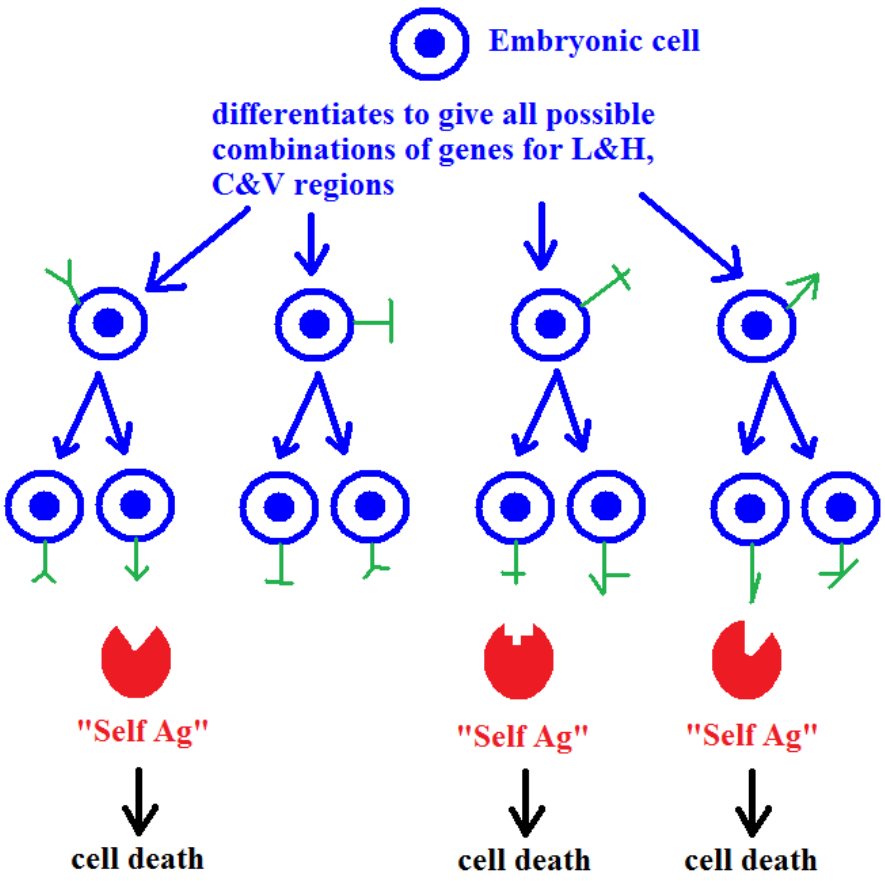
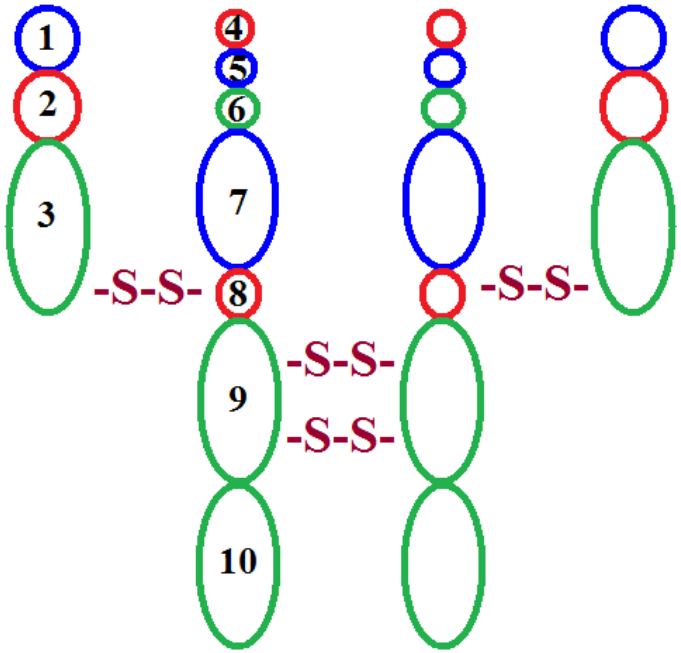
Immunogenetics for Microbiology

- How is it that 5 classes of antibodies regulate more than 10 billion different antigens without destroying the human body?
- Part of the answer came from Macfarlane Burnett in the 1950's when he proposed his Clonal Selection Model.
- This model suggests how the reticulo-endothelial system recognizes "self" from "non-self".
- In a nutshell, he proposed that germ line cells (endoderm, mesoderm and exo/ectoderm; you learned about these layers in the trilaminar disc in fetal development in A&P II) differentiate during embryological development into various embryological cells.
- The DNA of the antibody genes undergoes rearrangement.
- Those cells with the genes that recognize "self" die.
- Those cells with the genes to recognize "non-self" survive and begin our immune system.

- Figure, right, illustrates in a crude way in which cells that recognize "self" antigens die and those with "non-self" recognition genes survive.
- Note: the embryonic cell differentiates to give all possible combinations of genes for the light and heavy and constant and variable regions of antibodies.
- The bottom line is that cells with genes for "non-self" recognition survive embryological differentiation and permits "self" to be protected.
- As mentioned, above, "left over" cells in the developing, immature system can recognize ANY foreign substance very specifically following maturation.



- This old model is more or less correct and answers most of the question. The rest of the answer focuses on the genetic rearrangement of genes required for antibody synthesis. There are genes that regulate the production of each of the amino acid sequences of each antibody, Figure, right (antibody NOT in "normal" conformation). Most of the letter abbreviations in Figure, following, are already known.
- **"J" is not and stands for "Joining".**
- **"D" stands for "Diversity".**
- **"L" stands for "Leader".**
- **Each amino acid sequence represents an exon, by the way.**



V_L	V_H	C_H
1-V 2-J 3-C	4-V 5-D 6-J	7-C_1 8-Hinge 9-C_2 10-C_3

- The heavy chain classes come from **chromosome 14** and are γ , μ , α , δ and ε .
- They are about 400-500 amino acids in length.
- The variable region represents about the first 110 amino acids and the last 290-390 amino acids are for the constant region.
- In terms of the light chain classes, there are two: κ and λ.
- The former is from **chromosome 2** and the latter is from **chromosome 22q**.
- The light chain consists of 200-300 amino acids.
- The variable region is the first 110 amino acids and the constant region is the remaining 90-190 amino acids.
- Since there are 2 classes of light chains and 5 of heavy chains, this means that there are really 10 different classes of antibodies and not 5 as we discussed, earlier.

The table below lists the possible tetramers of the 10 classes of antibodies:

IgG	IgA	IgM	IgD	IgE
$\kappa_2\gamma_2$	$\kappa_2\alpha_2$	$\kappa_2\mu_2$	$\kappa_2\delta_2$	$\kappa_2\varepsilon_2$
$\lambda_2\gamma_2$	$\lambda_2\alpha_2$	$\lambda_2\mu_2$	$\lambda_2\delta_2$	$\lambda_2\varepsilon_2$

Note that there are 2 of each Ig class.

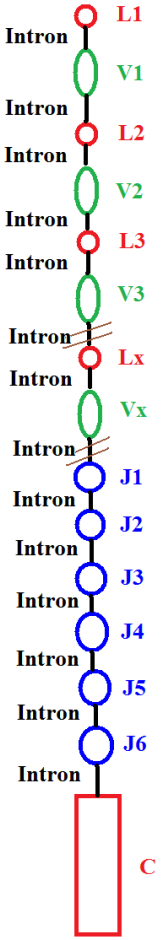
The Recombination Theory is another theory that helps us answer our question. In a nutshell, this theory says that in spite of there being:

	VJ recombination	V	D	J	V(D)J recombination
L chain	10	150	----	5	----
H chain		80	50	6	100

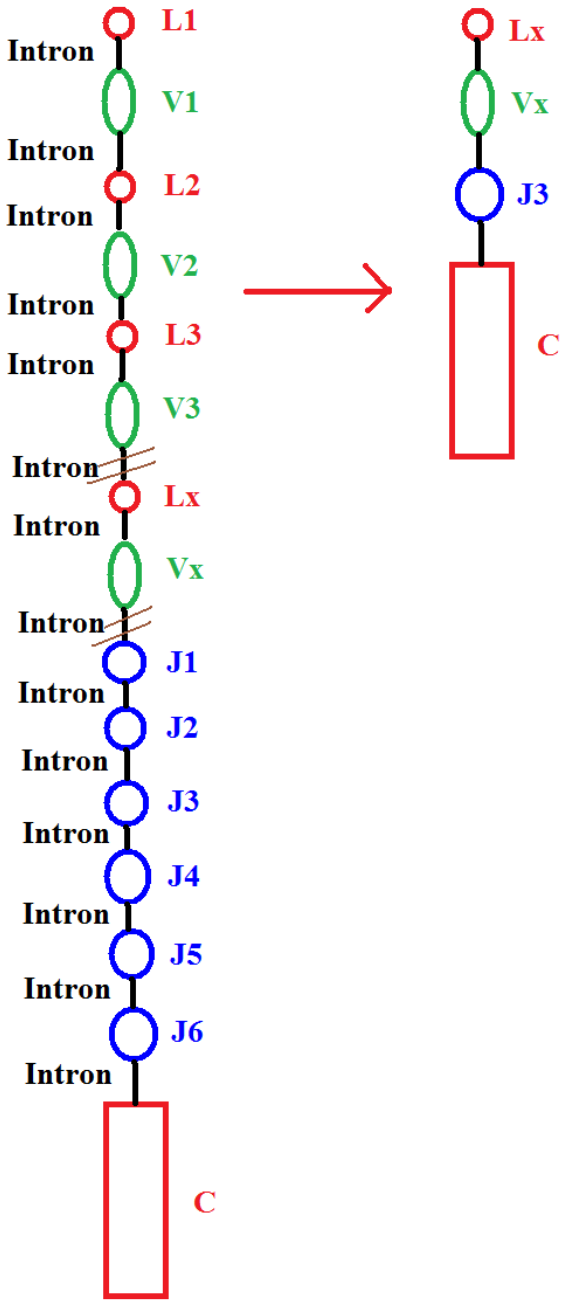
numbers of genes, as B cells become immunocompetent, some unknown mechanism causes the DNA to recombine in such a manner that **each B cell is able to code for, synthesize and release ONLY 1 antibody.** This is VERY specific. It is unknown whether this mechanism is in the "bursa equivalent" or the bone marrow. This rearrangement allows for the production of more than 100,000,000 antibodies.

An Example of the Synthesis of One (1) Human κ Light Chain

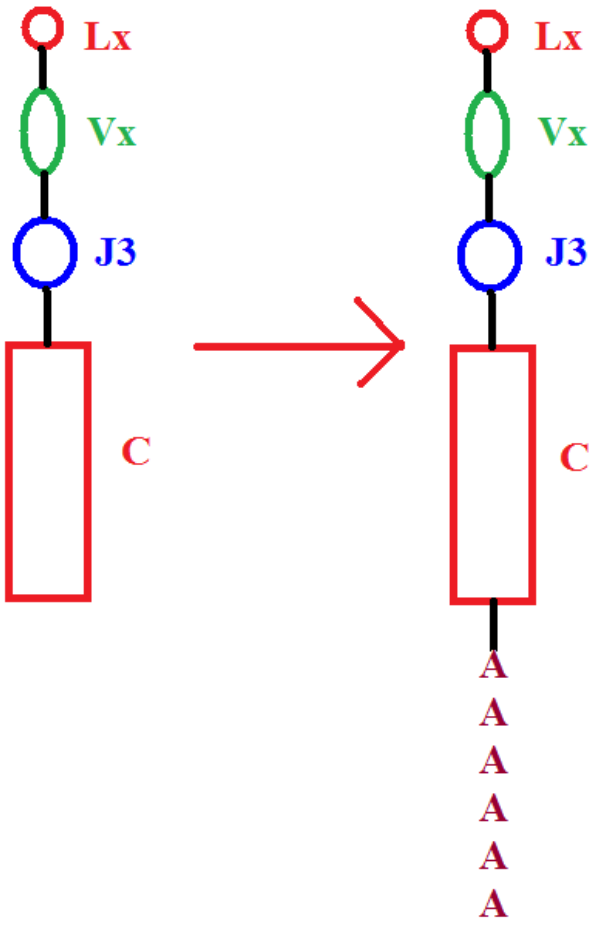
- The synthesis of 1 human κ light chain starts in the germ cell with **pre-B cell DNA** that contains L (leader), V, J and C regions, right.
- Step 1

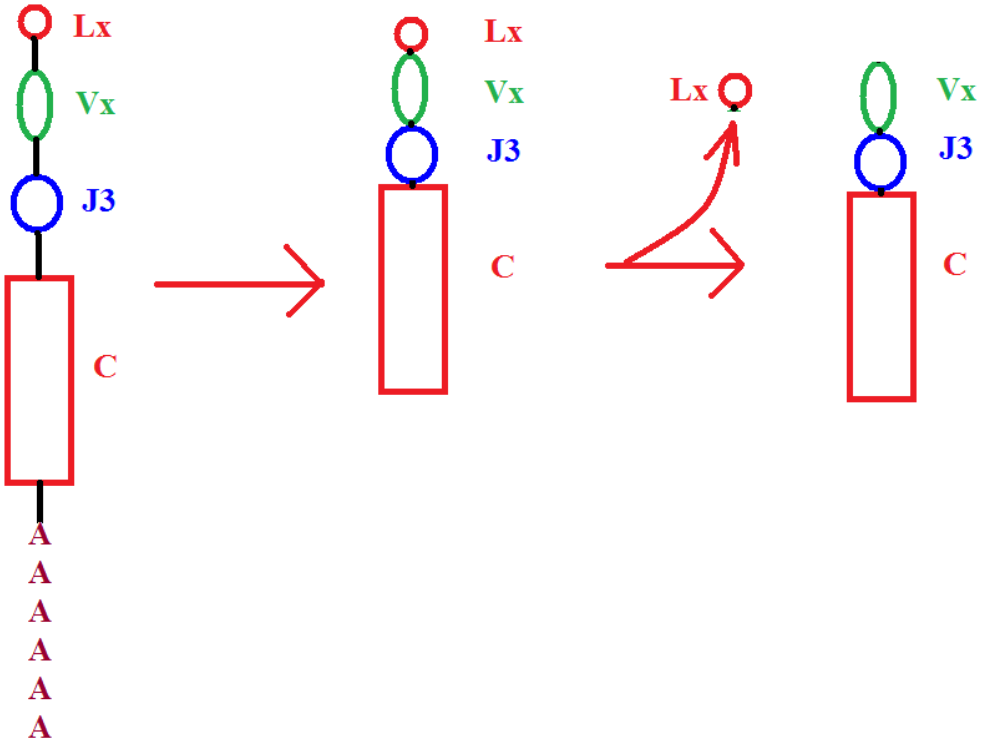


- As immuno-competence is conferred upon the B cell and it matures, the DNA is clipped and snipped in a rather imprecise manner, which causes MORE diversity amongst antibodies.
- Also gives each B cell its own DNA.
- Step 2



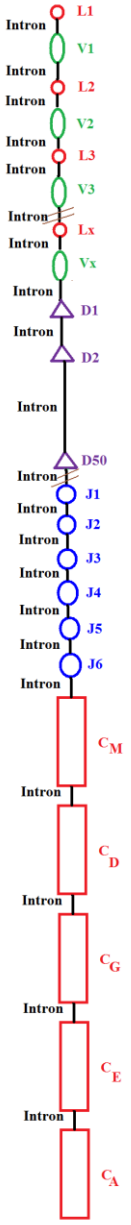
- Remembering that each B cell makes ONLY 1 kind of antibody, note that in Figure, right, the B-cell DNA has four (4) transcribable exons (Lx **[L is the leader sequence]**, Vx, J3 and C in Figure).
- It is transcribed to the primary transcript (hn RNA) and poly-adenylylated.
- Step 3



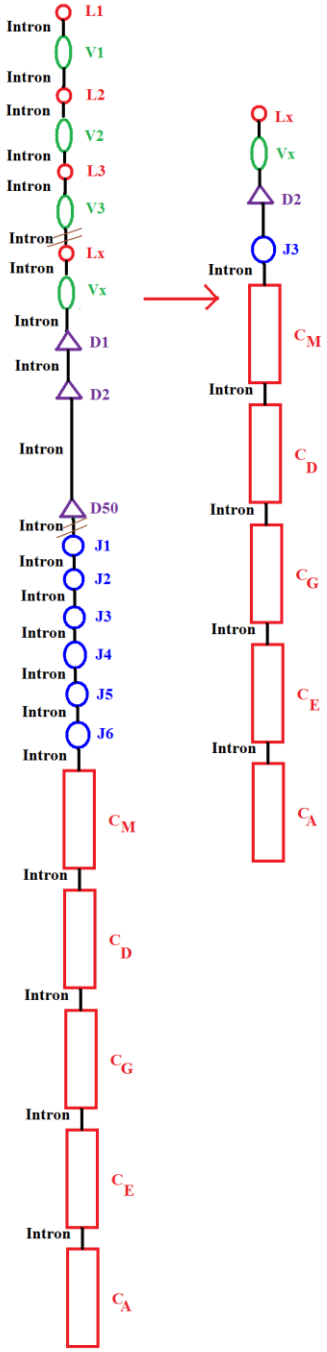


- The hn RNA is then spliced (left), then translated and processed (right) to give one (1) L chain (Vx, J3, C).
- Unprocessed Ab L chain (RNA) is in middle of sequence, above.
- Step 4

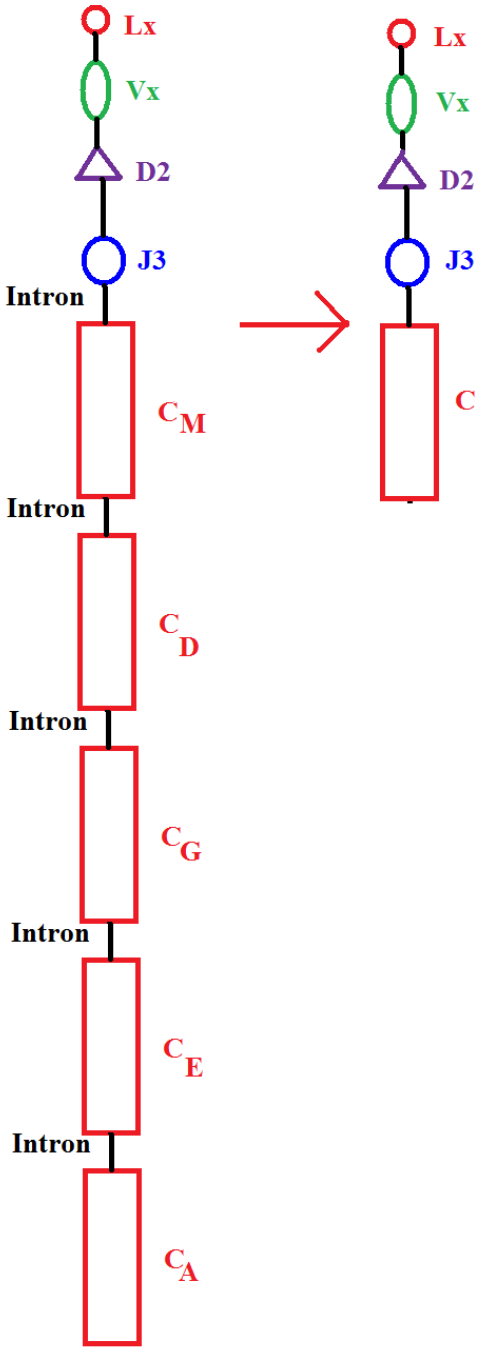
- H Chain Synthesis …
- … is in much the same way as L chain synthesis.
- Pre-B cell DNA contains numerous leader sequences, variable sequences, diversity sequences and constant sequences.
- There may be as few as 10 D sequences or as many as 50.
- Step 1



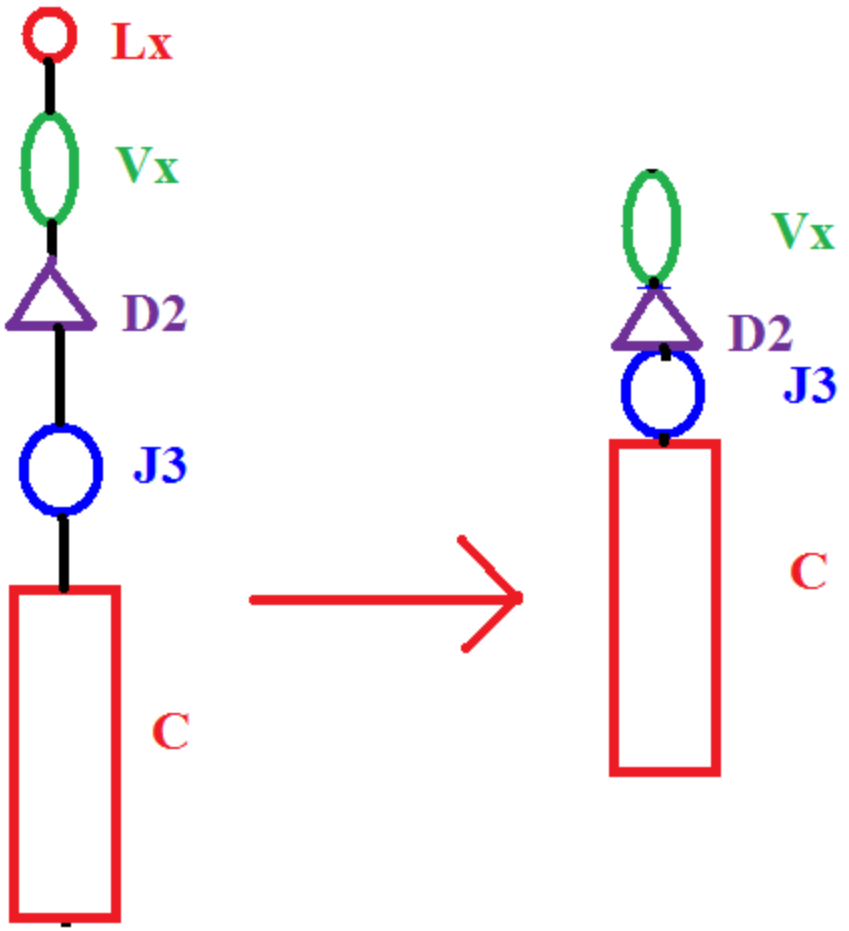
- This DNA is processed as the B cell matures and increases its immunocompete nce conferring ability.
- The "young B cell" DNA has been clipped and snipped to provide only one (1) L, V, D, J sequence per B cell, yet still has the 5 C regions.
- Step 2



- The "young B cell" DNA is then clipped and snipped to provide only one (1) constant sequence per “grown up” B cell.
- Step 3

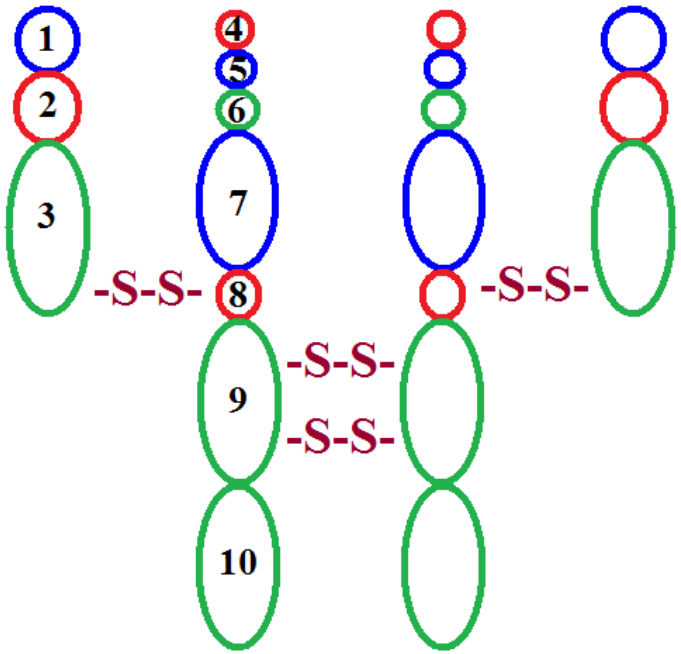


- Following transcription and translation the H chain is ready to be combined with the L chain.
- Step 4



H and L Chain Combination

- The 2-H chains and 2-L chains then combine as shown in Figure, right. This figure demonstrates that regions specific for each antibody are coded for by one exon per sub-unit. Remember, too, that the antibody is much like the human body: it is bilaterally symmetrical. Each sub-unit identified in the Figure on the right side has a matching sub-unit on the left.



V_L	V_H	C_H
1-V 2-J 3-C	4-V 5-D 6-J	7-C_1 8-Hinge 9-C_2 10-C_3

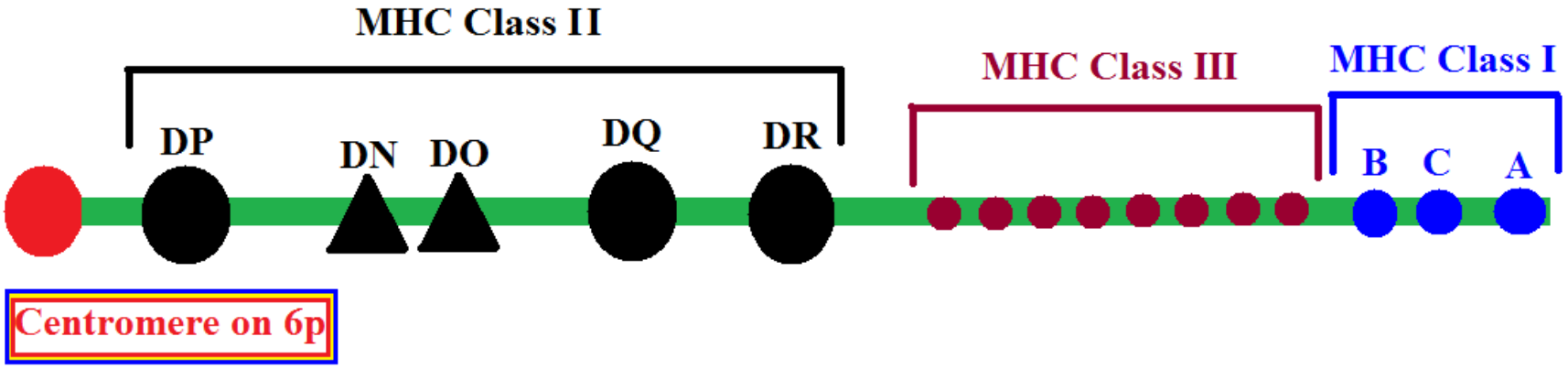
- This still doesn't answer the whole question.
- Some more of the answer occurs during the period of embryological development and growth: T cells receive information from the thymus in such a manner that if the T cell recognizes a "self" antigen, it is killed and the only T cells that survive are those T cells that have been "taught" to recognize foreign antigens.
- The foreign antigen recognition units in the case of the T cells, are of ANOTHER group of proteins in the immune system called the Major Histocompatibility Complex (MHC) or Human Leukocyte Antigens (HLA) – all of which follow in discussion after this section.

Major Histocompatibility Complex (MHC or HLA) and T Cell Receptors

- To get through this section, it is important to grasp some new terms and definitions.
- Alloantigens are antigens that are present only on the cells of some people.
- HLA stands for human leukocyte antigen and determines the rate of success of organ transplants, e.g., host vs. graft disease.
- HLA-A1 is the number 1 allele at the HLA-A gene on 6p.

“Genetic Map” of MHC’s on 6p

- The centromere is on the left and the telomeric end is to the right. Note that the class III MHC's are "nestled" between the class II and Class I MHC's.
- MHC III is the Complement System



A haplotype is one of the two chromosomes containing the HLA loci, therefore, each child will inherit one HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DO, HLA-DR and HLA-DN from each parent, e.g., Punnett square, below:

	Male haplotypes →	
Female haplotypes ↓	A3, B7, Cw2	A9, B40, Cw7
A32, B51, Cw2	A3, B7, Cw2 A32, B51, Cw2	A9, B40, Cw7 A32, B51, Cw2
A1, B27, Cw11	A3, B7, Cw2 A1, B27, Cw11	A9, B40, Cw7 A1, B27, Cw11

- Each of the gametes contains 1 haplotype; each zygote contains 2 haplotypes.
  - 2 siblings have a 25% chance of having the identical HLA;
  - 2 siblings have a 50% chance of having 1 identical HAL haplotype;
  - 2 siblings have a 25% chance of having NO identical haplotype.
- HLA-typing is the next step after blood grouping evidence if blood group data is incompatible.
- HLA typing is used to predict BEST tissue matches
- This works well since there is so little space (introns) between loci in the HLA region that there is virtually no crossing over during meiosis.

Example

- Based upon the genotypes below which child would be the best kidney donor to child #3?

	Male haplotypes →	
Female haplotypes ↓	A3, B7, Cw2	A9, B40, Cw7
A32, B51, Cw2	1) A3, B7, Cw2 A32, B51, Cw2	2) A9, B40, Cw7 A32, B51, Cw2
A1, B27, Cw11	3) A3, B7, Cw2 A1, B27, Cw11	4) A9, B40, Cw7 A1, B27, Cw11

Solution

	A antigens				B antigens				Cw antigens		
	1	3	9	32	7	27	40	51	2	7	11
Father		+	+		+		+		+	+	
Mother	+			+		+		+	+		+
1st kid		+		+	+			+	+		
2d kid			+	+			+	+	+	+	
3d kid	+	+			+	+			+		+
4th kid	+		+			+	+			+	+

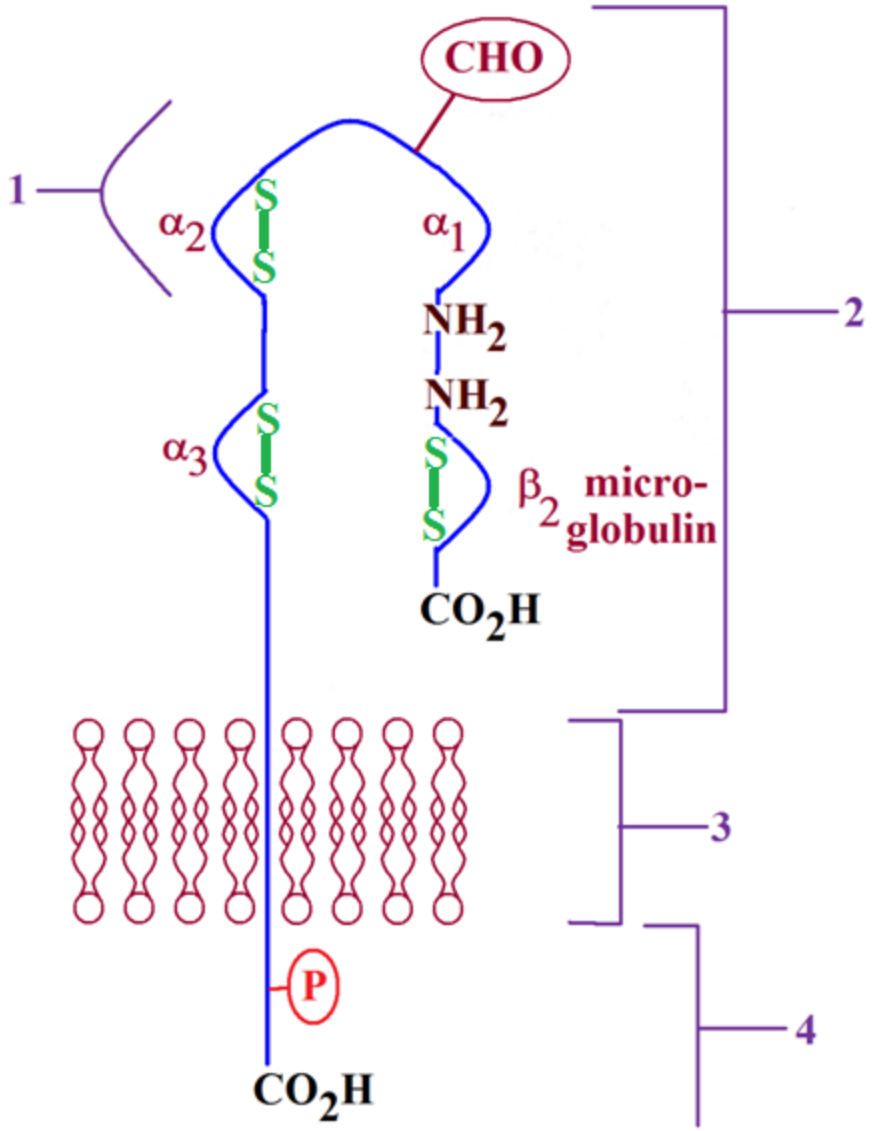
- CHILD #1. Child #2 has 5 HLA's different from #3 (in green); child #4 has 3 HLA's different from #3 (blue); Child #1 has only 2 HLA's different from #3 (red).
- NOTE: "different from" means "foreign to"
- ASIDE: the mother would be an equal donor as she has only 2 HLA's different from #3, as well (violet).

The classes of HLA's are tabulated below.
Data regarding each class is summarized, as well.

Classes of HLA's		
MHC-I	**MHC-II**	**MHC-III**
On almost every human cell	On immunocompetent cells: B cells, T cells, macrophages, monocytes, dendritic cells	In plasma (**complement**)
1.presents foreign antigen (e.g., virus) to cytotoxic T lymphocytes (CTL's): **T_8 cells** 2.Class I's are the primary antigen recognized by the HOST'S CTL's during graft vs. host disease	Recognized by/through **T_4 cells**; class II on DONOR cells initiate graft vs. host disease	Complement immune system

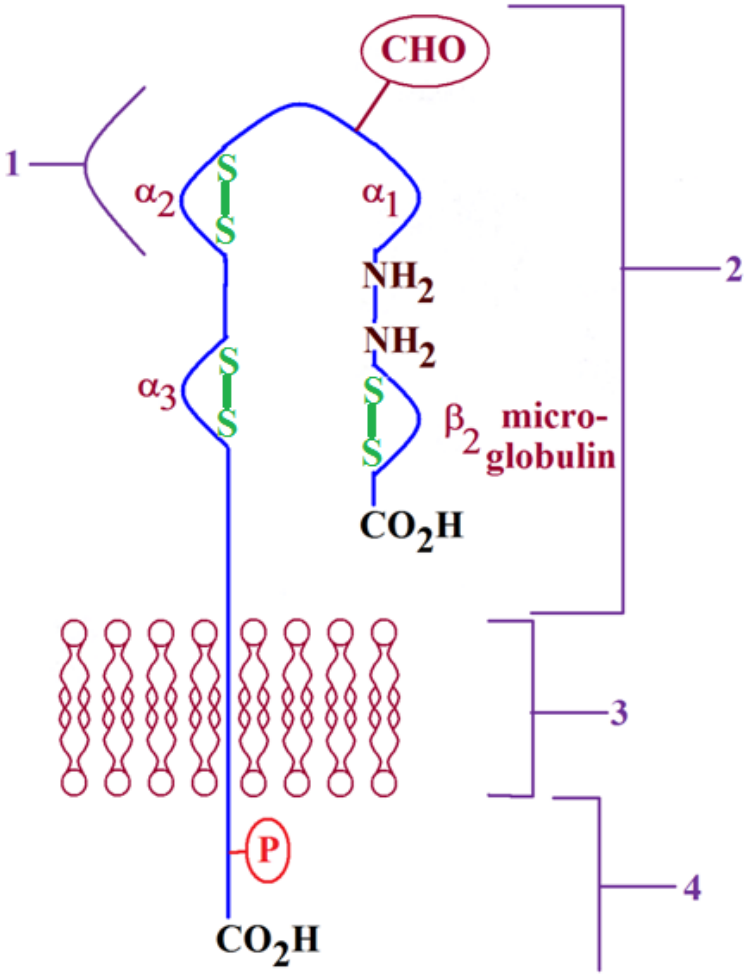
The structure of MHC-I proteins

- 1) Resembles a mushroom shape
- 2) Extracellular hydrophilic region
- 3) Transmembrane hydrophobic region
- 4) Intracellular hydrophilic region



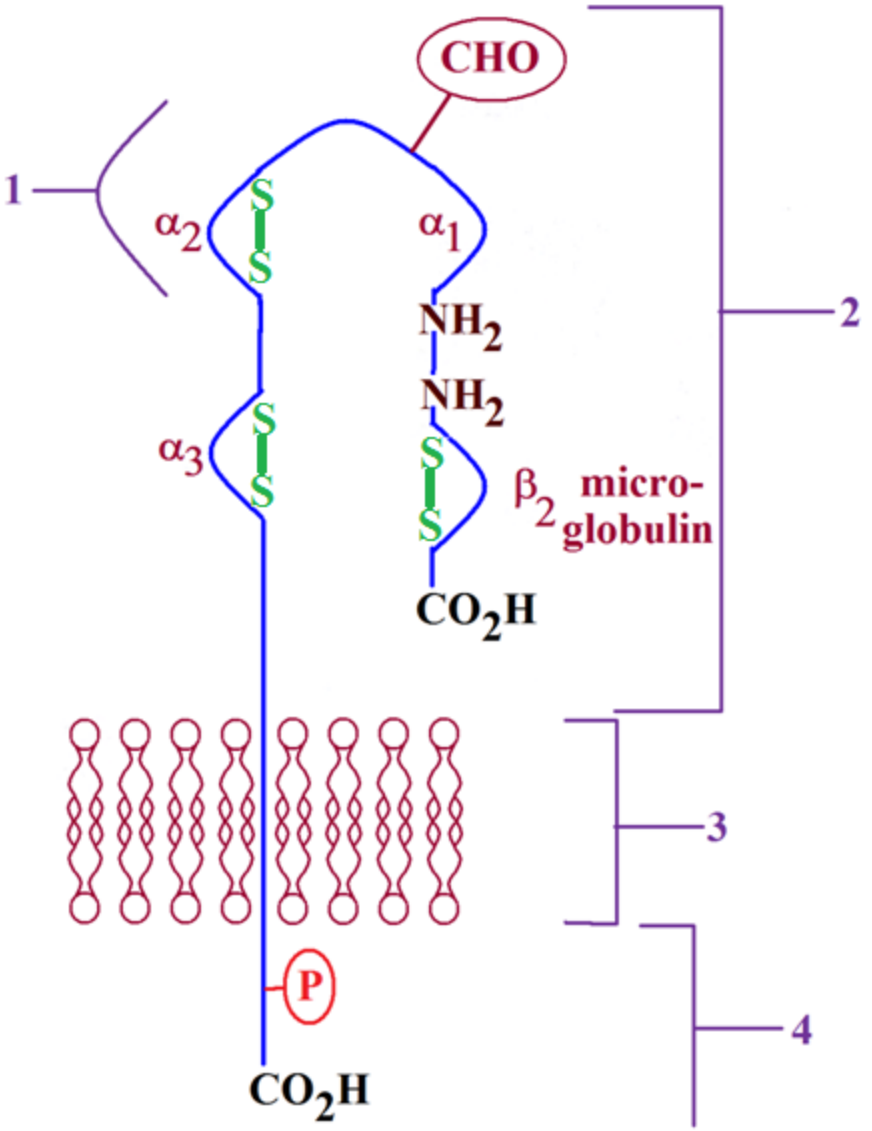
The structure of MHC-I proteins

- The H chain is a glycoprotein of molecular weight 44,000. It consists of 3 α chains.
- Associated with these three chains is the β2 microglobulin. This protein has a molecular weight of 12,000 and **is coded for on chromosome #15, NOT on 6p.** The whole structure is anchored in the cell membrane by the H chain. The α3 and β2 microglobulin are structurally similar to the C region of immunoglobulins.
- MHC class I's are members of the immunoglobulin supergene family. The α1 and α2 regions contain most of the alloantigenic determinants that are recognized by antibodies and T cells.
- These same regions (or domains) bind foreign antigens and then are acted upon by very specific T cells.



The structure of MHC-I proteins

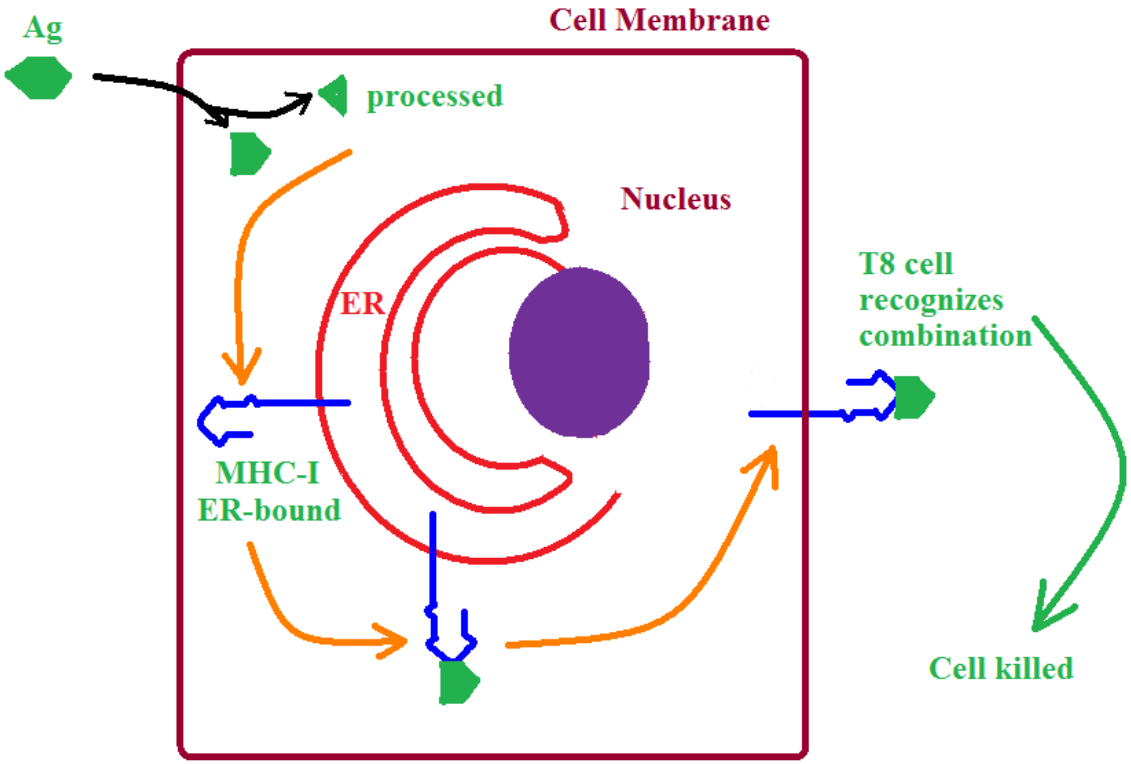
- The intra-cellular-most portion is **phosphorylated** and may be an intracellular switch.
- To code for this glycoprotein, 7 exons on the DNA are typically required.



MHC-I

- The α_1 and α_2 regions when they are flipped. This region appears like a clam-shell or a Venus fly trap. This shape is functional, as it "clamps" antigens for presentation to T_8 cells.

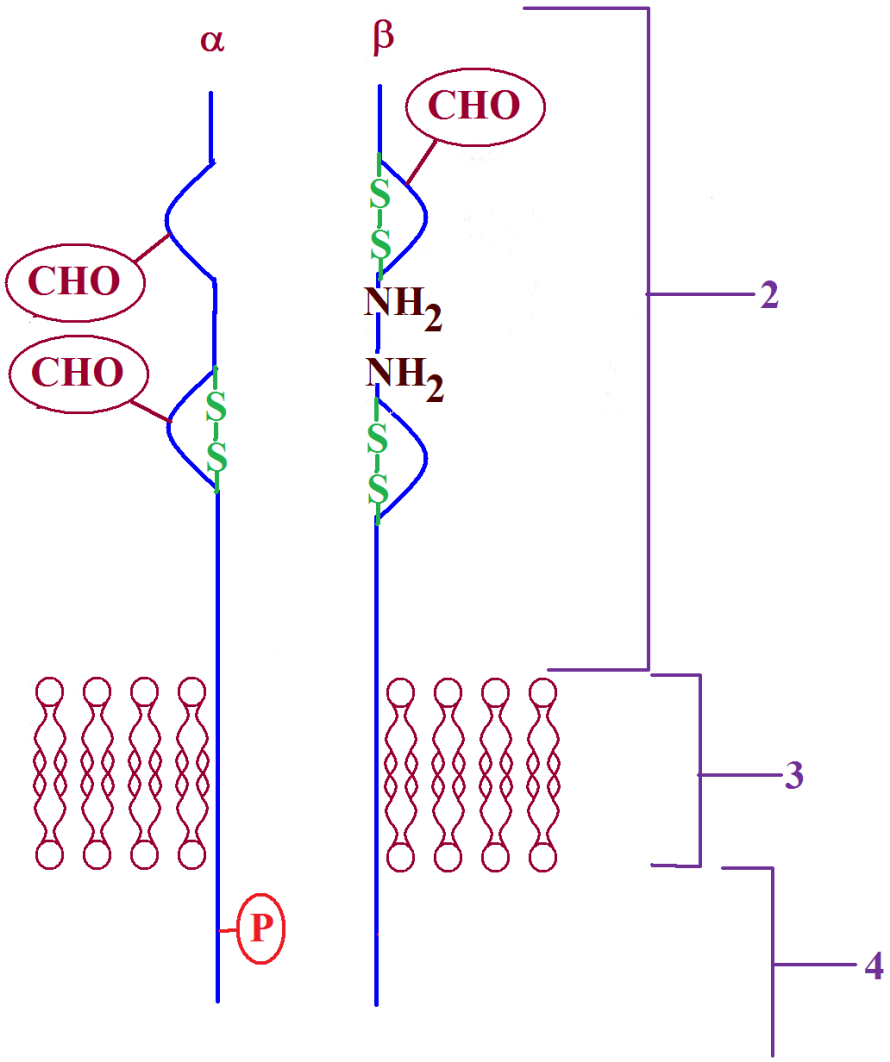
- Like B cells, T cells are also "educated" early on in embryogenesis to seek only 1 kind of antigen in association with Class I MHC proteins for presentation to CTL precursors.
- Once the CTL precursors meet with the antigen and MHC-I, the specific precursors divide and differentiate to mature CTL's (T_8).
- These CTL's, then, are said to be restricted in their activities to those cells in the body that have the SAME MHC-I and antigen as began the dividing and differentiating of the CTL precursors. More on this shortly
- CTL killing of the [target] human cell is at once 1) antigen-specific and 2) "Class I restricted".
- Remember that MHC-I glycoproteins are found on virtually every cell in the body.



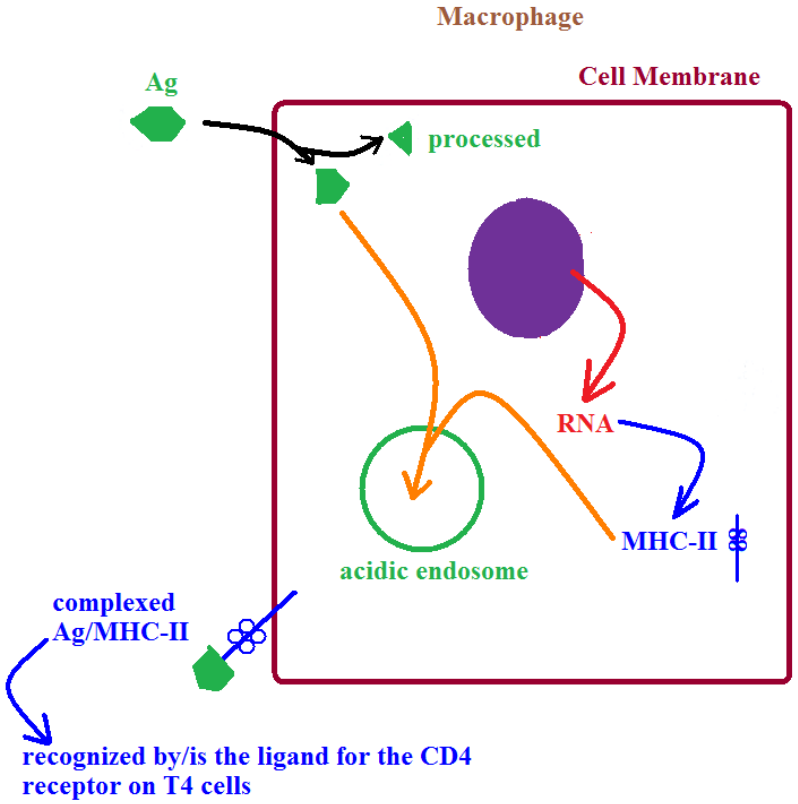
- It seems that MHC I glycoproteins REQUIRE intracellular binding with antigen in order to be transported to/through/into the cell membrane, Figure, above.
- The antigen is internalized and binds to the ER-bound MHC-I.
- The MHC-I-Antigen complex is then partially ejected through the cell membrane.
- A T_8 cell recognizes the complex and the T_8 cell kills the cell.

The structure of MHC-II proteins

- This glycoprotein consists of an α chain and a β chain. The former has a molecular weight of 34,000 and the latter of 29,000. The lower domains on each chain (α2 chain and β2 chains) show C chain homology (as we saw with MHC-I's α3 and β2 microglobulin domains).
- The upper domains of each chain (α1 and β1 domains) interact with foreign antigens. The structure of the MHC-II also seems to be that of a clamshell or Venus fly trap.
- MHC-II glycoproteins on DONOR cells initiate the immune reaction with host T4 cells.



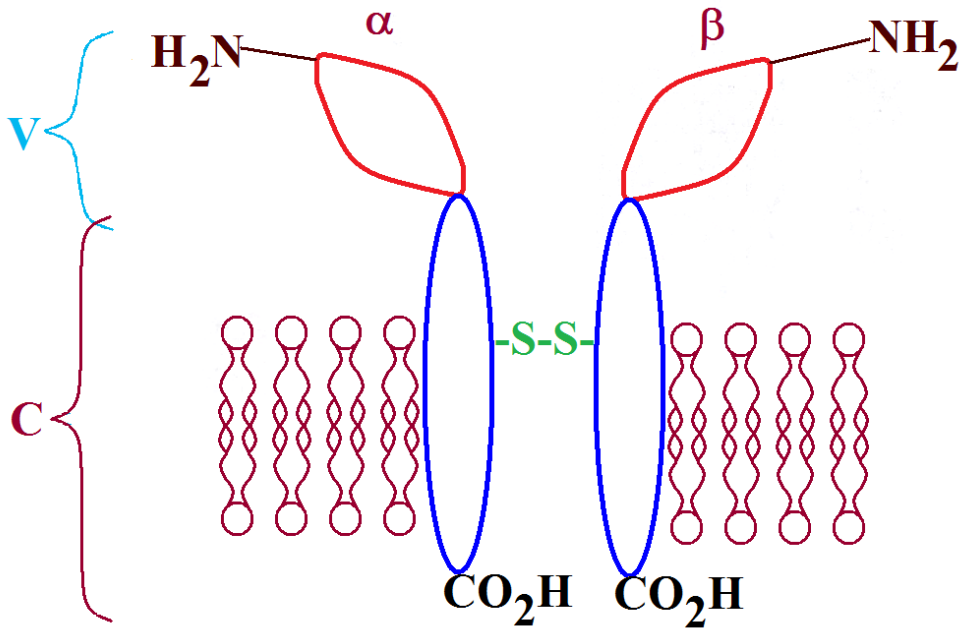
- Class II proteins work differently than Class I: **Class II glycoproteins are found only on immunocompetent cells.** It seems that when antigens come into contact with an immune cell, e.g., macrophage, the antigen is internalized and partially processed, Figure, right.
- The partially processed antigen and the newly synthesized MHC-II are enclosed in an acidic endosome and transported to the cell membrane where the MHC-II-antigenic particle are ejected part way through the cell membrane. The complex is recognized by/is the ligand for the CD4 receptor on T4 cells.
- This complex initiates T4-mediated immune responses. Like T8-MHC-I restriction, the **T4 cells are MHC-II restricted** in T4 recognition of antigens.



Major Histocompatibility Complex (MHC or HLA) and T Cell Receptors

T-Cell Receptors

T cells are differentiated (identified) by various receptors on their surfaces: TCR's

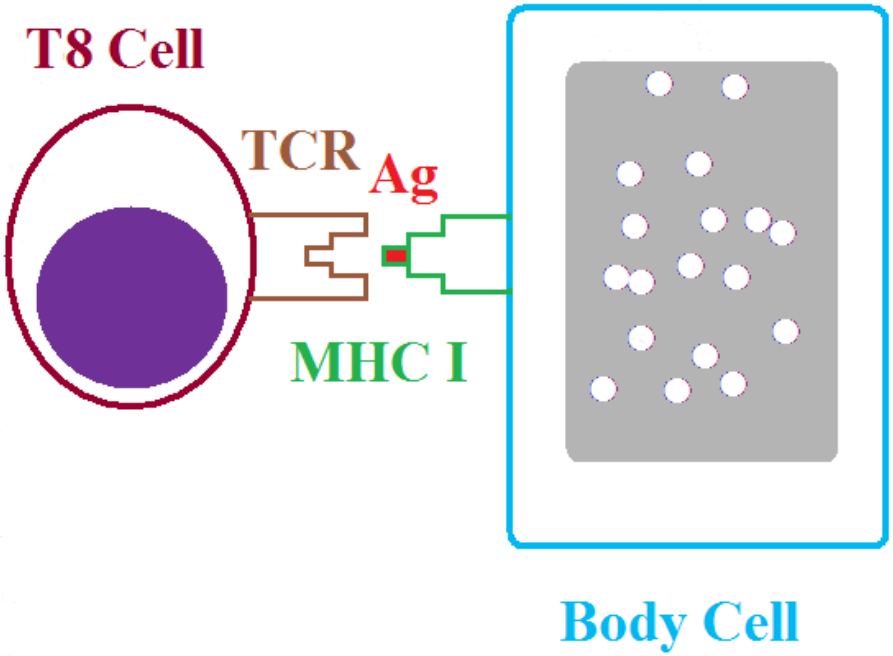


- Figure, above, illustrates a generic T cell receptor. Note that it consists of two chains: an α chain and a β chain. Note the similarity of the T cell receptor to antibody and MHC-II (Slide 26) general structures.
- Although the actual values vary by author and study, for the β chain, there are around 20-25 different genes for the V region; 1-2 for the D region; 6-12 for the J region; 2 different genes for the C region.

- The diversity of TCR's comes from VDJ recombination (like Ig and MHC's) and imprecise snipping. NOTE: the **diversity** of TCR's is much, much less than for the Ig genes.
- One T cell may have 1 of 2 kinds of proteins in their receptors: $\alpha\beta$ or $\gamma\delta$. In spite of the fewer genes for TCR's, T cells recognize just as many antigens as do B cells. This is due to the unusually high rate of mutation in TCR genes.
- Both Ig and TCR's undergo VDJ recombination. Numerous proteins are needed for this process. Two loci, RAG-1 and RAG-2 (recombination-activation gene) on chromosome #11, are next to each other. They are transcribed simultaneously and have NO introns. Typically, RAG-1 and RAG-2 are expressed in B and T cells -- ONLY during VDJ recombination.
- **This mechanism of action is not fully understood.**

MHC-I and TCR's

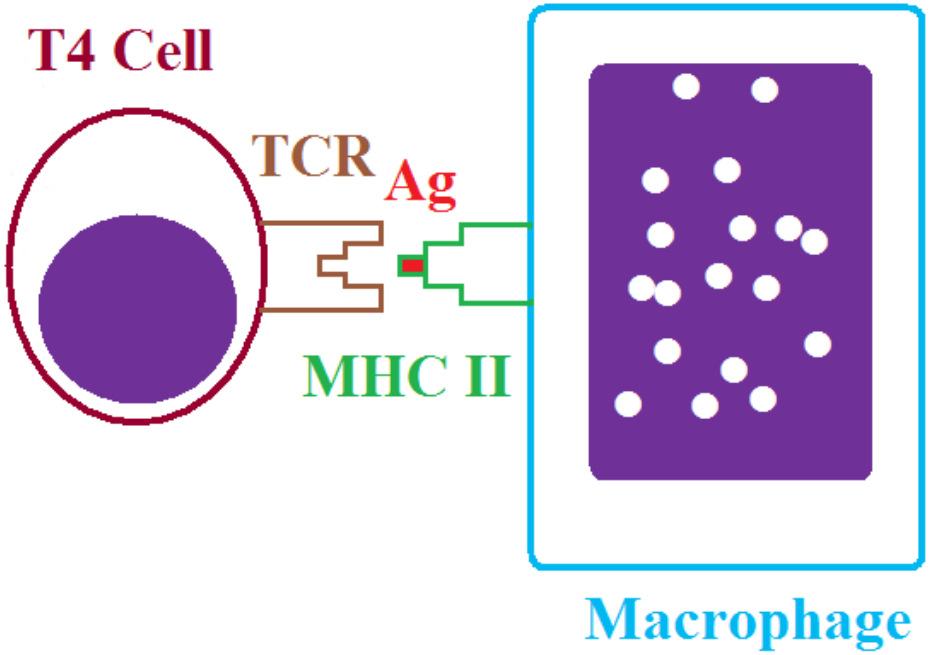
MHC-I
On almost every human cell
1.Presents foreign antigen (e.g., virus) to cytotoxic T lymphocytes (CTL's): **T_8 cells** 2.Class I's are the primary antigen recognized by the HOST'S CTL's during graft vs. host disease



- The bottom line is that with the action of MHC-I and TCR's, the body cell is killed by T_8 toxins.

MHC-II and TCR's

MHC-II
On immunocompetent cells: B cells, T cells, macrophages, monocytes, dendritic cells
Recognized by/through T_4 cells; class II on DONOR cells initiate graft vs. host disease



- The combined action of MHC-II and TCR's is to turn on a macrophage to destroy the antigen in the cell and does NOT kill the cell (VERY different from MHC-I).