diabetes	Pancreas	Pancreatic islet beta cells	Immunofluorescence
	Insulin resistant diabetes	Systemic	Insulin receptor	Competition for receptor
	Atopic allergy	Systemic	beta-adrenergic receptor	Competition for receptor
	Myasthenia graves	Muscle	Muscle, acetyl choline receptor	Immunofluorescence, competition for receptor
	Goodpasture's Syndrome	Kidney, lung	Renal and lung basement membrane	Immunofluorescence (linear staining)
	Pemphigus	Skin	Desmosomes	Immunofluorescence
	Pemphigoid	Skin	Skin basement membrane	Immunofluorescence
	Phacogenic uveitis	Lens	Lens protein	Passive hemagglutination
	AI hemolytic anemia	Red cells	Red cells	Direct Coomb's test
	Idiopathic Thrombocytopenia	Platelet	Platelet	Immunofluorescence

Table 2. Spectrum of autoimmune diseases, target organs and diagnostic tests (continued….).

Disease		Target	Antibody to	Diagnostic Test
	Primary biliary cirrhosis	Liver	Mitochondria	Immunofluorescence
	Idiopathic neutropenia	Neutrophils	Neutrophils	Immunofluorescence
	Ulcerative colitis	Colon	Colon lipopolysaccharide	Immunofluorescence
	Sjogren's syndrome	Secretory glands	Duct tissue	Immunofluorescence
	Vitiligo	Skin	Melanocytes	Immunofluorescence
	Rheumatoid arthritis	Joints	IgG	IgG-latex agglutination
Non-organ Specific	Systemic lupus erythematosus	Skin, kidney, joints, etc.	DNA, RNA, neucleoproteins	RNA-, DNA-latex agglutination, IF (granular in kidney)

Cross reactive antigens: Antigens on certain pathogens may have determinants which cross react with self antigens and an immune response against these determinants may lead to effector cells or antibodies against tissue antigens. Post streptococcal nephritis and carditis, anticardiolipin antibodies during syphilis and association between Klebsiella and ankylosing spondylitis are examples of such cross reactivity.

Diagnosis:

Diagnosis of autoimmune diseases is based on symptoms and detection of antibodies (and/or very rarely T cells) reactive against antigens of tissues and cells involved. Antibodies against cell/tissue-associated antigens are detected by immunofluorescence. Antibodies against soluble antigens are normally detected by ELISA or radioimmunoassay (see table above). In some cases, a biological /biochemical assay may be used (e.g., Graves diseases, pernicious anemia).

Treatment:

The goals of treatment of autoimmune disorders are to reduce symptoms and control the autoimmune response while maintaining the body's ability to fight infections. Treatments vary widely and depend on the specific disease and symptoms: Anti-inflammatory (corticosteroid) and immunosuppressive drug therapy (such as cyclophosphamide, azathioprine, cyclosporine) is the present method of treating autoimmune diseases. Extensive research is being carried out to develop innovative treatments which include: anti-TNF alpha therapy against arthritis, feeding antigen orally to trigger tolerance, anti-idiotype antibodies, antigen peptides, anti-IL2 receptor antibodies, anti-CD4 antibodies, anti-TCR antibodies, etc.

Models of autoimmune diseases:

There are a number of experimental and natural animal models for the study of autoimmune diseases. These experimental models include experimental allergic encephalomyelitis(EAE) which mimics Multiple Sclerosis, experimental thyroiditis, adjuvant induced arthritis, etc.

Naturally occurring models of autoimmune diseases include hemolytic anemia in NZB mice, systemic lupus erythematosus in NZB/NZW (BW), BXSB and MRL *lpr*/*lpr* mice and diabetes in NOD (non-obese diabetic) mice.

You should know:

Mechanisms of tolerance induction to self.
Different autoimmune diseases and organs/antigens involved in these conditions.
Type of immunologic tests normally used to diagnose different autoimmune diseases.
Possible etiology of autoimmune diseases and major experimental models.

Hypersensitivity reactions

TEACHING OBJECTIVES:

1. Understand the classification of hypersensitivity reactions
2. Know the diseases associated with hypersensitivity reactions
3. Understand the mechanisms of damage in hypersensitivity reactions
4. Know the methods for diagnosing conditions due to hypersensitivity
5. Know the modes of treating disease due to hypersensitivity and their rationale

Hypersensitivity refers to excessive undesirable (damaging, discomfort producing and sometimes fatal) reactions produced by the normal immune system. Hypersensitivity reactions require a pre-sensitized (immune) state of the host. Hypersensitivity reactions can be divided into four types: type I, type II, type III and type IV, based on the mechanisms involved and time taken for the reaction. Frequently, a particular clinical condition (disease) may involve more than one type of reaction.

Type I Hypersensitivity

It is also known as **immediate** or **anaphylactic** hypersensitivity. The reaction may involve skin (urticaria and eczema), eyes (conjunctivitis), nasopharynx (rhinorrhea, rhinitis), bronchopulmonary tissues (asthma) and gastrointestinal tract (gastroenteritis). The reaction may cause from minor inconvenience to death. The reaction takes 15-30 minutes from the time of exposure to the antigen. Sometimes the reaction may have a delayed onset (10-12 hours).

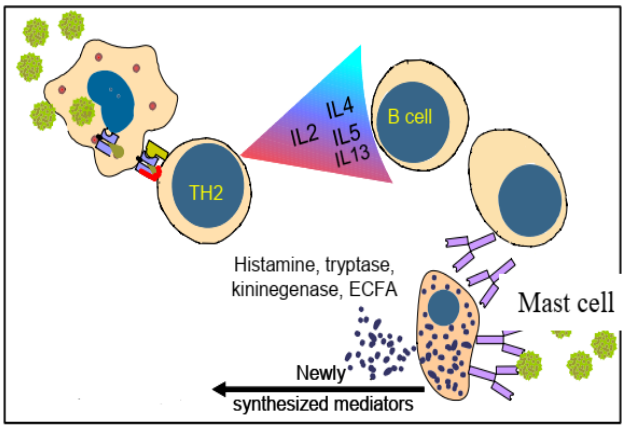

Figure 1: Induction and effector mechanisms in type I hypersensitivity

Type I hypersensitivity is mediated by **IgE**. The primary cellular component in this hypersensitivity is **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** (Figure 1). 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 (Figure 1). 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 of mast cells, whereas, agents which deplete cytoplasmic Ca^{++} suppress degranulation.

The agents released from mast cells and their effects are listed in Table 1. Mast cells may be triggered by other stimuli such as exercise, emotional stress, chemicals (*e.g.*, photographic developing medium, calcium ionophores, codeine, *etc.*), anaphylotoxins (*e.g.*, C4a, C3a, C5a, *etc.*). These reactions mediated by agents without IgE-allergen interaction **are not typical hypersensitivity reactions**, although they produce the same symptoms.

Table 1. Pharmacologic Mediators of Immediate Hypersensitivity

mediator	Physiological effect
preformed mediators in granules	
histamine	bronchoconstriction, mucus secretion, vasodialatation, vascular permeability
tryptase	proteolysis
kininogenase	kinins and vasodialatation, vascular permeability, edema
ECF-A (tetrapeptides)	attract eosinophil and neutrophils
newly formed mediators	
leukotriene B_4	basophil attractant
leukotriene C_4, D_4	similar to histamine but 1000x more potent
prostaglandins D_2	Eosinophil and basophil chemotactic, histamine-like but more potent edema and pain
PAF	platelet aggregation and heparin release: microthrombi

The reaction is amplified by PAF (platelet activation factor) that 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. Eosinophil may also control the local reaction by releasing arylsulphatase, histaminase, phospholipase-D and prostaglandin-E, although this role of eosinophils is now in question.

Cyclic nucleotides appear to play a significant role in the modulation of immediate hypersensitivity reaction, although their exact function is ill understood. Substances which alter cAMP and cGMP levels significantly alter the allergic symptoms. Thus, substances that increase intracellular cAMP seem to relieve allergic symptoms, particularly broncho-pulmonary ones, and are used therapeutically (Table 2). Conversely, agents that decrease cAMP or stimulate cGMP aggravate these allergic conditions.

Table 2: Relationship between allergic symptoms and cyclic-nucleotides

Lowering of cyclic-AMP	**Elevation of cyclic AMP**
stimulation of α-adrenergic receptor (nor-epinephrin, phenyl-epinephrin) or blocking of β-adrenergic receptor (propanolol)	stimulation of β-adrenergic receptor (epinephrine, isoproterenol) blocking of α-adrenergic receptor (phenoxybenzamine)
Elevation of cyclic GMP	inhibition of phosphodiesterase (theophylline)
stimulation of γ-cholinergic receptor (acetyl choline, carbacol)	binding of histamine-2 or PGE to their receptors
WORSENING OF SYMPTOMS	**IMPROVEMENT OF SYMPTOMS**

Diagnostic tests for immediate hypersensitivity include skin (prick and intradermal) tests resulting in wheal and flare reaction, 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). Increased IgE levels are indicative of atopic condition, although IgE may be elevated in some non atopic diseases (*e.g.*, myelomas, helminthic infection, *etc*.).

There appears to be a genetic predisposition for atopic diseases and there is evidence for HLA (A2) association.

Symptomatic treatment is achieved with antihistamines that 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, Lukast) or inhibitors of 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.

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.

Hyposensitization (immunotherapy or desensitization) is another treatment modality which is successful in a number of allergies, particularly to pollen. The mechanism is not clear, but there is

a correlation between appearance of IgG (blocking) antibodies and relief from symptoms. Suppressor T cells that specifically inhibit IgE antibodies may play a role.

Type II Hypersensitivity

It is also known as cytotoxic hypersensitivity and may affect a variety of organs and tissues. The antigens are normally endogenous, although exogenous chemicals (haptens) that 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. It is mediated, primarily, by antibodies of IgM or IgG class and complement (Figure 2). Phagocytes and K (killer)cells may also play a role.

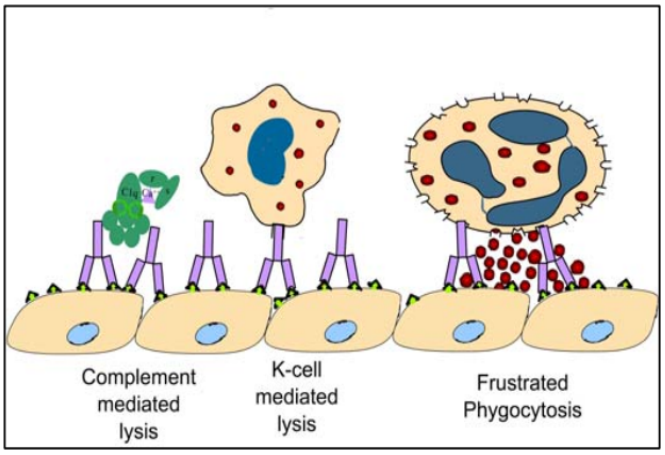

Figure 2: Type II hypersensitivity mechanisms

The lesion contains antibody, complement and neutrophils. Diagnostic tests include detection of circulating antibody against 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

It 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 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 eythematosus-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 (Figure3).

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

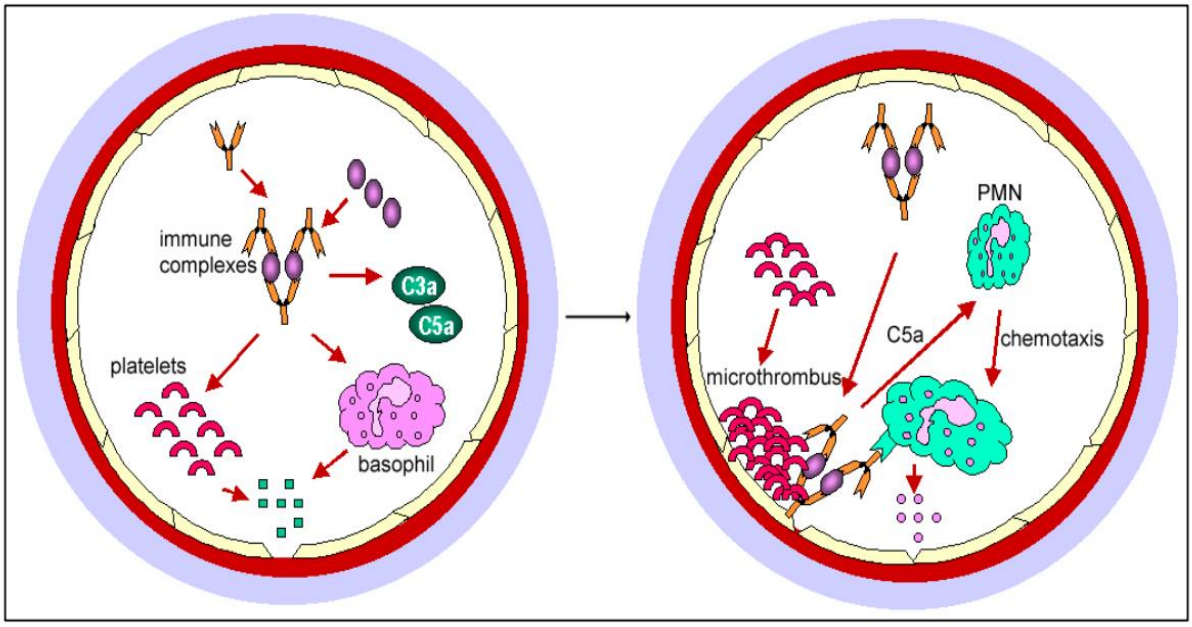

Figure 3: Mechanism of damage in type-III hypersensitivity

The affinity of antibody and size of immune complexes are important in production of disease and determining the tissue involved. Diagnosis involves examination of tissue biopsies for deposits of Ig and complement by immunofluorescence. The immunofluorescent staining in type III hypersensitivity is granular (as opposed to linear in type II: Goodpasture). Presence of immune complexes in serum and depletion in complement level are also diagnostic. Treatment includes anti-inflammatory agents.

Type IV Hypersensitivity

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

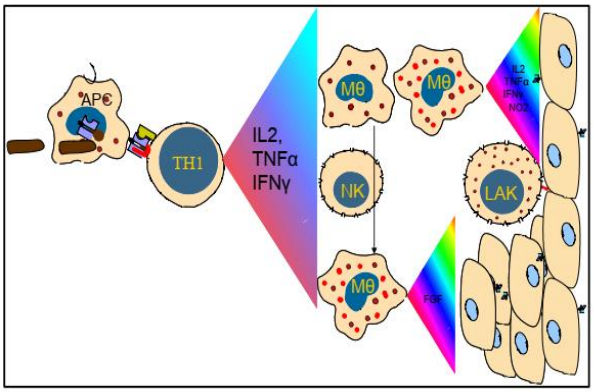

Figure 4. Mechanisms of damage in delayed hypersensitivity

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).

Mechanisms of damage in delayed hypersensitivity include T lymphocytes and monocytes and/or macrophages. The pathogenesis is triggered primarily by helper T (TH1) cells that secrete cytokines that activate and recruit macrophages, which cause the bulk of the damage (Figure 3). The delayed hypersensitivity lesions mainly contain monocytes and T cells.

Major lymphokines involved in delayed hypersensitivity reaction include monocyte chemotactic factor, interleukin-2, interferon-γ, TNF α, *etc*.

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*.)

Diagnostic tests *in vivo* include delayed cutaneous reaction (*e.g*. Montoux test) 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.

A comparative summary of all four types of hypersensitivity reactions have been presented in Table 4 below.

Table 4. 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	wheal & flare	lysis and necrosis	erythema and edema, necrosis	erythema and induration
histology	basophils and eosinophil	antibody and complement	complement and neutrophils	macrophages and T cells
transferred with	antibody	antibody	antibody	T-cells
examples	allergic asthma, hay fever	erythroblastosis fetalis, Goodpasture's nephritis	SLE, farmer's lung disease	tuberculin test, poison ivy, granuloma

You have learned:

Distinctions between different types of hypersensitivity.
Mechanisms of immune-mediated damages.
Examples of different types of hypersensitivity and overlap among them.
Diagnostic test for hypersensitivity diseases and treatments.

Immunodeficiency

Objectives
1. Understand Primary and Secondary immunodeficiencies
2. Characterization of various immunodeficiencies
3. Studies on HIV and Development of AIDS
4. Analysis of Strategies for Prevention and Treatment of AIDS

Immunodeficiency is the failure of the immune system to protect against disease or malignancy.
Primary Immunodeficiency is caused by genetic or developmental defect in the immune system. These defects are present at birth but may show up later on in life.
Secondary or Acquired Immunodeficiency is the loss of immune function as a result of exposure to disease agents, environmental factors, immunosuppression, or aging.

Primary Immunodeficiency

Defect in the early hematopoiesis which involves **stem cells** results in **reticular dysgenesis** that leads to general immune defects and subsequent susceptibility to infections. This condition is often fatal.

Lymphoid lineage immunodeficiency:

If the lymphoid progenitor cells are defective, then both the T and B cell lineages are affected and result in the **severe combined immunodeficiency (SCID).** They are less common but more severe. Infants suffer from recurrent infections especially by opportunistic microrganisms. These include several disorders.

Patients having both T and B cell deficiency lack **recombinase activating genes** (RAG1 and 2) that are responsible for the T cell receptor and Ig gene rearrangements. These patients are athymic and are diagnosed by examining the T cell receptor (TCR) gene rearrangement. Defects in B cells are not observed in early infant life because of passive Abs obtained from mother. NK cells are normal.

In some SCID patients, T cells may be present but functionally defective because of deficiency in signaling mediated by CD3 chain that is associated with the TCR.

Interleukin-2 Receptor common gamma chain **(IL-2Rγc)** may be lacking in patients thereby preventing signaling by IL-2, 4, 7, 9 and 15. These patients are T and NK cell deficient.

Adenosine deaminase (ADA) is responsible for converting adenosine to inosine. **ADA deficiency** leads to accumulation of adenosine which interferes with DNA synthesis. The patients have defects in T, B and NK cells.

B cell deficiency may result from absence of B cells, plasma cells, total Igs or selective Igs. These patients have recurring infection with extracellular bacteria, but have normal immunity against intracellular bacterial as well as normal anti-viral and anti-fungal immunity.

X-linked Agammaglobulinemia (X-LA) remain in pre B cell stage with H chains rearranged but not L chains. They have low or no Igs and suffer from recurrent bacterial infections. Also known as **Bruton's hypogammaglobulinemia** as the patients have a defect in Bruton's tyrosine kinase (Btk) involved in signal transduction.

X-linked hyper-IgM Syndrome: Deficiency in IgG, IgA and IgE but elevated IgM. This is due to a defect in CD40L expression.

Common Variable Immunodeficiency: Late onset hypogammaglobulinemia. Treatment is with Igs. B cells fail to mature into plasma cells. Often confused with acquired immunodeficiency.

Selective Deficiency of Ig classes: IgA deficiency results in respiratory and genitourinary tract infections. IgM deficiency leads to infections or malignancy.

T cell deficiency affects both cell-mediated and humoral immunity. The patients are susceptible to viral, protozoal and fungal infections. Infection with viruses such as cytomegalovirus or attenuated measles vaccine can be life-threatening in these patients.

DiGeorge Syndrome or congenital thymic aplasia patients lack a thymus. Results from deletion of chromosome 22 region during development of 3rd and 4th pharyngeal pouch is also responsible for facial and heart defects.

Bare Lymphocyte Syndrome (BLS) is due to absence of MHC class II molecules.

Wiskott-Aldrich Syndrome (WAS) is an X-linked disorder involving T and B cells due to defect in a cytoskeletal glycoprotein, CD43.

Myeloid Lineage deficiency:

Affects innate immunity. Inasmuch as, phagocytosis is affected, the patients are susceptible to microbial infections.

Chronic Granulomatous Disease (CGD):
Defective ROS production which kills phagocytosed bacteria. Thus, they exhibit inflammatory reaction and granulomas. Defect in Ag presentation cells. Treatment is with IFN-γ.

Chediak-Hegashi Syndrome:
Defective intracellular trafficking of proteins to lysosomes and thus unable to kill bacteria.

Leukocyte adhesion Deficiency (LAD):
Lack of CD18 (β chain) on LFA-1 and Mac-1(which have additionally CD11a and b respectively). Impairs adhesion of leukocytes to endothelium thereby preventing inflammation.

Complement defects:
Defects in C components results in immunodeficiency or immune complex disease. The patients are susceptible to infections.

Treatment:
Treatment is by replacing missing protein (e.g.Ig, rIL-2, rIFN-γ), cells (stem cells) or gene (e.g. ADA).

Acquired or Secondary Immunodeficiencies:

Acquired Hypogammaglobulinemia differs from the common variable immunodeficiency which is a genetic disorder
Agent-induced immunodeficiency: Due to drugs such as corticosteroids and other immunosuppressive agents.

All acquired immunodeficiencies have been outdone by the AIDS that is caused by **Human Immunodeficiency Virus** (HIV)-1. It was first discovered in 1981 and the patients exhibited fungal infections with opportunistic organisms such as Pneumocystis carinii and in other cases, with a skin tumor known as Kaposi sarcoma. HIV-1 and 2 have been discovered with the strain frequently found in N. America being HIV-1. HIV is spread through heterosexual intercourse, infected blood and body fluids as well as by delivery from mother to offspring. HIV was discovered by Luc Montagnier in Paris and Robert Gallo in Bethesda in 1983. It is a retrovirus with RNA that is reverse transcribed to DNA by reverse transciptase (RT) following entry into the cell. The DNA is integrated into the cell genome as a provirus that is replicated along with the cell. HIV-1 does not replicate in most other animals but infects chimpanzees although it does not induce AIDS in them. Severe combined immunodeficient mice (SCID) reconstituted with human lymphocytes can be infected with HIV-1.

HIV-1 virion consists of viral envelope made up of the outer lipid bilayer of the host cell in which are embedded glycoproteins composed of the transmembrane gp41 along with the associated gp120. The gp120 binds the CD4 expressed on host cells. Within the viral envelope is the viral core or nucleocapsid consisting of a layer of matrix protein composed of p17 and an inner capsid made up of p24. The viral genome consists of 2 ssRNA strands associated with 2 reverse transcriptase (RT) molecules as well as other enzymes including a protease and an integrase.

Replication cycle and targets of therapy:
The virus attaches to the CD4 molecule on Th cells, monocytes and dendritic cells through the gp120 of HIV. For HIV infection, a coreceptor is required. The coreceptor is a chemokine receptor such as CXCR4 or CCR5. CCR5 expressed predominantly on macrophages and CXCR4 on CD4+ T cells serve as coreceptors for HIV infection. After the fusion of HIV envelope and the host membrane, the nucleocapsid enters the cell. The RT synthesizes viral DNA which is transported to the nucleus where it integrates with the cell DNA in the form of a provirus. The provirus can remain latent till the cell is activated when the provirus also undergoes transcription. The virions consisting of the transcribed viral RNA and proteins are produced. These are budded out of the host cell membrane from where they acquire the envelope. Thus, therapeutic agents have been developed that target viral entry and fusion, as well as serve as RT, protease and integrase inhibitors. Highly active anti-retroviral therapy is a cocktail of 3 or more such agents.

Immunological Changes:
The virus replicates rapidly and within ~2 weeks the patient develops fever. The viral load in the blood increases significantly and peaks in 2 months after which there is a sudden decline because of the latent virus found in germinal centers of the lymph nodes. CTL develop very early whereas antibodies can be detected between 3-8 weeks. The CTL killing of Th cells around 4-8 weeks leads to decrease in CD4+ T cells. When the CD4+ T cell count decreases below 200/mm^3, full blown AIDS develops.

Immunotherapy:
There are several barriers to development of an effective HIV vaccine.

1) Attenuated vaccine may induce the disease
2) CD4+ T cells may be destroyed by the vaccine
3) Antigenic variation of HIV
4) Low immunogenicity of the virus by downregulation of MHC molecules
5) Lack of animal models
6) Lack of in vitro tests

The following reagents have been considered in developing vaccines

1) Immunization with Deletion mutants to reduce pathogenicity
2) Vaccination with Recombinant proteins
3) Gene encoding proteins introduced into virus vectors may be used for vaccination
4) Chemokines that compete for the co-receptors.
5) IL-2 to boost the Th cells.

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