Immunological Tolerance

Introduction:

Immunological tolerance is the other face of autoimmunity. Autoimmunity and tolerance are two faces of a coin. Exposure to an antigen may or may not lead to an immune response, depends on the presence of immune response genes and characteristics of the substance. If the antigen is recognized as foreign antigen an immune response will be generated, if the body fails to recognize the antigen as a foreign object no immune response is generated leading to a state of tolerance.

And different forms of the same antigens can cause different outcomes. Certain antigens will promote a humeral antibody response, other will promote cell mediated immunity, and some cells can promote both cell types of an immune response. But all of these are govern by innate property of the immune system which is its ability to discriminate between self and non-self, so somehow the immune system is equipped with the mechanism that enables the discrimination between self and non-self.

We inherit the capacity to mount an immune response against self. We said that repertoires of immune cells that carry different receptors have the receptors as a consequence of gene rearrangement that recognize self but those cells that carry such receptors are deleted physically or functionally so this matter is dealt with the phenotypic level.

After expression of BCR/TCR auto-reactive cells are deleted (some cells escape deletion). Other mechanisms that operate peripherally are activated during generation of an immune response to get rid of auto-reactive cells that escape deletion. Failure of these mechanisms leads to autoimmunity.

Balancing lymphocyte activation and control:

In healthy individuals there is a balance between lymphoid activation and control in the periphery, we have two mechanisms that operate with restrict balance, these are: the effector mechanism and regulatory mechanism of the immune system.
 ✓ Effector mechanisms: if activated it will mount response against foreign substances “Pathogens” and as a consequence they promote inflammation. Inflammatory disease will be involved as a result of the reaction with self, because the inflammatory system doesn’t discriminate between self and non-self.

 ✓ Regulatory mechanisms: modulate the immune system, reduce response against foreign substances to maintain response against self. Controlled response to antigen.

Now, why immune Regulation operates in such balance?

1. The immune regulation is important to avoid excessive Lymphocyte activation and tissue damage during normal protective responses against infection... Whenever the response takes place the host’s immune response is exaggerated and destroys self-tissue. A phenomenon which is referred to as hypersensitivity. So immune Regulation tries to avoid such process, but this takes place and that’s why we have four the different types of the hypersensitivity reactions.
   Types of hypersensitivity reactions:
   - TYPE I: immediate or anaphylactic hypersensitivity.
   - TYPE II: cytotoxic hypersensitivity
   - TYPE III: immune complex hypersensitivity
   - TYPE IV: cell mediated or delayed type hypersensitivity

2. To prevent inappropriate reaction against self “self-tolerance”, so the establishment of self-tolerance especially at the peripheral level is the function of immune regulation.
3. Failure of control mechanisms is the underlying cause of immune-mediated inflammatory disease.

There are general mechanisms that operate during mounting immune response, response against pathogens declines as the infection is eliminated. If the infection is eliminated cellular or humeral mechanisms act to prevent the continuous response against foreign substance and this is mediated by apoptosis of lymphocytes, so those lymphocytes that were recruited die, and memory cells in this case will survive.

**Immunological tolerance**

Specific unresponsiveness to an antigen that is induced by exposure of lymphocytes to that antigen (tolerogen vs. immunogen).

Tolerogen is an antigen that does not induce in immune response.

Tolerance requires the introduction of an antigen to the host and requires the exposure of lymphocytes to those antigens. If no response takes place, then we can say this is a
state of tolerance but without the introduction of an antigen we can’t label a substance as tolerogen.

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 (which is associated with expression of new antigens to the host similar to transplantation).

But the immune system is capable of maintaining responses against foreign substances and destructive responses are prevented by a variety of mechanisms, more than one mechanism that operate during the development of immune system which we call central tolerance or during the generation of each immune response and that’s the peripheral tolerance.

**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 (Lymphocytic choriomeningitisvirus) in utero into mice producing an infection that was long-lived. Basically LCMV was injected during maturation of the immune system; it was recognized as self leading to a tolerance and a long-lasting infection. (since no immune response will be generated against LCMV)

(1945) Probably, the strongest evidence for tolerance was instructed by Owen, who demonstrated that dizygotic twin claves accepted mutual skin grafts on adulthood. As you know twins can be dizygotic or monozygotic each one can have its own placenta or they share the same placenta.

Owen has introduced that skin grafts taken from either animals was mutually accepted. No rejection of grafts took place, although, they are not identical twins and differ in their antigenic make up, they accepted skin grafts from each other. And he explained this on the principle of “parabiosis” which is sharing of the same blood supply and as a consequence antigens from both were exposed to each other during the fetal life so blood borne antigen were introduced into these animals and as a consequence these accepted grafts from each other (skin grafts) when they become adults, became immunologically mature they accepted grafts from each other.

Tolerance resulted from sharing the same blood supply in fetal life.
(1949) Macfarlane Burnet: (who proposed the clonal selection theory) 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. He proved the work of Medwar in chicken.

- In Humans, an immune response to non-inherited paternal antigens but no response to non-inherited maternal antigens. Non-inherited maternal antigens are introduced during fetal life (during maturation of the immune response), the self recognizes the antigens are self-leading to tolerance to the antigens.

**Medawar’s Experiment:**

In animals -this is the simple experiment of Meduwar for which he was rewarded - he took two strains of mice “A” and “B” and group A mice were divided to two subgroups. One group received splenocytes from strain “B” and the other didn’t receive these splenocytes, these mice that were not injected with splenocytes rejected skin grafts when they become adults from the other group whereas these mice that were injected with splenocytes as neonates accepted the grafts later (so the grafts was accepted because were injected with splenocytes.

He demonstrated that exposure to an antigen during immunological immaturity leads to a state of tolerance.

So tolerance is a phenomenon that is established when an individual is exposed to an antigen during immunological immaturity, and mechanisms that operate to establish immune state of tolerance are variable some of them which are central others are peripheral.
We have 2 types of tolerance: Central tolerance and Peripheral tolerance

1. Central Tolerance (Takes place in the thymus and bone marrow during maturation of lymphocytes).

   - 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 cells 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.

   Repertoires of T-cell that can mount a response against self will be eliminated in the thymus and this involves of course presentation of the antigens via dendritic cells in the thymus, so we know now that T cells that leave the thymus are MHC distributed because the process of positive selection, and if they fail to do positive selections they will be regulated, and lymphocytes that recognize self-antigens will be eliminated by the process of negative selection in the thymus.

   Negative selection during B cell development:

   Now during B cell development similar process takes place as mentioned previously, B cell interact with an antigen if the antigen is foreign B, cell can’t interact with it, it will not cause B cell receptor cross linking and the B Lymphocytes will undergo selection.
However, B lymphocytes that interact with antigens in the bone marrow will be treated according to the interaction. If the antigen is multivalent, it will result in multivalent cross linking of T-lymphocytes with high affinity, and those B-lymphocytes will be given the chance to change their receptor which is “Receptor Editing” if they succeed they will continue maturation if not they will be negatively selected. whereas lymphocytes that recognize soluble antigens with low affinity will undergo anergy and Clonal Ignorance, which they are clonally ignorant B lymphocytes because they don’t recognize self-antigens with high affinity, those antigens are present in very low concentrations and bind with low affinity B cells and that’s why even though B-lymphocytes carry receptors that can be recognized But they don’t mount an Immune Response

-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.

remember to induce B cell tolerance these should be cross linking with high enough concentrations of multivalent antigens and Ig receptor with high enough affinity and B cell tolerance is not as T cell tolerance B cell tolerance is short lived and its more easily into induced as compared to T cell tolerance, T cell tolerance is difficult to induce as it is longer lasting than B cell tolerance. B cell tolerance can be lost with time whereas T cell tolerance is long lasting.
The dynamics of this tolerance kinetics and waning of tolerance induction is different in T and B lymphocytes. However, if we are talking about tolerance remember we are talking about those clones of B or T lymphocytes that recognize an antigen so tolerance present as long as the clone carry the receptor of that antigen persisting in circulation.

Central tolerance does not remove all auto-reactive cells; some auto-reactive cells can escape the process because of:

1. Lack of efficient interactions.
2. Failure to introduce antigens to auto-reactive maturing cells.

Peripheral tolerance acts on these auto-reactive cells that escape.

2. **peripheral tolerance**: The other form of tolerance and immuned to the level of mature cells, the most important type of tolerance is clonal anergy. We know that an immune response requires two segments (1) cross linking (2) Co-Stimulatory segment. The co-stimulatory segment can be of more than one type, but the most important is the interaction between CD28 and CD86 or CD80.

1. Clonal Anergy
2. Activation Induced Cell Death
3. Immune Deviation
4. CD8+ T cell Tolerance
**Clonal Anergy** (Functional deletion of lymphocytes)

Lymphocyte activation:

- TCR occupancy and CD28 binding to B7-1 (CD80) or B7-2 (CD86) known as the co-stimulatory 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**.

Peptide antagonists bind with low affinity to the TCR leading to anergy.

Sometimes clonal anergy induces apoptosis leading to cell death.

**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.

So activated T cells are removed to make room for memory cells and to new cells that are generated due to subsequent exposure to new antigens.

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

Persistent infection is due to persistent antigens = repeated stimulation of T lymphocytes resulting in cell death and the maintenance of the infection.

**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 (Th1 mediated) and low affinity and large doses stimulating Ab production (Th2 mediated).

Basically activation of one subset of T helper cells blocks the activation of the other.
- Due to a reciprocal regulation between two interacting T cell populations specific for the same antigen but with different effector functions.

- Th1 → IFNγ, TNFα, and IL-12 → DTH

- Th2 → IL-4, IL-5, IL-6, and IL-10 → Ab production

- 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 α 3 domain of the MHC class 1 molecule on the recognizing cell as signaling through MHC along with TCR signaling leads to apoptosis.

Different subset activation = different outcomes for the same disease.

p.s : the doctor read the table in slide 22

- **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. The CD8+ T cell in this case induces tolerance.
- CD8+ T cells produce cytokines that block the activation and functions of effector T lymphocytes.

The tolerogenic T cell interacts with another CD8+ T cell mounting a response to the antigen and stops it.

- Veto cells: Carry CD8 that engage the α 3 domain of the MHC class 1 molecule on the recognizing cell as signaling through MHC along with TCR signaling leads to apoptosis.

Prevent cytotoxic killing by the other CD8+ T cell.

CD4+ CD25+ Treg cells need antigen presentation by professional APC (just like effector T cells), this leads to it's activation which suppresses another CD4+ T cell that is already engaged.

**Properties of regulatory T cells (Treg 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 (for T-cells)).

P.S:

Check the tables that show the factors which affect immune response and tolerance.

➢ 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)
D- Mothers who give birth to D+ infant will produce and immune response to the 2nd D+ infant, this is prevented by injecting the mother with Anti-D antibodies right after giving birth to the 1st infant, these Anti-D antibodies will neutralize infant’s D+ antigens and prevent the processing and presentation of these antigens = no future immune response generated against D+ antigens.

- **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.

- **IMMUNOREGULATION**
  - 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 of immune regulation:**

1. immune Deviation
2. Oral Tolerance
   - Develops in relation to protein antigens and is a T-cell mediated phenomenon.

Remember M-cells and γδ-T cells are responsible for oral tolerance.

   - 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?) (Treg)
4. Antibody – Mediated Tolerance (original antigenic sin)

The best example is influenza; the immune system may not be able to recognize the difference between the antigens presented by a certain strain in a recent infection and the antigens that
were presented by a similar strain in a previous infection, resulting in generation of the immune response that was generated against the previous infection which may be less efficient against the new strain.

This is mediated by memory B lymphocytes.

5. Antidiotypic B-cell Regulation

6. Anti-idiotypic 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 (Bone), presence of special barriers (Placenta blood-testis barrier), 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.