

The Osler Institute

Excellence in Continuing Medical Education

Best of Series **Pathology** *Course Syllabus*

Part II

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Humoral and Cellular Immunity

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I. Humoral and Cellular Immunology

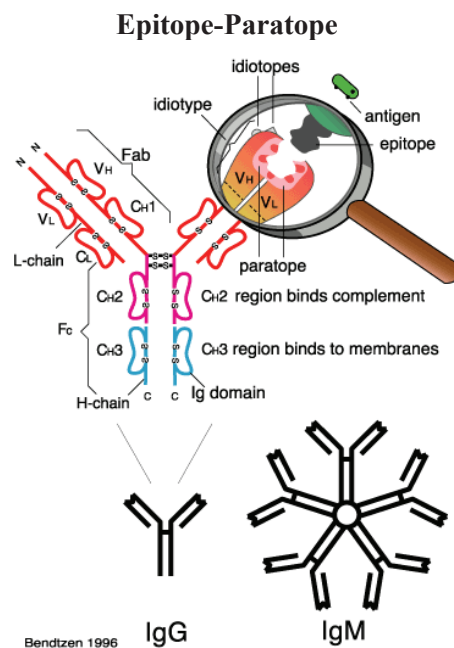
A. The immune system – why?

1. To protect us from disease?
 - It does that
 - Also protects us from beneficial organ transplants and blood transfusions
 - Also causes problems by responding to innocuous allergens
 - Autoimmune disease exists.
2. Protection from disease is not a good answer.
3. Two current theories:
 - Self – non-self discrimination
 - Danger hypothesis
 - a. Self – non-self
 - Differentiates what belongs and what doesn't
 - Thus self and non-self must have some type of appearance (antigenic structure).
 - The immune system must have some type of eyes to recognize what is self or non-self (antibodies, T cell receptors).
 - When self is recognized, it is protected.
 - When non-self is recognized, it is removed.
 - How does the system know what is “self”? Good question!
 - b. Danger hypothesis
 - The immune system does not know what is self or non-self.
 - The immune system responds to “danger” signals triggered primarily through the innate immune system.
 - Anything that triggers this danger signal will be eliminated.
4. The innate immune system
 - a. Cells and proteins that act to protect the body from danger, or an intruder.
 - b. Cells
 - Neutrophils
 - Monocytes/macrophages
 - Dendritic cells
 - c. Proteins involved
 - “Inflammatory” cytokines, eg, IL-1, 6, TNF
 - Complement?

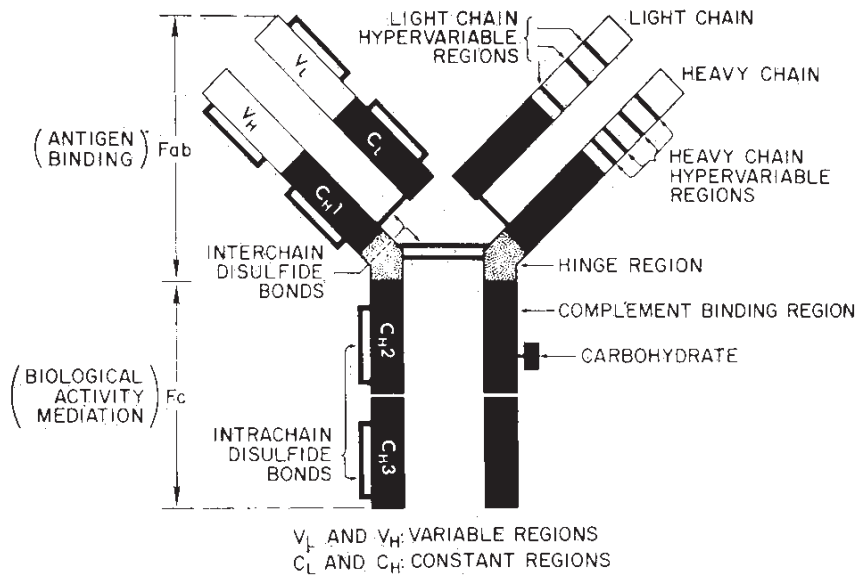
- d. How are foreign/danger molecules recognized?
- Binding to toll like receptors
- Cell surface proteins, similar to a “toll” protein in *Drosophila*.
 - Recognize bacterial motifs

B. Antigens

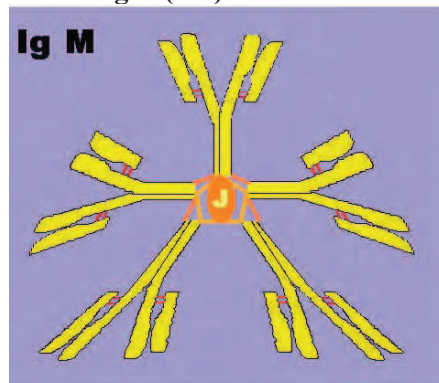
- Elicits and combines with an immune response
 - Self or non-self
 - Large molecules
1. Epitope
 - Portion of an antigen which combines directly with the antigen binding regions of an antibody (paratope) or T cell receptor
 - T cell epitopes presented by APC
 - Hapten – slightly larger than an epitope, but not large enough to elicit a response
 2. T dependent – requires T cell help: usually proteins or nucleic acids
 3. T independent – binds directly to B cells: usually repeating polysaccharide chains
 4. Idiotypes
 - Three dimensional structure of the variable regions of an immunoglobulin
 - Individual structures on the variable region are called **idiotopes**.
 - The collection of all the idiotopes on an immunoglobulin is called the idiotype.
 - Idiotopes can be antigenic.



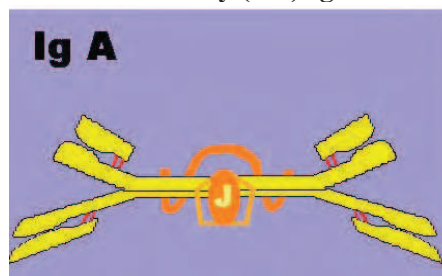
5. B cells
 - a. B = bursa of Fabrecious: B cell development organ in birds
 - b. Bursal equivalent
 - Fetal liver and spleen
 - Adult bone marrow
 - c. B cells produce an antigen receptor (immunoglobulin).
 - d. The immunoglobulin can be either secreted or expressed on the cell surface.
 - e. Immunoglobulins have antigen specificity, determined by a complex process of gene rearrangement and mutation.



IgM (19s) Pentamer



Secretory (11s) IgA

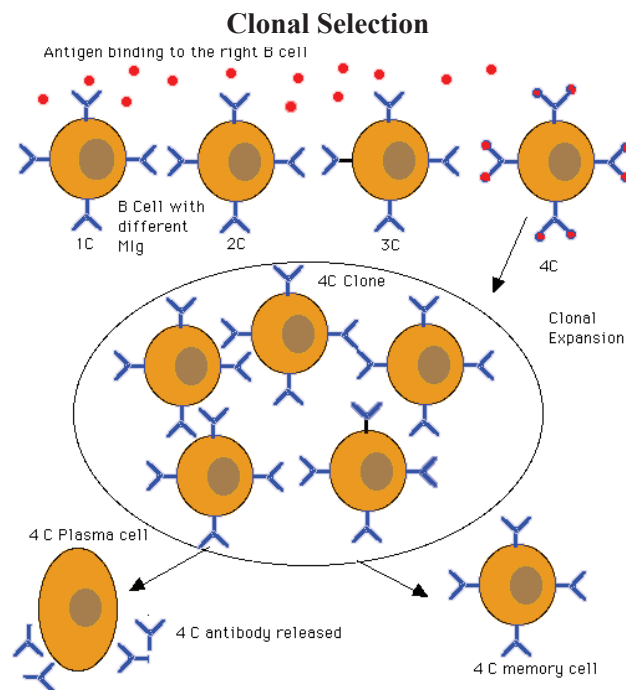


C. Instructional theories

- Antigen instructs lymphocytes to make an antibody which will bind to the antigen.
- Pro – affinity maturation during an immune response
- Con – no real molecular basis for this

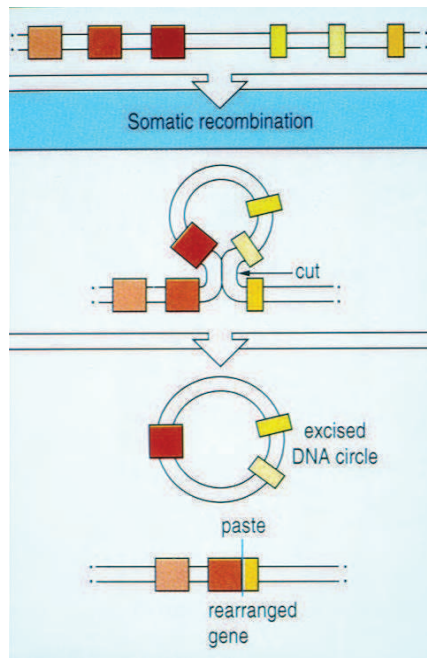
1. Selective theories

- Lymphocytes have antigen binding sites (paratopes) determined prior to antigen contact.
- Pro – experimental and molecular evidence supports this concept.
- Con – number of genes required exceed genome size.
- Each paratope (antibody binding region) is not the product of one gene, but is the product of rearrangement and/or mutation of many genes.
- Imprecise gene rearrangement results in greater diversity.

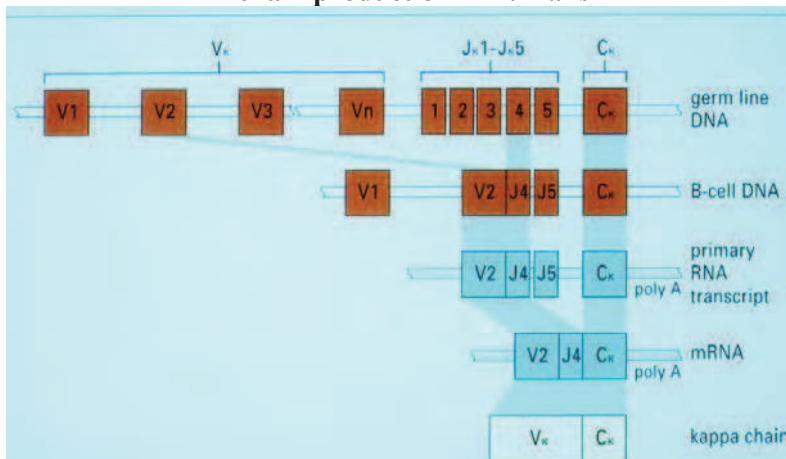


2. Light chain genes

- a. Variable and joining regions
 - 76 kappa V genes
 - 5 kappa J genes
- b. Separate V and J genes for kappa and lambda
- c. One V will combine with one J, then this VJ construct will be coupled to the C gene.



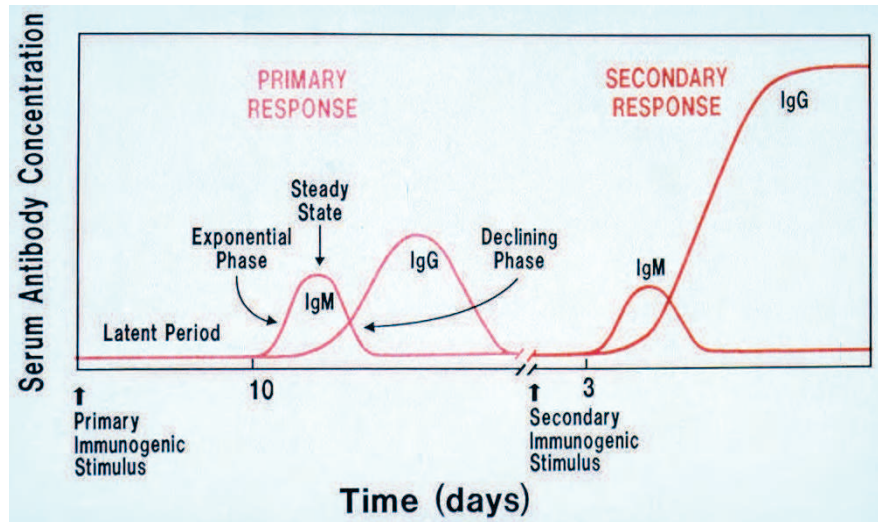
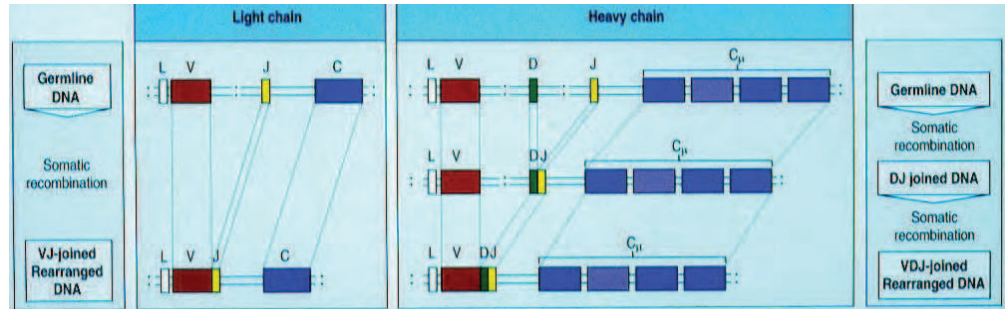
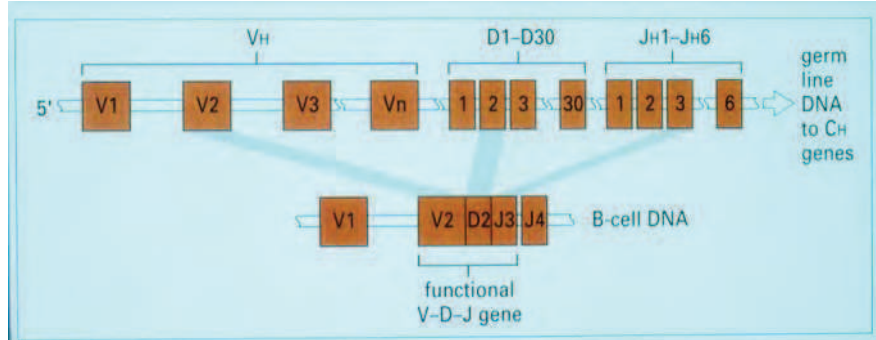
κ chain production in humans



3. Heavy chain genes

- V, D and J regions
- 100-200 V genes
- 6 J genes
- App 30 D(iversity) genes
- V-D-J joining occurs.
- Complete VDJ gene combines with the C gene.

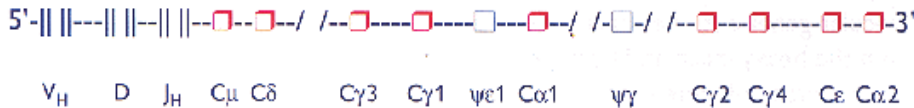
V-D-J recombination in humans



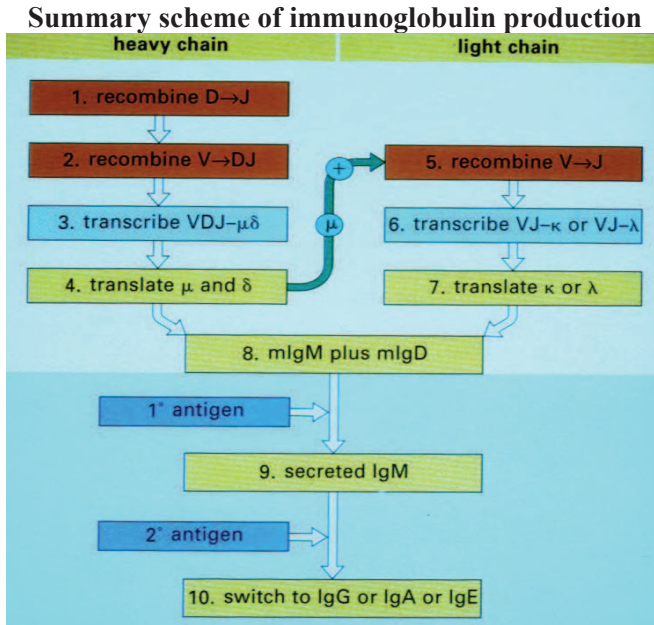
4. Isotype switching

- First Ig expressed by a cell is IgM.
- T cell interactions and cytokines stimulate isotype switching.
- Isotype switching occurs by C_h gene rearrangement to a “downstream” gene.

Heavy Chain Constant Region Genes



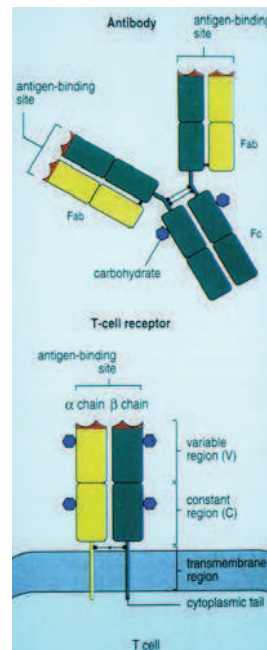
Schematic representation of the arrangement on chromosome 14 of the gene loci governing the constant region of the human immunoglobulin heavy chain. V_H denotes variable segments, D the diversity gene segments, J_H the joining gene segments. C_μ denotes IgM- C_H gene, C_δ the IgD- C_H gene, $C_{\gamma 3}$ the IgG3- C_H gene, $C_{\gamma 1}$ the IgG1- C_H gene, $\omega\epsilon 1$ a pseudogene (not expressed), $C_{\alpha 1}$ the IgA1- C_H gene, $\omega\gamma$ a pseudogene (not expressed), $C_{\gamma 2}$ the IgG2- C_H gene, $C_{\gamma 4}$ the IgG4- C_H gene, C_ϵ the IgE- C_H gene and $C_{\alpha 2}$ the IgA2- C_H gene.



5. Many from few
 - a. Human kappa chain genes
 - 76 V region genes
 - 5 J region genes
 - b. If any V can combine with any J, then the total possible combinations 76×5 or 380, using 81 genes
 - c. Mutations/imprecise joining can increase this number.
 - d. Heavy chain
 - 100 V genes
 - 30 D genes
 - 6 J genes
 - e. Total combinations = $100 \times 30 \times 6 = 18000$ using 136 genes.
 - f. Mutations/imprecise joining will increase this number.
 - g. Total kappa = 380
 - h. Total heavy chain = 18000
 - i. Total possible combinations if each kappa can combine with any heavy chain gene = $380 \times 18000 = 6,840,000$ using 217 genes.
 - j. Mutations and imprecise joining increase this number.

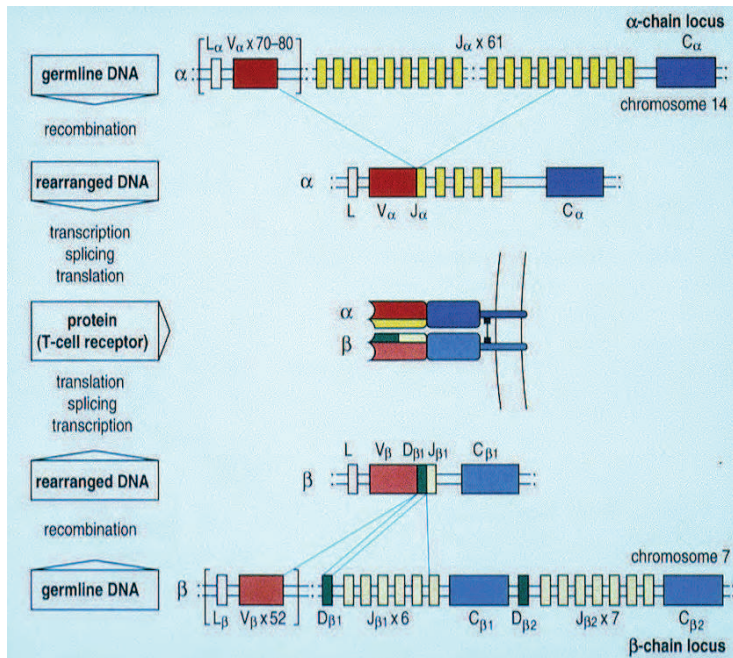
6. Mechanisms for diversity
 - a. Genetic recombination
 - Multiple germ line V genes
 - V-J and V-D-J recombinations
 - b. Imprecise joining
 - c. Somatic point mutation

	94	95	96	97
	SER	PRO		
V	T C T	C C T	C C C	A C A
J	C G T	T G G	T G G	A C G
			TRP	THR
	SER	PRO		
V	T C T	C C T	C C C	A C A
J	C G T	T G G	T G G	A C G
			TRP	THR
	SER	PRO		
V	T C T	C C T	C C C	A C A
J	C G T	T G G	T G G	A C G
			ARG	THR
	SER	PRO		
V	T C T	C C T	C C C	A C A
J	C G T	T G G	T G G	A C G
			PRO	THR



7. T cell antigen receptor
 - a. Alpha-beta receptor
 - Alpha – V, J, C
 - Beta – V, D, J, C
 - 95% of circulating T cells
 - b. Gamma delta receptor
 - Gamma – V, J, C
 - Delta – V, D, J, C
 - Limited antigenic repertoire
 - First T cells to mature

- CD3 +, CD4 and 8 -
- 5% of circulating T cells
- Found in the skin, appearance resembles dendritic cells; may



D. Cluster of Differentiation (CD) Proteins

1. CD45
 - Present on all leukocytes
 - Used as a “gating” parameter on flow cytometry
 - CD45RA on naive lymphocytes
 - CD45RO on memory cells
2. CD markers on T cells
 - CD3 – mature T cells
 - CD4 – on “helper/inducer” cells
 - CD5 – circulating T cells and CLL
 - CD7 – on all T cells
 - CD8 – on T “cytotoxic” cells
3. CD antigens on B cells
 - CD19 – from Pre B cell on
 - CD20 – mature B cells, also on some T cell lymphomas
 - CD21
 - CD22
 - HLA-DR – MHC, class II
4. CD antigens on LGL
 - CD16 – Fc gamma receptor
 - CD56
5. Cytokines
 - a. Interleukin (IL)-1
 - Produced by macrophages, activated Th cells
 - Endogenous pyrogen

Pathology Review Course

- b. IL-6: produced by macrophages, proinflammatory cytokine
 - c. IL-2
 - Produced by activated Th1 cells
 - Augments T, LGL and B cell activity
 - d. IL-12
 - Produced by Th1 cells
 - Suppresses Th2 activity
 - e. IL-4: produced by Th2 cells, activates B cells.
 - f. IL-5: produced by Th2 cells, activates B cells.
 - g. IL-10: produced by Th2 cells, increases humoral immunity, decreases CMI.
 - h. IL-13: produced by Th2 cells, activates B cells similar to IL-4.
6. Interferons
- Interferon (IFN) alpha: leukocyte produced, inhibits viral replication. Used to treat Hepatitis C
 - IFN beta: fibroblast produced, inhibits viral replication. Used to treat multiple sclerosis.
 - IFN gamma: Th1 cell produced, increases CMI.
7. Tumor necrosis factors
- a. Tumor necrosis factor (TNF) alpha
 - Macrophage produced, similar to IL-1
 - Causes vascular thromboses, tumor necrosis
 - b. TNF beta: T cell produced, similar activity to TNF alpha
 - c. IL-1, IL-6 and TNF are all part of the acute phase response.
8. CD8 positive cells
- Mediate cell mediated cytotoxicity
 - May suppress responses, but mechanism is unclear, may be a cytotoxic function directed against idiotypes on antibodies and T cell receptors
 - Interact with an antigen-MHC class I combination

E. MHC

- Major histocompatibility complex
- Human leukocyte Ag (HLA)
- On 6th chromosome
- Class I, II and III genes
- Class I and II genes have many allelic variants.

Table 8.13. Recognized HLA Specificities

A. Specificities Based on Classical Serological Alloantisera Typing⁹³⁶

Class I Proteins				Class II Proteins					
HLA-A		HLA-B		HLA-C	HLA-D		HLA-DR	HLA-DO	HLA-DP
A1 A28	B5 B38	BS102 B65	Cw1	Dw1 Dw14	DR1 DR12	D01	DPw1		
A2 A29	B7 B39	BS103 B67	Cw2	Dw2 Dw15	DR103 DR13	DO2	DPw2		
A203 A30	B703 B3901	B52 B70	Cw3	Dw3 Dw16	DR2 DR14	DO3	DPw3		
A210 A31	B8 B3902	B53 B71	Cw4	Dw4 Dw17	DR3 DR1403	DO4	DPw4		
A3 A32	B12 B40	B54 B72	Cw5	Dw5 Dw18	DR4 DR1404	DO5	DPw5		
A9 A33	B13 B40	B54 B72	Cw6	Dw6 Dw19	DR5 DR15	DO6	DPw6		
A10 A34	B14 B41	B55 B73	Cw7	Dw7 Dw20	DR6 DR16	DO7			
A11 A36	B15 B44	B57 B76	Cw8	Dw8 Dw21	DR7 DR17	DO8			
A19 A43	B16 B45	B58 B77	Cw9	Dw9 Dw22	DR8 DR18	DO9			
A23 A46	B17 B46	B59 B7801	Cw10	Dw10 Dw23	DR9 DR51				
A24 A68	B18 B47	B60		Dw11 Dw24	DR10 DR52				
A25 A69	B22 B48	B61 Bw4		Dw12 Dw25	DR11 DR53				
A26 A74	B27 B49	B62 Bw6		Dw13 Dw26					
	B35 B50	B63							
	B37 B51	B64							

Total number of protein types = 26 + 57 + 10 + 26 + 24 + 9 + 6 = 158 types

Total number of combinations = 26 x 57 x 10 x 26 x 24 x 9 x 6 = 499,374,720 combinations

B. Specificities Based on Modern Nucleotide Sequence Typing¹¹⁰⁹

Class I Proteins				Class II Proteins			
Locus	Number of Alleles	Locus	Number of Alleles	Locus	Number of Alleles	Locus	Number of Alleles
HLA-A	67	HLA-F	1	HLA-DRA	2	HLA-DPA	8
HLA-B	149	HLA-G	6	HLA-DRB	179	HLA-DPB	69
HLA-C	39			HLA-DQA	18	HLA-DMA	4
HLA-E	5			HLA-DQB	29	HLA-DMB	5
		Total:	266			Total:	314

Number of Class I sets: 11,689,110 six allele sets

Number of Class II sets: 2,063,111,040 eight allele sets

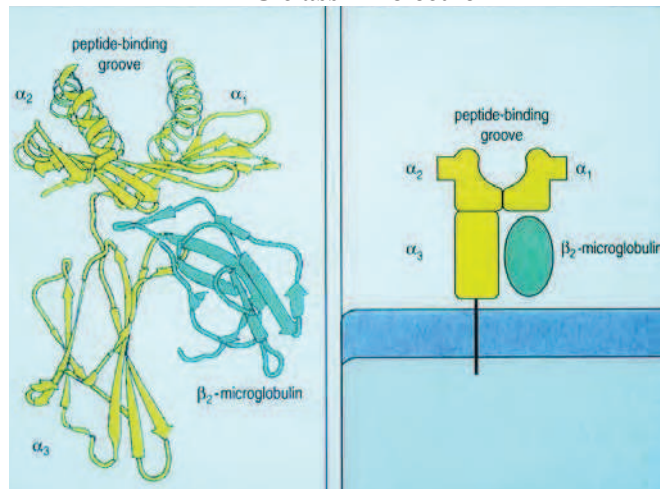
Total number of combinations = 2.41 x 10¹⁶ fourteen-allele combinations

Robert A. Freitas Jr., Nanomedicine, Volume I: Basic Capabilities, Landes Bioscience, Georgetown, TX, 1999

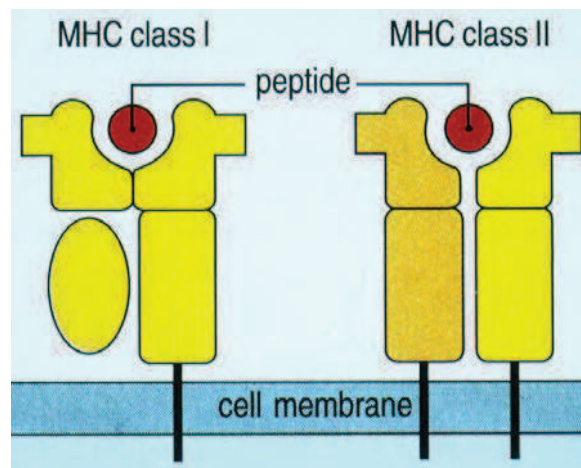
1. HLA, class I

- HLA A, B, C, E, G
- Two chain molecule
 - Single polypeptide chain, MW 44,000 encoded by MHC
- Coupled to the second chain, Beta-2 microglobulin, not encoded by MHC
- Present on all nucleated cells
- Class I proteins bind and express on the cell surface endogenous antigen produced by the cell.

MHC class I molecule

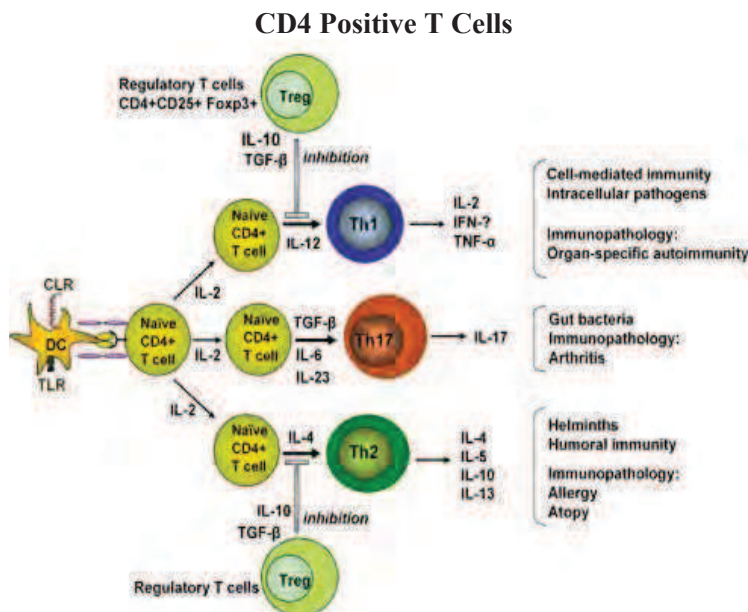


2. HLA, class II
 - a. HLA DR, DQ, DP and DZ
 - b. Two polypeptides for each molecule encoded by separate genes in the HLA region, eg, HLA DR alpha and HLA Dr beta genes
 - Alpha encoded peptide – 34,000 MW
 - Beta encoded peptide – 28,000
 - c. Limited cellular expression of class II proteins
 - B cells
 - Monocytes
 - Activated T cells
 - Vascular endothelial cells
 - d. Class II proteins bind and express exogenous Ag processed by the cell

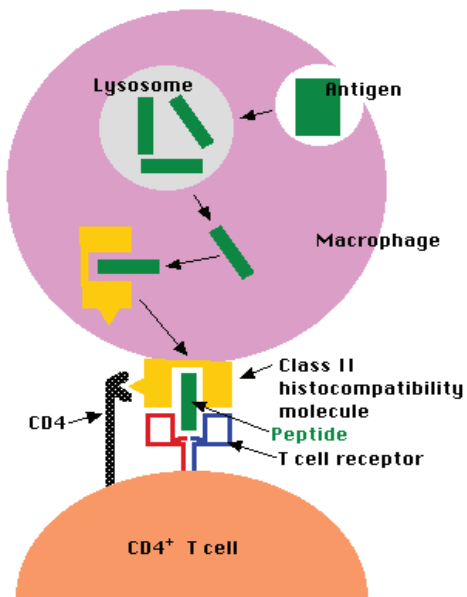


3. HLA nomenclature
 - a. HLA encoded cell surface proteins are identified by their genetic region (eg, A, DR) followed by a single or two digit number.
 - b. The number refers to an allelic specificity determined by antibody typing, eg, A4, B27.

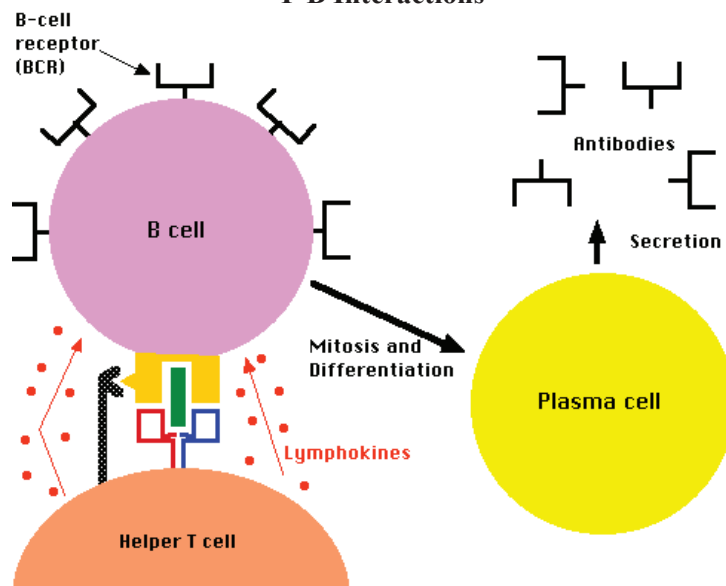
- c. A letter with an asterisk (*) followed by a 4 digit number refers to a specific genetic sequence, eg, HLA A*2901, HLA B*0702.
 - The first two numbers refer to the serologic (protein structure) specificity.
 - The second two numbers refer to the specific genetic sequence encoding that protein.
 - d. The * designations are determined by DNA sequencing.
 - e. An 'N' indicates a non-expressed allele, eg, HLA B*5201N.
4. T cell helper subclasses
- Th1 cells; produce IL-2, 12 and IFN: promote anti infectious organism immunity, primarily cell mediated
 - Th2 cells; produce IL 4, 5 and 10: promote humoral immunity associated with allergic (IgE) responses some autoimmune diseases and some infections.
 - Th0 cells: produce IL2, 4 and IFN.
 - Th17 cells: produce IL 17, activated by IL-23.
 - Memory aid: **Principal role of T cells is CMI. Thus, Th1 help CMI, Th2 help Humoral Immunity (a secondary T cell function).**
5. T regulator cells
- CD4, CD25 (IL-2 receptor) positive
 - Responsible for suppressing autoimmune responses
 - May be involved in controlling antigraft responses
 - The elusive “suppressor cell”?



6. CD4 positive Cell Ag recognition: recognize Ag in the context of an MHC class II molecule.



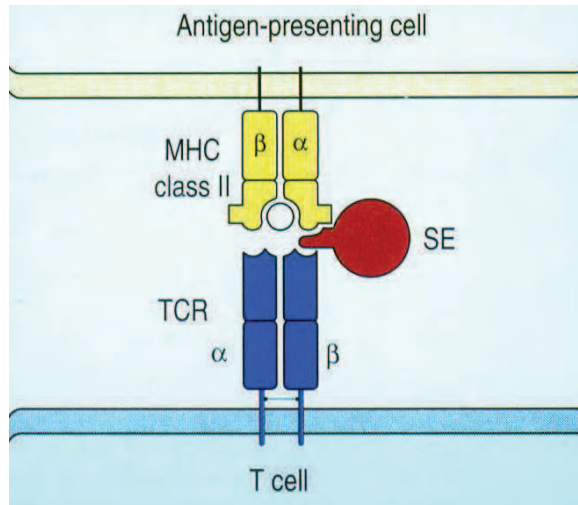
T-B Interactions



F. Superantigens

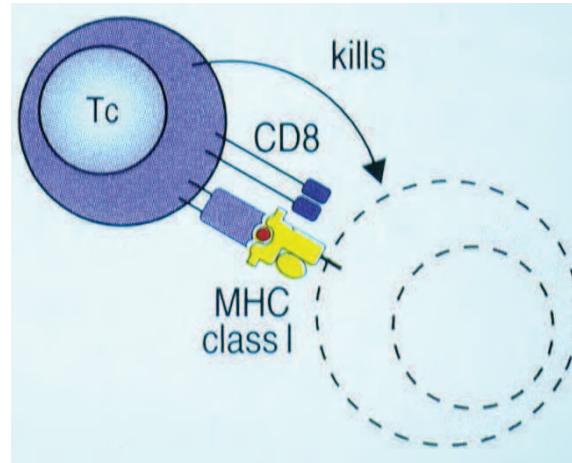
- Protein which binds directly to a V beta region of a T cell receptor and an MHC antigen
- Not processed by APC
- Up to 10-20% of T cells may share one V beta.
- Staph TSST-1

Bacterial superantigens bridge the T-cell receptor β -chain V region to the MHC class II α chain

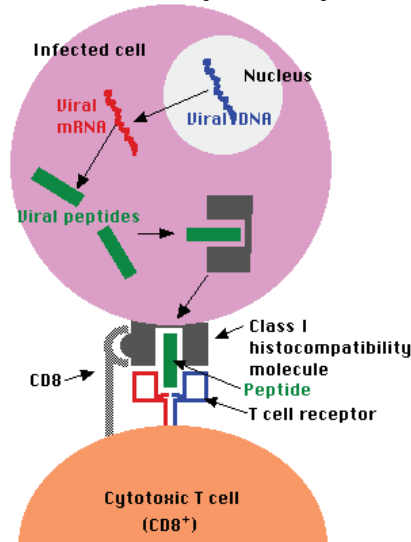


1. T cell suppression
 - Classically CD8 positive cells
 - May be cytotoxic response against idiotype positive cells
 - Suppression is mediated by Treg (CD4 positive).
 - CD8 cells should be called T cytotoxic cells, not T suppressor cells.
2. T cell cytotoxicity
 - a. CD8 positive cells recognize Ag presented by MHC, class I.
 - b. CD8 molecule binds to MHC, class I.
 - c. T cell releases enzymes.
 - Direct lysis
 - Induce target to enter apoptosis
 - d. Principal line of defense against
 - Virus infected cells
 - Intracellular bacteria
 - Fungi
 - Tumors

Cytotoxic T cell recognizes complex of viral peptide with MHC class I and kills infected cell



T Cell Cytotoxicity



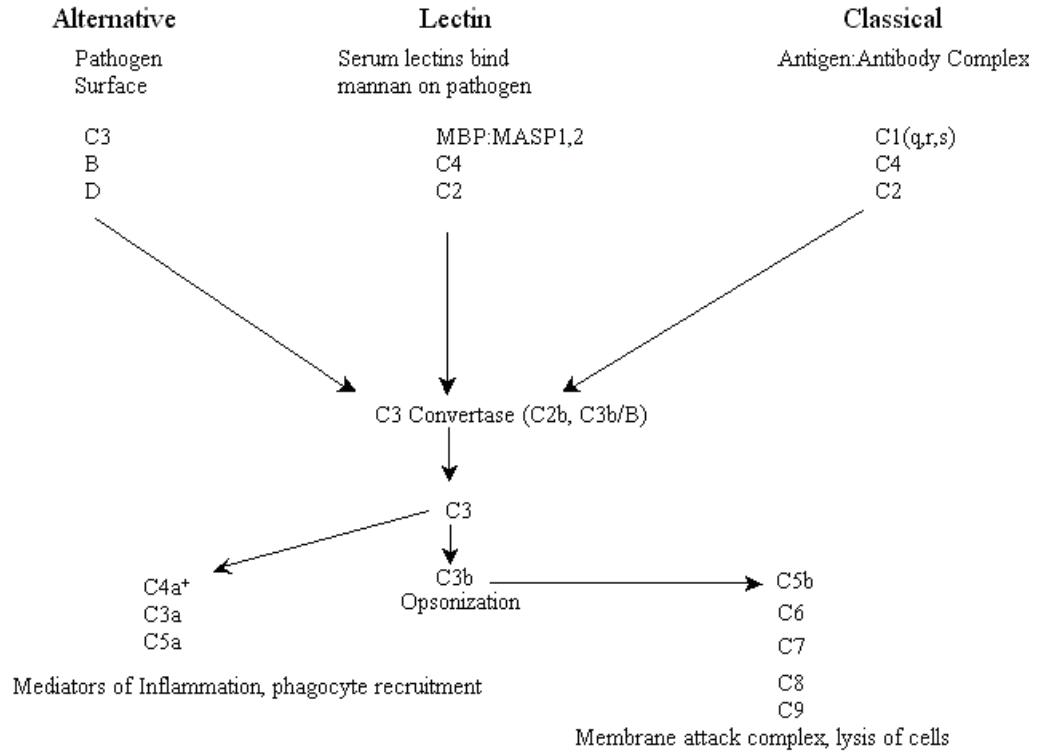
3. Memory aid
 - CD4 and CD8 subsets bind to antigen presented by different MHC class molecules.
 - CD4 binds to MHC class II, CD8 binds to MHC class I.
 - **The product of the CD number times the MHC class number is always 8.**
4. NK T cells
 - T cells which share NK markers (eg, CD16)
 - Recognize glycolipid Ag/MHC class I combination
 - Produce Th1 and Th2 cytokines
 - May have a role in immune regulation, tumor immunity and autoimmunity

G. Delayed type hypersensitivity (DTH)

- Mediated by CD4 cells: recent work implicates a CD8 role.
 - Occurs at the site of Ag location
 - Macrophage-T cell interaction: IL-1, IL-6, IL-8
 - Reactions reach a maximum at 48-72 hours.
1. Clinical examples

- Poison ivy
- PPD skin test
- 2. Stimulation assays
 - a. Activate an entire class of lymphocytes
 - b. T cell mitogens
 - PHA, Con A
 - Cylex ImmuKnow Assay
 - c. B cell mitogens
 - Pokeweed mitogen (T dependent, B cell mitogen)
 - *Staph aureus* protein A – B cell specific
 - d. Antigen stimulation – quantiferon in tube TB assay, or T Spot
 - In vitro ag stimulation
 - More specific for Mtb than PPD assay
- 3. Complement
 - a. A series of over 30 proteins
 - Work with antibody to effect lysis of target cells
 - Involved in the inflammatory response
 - b. Three methods of activation
 - Classical
 - Alternative
 - Mannose binding
 - 1) **Classical activation**
 - AgAb complexes with IgM or IgG 1 or 3
 - Complex binds to C1q.
 - Activation sequence: C1, 4, 2, 3, 5, 6, 7, 8, 9
 - 2) **Alternative pathway**
 - Properdin factor B/Zymogen induced C3 cleavage.
 - Once C3 is cleaved, the rest of the pathway will continue.
 - Fluid phase C3 convertase continues this pathway.
 - 3) **Mannose lectin binding**
 - Mannose binding lectin associated proteases (MASP) 1 and 2 can cleave C4.
 - This starts the classical pathway from C4 on.
 - Associated with *Salmonella*, *Listeria*, *Neisseria*, *Candida* and *Cryptococcal* infections

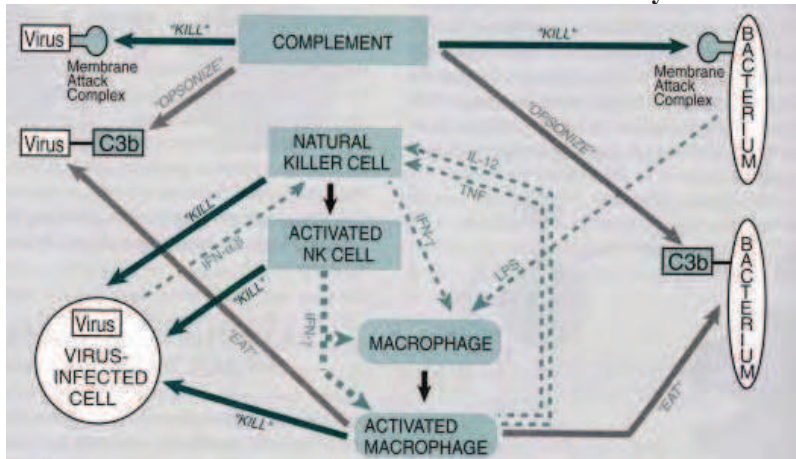
Complement Activation Pathways



c. Complement assays

- 1) Quantitative – C3, C4, commonly by nephelometry
- 2) Functional
 - CH50 – titer of serum that contains sufficient complement to lyse 50% of a known amount of Ab coated RBC.
 - AH50 – similar, measures C3-C9

Effector Mechanisms of the Innate Immune System



Downloaded from http://www.mse.cornell.edu/courses/mse461/assets/images/innate_immune_sm.JPG