

# Tolerance and Autoimmunity

lecture: 16-18

## 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

## READING:

Male, Brostoff, Roth and Roitt : Immunology, 7th ed., Chapt. 19 and 20.

# 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  $\alpha\beta$  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 lose 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 carry out 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-idiotypic 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- $\beta$  and IL-10. Genetic mutations in Foxp3 in humans leads to development of a severe and rapidly fatal autoimmune disorder known as **Immune dysregulation, Polyendocrinopathy, Enteropathy, 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 ( <i>e.g.</i> 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-idiotypic 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 2). The antigen involved, in most autoimmune diseases is evident from the name of the disease (Table 2).

## 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
Non-organ Specific	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
	Systemic lupus erythematosus	Skin, kidney, joints, etc.	DNA, RNA, nucleoproteins	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-idiotypic 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.