**Establishment and Maintenance of Tolerance**

**The term tolerance** applies to the many layers of protection imposed by the immune system to prevent the reaction of its cells and antibodies against host components.

* In the first step of this process, a phenomenon **termed central tolerance** deletes T- or B-cell clones before the cells are allowed to mature if they possess receptors that recognize self antigens with high affinity. Central tolerance occurs in the primary lymphoid organs: the bone marrow for B cells and the thymus for T cells.
* Because central tolerance is not perfect and some self-reactive lymphocytes find their way into the periphery and secondary lymphoid tissues, additional safeguards limit their activity. These backup precautions include **peripheral tolerance**, which renders some self-reactive lymphocytes in secondary lymphoid tissues inactive and generates others that actively inhibit immune responses against self.
* Antigens that induce tolerance are called **tolerogens** rather than immunogens. . Here, context is important; the same chemical compound can be both an immunogen and a tolerogen, **depending** on how and where it is presented to the immune system. **For instance**, **(1)** an antigen presented to T cells without appropriate costimulation results in a form of tolerance known **as anergy** (unresponsiveness to antigenic stimulus), whereas the same antigen presented with costimulatory molecules can become a potent immunogen**.(2)** When some antigens are introduced orally, tolerance can be the result, whereas the same antigen given as an intradermal or subcutaneous injection can be immunogenic. In other instances, mucosally administered antigens provide protective immunity, such as in the case of Sabin’s oral polio vaccine.

**Factors that promote tolerance rather than stimulation of the immune system by a given antigen include the following:**

• High doses of antigen

• Long-term persistence of antigen in the host

• Intravenous or oral introduction

• Absence of adjuvants (compounds that enhance the immune response to antigen)

• Low levels of costimulation

• Presentation of antigen by immature or un activated antigen-presenting cells (APCs)

**Antigen Sequestration Is One Means to Protect Self Antigens from Attack**

In addition to the various mechanisms of central and peripheral tolerance, an effective means to avoid self-reactivity is sequestration or compartmentation of antigens.

* sequestration or compartmentation of antigens.**For example,**the anterior chamber and lens of the eye are considered sequestered sites, without lymphatic drainage and possessing tissue-specific privileged antigens that are normally isolated from interaction with immune cells.
* one possible consequence of sequestration is that the antigen is never encountered by developing lymphocytes, and thus active tolerance to the sequestered antigen is not established.
* **Trauma to one eye that allows entry of immune cells can result in inflammation in that eye, leading to tissue destruction and impaired vision**. In these cases, the other eye may also become inflamed due to the sudden entry of clones of these recently activated immune cells recognizing newly discovered tissue-specific antigens.

**Central Tolerance Limits Development of Autoreactive T and B Cells**

One mechanism strongly influencing central tolerance is the deletion during early stages of maturation of lymphocyte clones that have the potential to react with self components later. Generation of variable regions that react with self antigens is almost inevitable (sure to happen). If this were allowed to occur frequently, such TCR or Ig receptors could produce mature functional T or B cells that recognize self antigens, and autoimmune disease would ensue.

**cells undergo a developmentally regulated event**:

* **negative selection**. This results in the induction of death (apoptosis) in some, but not all, cells that carry potentially autoreactive **TCR or Ig receptors**.
* **receptor editing** work to eliminate many autoreactive T cells in the thymus and autoreactive B cells in the bone marrow in central tolerance. In this process, the antigen-specific V region is “edited” or switched for a different V-region gene segment via V(D)J recombination, sometimes producing a less autoreactive receptor with an affinity for self antigens below a critical threshold that would lead to disease, allowing the cell to survive (central tolerance processes)
* More recently, it has been discovered that some of these self-reactive T cells in the thymus may be spared, and that these cells may function in the periphery as antigen-specific **regulatory cells** (TREG)working to dampen(diminish) immune responses to the antigens that they recognize.

All these are now recognized as mechanisms that lead to central tolerance in developing B cells. By similar mechanisms, T cells developing in the thymus that have a high affinity for self antigen are deleted, primarily through the induction of apoptosis.

**Peripheral Tolerance Regulates Autoreactive Cells in the Circulation**

lymphocytes with specificity for self antigens are not uncommon in the periphery. Two factors contribute to this:

(1) not all self antigens are expressed in the central lymphoid organs where negative selection occurs, and

(2) there is a threshold requirement for affinity to self antigens before clonal deletion is triggered, allowing some weakly self-reactive clones to survive the weeding-out process.

Just like central tolerance, the mechanisms that control peripheral tolerance have been demonstrated by a variety of experimental strategies.

**IN T Cells:**

* In order for **T cells** to become activated, the TCR must bind antigen presented by self-MHC (major histocompatibility complex molecules) **(signal 1),**
* while at the same time the T cell must undergo costimulatory engagement **(signal 2)**.
* when CD4+ T-cell clones are stimulated in vitro through the TCR alone, without costimulation, they become **anergic**. Subsequent data showed that the interaction between CD28 on the T cell and CD80/86 (B7) on the APC provided the costimulatory signal required for T-cell activation. **This led to a careful examination of costimulation, revealing the existence of other molecules that could bind to CD80/86 and the discovery of a related molecule, called CTLA-4**. [This molecule inhibits rather than stimulates T-cell activation upon binding CD80/86]. We now appreciate that many such molecules deliver supplementary signals during T-cell activation, and the group of molecules that regulate T-cell behavior are now often referred to as **immunomodulatory**, to cover both costimulatory and inhibitory behavior . **-🡪**: CTLA-4 expression is induced only after T cells are activated, providing a mechanism to dampen T-cell activity and regulate the immune response. Mice lacking CTLA-4 display massive proliferation of lymphocytes and widespread autoimmune disease, suggesting an essential role for this molecule in maintaining peripheral tolerance.

**IN B Cells:**

Peripheral tolerance in B cells appears to follow a similar set of rules. For instance:

* experiments with transgenic mice have demonstrated that when mature B cells encounter most soluble antigens in the absence of T-cell help, they become **anergic and never migrate to germinal centers**. [In this way, maintenance of T-cell tolerance to self antigens enforces B-cell tolerance to the same antigens].
* In T cells, a third mechanism for maintaining tolerance, in addition to T-cell anergy and apoptosis, is through the activity of regulatory T cells (TREG cells). Acting in secondary lymphoid tissues and at sites of inflammation, TREG cells recognize specific self antigens, and sometimes foreign antigens, via TCR interactions. However, they down-regulate immune processes when they engage with these antigens in the periphery.

**How TREG cells can be generated??**

They can generated both naturally, in the thymus (nTREG cells,), and after induction by antigen in the periphery (iTREG cells;). In fact, many of the circulating T cells with specificity for self antigens may be such regulatory cells. nTREG cells specializing in regulating responses against self antigen to inhibit autoimmune disease and iTREG cells controlling reactions against benign foreign antigens at mucosal surfaces, where the immune system comes in constant contact with the outside world (e.g., gut commensals or respiratory allergens).

1. **TREG cells were most recently characterized as a unique subset of CD4+ T cells** that express high levels of the IL-2R α chain (CD25), the low-affinity receptor for this cytokine.

* **Naturally occurring nTREG cells arise from a subset of T cells expressing receptors with intermediate affinity for self antigens in the thymus**. Certain of these cells up-regulate the transcription factor FoxP3 and then develop into cells that migrate out of the thymus and are capable of suppressing reactions to self antigens.
* CD4+ TREG cells have also been found to suppress responses to some nonself antigens. For example, these cells may control allergic responses against innocuous (causing no injury) environmental substances and/or responses to the commensal microbes that make up the normal gut flora. depletion of this CD4+ T cell subset increase the susceptibility to disease, suggesting that these regulatory cells play a role in suppressing autoimmunity.
* The importance of FoxP3, which appears to be both essential and sufficient for the induction of immunosuppressive function.
* Naïve T cells that have escaped to the periphery also can be **induced** to express FoxP3 and acquire regulatory function (**iTREG cells**). **Factors that favor the development of iTREG cells include**
* the presence of certain cytokines during antigenic stimulation,
* chronic low-dose antigen exposure, and
* lack of costimulation or the presentation of antigen by immature dendritic cells (DCs).

Q// In studying the mechanisms by which TREG cells inhibit immune responses,HOW??both contact-dependent and contact independent processes have been observed.

1. CD4+ TREG cells have been shown to kill APCs or effector T cells directly, by means of granzyme and perforin.
2. TREG cells may also modulate the function of other cells responding to antigen by surface receptor engagement. One prime example of this is the expression of CTLA-4. TREG cells express high levels of this immune inhibitory receptor. interaction of CTLA-4 on TREG cells with CD80/86 on an APC can lead to inhibition of APC function, including reduced expression of costimulatory molecules and proinflammatory cytokines, such as IL-6 and tumor necrosis factor-α (TNF-α). At the same time, these targeted APCs begin to express soluble factors that inhibit local immune cells.
3. TREG cells themselves also secrete immune inhibitory cytokines, such as IL-10, TGF-β, and IL-35, suppressing the activity of other nearby T cells and APCs.
4. Finally, because TREG cells express only the low-affinity IL-2R α (CD25) but not the β or γ subunits, which are required for signal transduction prevent expansion of local immunostimulatory effector T cells.
5. **CD8+ TREG** cells can play a role in inhibiting responses to self antigens is now fairly well established,unlike in the case of CD4+TREG cells, the contribution to this population from thymic selection (nTREG cells) is likely very small. However, in the presence of antigen and TGF-β, CD8+ regulatory T cells expressing FoxP3 can be induced (iTREG).

**Like with CD4+ TREG cells,** **CD8+ TREG cells have three main pathways seem to exist:**

* APC lysis,
* inhibition of APC function, and
* regulation of effector T cells that share cognate antigen with the CD8+ TREG cell.