Endogenous antigen is the antigen that's produced within the cell and can be a protein of the cell or foreign like a virus or intracellular bacteria.

synthesis of MHC I molecules:

there's a gene of chromosome 6 produces the alpha chain and another gene on chromosome 15 which goes for beta2m. these will be forming MHC class 1 molecule on the endoplasmic reticulum.. at the same time, within the ER there're the cell proteins or foreign proteins, these proteins are broken down by proteosomes (subunits of enzymes the break down proteins into small peptides).

so how are these small peptides associated with MHC class 1 molecule?

TAP 1 and TAP2 (transporter of antigenic peptide), they transport these peptides to the peptide binding groove of MHC class 1 molecule, so now we have MHC class 1 molecule with a peptide and under normal conditions it's a self peptide or viral antigenic peptide when infected with a virus or bacterial when infected by bacteria.. after that they are transported into golgi apparatus and vesicles are formed , and the antigenic peptides are displayed on the surface of MHC class 1 molecule.

MHC class 2 molecules: same story of class one but here we don't want an endogenous peptide so anything coming with TAP 1 and 2 won't get into the peptide binding groove, how do we know that? There's another peptide which is called the invariant chain that goes and bind the peptide binding groove preventing peptides that are coming through TAP 1 and TAP 2 from binding..

then MHC class 2 molecules goes to golgi apparatus and then it's exported to the cytoplasm within a vesicle….

the cell is a phagocytic cell, when there's a phagocytosis either of a self dead cells or break down protein or a virus or bacteria, the phagosome fuses with lysosome forming pahgolysosome where proteins are broken down into peptide.

now the phagolysosome is fused with the vesicle that contain the MHC class 2 molecules , and there's an enzyme that breaks down the invariant chain , takes it off and replaces it with a peptide coming from the phagolysosome (the peptide might be either self or foreign antigenic peptide), so now we have alpha and beta chains and peptide of its groove that comes up and is displayed on the surface of the cell for the benefit of helper t cells.

inheritance of MHC gene:

we inherit it in haplotype, which means;

- you take the full package (one haplotype) from your father, and take the full package (one haplotype) from your mother
- also, there is no cross over during meiosis

In Biology, we take that during meiosis, cross over occur, and then you get genes coming from one and others come from the other.. In haplotype this does not happen ..

So here you get MHC class **I**, class ***II***, ... ALL TOGETHER

here, you inherit all of the genes as one gene

when you examine siblings, if you look at their MHC molecules and genes, they have exactly the same multi types of these genes

Q: for example, if you need a transplant, what's the percentage that your brothers' and sisters' tissues will be identical with yours'??

- 0.25, one in four (0.25) has to be identical completely (the two haplotypes; the one that from the father, and the one that from the mother, of you and your brother, are identical)
- two in four (0.50) have a half identical (one haplotype is identical)
- one in four (0.25) has to be non identical completely (none haploid is identical)

clinical significance of MHC molecules:

- we talked about responders and non responders
  in animals, we said that there are animals that respond to certain antigens, others do not
  that is the function of what's known **Immune response genes (IRGs)**
  they are the genes that courage the response for certain antigen (responders) ..
  and in some others (non responders), they are not present
  -immune response genes = MHC molecules
  it does not make sense, because after all, you have certain number of HLA antigens. But some people have the ability to fit the antigen peptide within the MHC molecule while others don't.
  that's why some people are susceptible to infections more than others
  so according to MHC molecules they have, they do mount the immune response, some are infected, other don't
  MHC molecules play a role in immune response
- there is what's known as **disease association**
  which means that: if you have certain HLA gene, you more likely to have a disease, and most of these diseases are also immune
- for example, Ankylosing spondylitis disease,

which is a chronic disease of the spinal column, and it's very often associated with HLA-27
majority of these people that have Ankylosing spondylitis have HLA-27

- Q: does that mean that HLA-27 allele takes the antigen of spinal column and presenting them in pathological way, to produce immune rxn, to attack your spinal column??
  maybe but maybe it's not true,
  because if it's true, ever body has HLA-27 should have the disease
- Q: HLA presents in all cells, so why this disease is restricted to spinal column??
  it's an auto-immune disease, and any immune disease can be negligible, or can be very generalized, but not every cell in the body is affected
- other theory propose that maybe there is a gene, that is responsible for Ankylosing spondylitis, that happens to be associated with HLA-27,
  it's very unlikely to separate one gene from another, for ex: mouse or dog (linking disequilibrium)
  So it's a gene that is associated with HLA-27, and get stuck to it

- another example: Behget' disease
  again it's an autoimmune disease, which associated with HLA-B51
  who discover it, was known Bahjat, he is Turkish
  this disease affects many things; produces ulcers
  some cells in the mouth, eyes, mucus membranes and might affect the brain

let's begin with today topic

Development of B- and T-cells

we already take about them at the beginning of the course BUT here in details

B-Cells:

- they developed in bone marrow
- of course, they come from stem cells
- the earliest cell, that come from stem cell, and committed to produce B-cell from the stem cell >> PRO B-cell
  this can be identified by the presence of 2 markers:
  - CD-19
  - CD-10 (CALLA)
    CALLA: common acute lymphoblastic leukemia-associated antigen
- next stage, RAG1 and RAG2 are going to be activated, produce rearrangement of the genes, then production of heavy chain (first),
- heavy chain that is going to be produced have to be (μ)
  so now μ-chains will appear in cytoplasm
- and further development of the cell to occur; you need a receptor on the surface to signal to the inside, to carry on maturation
- by that time, you only have μ-chains, and don't have light chains (not assembled yet)
- there's a chain known as Surrogate chain, which happen to be produced inside the cell, and this actually act like light chain and takes it's place
- corrugate: when mother of baby die, the women that come to lactate the baby is called so
- now, compination of corrugates (light chain) with μ (heavy chain) occur to produce **<u>temporary receptors</u>**
- they appear on the surface of the cell
- they are known as PRE B-cell receptor

first of all, PRO B-cell
next stage, PRE B-cell

- Q: how do we recognize these PRE B-cells??
  - they have μ-chain in the cytoplasm
  - also, they have PRE B-cell receptors on their surface, made of: *heavy chain μ
    *light chain (corrugate)
- the receptor will signal to the inside, so you can carry on maturing, and you will get the production of the light chains
- then, the light chain will associate with the heavy chain, and they will produce IgM, which is appear on the surface
- of course, these receptors are associated with Igα and Igβ

DON'T FORGET: *TCR associated with CD3
*B-cells receptors (IGs) always associated with Igα and Igβ

- now, you get IgM on the surface
- RAG1 and RAG2 are shut off (inactivated),
  we have rearranged the μ (heavy chain),
  and we have rearranged the (light chain)
  so we don't need them any more
- now, this cell is known as >> **Immature B-cell**
- it has IgM receptor on its surface BUT does not have IgD yet
- next step in maturation (development):
  the appearance of IgD on the surface
- and now, it's a **fully mature B-cell**
- it leaves the bone marrow, and goes to the circulation to populate in secondary lymphoid tissues
- during the development, at the stage of Immature cell, we have IgM receptor
  this receptor can interact with all molecules and antigens in the surroundings, (some of the antigens in the bone marrow are going to be recognized by some of IgMs), because we have so many specificities produced
- recognition of the antigen (self antigens in the bone marrow) at the stage of immature cell (has IgM and does not have IgD), instead of activating the cell, actually send a message to inside it, to kill it by apoptosis (so it's deleted)
- the signal depends on the stage of maturity of the cell, it can be either positive or negative, so it will be negative at immature stage
- stimulate the B-cell while it's Immature by any antigen present in bone marrow, will kill the cell >> self tolerance
- also, there is sth may happen instead of deletion,
  once the cell is immature (has IgM), when IgM recognize the antigen, what will happen is that, the genes which assemble to this kind of antibody will be deleted
- they do not die but their ability to express IgM on their surfaces is lost

  for example, it occurs with kappa

DON'T FORGET: kappa come from one constant gene, V and J (assemble them together)

at this type, at this stage, there are the mechanism by which you can cancel this assemble and delete it
then, you can start again with the light chain
>> reactivate RAG1 and RAG2
and try to produce a light chain

hoping that the new light chain + old heavy chain >> won't be cell reactive

this is known as receptor editing.

of course, if you change the light chain, and still it's self reactive, it will be taken out

- receptor editing occurs only in the kappa chain, there are special region on kappa chain that will delete the gene that is produced
- BUT when we reach lambda, this mechanism won't occur, and the cell has to be killed
- That doesn't mean that every auto reactive antigen in your body is going to be in your bone marrow
    - so maybe we remove the majority of B-cells that are going to be self reactive
    - BUT there will be some that will go to the periphery and still can be potentially auto-reactive
    - and those have to be deleted by sth else
- deletion of B-cells in the bone marrow >> **central tolerance**
- others which escape, despite they are auto-reactive, will be inactivated by another means >> **peripheral tolerance**
- one more thing about B-cells, out of their population in your body, there is a certain number (about 5%)
    - known as **CD5 positive cells or B1 B-cells**
    - these are mainly found in GI tract
    - mainly associated with membranes
    - they produce antibodies (usually IgM), which:
      *have low affinity
      *and happened to be auto-antibodies
- Q: what's the function of these CD5 B-cells??
  it's not really very clear
- Q:why do they produce auto-antibodies??
  these auto-antibodies that are produced are not harmful, because they have very low affinity

antibodies that are harmful mostly of IgG type not IgM

- sometimes, they call these antibodies as >> **natural antibodies**
- these cells are just sitting, taking our own antigen >> producing antibodies against them >> they keep them preserved
- so that, if the pathogen comes to GI tract, we can have them as first line defense against them
- so they produce them by using our own antigen as a template, that's why they are auto-antibodies
  BUT their purpose not to kill our own cells

instead they are ready in case that a kind of bacteria comes in, some of these antibodies will be able to join them and stop them as first line defense, until we mount the immune response

T-Cells:

- they are produced in bone marrow
- then the leave it and go into thymus
- as they go into thymus, they have no markers; no TCR, CD4 and CD8 ....
- so they are really known as >> **naked or double negative**
- they have no molecules on their surface
- and as they go inside the cells of thymus, they go to the **cortex,** which will producing their receptors: TCR
- β-chain is the first to be assembled in the TCR, and before α-chain is assembled, Surrogatechain will be assembled and take place of the light chain, just the same story as that of immunoglobulins, and this will appear on the surface of the cell
- β-chain + Surrogate chain + and, of course, CD3 this is known as **three T-cell receptor**
    - it's temporary receptor
- if it's not assembled, cell will actually die
- now you will have the production of α-chain, and you have TCR on the surface
- at the same time, and on the same cell, you will develop CD4 and CD8
- this cell known as **double positive**
- double positive cells: CD4 positive, CD8 positive, TCR positive, and of course CD3 positive, all these molecules are present on these cells
- these cells will interact with the cells in the **cortex**
    - majority of cells in the cortex are: thymic epithelial cells
    - also, there are some dendritic cells, and some macrophages
- thymic epithelial cells have MHC class **I** molecules on them, also they have MHC class **II** molecules
- MHC molecules are also present in APC (dendritic cells and macrophages)
- so both have MHC class **I** molecules and MHC class **II** molecules
- we know that recognition of antigen by TCR is MHC restricted
- so this means that TCR of T-cells should be able to recognize antigens and **MHC molecules**
- so these double positive T-cells interact with MHC molecules, could be class **I** or class **II**, that are on the epithelial cells
- after recognition, they will stick here,

- if this recognition of MHC by TCR of Thymocytes, this is something useful
  in this case what will happen is that: the signal will be given to the thymocytes, to carry on maturing

Thymocytes: developing T-cells passing through thymus

- if that TCR can't recognize an MHC molecule on any epithelial cell in the thymus, it will be neglected
  and it won't be given a sign (signal) to further mature and it will die by apoptosis

this is known as **positive selection**

- positive selection: if the thymocyte TCR can recognize the MHC either class **I** or class **II**, it will be nourished, and it will have the chance to grow
- if it can't recognize the MHC, it will be neglected
- this is positive selection of thymocytes in the cortex, and occurs at double positive stage
- after that, they are going to move forward, to medulla,
  now, it gets a little bit confusing! it's not very clear!
  books say different things about that!
- as these cells go through junction between the medulla and the cortex, and as they go into medulla, they interact with its cells
- now, cells in the medulla,
  - the majority of them are dendritic cells and macrophages
  - and the amount of thymic epithelial cells is actually less than that in the cortex

- nevertheless, again, when we have interactions, (it's believed that most interactions are going to occur between thymocytes and dendritic cells), they will undergo a **negative selection**

negative selection: occurs probably in junctions between the cortex and the medulla, some book say that it occurs at single positive stage, others say that it occurs at double negative

- once the T-cell recognizes MHC class **II**, and it was positively accepted >> it's going to develop CD4
- if it recognizes MHC class **I** >> it should develop CD8, and the other one (CD4) should be neglected and go far away
- so by the time, you do this, you are ending up with single positive; there is only CD4 or CD8
- once T-cell binds an antigen presenting cell, MHC class **I** or MHC class **II,** interacts to the T-cells, because T-cells already know the MHC

- now, the new cell will find how much attractive (affinity) between TCR and MHC class **I** or **II**
- the strength of attraction between the two will determine whether the cell will be destroyed or not, because;
    - if the attraction is too strong, it means that this cell is potentially auto-reactive, and it will send a signal to kill it
    - if the attractive is very light, (affinity is very law), this is useful... why??
      because it recognizes MHC,
      if they change peptide from self to a foreign peptide, then the attraction and activity are going to be big, and they will be killed (deleted)
- single positive rather than single negative,
  because if the T-cell recognizes MHC class **II >>** it should engage CD4 molecules, this engagement allow it to stay
  CD8 is not engaged >> it will be lost
- after that, they move to medulla, and that cell that has CD4 >> would interacts with MHC class **II**
    - and if the attraction is too strong, the signal will be sent to be killed
    - if it's low, it would allowed to carry on, and come mature T-cell
    - Q: what happens if the attraction was median (median affinity)?? it's believed that these cells which have median affinity, mature and become as **T-regulator cells**; which is type of T-cells suppress the immune response (will be discussed later)
- T-cells types:
    - cytotoxic T-cells
    - helper T-cells
    - regulator T-cells (Reg T-cells)
- the deletion of these cells in negative selection >> **central tolerance** of the thymocytes
- some of them may escape to the periphery >> and their will have their own **peripheral tolerance**
- of course, you don't have all the antigens in your thymus, but here we have an extra advantage; when you get to the medulla, thymic epithelial cells in the medulla have a gene which is known as **(AIRE - autoimmune regulator gene)**; which means than this gene has the ability to switch on genes that manufacture various antigens that can present outside thymus, they can produce **antigens of insulin.**
- the cell which recognize the antigen will be deleted

- it's very important in prevention of autoimmune diseases
- if you have a defect in this gene >> you will get **poly autoimmune diseases syndrome** ; a syndrome with multiple , several autoimmune diseases occur at the same time
  ex: autoimmune in thyroid, parathyroid ...
- and it's believed that cells that have lost their AIRE gene, and stop working, they will die, and they produce what's known as hassall corpuscle ; they are special cells in the medulla of thymus (seen when you study the histology of thymus), some people say that they are dead cells that have lost the ability of AIRE gene
- still there are other cells come out; cytotoxic T-cells, helper T-cells, T-regulators
- there are other type called **NK T-cells**
  - they are discovered newly
  - they consist 0.1% of T-lymphocytes of your body (very few)
  - natural killer T-cells: on top of TCRs, they have markers of NK cells; NK1.1, It's present on NK cells BUT happens to be on these cells, that's why they called NK T-cells
  - they recognize antigens through a different mechanisms other than MHC class molecules