IMMUNOLOGICAL TOLERANCE
Introduction

- Exposure to an antigen may or may not lead to an immune response.

- Different forms of the same antigen can cause different outcomes.

- A unique property of the immune system is its ability to discriminate between self and nonself.

- Ability to respond to self is inherited but it is regulated at the phenotypic level by induction of tolerance.
Balancing lymphocyte activation and control

Activation of Effector T cells
- Normal: reactions against pathogens
- Inflammatory disease, e.g. reactions against self

Activation of Regulatory T cells
- No response to self
- Controlled response to pathogens
The importance of immune regulation

• To avoid excessive lymphocyte activation and tissue damage during normal protective responses against infections

• To prevent inappropriate reactions against self antigens (“self-tolerance”)

• Failure of control mechanisms is the underlying cause of immune-mediated inflammatory diseases
General principles of controlling immune responses

• Responses against pathogens decline as the infection is eliminated
  – Apoptosis of lymphocytes that lose their survival signals (antigen, etc)
  – Memory cells are the survivors

• Active control mechanisms may function to limit responses to persistent antigens (self antigens, possibly tumors and some chronic infections)
  – Often grouped under “tolerance”
Immunological tolerance

• Definition:
  – specific unresponsiveness to an antigen that is induced by exposure of lymphocytes to that antigen (tolerogen vs. immunogen)

• Significance:
  – All individuals are tolerant to their own antigens (self-tolerance); breakdown of self-tolerance results in autoimmunity
  – Therapeutic potential: Inducing tolerance may be exploited to prevent graft rejection, treat autoimmune and allergic diseases, and prevent immune responses in gene therapy
Tolerance is a state of unresponsiveness that is specific for a particular antigen which is induced by prior exposure to that antigen.

Tolerance is best defined as “a physical state in which the immune system does not react destructively against the host that harbors it.”

Destructive responses are prevented by a variety of mechanisms that operate during the development of the immune system and during the generation of each immune response.
Experimental Evidence of Tolerance

- **1901: Ehrlich and Morgenroth**
  - Immunized goats with RBC from another goat to conclude that host immune system responds to foreign antigens.
  - They coined the Latin phrase: *horror autoxicus*

- **1938- Traub** induced tolerance by injecting LCMV in utero into mice producing an infection that was long-lived.

- **1945- Owen** demonstrated that dizygotic twin claves accepted mutual skin grafts on adulthood.
Tolerance

Tolerance in non-identical cattle twins
1949- Macfarlane Burnet: postulated that the age of the animal at the time of the first encounter with antigen was the critical determinant in the induction of tolerance.

1953: Medawar induced immune tolerance to skin allografts in mice by neonatal injection of allogeneic cells.

Hasek confirmed The concept that tolerance was an acquired state in chicken.

IN Humans, an immune response to noninherited paternal antigens but no response to noninherited maternal antigens.
Medawar’s experiment demonstrating neonatal tolerance induction (Nobel Prize)
Central Tolerance

- **Negative Selection During T cell Development**
  - Mature T cells are self tolerant, MHC restricted and responsive to foreign antigens.
  - The fundamental steps of T cell self tolerance occur in the thymus.
  - T cells undergo expansion, rearrangement of TCR genes and begin to express surface markers.
  - Thereafter, they undergo positive selection (MHC restriction) and negative selection (deletion of self reactive cells).
Immature T cell encountering their antigen during development are clonally deleted.

All T cells are susceptible to the process of negative selection.

Antigen presenting cells for negative selection are most likely dendritic cells.

The molecular mechanisms responsible for inducing apoptosis are not fully known.
Central Tolerance
Negative Selection During B cell Development

- If B cells recognize an antigen for the first time as immature, they can be eliminated or inactivated.

- Functional deletion is more likely to occur.

- There is uniform agreement that sufficient engagement of the Ig receptor on immature B cells can lead to maturation arrest which may be followed by cell death.

- B cells can undergo light chain gene rearrangement, a process called “receptor editing” and be rescued.
Receptor editing in B cells is mediated by reactivating their RAG1 and RAG2 genes and expressing a new Ig light chain, thus acquiring a new specificity.

To induce B cell tolerance, the antigen has to be multivalent (signaling requires cross linking), be present at high enough concentration and react with Ig receptor with a high enough affinity.

B cell tolerance is short-lived.
Tolerance Exists in Both T and B Cells

However, the Kinetics and Waning of Tolerance Induction Differs in T and B Lymphocytes
Peripheral Tolerance

- Tolerance Induction In Mature T cells: Clonal Anergy

- Lymphocyte activation:
  - TCR occupancy and CD28 binding to B7-1 (CD80) or B7-2 (CD86) known as the costimulatory signal.
  - Clonal anergy results from the lack of a second signal or from partial agonist peptide in the presence of costimulation.
  - Antigen contact is necessary.
If CD4+ T cells specific for a peptide antigen encounter a mutated form of the antigen in which amino acid residues that contact the TCR are altered, the cells may be rendered anergic.

Such mutated antigens are called peptide antagonists and they belong to a class of antigens called altered peptide ligands.
2- Activation Induced Cell Death

- More than 90% of T cells responding to antigens die due to homeostatic regulation of the immune system, rather than antigen specific tolerance per se.

- Room must be maintained for the influx of new cells as well as the preservation of memory T cells for an extended period of time.

- Repeated stimulation of T lymphocytes by persistent antigens result in death of the activated cell by a process of apoptosis.
3- Immune Deviation

- A general reciprocal relationship between antibody production and DTH reactions as a function of affinity and antigen dose; high affinity and small doses favoring DTH and low affinity and large doses stimulating Ab production.

- Due to a reciprocal regulation between two interacting T cell populations specific for the same antigen but with different effector functions.

- $\text{Th}_1 \rightarrow \text{IFN}_{\gamma}, \text{TNF}_{\alpha}, \text{and IL-12} \rightarrow \text{DTH}$
- $\text{Th}_2 \rightarrow \text{IL-4, IL-5, IL-6, and IL-10} \rightarrow \text{Ab production}$
<table>
<thead>
<tr>
<th>Disease</th>
<th>Th1</th>
<th>Th2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental Leishmaniasis</td>
<td>Cure</td>
<td>Progression</td>
</tr>
<tr>
<td>Experimental autoimmune encephalomyelitis</td>
<td>Progression</td>
<td>Prevention</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>Cure/Prevention</td>
<td>Progression</td>
</tr>
<tr>
<td>Atopy</td>
<td>Prevention</td>
<td>Progression</td>
</tr>
<tr>
<td>Type 1 Diabetes (NOD)</td>
<td>Progression</td>
<td>Prevention</td>
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4- CD8$^+$ T cell Tolerance

- CD8$^+$ T cell activation by antigen specific (signal 1) alone in the absence of CD4$^+$ T cell help (IL-2) is tolerogenic

- CD8$^+$ T cells produce cytokines that block the activation and functions of effector T lymphocytes

- **Veto cells**: Carry CD8 that engage the α3 domain of the MHC class 1 molecule on the recognizing cell as signaling through MHC along with TCR signaling leads to apoptosis.
The principal fate of lymphocytes that recognize self antigens in the generative organs is death (deletion), **BUT:**

- Some B cells may change their specificity (called “receptor editing”)
- Some CD4 T cells may differentiate into regulatory (suppressive) T lymphocytes
Suppression of autoreactive T cells by regulatory T cells requires them to interact with the same antigen-presenting cell.

Figure 11-21 The Immune System, 2/e (© Garland Science 2005)
Properties of regulatory T cells

- Phenotype: CD4, high IL-2 receptor (CD25), low IL-7 receptor, Foxp3 transcription factor; other markers

- Mechanisms of action: multiple
  - secretion of immune-suppressive cytokines (TGFβ, IL-10, IL-35),
  - inactivation of dendritic cells or responding lymphocytes
Regulatory T cells

• Explosion of information about the generation, properties, functions and significance of these cells
  – Some autoimmune diseases are associated with defective generation or function of Tregs or resistance of effector cells to suppression by Tregs

• Will cellular therapy with ex vivo expanded Treg become a reality?

• Therapeutic goal: selective induction or activation of Treg in immune diseases
Tolerance Induction in Mature B Cells

1- Receptor blockade and Antigen Characteristics
   (Poor degradability, extremes of Ag concentration)

2- B-Cell anergy and Death
   - Activation in the absence of T cell help
   - A characteristic feature is a 90% reduction of surface IgM
   - It is reversible and anergic B cells are short lived (3-4 days Vs 4-5 weeks).
Factors affecting tolerance: role of antigen

<table>
<thead>
<tr>
<th>Factors which affect response</th>
<th>Favor immune response</th>
<th>Favor tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of responding animal</td>
<td>Adult, immunological mature</td>
<td>Newborn (mice) Immunologically immature</td>
</tr>
<tr>
<td>Differentiation state of cells</td>
<td>Fully differentiated, Memory</td>
<td>Undifferentiated B cell with only IgM, T cells in the thymic cortex</td>
</tr>
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</table>
## Factors affecting tolerance: role of antigen

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<th>Favor immune response</th>
<th>Favor tolerance</th>
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<tr>
<td>Physical form of antigen</td>
<td>Large, aggregated, complex molecules</td>
<td>soluble, aggregate-free, simple small molecules</td>
</tr>
<tr>
<td>Antigen processing</td>
<td>properly processed</td>
<td>improperly processed</td>
</tr>
<tr>
<td>Route of injection</td>
<td>Subcutaneous or intra-muscular</td>
<td>Oral or, sometimes, intravenous</td>
</tr>
<tr>
<td>Dose of antigen</td>
<td>Optimal dose</td>
<td>Very large or very small dose</td>
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Inhibition by Antibody Feedback

- Passively administered antibody can prevent an antibody response

- Antibody produced during an immune responses leads to elimination of antigen (stimulus)
  - Less antigen available to stimulate specific cells
  - Immune complexes can bind to inhibitory receptors

Application: Anti-D (RhoGam) for Hemolytic Disease of the Newborn (HDN)
IN Conclusion, Tolerance is

- A state of functional unresponsiveness for a particular antigen (Antigen specific) that may occur in the context of a non-inflammatory immune response.

- An active process that is not inherited but somatically acquired

- Achieved through clonal deletion or clonal anergy and may be overcome leading to autoaggressive immune responses.
Many states of unresponsiveness observed following the introduction of antigens to a mature immune system are actually the result of negative regulation of one type of immune response by another. This state is sometimes called “Split” tolerance.
Mechanisms

1- Immune Deviation

2- Oral Tolerance

- Develops in relation to protein antigens and is a T-cell mediated phenomenon.

- Other factors that influence the development of oral tolerance include antigen dose, genetic makeup, prior immunization and the level of overall immunologic activation.
3- Suppression (Suppressor T cells?)

4- Antibody – Mediated Tolerance (original antigenic sin)

5- Antidiotypic B-cell Regulation

6- Antidiotypic T-cell Regulation.
   The generation of regulatory T cells that could suppress an immune response by recognizing the receptor on responding T cells (recognition of unique peptides derived from TCR).
Immune Privileged Sites and Tissues

- Certain areas in the body are more favorable for grafting than others (the brain, the cornea, the anterior chamber of the eye, the uterus, and the testis).

- Likewise, certain tissues are more suitable for transplantation than others; including cornea, brain cells, bone, cartilage, heart valves, and fetus.

- Mechanisms are; lack of dendritic cells (APCs), lack of lymphatic drainage, lack of MHC molecules, resistance to vascularization, presence of special barriers, and expression of Fas ligand.
The Fetal – Maternal Relationship

- It is a form of immune privilege that was suggested to be due to the lack of expression of histocompatibility antigens by the placenta.

- Other mechanisms include, expression of Fas ligand in the placenta and the production of cytokines and hormones by the placenta which would inactivate T cells or deviate them towards a Th2 response.
Breaking of Tolerance

- The immune system is not fully tolerant to all self antigens, therefore autoimmunity could develop in normal individuals.

- Tolerance at the B cell level is readily broken (use of cross-reacting antigens).

- At the T cell level, however, the immune system appears to take extra precautions to ensure that autoreactive T-cell clones are deleted in the thymus.