SHEET NO. 15

IMMUNOLOGY

DOCTOR 2019 | MEDICINE | JU

DONE BY : Doctor 2018

SCIENTIFIC CORRECTION :

GRAMMATICAL CORRECTION :

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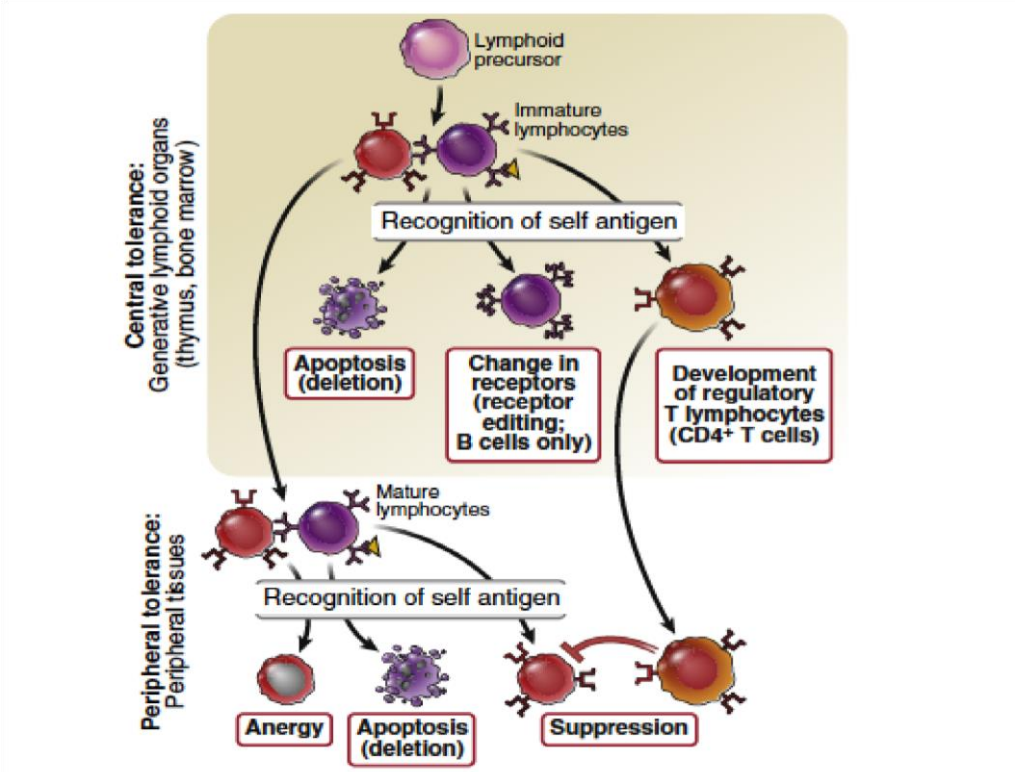
Immunological Tolerance(IT) and Autoimmunity(AI)

- We will start with the definition of IT , which is the ability of the immune system to discriminate between self and non-self antigens , that's it , when your body doesn't make antibodies against self antigens , it's said to be tolerant. When it does so , Autoimmunity will develop as we'll see later.
- Another definition for IT is the lack of immunological response to an antigen , induced by the exposure of lymphocytes to this antigen previously.
- *There're some terms that you should be familiar with :-*
  - Immunogenic antigen : An antigen that elicits an immune response (bacterial and viral antigens for example).
  - Tolerogenic antigen : An antigen that induces tolerance , where the lymphocytes become functionally inactive or they're killed.(such as self antigens)
  - Immunological Ignorance : As the term implicates , here the lymphocytes IGNORE the presence of the antigen(either self or non self antigen). This phenomenon is seen in Immune Privileged Sites (IPSs) such as Brain , Testes and lens of the Eye. You should notice that these areas are physically separated from antigens (ie; there's no Lymphatic drainage) and the idea behind this separation is to protect these IPSs from damage.
- IT is an important subject because (1) its failure is the underlying cause of AI , (2) also , if it's well studied , there'll be production of tolerance against specific antigens and thus prevention of unwanted immune responses as in cases of transplantation .(3) Strategies for inducing tolerance are being tested to treat allergic and
- autoimmune diseases and to prevent the rejection of organ transplants.(4) Another application of this is "Gene therapy" to prevent immune responses against products of newly expressed

genes or vectors and even for stem cell transplantation if the stem cell donor is genetically different from the recipient.

- There're two types of IT :- **Central IT** (In central (generative)lymphoid organs , ie; Bone Marrow and Thymus) and **Peripheral IT** (In peripheral(secondary) lymphoid organs).

  *** Remember that Central tolerance isn't perfect as some cells can escape. Another difference between central and peripheral tolerance is that the earlier occurs at the level of immature lymphocytes while peripheral IT occurs at the level of mature lymphocytes.



- This scheme is very important as it summarizes what happens.
- In the upper part of the diagram you see the central tolerance , it occurs at the level of immature lymphocytes that recognize self antigens , they either *(1) Die by apoptosis , (2) Change in receptors*** , *(3) Development of regulatory T lymphocytes (CD4+ T cells)* or *(4) Anergy for B cells ONLY***

** The change in receptors happens ONLY in B cells through what's called *Receptor editing*. During which B cells increase the expression of Recombinant Activating Genes (RAGs) and they change their LIGHT chain (if it was κ-kappa they'll change it to λ-lambda and vice versa)and therefore the specificity of the receptor toward the antigen will change (heavy chain doesn't change here), in order not to die by apoptosis (During –ve selection) and this is the most common method in removing self reactive B cells.

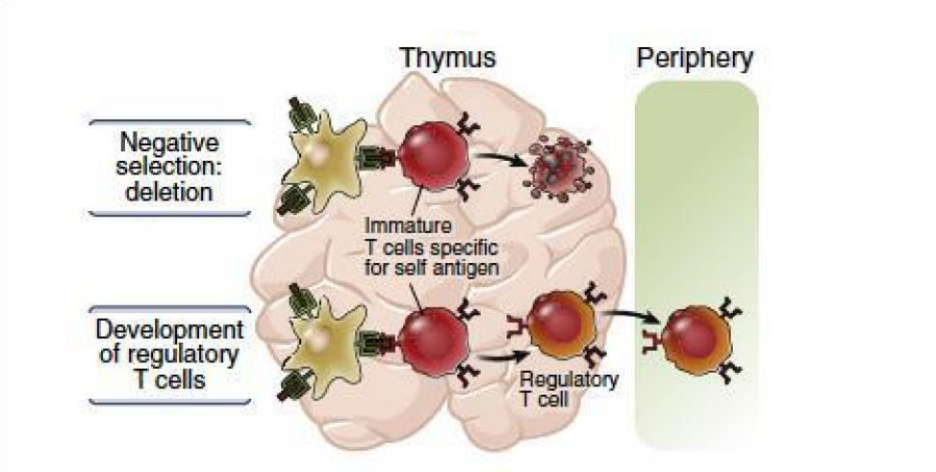
** Anergy is known as Long-standing functional unresponsiveness that is induced when these cells recognize self antigens , ie ; lymphocytes are viable but they're non-functional , I'll will talk about it in more details later on.

- In the lower part of the diagram you see the peripheral tolerance where self reactive MATURE lymphocytes have multiple fates : (1) Anergy for BOTH B and T cells , (2) Apoptosis or (3) Suppression by Regulatory T cells.

- ***<u>Central T lymphocytes Tolerance(CTLT):-</u>***

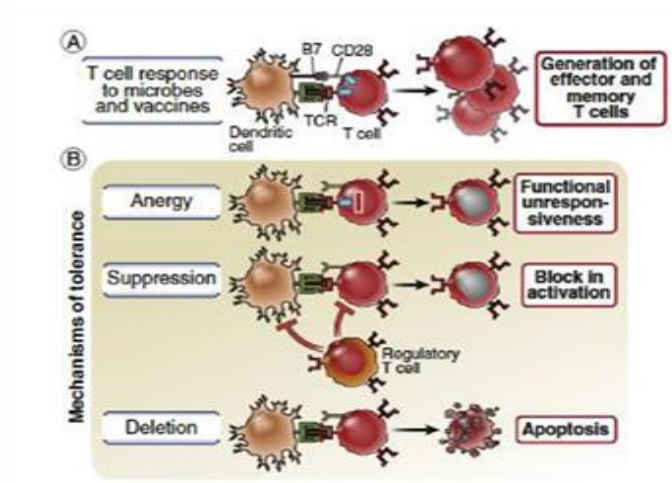
- If you refer to the diagram you'll see that the principal mechanisms for CTLT are death of immature T cells (negative selection) and the generation of CD4+ regulatory T cells .
- Immature lymphocytes may interact strongly with an antigen if the antigen is present at high concentrations in the thymus and if the lymphocytes express receptors that recognize the antigen with high affinity. Antigens that induce negative selection may include proteins that are abundant throughout the body, such as plasma proteins and common cellular proteins.
- Remember that self antigens play a vital role in –ve selection of T cells. (because foreign antigens are not found in central lymphoid organs ,only self antigens are there so they determine –ve selection in central IT)

- These antigens are triggered (Expressed in the thymus) by AIRE (AutoImmune REgulator) protein.
- This process happens in the thymus for T cells.



- ***<u>Preipheral T lymphocytes Tolerance (PTLT):-</u>***

 Peripheral tolerance is induced when mature T cells recognize self antigens in peripheral tissues, leading to-as you can see in the diagram- :1-functional inactivation (anergy) or 2-death, or 3- when the self-reactive lymphocytes are suppressed by regulatory T cells.(Antigen recognition without adequate co-stimulation results in Tcell anergy or death, or makes T cells sensitive to suppression by regulatory T cells)



Anergy :-

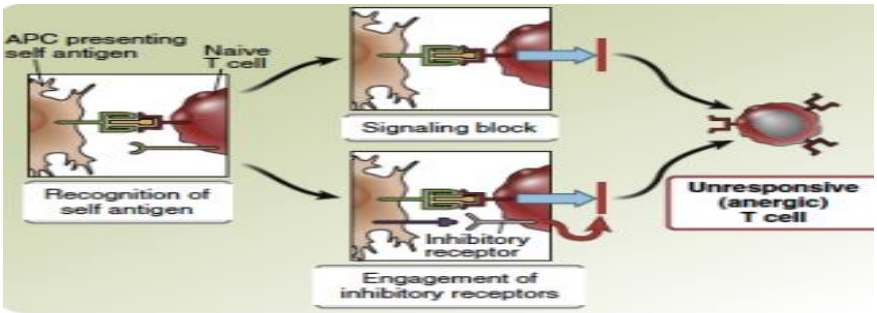
- As we said before , Anergy is … ?
- To understand this , let me remind you that a Naive lymphocyte needs two signals in order to become activated :

  (1) The binding of the antigen to its receptor on the lymphocyte (whether it's present on MHC for T cells or Free for B cells)

  (2) Co-stimulatory signal , which is achieved by the binding of B7 protein on APCs with its receptor on the lymphocyte , CD28. This binding should be strong in order to activate the lymphocyte.
- If the Co signal is weak , the first signal will lose its ability to transmit activating signals , and sometimes , the first signal will activate some enzymes (eg; ubiquitin ligases) that target signaling proteins for intracellular degradation by proteases.
- Also-On recognition of self antigens-, if the binding is weak , T cells preferentially engage one of the inhibitory CD28 receptors family , for example , Cytotoxic T Lymphocyte associated Antigen 4 (CTLA-4 or CD152) or Programmed Death protein 1 (PD-1). In these cases the signal is converted from B7-CD28 activation to either B7-CTLA4 inhibitory or B7-PD1 which induces apoptosis.
- CTLA-4 is expressed transiently on activated CD4+ T cells and constitutively on regulatory T cells.it functions to terminate activation of responding T cells and also mediates the suppressive function of regulatory Tcells . CTLA-4 works by blocking and removing B7 molecules from the surface of APCs, thus reducing costimulation and preventing the activation of Tcells.
- PD-1 is expressed on CD4+ and CD8+ T cells after antigen stimulation. It has an immunoreceptor tyrosine-based inhibitory motif(ITIM) typical of receptors that deliver inhibitory signals,

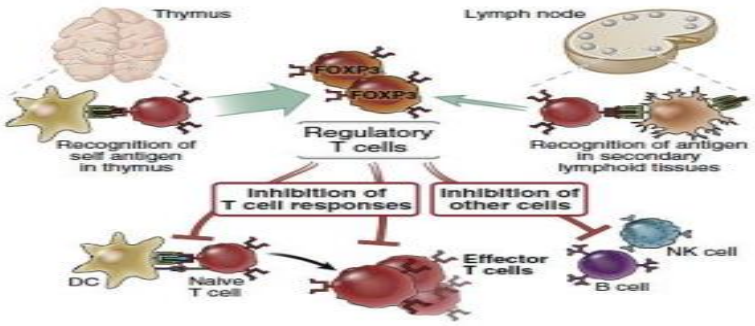
PD-1 terminates responses of Tcells to self antigens and also to chronic infections, notably virus infections.

** The process by which T cells determine whether they'll express CTLA-4 or PD-1 is complicated and not well understood.



- ***Immune Suppression by Regulatory T cells:-***

- Regulatory cells as we said before , either develop in the thymus (after they recognized self antigens but didn't die by –ve selection NOTE that we used a big arrow to represent this in the picture)or in the periphery (some cells differentiate into T reg cells)after their recognition of self antigens and the function of these cells is to regulate immune responses. ***Most regulatory T cells are CD4+***
- When flow cytometry is done we search for these things in T reg cells: 1-thsese cells express high levels of CD25 (which is an IL-2 receptor)and 2-they also express a transcription factor called FoxP3.
- As mentioned earlier CD25 is an IL-2 receptor , remember that IL-2 is an important cytokine for T cells activation and proliferation (ie; T cells also express CD25).



- There're many mechanisms by which T Reg cells suppress immune responses , of them are :

1- Production of some cytokines (immunosuppressive cytokines, eg; IL-10 and TGF-B) which are inhibitory cytokines for lymphocytes , DCs and Macrophages.

2- Expression of CTLA-4 and PD-1 .when they express CTLA-4, it may block or remove B7 molecules made by APCs

and make these APCs incapable of providing costimulation via CD28 and activating T cells. (The Co signal will be missed).

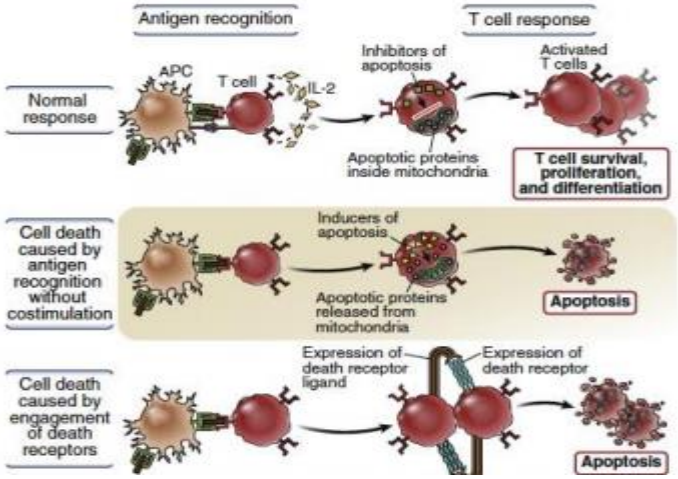
3- Also , highly expressed IL-2Receptor (CD25) reduces IL-2 levels, by binding and consuming most of the IL-2 ,thus reducing its availability for responding T cells so other T lymphocytes won't have enough IL-2 for their activation and differentiation.

- ***<u>Deletion (Apoptosis) :-</u>***

- When the lymphocytes recognize self antigens , pathways of production of pro-apoptotic proteins and receptors are activated.
- Let me remind you of the two pathways of apoptosis :-

⇨ Intrinsic pathway , happens when there's an imbalance between pro-apoptotic (BAK and BAX) and anti- apoptotic (BCL-2 and BCL-XL) proteins , this is followed by the leakage of Cyt C form mitochondria which in turn activates caspases.

⇨Extrinsic pathway , happens when Fas ligand bind to Fas Receptor (On the apoptotic cell) ;Recognition of self antigens may lead to the coexpression of death receptors and their ligands. This ligand-receptor interaction generates signals through the death receptor that culminate in the activation of caspases and apoptosis (Fas-FasL).

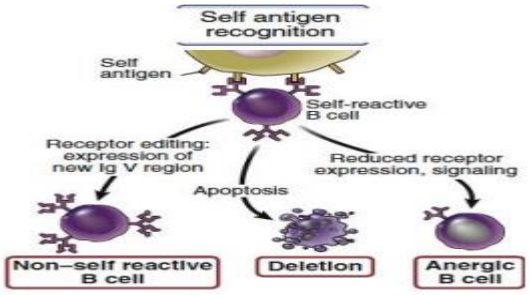


- Now let's talk about ***Central B Lymphocyte Tolerance:-***

- If you refer to the diagram in page 2 , you can see that When immature B lymphocytes interact strongly with self antigens in the bone marrow, the B cells either undergo Receptor Editing,apoptosis(deletion) or Anergy.
- 1-We've previously talked about Receptor editing but let's talk about it again. Self reactive immature B cells can escape death by altering their receptors(they may reexpress RAG genes, resume immunoglobulin (Ig) light-chain gene recombination, and express a new Ig light chain. This new light chain associates with the previously expressed Ig heavy chain to produce a new antigen receptor that may no longer be specific for the self antigen)
- 2-Deletion. If editing fails, immature B cells that strongly recognize self antigens receive death signals and die by apoptosis.

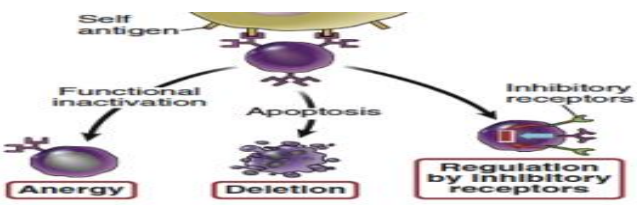
This process of deletion is similar to negative selection of immature T lymphocytes. As in the T cell compartment negative selection of B cells eliminate lymphocytes with high affinity receptors for abundant and usually widely expressed cell membrane or soluble self antigens.

3-Anergy. Some self antigens, such as soluble proteins, may be recognized in the bone marrow with low avidity. B cells specific for these antigens survive but antigen receptor is reduced, and the cells become functionally unresponsive(anergic)



- ***<u>Peripheral B Lymphocyte Tolerance :-</u>***

- If you refer to the diagram in page 2 , you can see that the mechanisms for PBLT are Apoptosis , Suppression by T Reg cells or Anergy.(note that we can have anergy of B cells in both central and peripheral tolerance but mainly in peripheral tolerance while for T cells we only have anergy in case of peripheral tolerance) **B cells express high levels of Fas then they wait for the signal of T cells that are expressing Fas-L which will bind to B cell and kill it.



Now one can ask , why we don't have immune responses against Normal Flora ? and why pregnant women don't have immune responses against Fetal Antigens??

- The answer for these questions is Tolerance, but the exact mechanism is unknown , an acceptable theory states that T Reg cells carry up this process.so we can consider these two conditions as an exception because although they are foreign antigens, the body will recognize them as self antigens and induce tolerance toward them.
- **Commensal Microbes(normal flora) reside in the intestinal and respiratory tracts and on the skin, where they serve many essential functions. Mature lymphocytes in these tissues are capable of recognizing the organisms but do not react against them ,so the

microbes are not eliminated,and harmful inflammation is not triggered .

- **the mother and her fetus are connected through the placenta thus there's bidirectional transfer of cells between both of them(microchimerism)and in this case ;fetus is immunodefficient while mother is immunocompetent therefore if tolerance is absent this will lead to"graft versus host disease" because mother's T cells will fight Fetus's antigens(since parental antigens are expressed in the fetus which are foreign to the mother) causing serious problems.

  ((there are two theories regarding microchimerism; the first one states that if the babies has higher microchimerism they have higher probability to develop autoimmune diseases in the future, while the second theory "hygiene theory" states that if the babies were exposed to more antigens ,their immune response will be better in the future))

- ***AutoImmunity :-***

- We already know that AI is an immune response against self antigens . AI is of two types , organ-specific affecting only one or few organs , or systemic affecting multiple tissues and clinical manifestations. tissue injury in autoimmune diseases may be caused by antibodies against self antigens or by T cells reactive with self antigens.
- As I previously mentioned , AI results when Tolerance fails to occur.
- **NOTE** :- AI differs from Uncontrolled immunity , in the latter , there's an antigen that elicits a strong immune response (Super Antigen) as seen in Mycobacterium TB's granuloma.
- autoimmunity is an important cause of disease, estimated to affect 2% to 5% of the population in developed countries, and the prevalence of several autoimmune diseases is increasing.(one of the reasons for this increase is hygiene theory)
- **Pathogenesis of AI:-**
  - ⇨ First of all , you should know that AI is a multifactorial process , it depends on Genetic factors , Environmental factors and failure of tolerance to occur (Interaction between these factors produces AI).
  - ⇨ Genetic Factors are attributed to multiple gene loci , most commonly MHC genes (MHC Loci).

Disease	MHC allele	Relative risk
Ankylosing spondylitis	HLA-B27	90
Rheumatoid arthritis	HLA-DRB1*01/*04/*10	4-12
Type 1 diabetes mellitus	HLA-DRB1*0301/0401	35
Pemphigus vulgaris	HLA-DR4	14

⇨ Notice that the presence of the gene only increases the risk and not necessarily means the presence of the disease.(we call this: genetic predisposition)

For example people who carry HLA-B27 allele have higher risk(90) to develop ankylosing spondylitis but this doesn't mean they will certainly develop it.

⇨ Other non-HLA genes polymorphisms are associated with various autoimmune diseases and may contribute to failure of self tolerance or abnormal activation of lymphocytes.

A

Genes that may contribute to genetically complex autoimmune diseases		
Gene(s)	Disease association	Mechanism
PTPN22	RA, several others	Abnormal tyrosine phosphatase regulation of T cell selection and activation?
NOD2	Crohn's disease	Defective resistance or abnormal responses to intestinal microbes?
IL23R	IBD, PS, AS	Component of IL-23 receptor; role in generation and maintenance of Th17 cells
CTLA4	T1D, RA	Impaired inhibitory checkpoint and regulatory T cell function
CD25 (IL-2Rα)	MS, type 1 diabetes, others	Abnormalities in effector and/or regulatory T cells?
C2, C4 (Complement proteins)	SLE	Defects in clearance of immune complexes or in B cell tolerance?
FCGRIIB (FCγRIIB)	SLE	Defective feedback inhibition of B cells

⇨ The Dr. just read the highlighted parts.

--Roles of non MHC genes in autoimmunity--

⇨ Single gene defects that cause AI (Mendelian diseases) :-

B

Single-gene defects that cause autoimmunity (mendelian diseases)		
Gene(s)	Disease association	Mechanism
AIRE	Autoimmune polyendocrine syndrome (APS-1)	Reduced expression of peripheral tissue antigens in the thymus, leading to defective elimination of self-reactive T cells
CTLA4	Autosomal dominant immune dysregulation syndrome	Impaired inhibitory checkpoint and regulatory T cell function leading to loss of B and T cell homeostasis
FOXP3	Immune dysregulation, X-linked polyendocrinopathy and enteropathy (IPEX)	Deficiency of regulatory T cells
FAS	Autoimmune lymphoproliferative syndrome (ALPS)	Defective apoptosis of self-reactive T and B cells in the periphery

⇨ This table is very important , the doctor read it and I think that it's required to know its info. ⇨ AIRE gene defects are AR.

⇨ Some notes about the table:

*in APS-1 disease ,antibodies will start targeting endocrine organs mainly the parathyroids ,adrenals and pancreas.

*first disease in this table(APS-1) is autosomal recessive

*the last 3 diseases are autosomal dominant

Role of Infections and Other Environmental Influences

- **Infections may activate self-reactive lymphocytes, thereby triggering the development of autoimmune diseases.** Clinicians have recognized for many years that the clinical manifestations of autoimmunity sometimes are preceded by infectious prodromes. This association between infections and autoimmune tissue injury has been formally established in animal models.

Role of Infections

- An infection of a tissue may induce a local innate immune response, which may lead to increased production of co-stimulators and cytokines by tissue APCs. These activated tissue APCs may be able to stimulate self-reactive T cells that encounter self antigens in the tissue. In other words, infection may break T cell tolerance and promote the activation of self-reactive lymphocytes.

- Some infectious microbes may produce peptide antigens that are similar to, and cross-react with, self antigens. Immune responses to these microbial peptides may result in an immune attack against self antigens. Such cross-reactions between microbial and self antigens are termed **molecular mimicry.**

Explanation of the role of tissue infection: if a microbe(bacteria,virus) caused inflammation there will be higher expression of B7 ,therefore there'll be higher probability of B7 engagement with CD28 thus, strengthen the co-stimulatory signal resulting in more activation of self reactive T cells which become more reactant to self antigens.

Example of molecular mimicry: infection caused by –group A beta hemolytic streptococci- which causes pharyngitis , people who get this infection will recover in few days ,but 2 weeks after the recovery they may develop rheumatic fever(why? Because this bacteria has a protein called M protein which looks a lot like myocin in cardiac muscle so after the immune response against M protein from the streptococci, antibodies will start attacking cardiac muscle resulting in rheumatic fever)

* We should know that there are some other factors that trigger autoimmune diseases such as: exposure to sunlight is a trigger for patients with SLE disease(systemic lupus erythematosus)

*80% of autoimmune diseases occur in females, we still don't know why, but it is thought that hormonal effect(estrogen, progesterone) is the reason ,but there's still no valid evidence why AI chance in women is higher and why they engage to an inhibitory receptor rather than an activation receptor.

Mechanisms by which microbes may promote autoimmunity.