Medical Genomics of the Human Immune System
For PMK Medical Student Y3

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Content

B1.4.7 Immunogenetics: MHC structure and function, erythrocyte antigens

B1.5.5 Diseases of the immune system

I. Molecular Immunogenetics

II. Clinical Immunogenetics

I. Molecular Immunogenetics

Innate Immunity

Non-specific responses such as phagocytosis

Adaptive Immunity

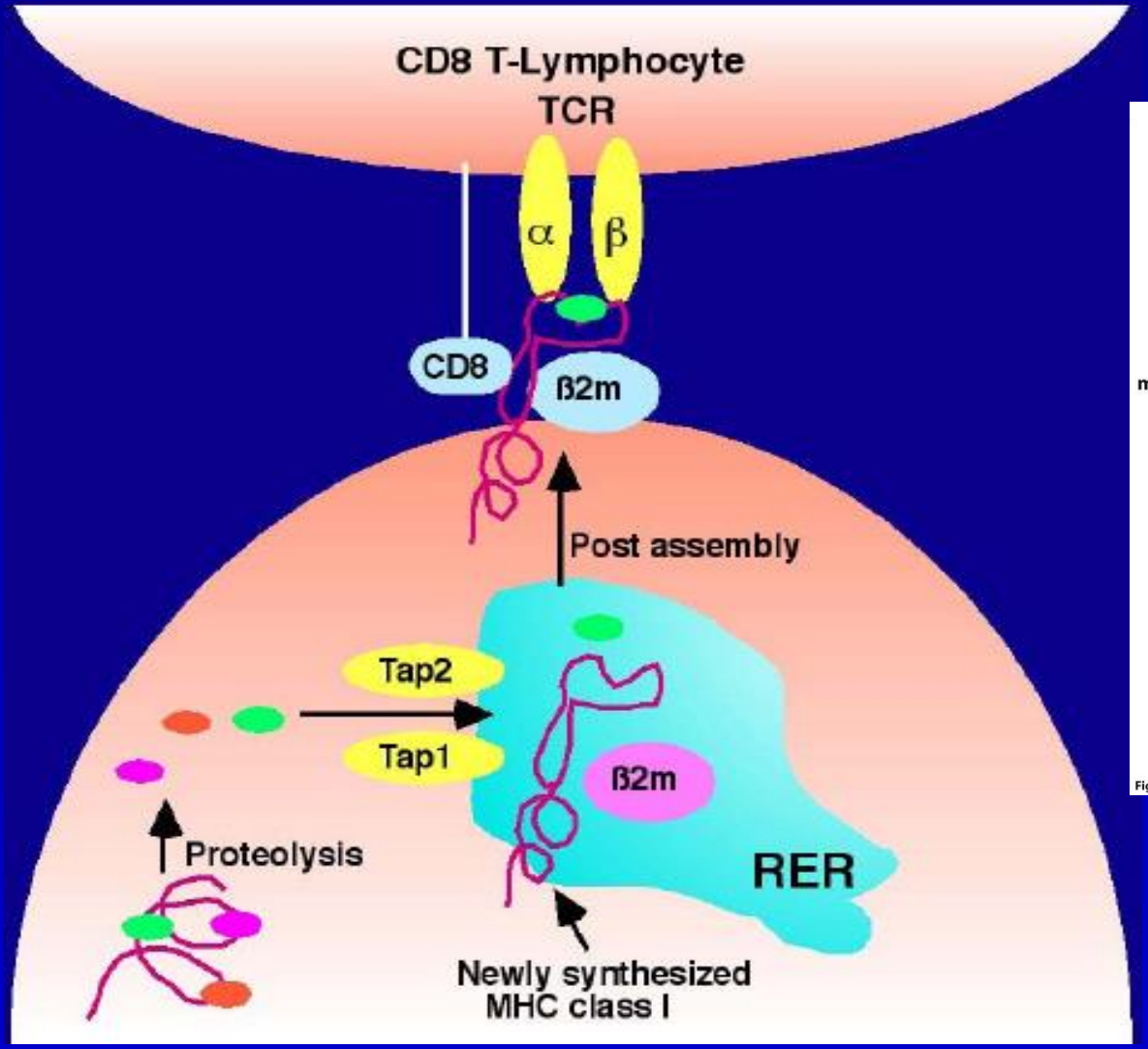
Specifically acquired T and B lymphocytic responses to particular antigens

Genes of Key Components of the Adaptive Immune Response

- **Major Histocompatibility Complex (MHC)**
- **Immunoglobulin (Ig)**
- **T-Cell Antigen Receptor (TCR)**

“ Immunoglobulin gene superfamily ”

MHC Class I Mediated Antigen Presentation



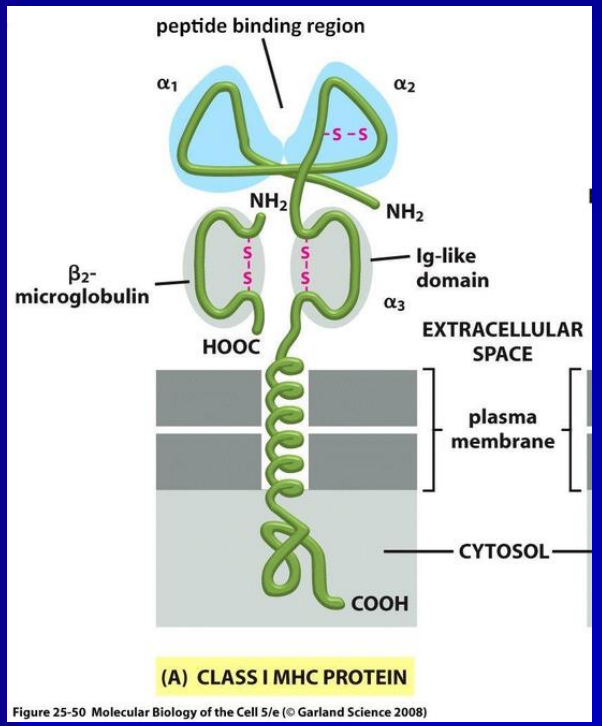
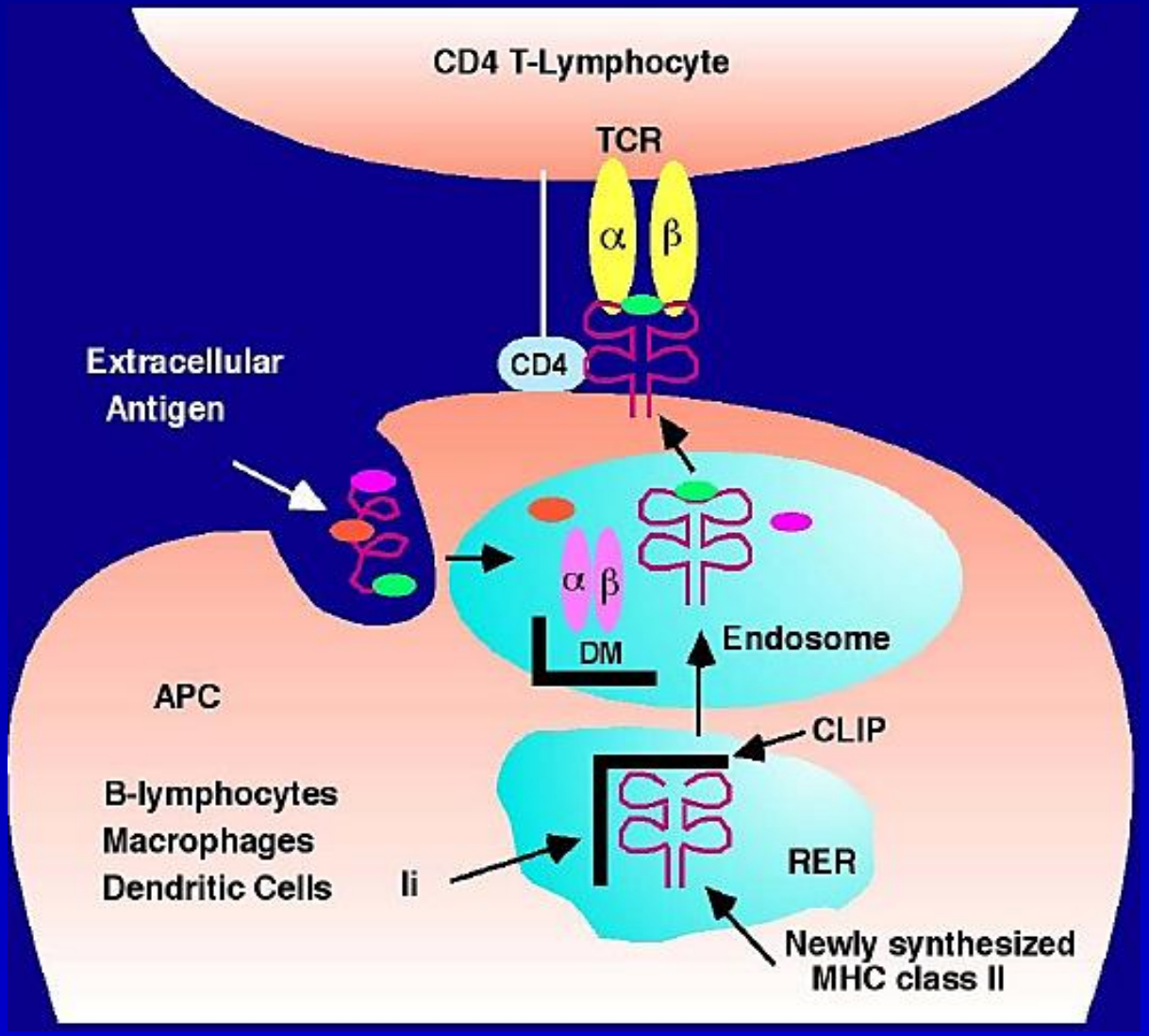
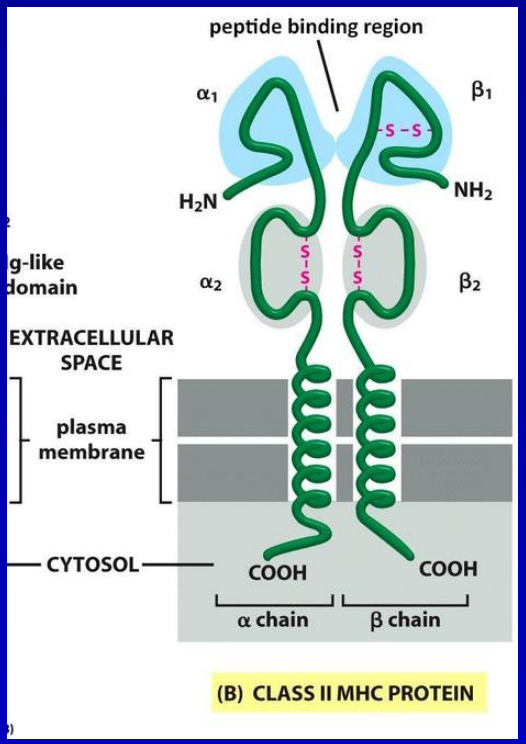


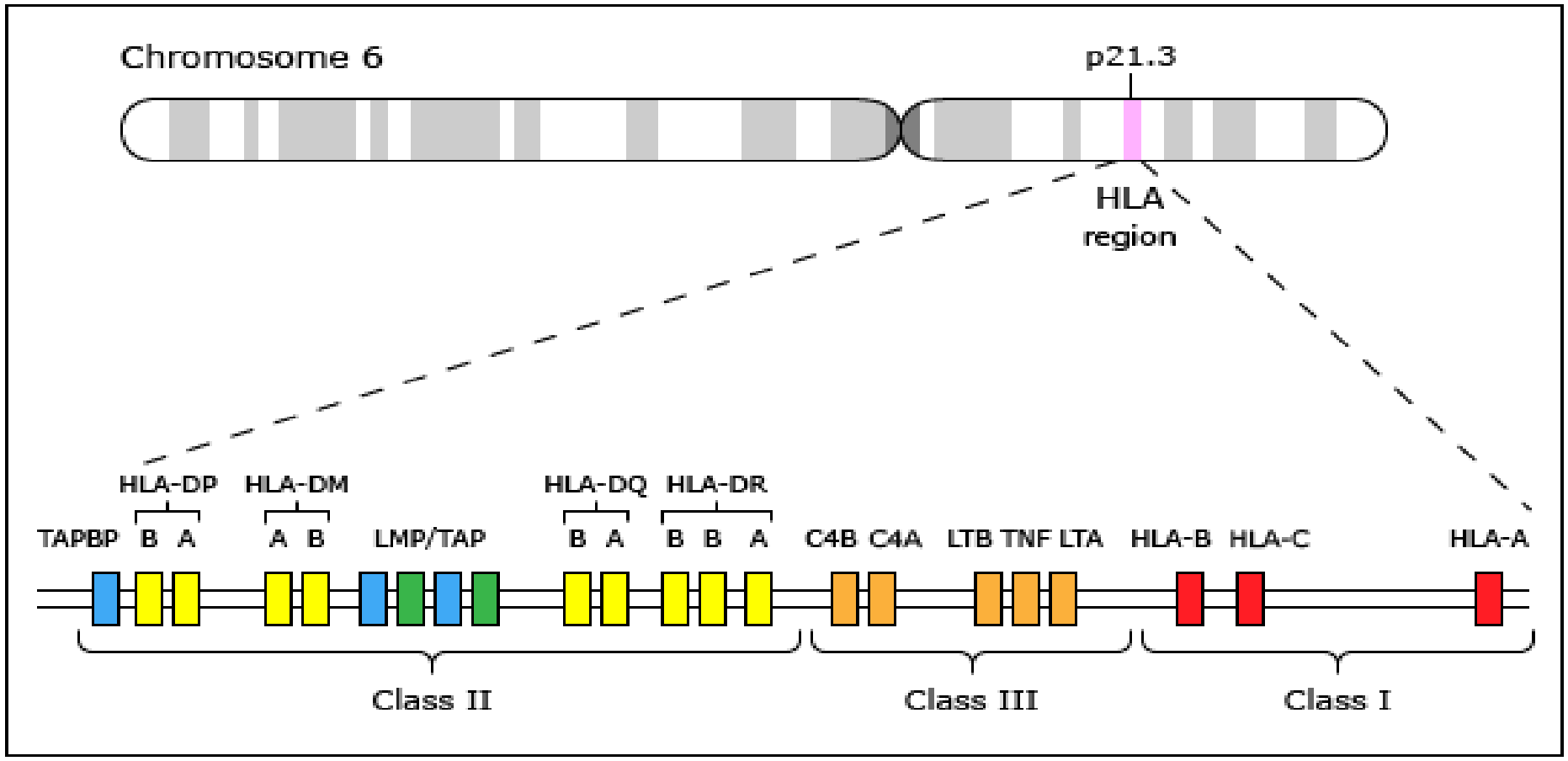
Figure 25-50 Molecular Biology of the Cell 5/e (© Garland Science 2008)

MHC Class II Mediated Antigen Presentation





Gene Structure of the Human MHC



Class I + II → Classical HLA

Major Histocompatibility Complex

MHC region:

- The MHC genomic region, located on the short arm of **chromosome 6 (6p21.3)**
- Contains the HLA (Human Leukocyte Antigen) genes
- At least 200 other genes (≈40% involved in some aspect of the immune response)
- Spanning ≈ 3.6 Mb (Shiina et al, 2009)
- It is the most gene-dense region of the genome (≈ 1 gene/16 kb)
- High levels of polymorphism
- Referred to as the **HLA region** (in humans)

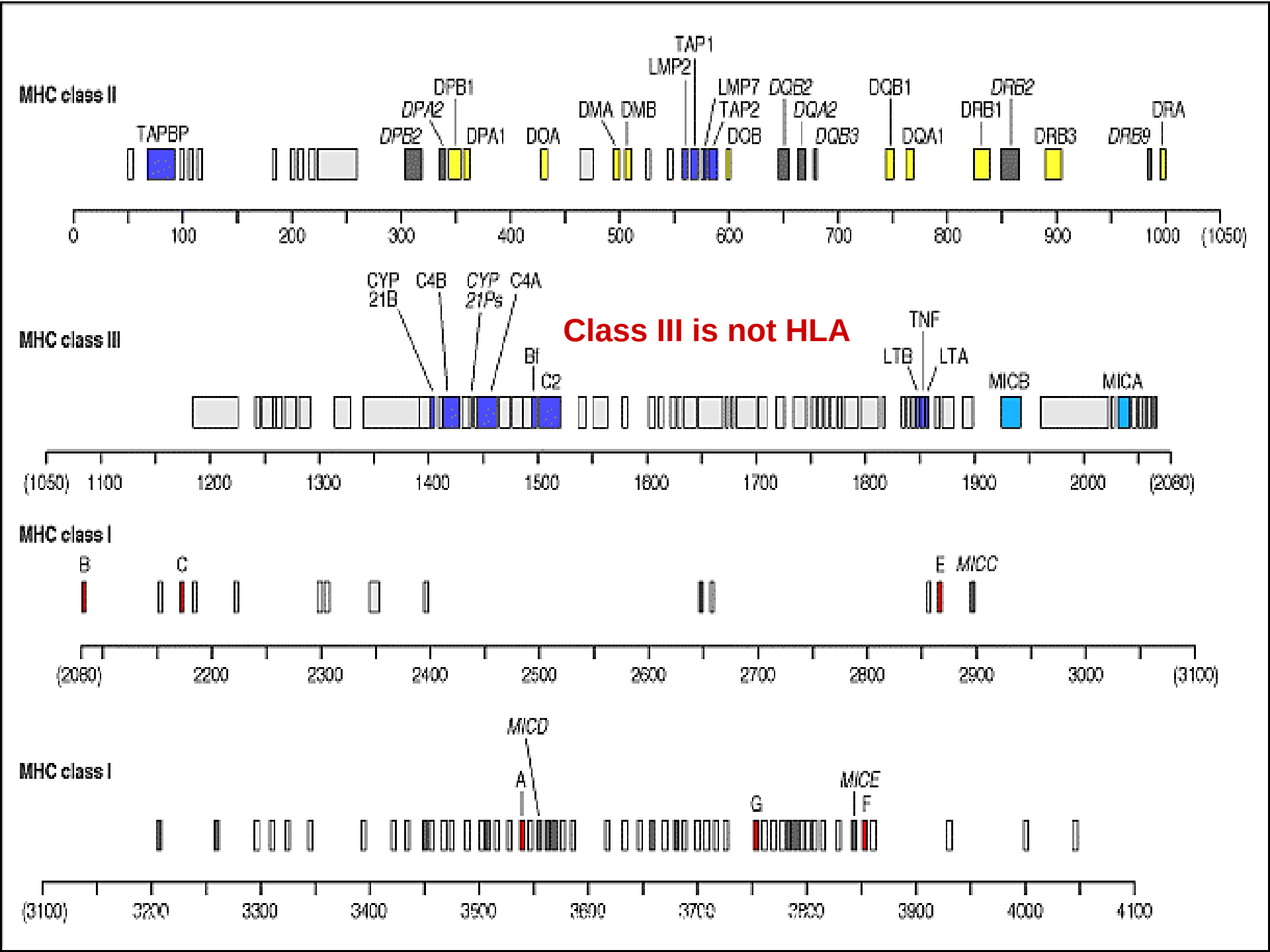
Major Histocompatibility Complex

Extended MHC region:

- ≈ 8 Mb of chromosome 6, containing over 250 protein-coding genes
- This region expands the MHC borders to segments with high linkage disequilibrium with the MHC
- Contains additional genes involved in the immune response (Horton et al, 2004; Shiina et al, 2009)

MHC / HLA Class

- **Class or loci of HLA genes:**
  - **Classical HLA genes** are the highly polymorphic loci that code for the proteins that present peptides to the T-cell receptors (*HLA-A, -B, -C, -DPA1, -DPB1, -DQA1, -DQB1, -DRB1, -DRA1*) → **Class I, II**
  - **Non-Classical genes** which have reduced polymorphism and do not have a role in peptide presentation. → **Class III**

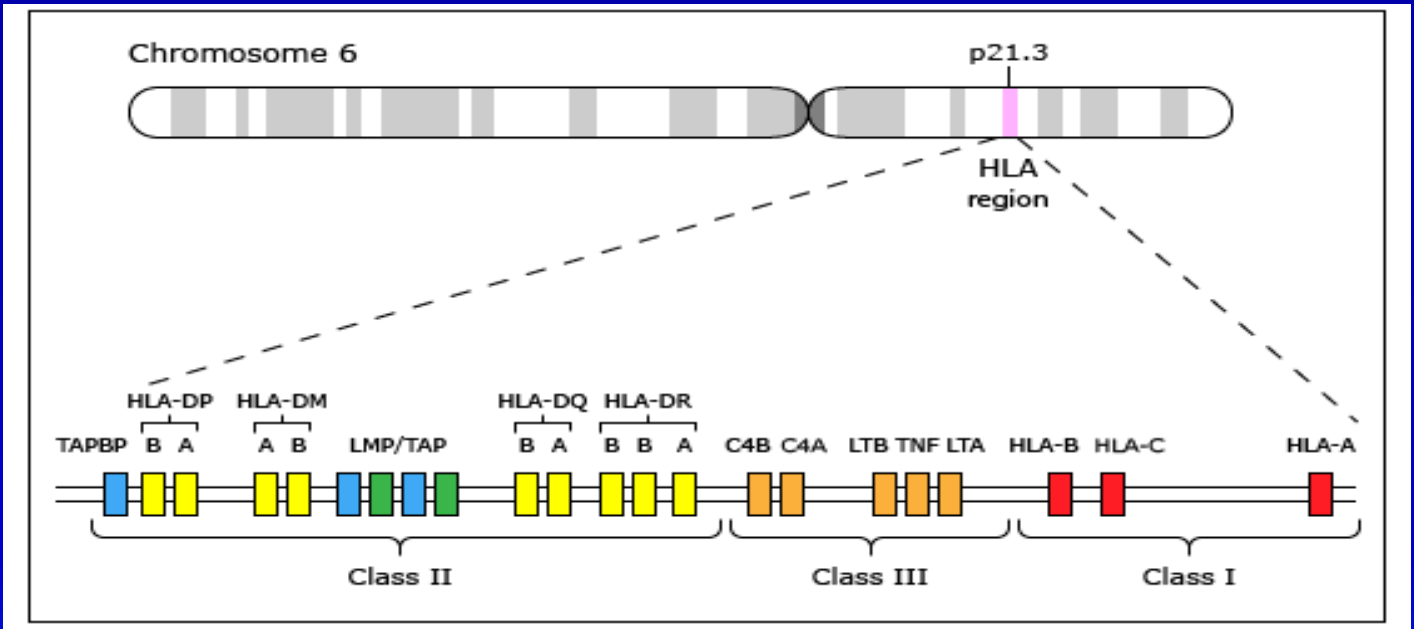

MHC class II
TAPBP
DPA2
DPB2
DPB1
DPA1
DOA
DMA
DMB
TAP1
LMP2
LMP7
TAP2
DOB
DQB2
DQA2
DQB3
DQB1
DQA1
DRB2
DRB1
DRB3
DRB9
DRA
0
100
200
300
400
500
600
700
800
900
1000
(1050)
MHC class III
CYP 21B
C4B
CYP 21Ps
C4A
Bf
C2
Class III is not HLA
TNF
LTB
LTA
MICB
MICA
(1050)
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
(2060)
MHC class I
B
C
E
MICC
(2060)
2200
2300
2400
2500
2600
2700
2800
2900
3000
(3100)
MHC class I
MICD
A
G
MICE
F
(3100)
3200
3300
3400
3500
3600
3700
3800
3900
4000
4100

HLA allele

- A specific DNA sequence at an HLA gene
- Can be thought of as a haplotype of SNP variants
- There are extraordinarily large numbers of HLA alleles, with **> 12,000 for class I** alleles and **> 4,000 for class II**.

HLA Haplotype

The combination of alleles at several HLA genes on a single chromosome of a given individual.



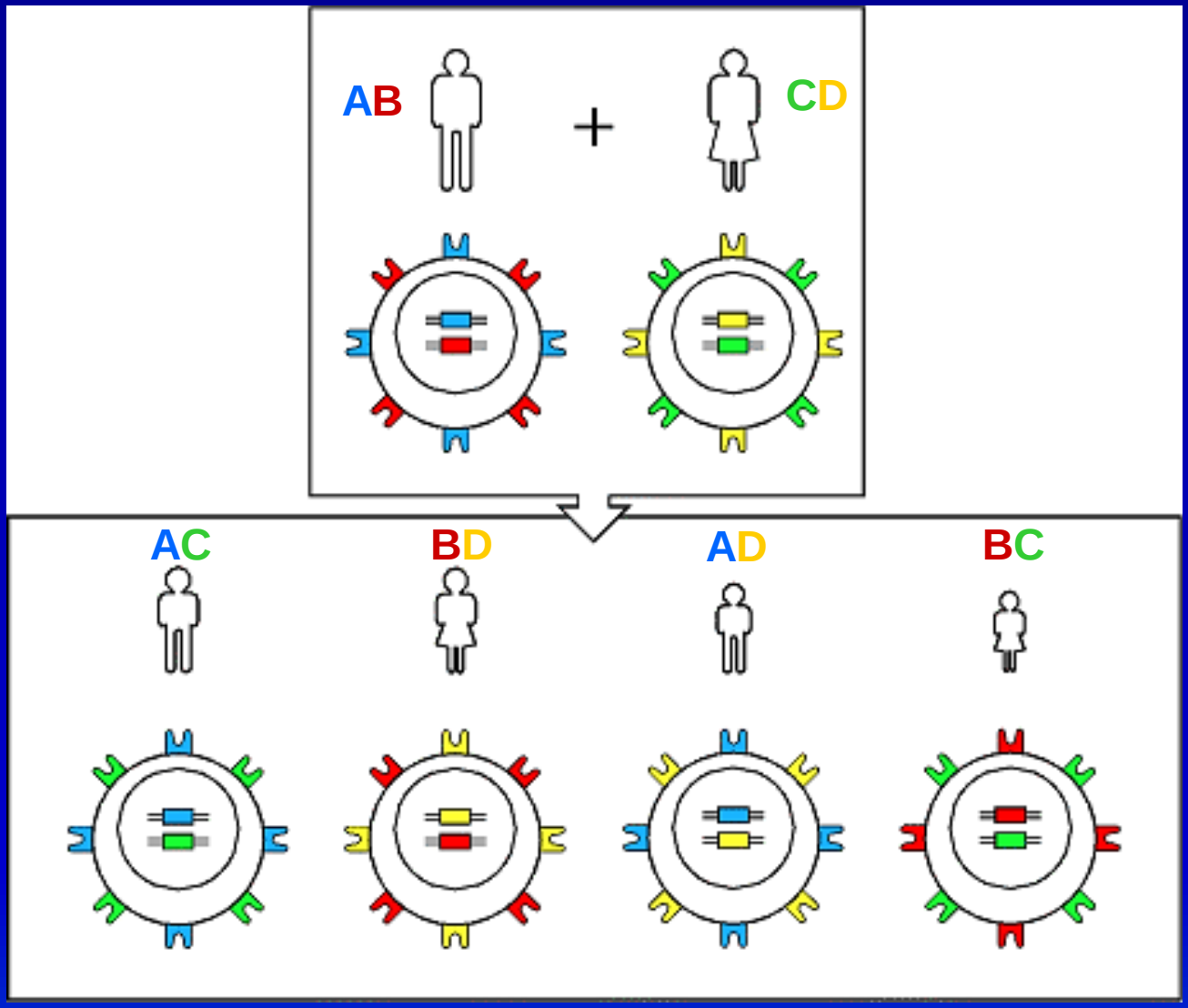
Protein & DNA Variation at HLA Loci

HLA Locus	Antigenic Variants	DNA Variants
HLA-A	25	83
HLA-B	53	186
HLA-C	11	42
HLA-DR	20	221
HLA-DQ	9	49
HLA-DP	6	88

HLA Function

- **HLA class I** molecules
  - Expressed in most cells, bind peptides of intracellular origin and present them on the cell surface to the TCRs of CD8+ T-cells
  - Initiating a cytotoxic response if they are recognized as foreign.
- **HLA class II** molecules
  - Expressed in antigen presenting cells, typically bind peptides of extracellular origin and present them to the TCRs of CD4+ T-cells
  - Triggering a signaling process that leads to the multiplication of T-helper cells
  - Leading to the stimulation of B-cells, which produce antibodies to the antigen that triggered the response.

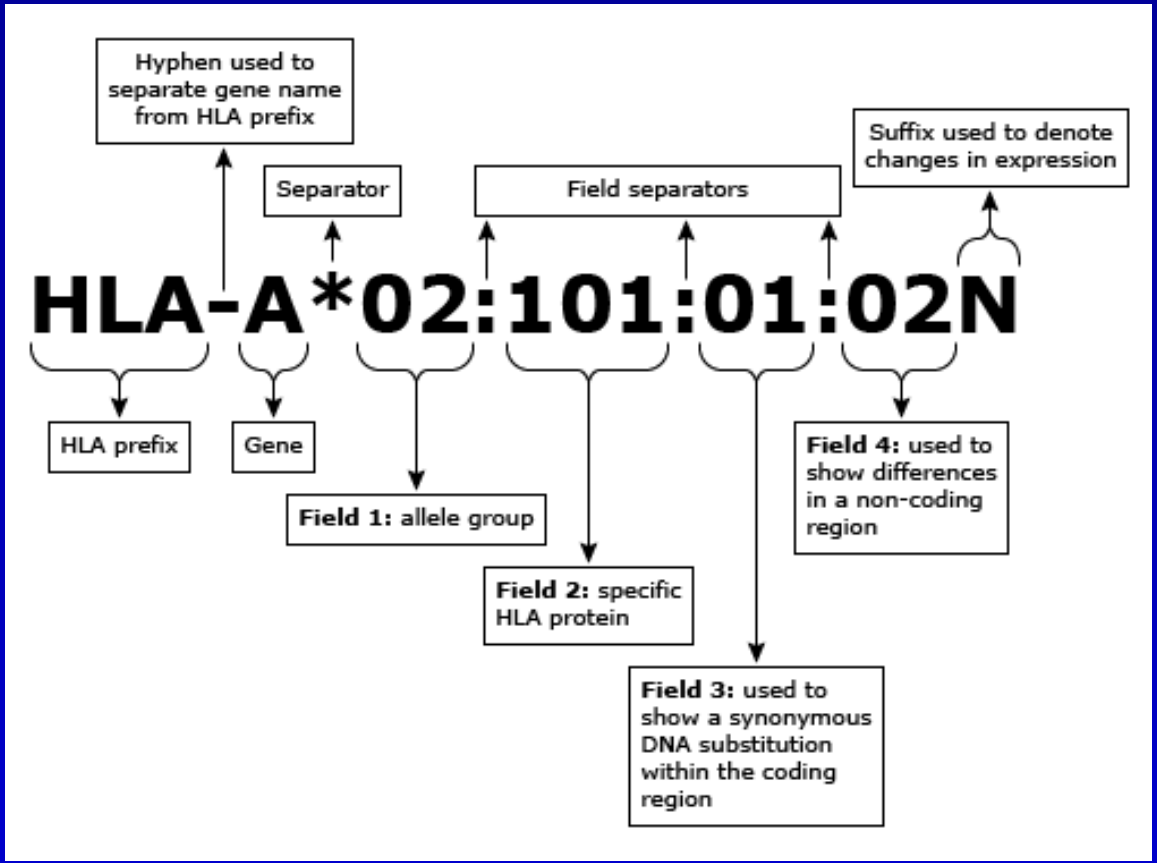
Inheritance of HLA Haplotypes



HLA Allele & Diseases Associations

Ankylosing spondylitis	B27
Reiter syndrome	B27
Acute anterior uveitis	B27
Nacrolepsy	DQ6
Celiac disease	DQ2
Hemochromatosis	A3
Congenital adrenal hyperplasia	Bw47

Nomenclature of HLA Allele



N = Null

L = Low

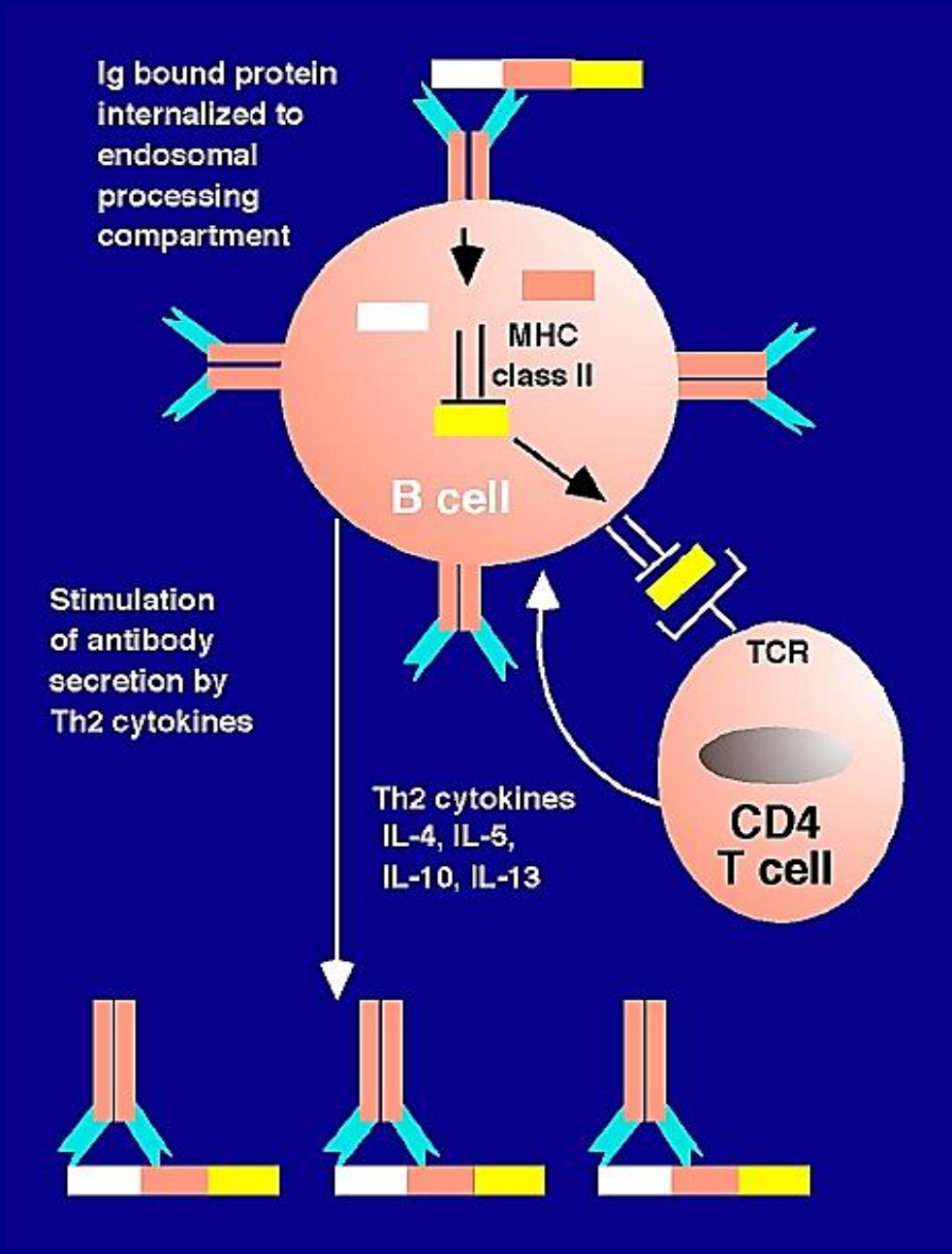
S = Secreted

C = Cytoplasm

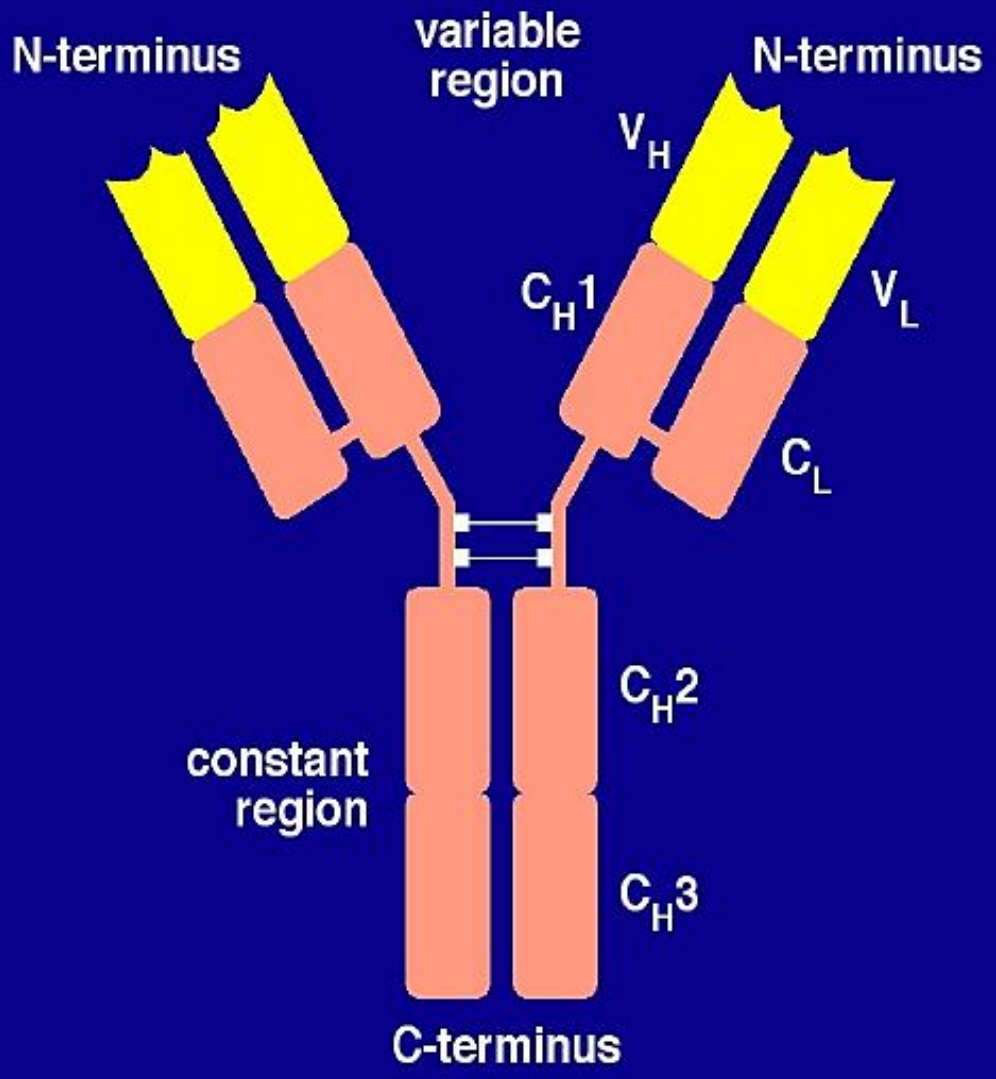
A = Aberrant

Q = Questionable

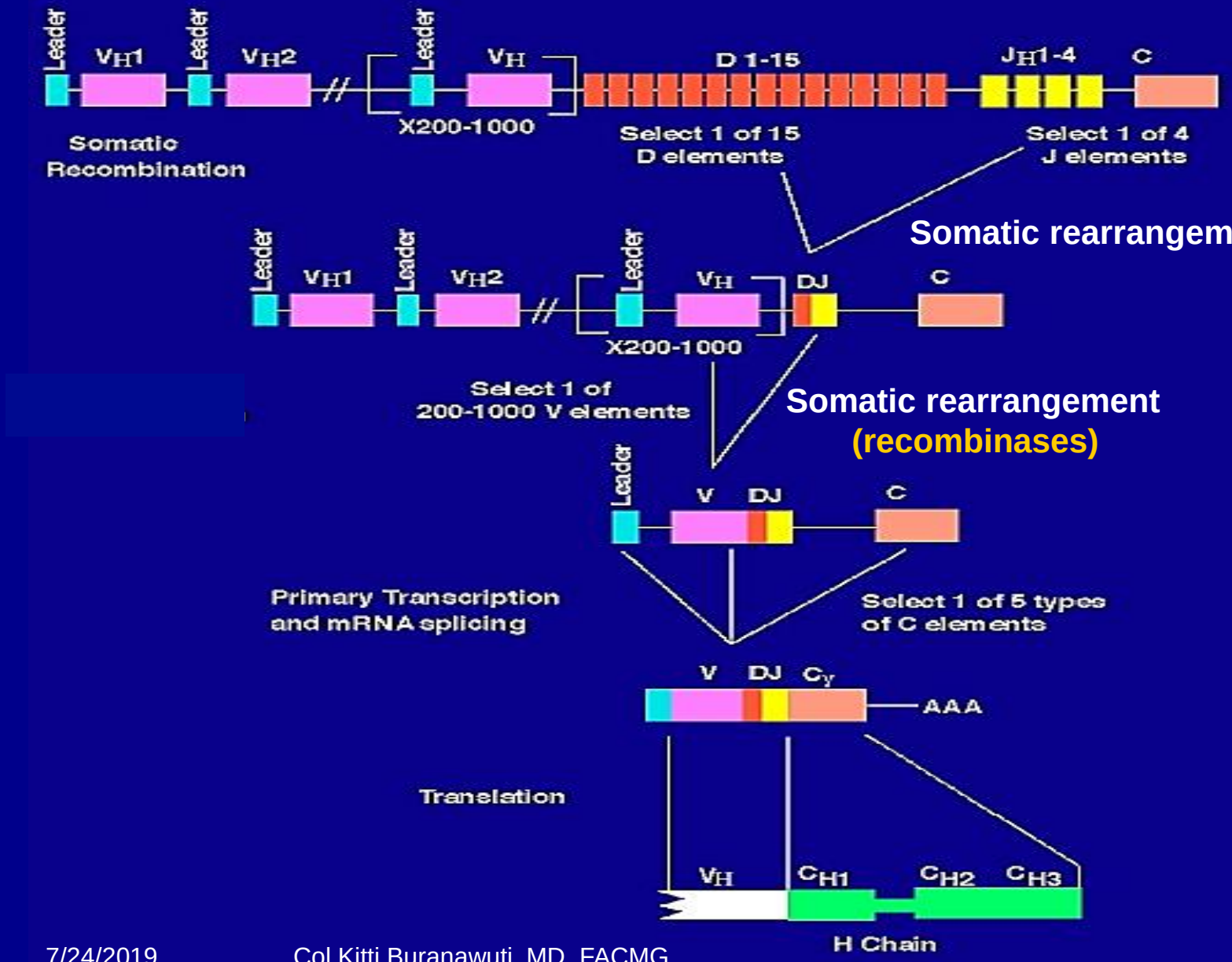
Immunoglobulin (Ig)



Immunoglobulin Structure

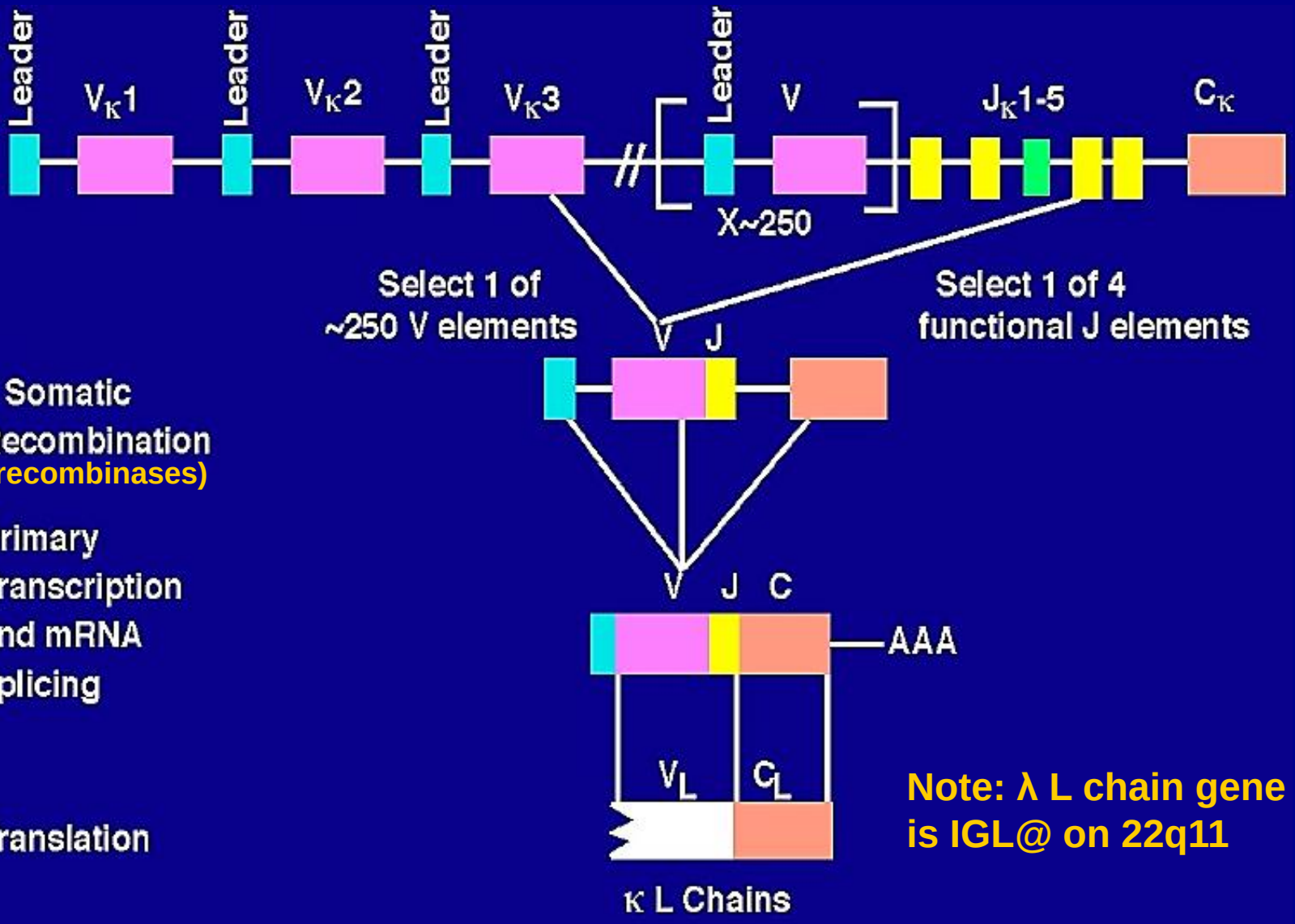


Ig H Chain Gene Rearrangements



IGH@ 14q32

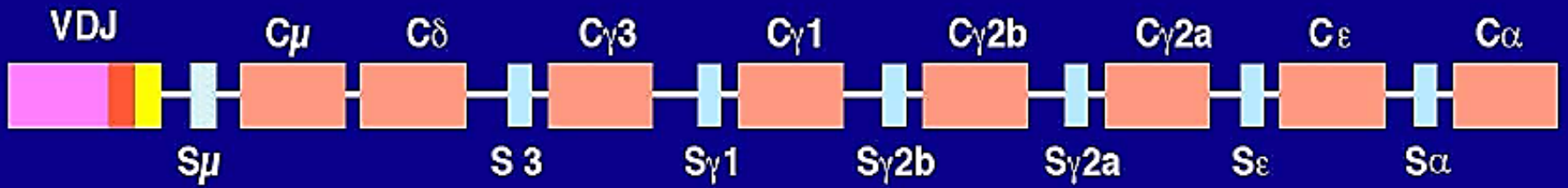
Ig L Chain Gene Rearrangements



IGK@ 2p12

Note: λ L chain gene is IGL@ on 22q11

Genes Controlling Isotype Switching of Antibodies



Constant Region of the H Chain Controls Antibody Function

Ig Isotype	Representative Immunological Functions
M	Complement activation
D	Unknown
G	Complement activation, placental transfer, binding to macrophage Fc receptors
E	High affinity binding to mast cells and basophils to elicit histamine/serotonin release
A	Mucosal membrane immunity

Additional factors contributing to antibody diversity

- **Junctional diversity**
  Results from the insertion of additional untemplated nucleotides to double stranded V, D, J sequences undergoing recombination

- **Imprecise joining**
  Results from variability in original points of DNA fragmentation in V, D, J sequences undergoing recombination

- **Combinatorial diversity**
  Results from the pairing of H and L chains produced by independent gene rearrangement events

Restricted Expression

- Isotypic exclusion

  Ig produced by single B cell contains only a single H isotype and a single L isotype

- Allelic exclusion

  Either the paternal or maternal allele for each H and L chain is expressed within single cell

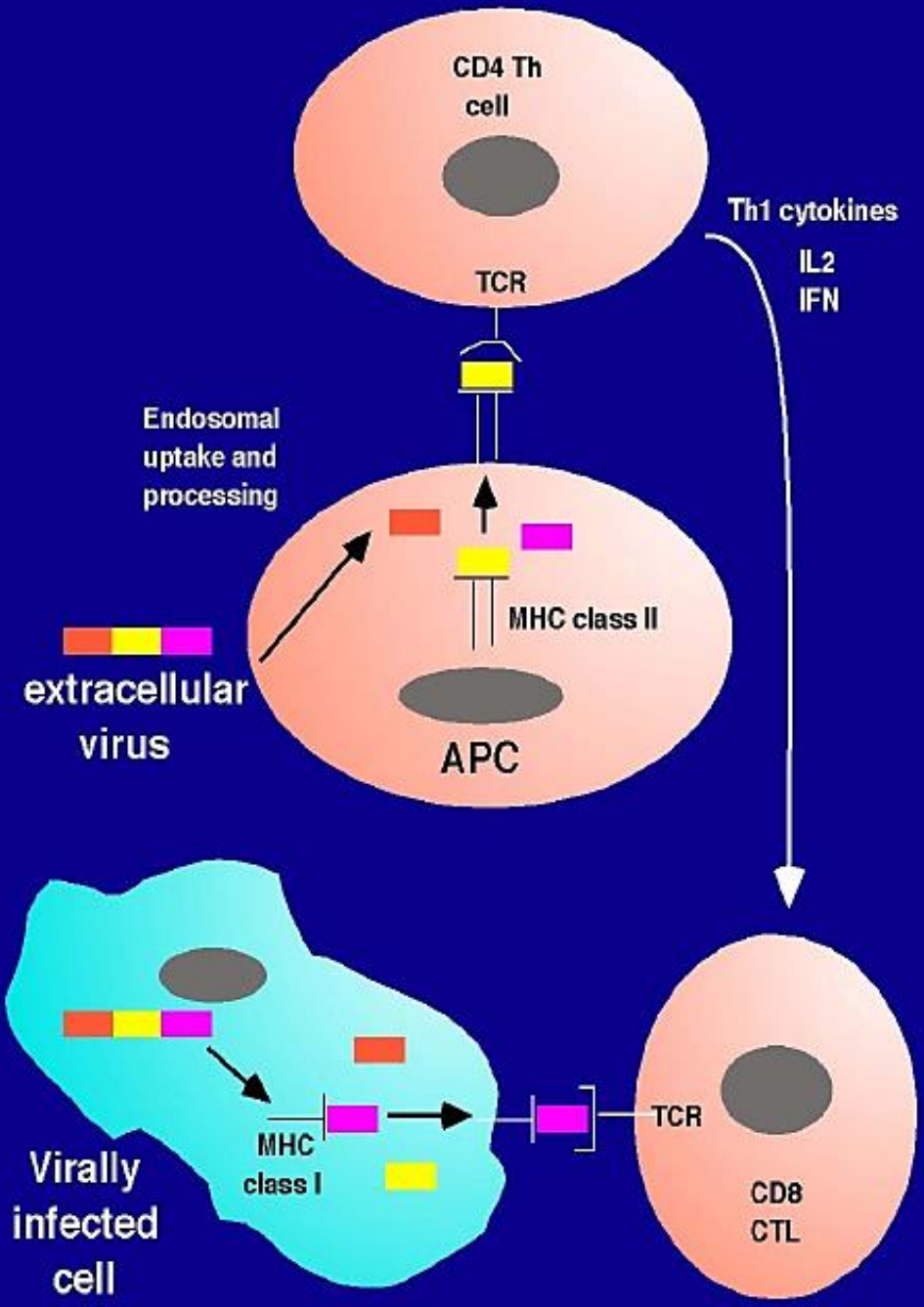
Generation of Antibody Diversity

Germline Genes	H	κ	λ
V	200	100	100
D	20	-	-
J	6	5	6
V x D x J	2.4×10^4	500	600
H x κ	1.2×10^7		
H x λ	1.4×10^7		
Total potential repertoire	$\sim 10^{11}$		

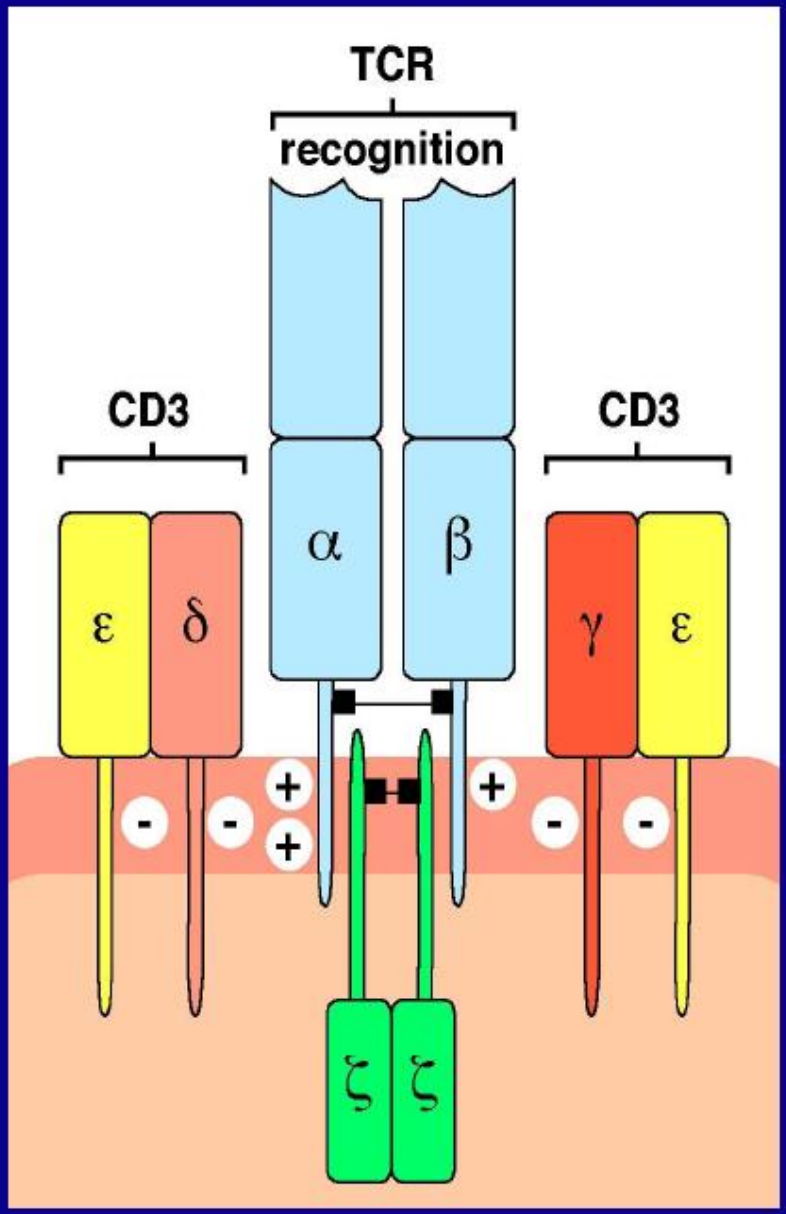
Immunoglobulin Diversity

- Unique somatic rearrangement of DNA sequence in lymphocyte precursor cells
- Can generate ~ 10^{11} different antibodies
- Protect against the large array of infectious organisms, toxins, cancer cells

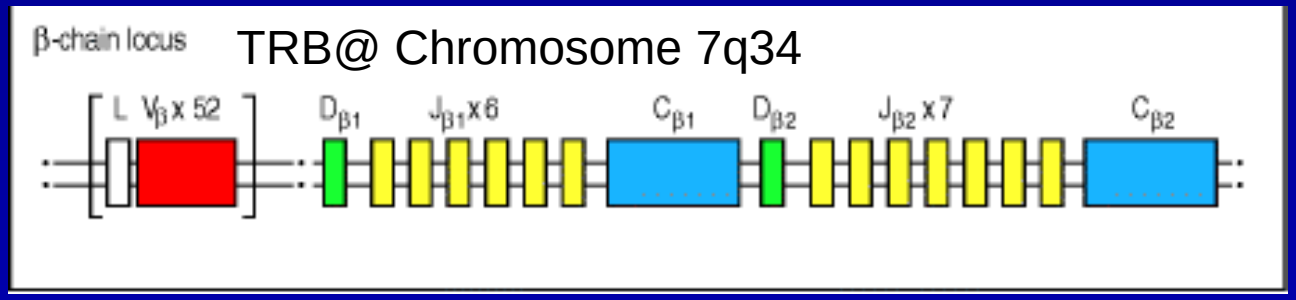
TH1 Cytokine Mediated Cooperation Between CD4 and CD8 T Cells

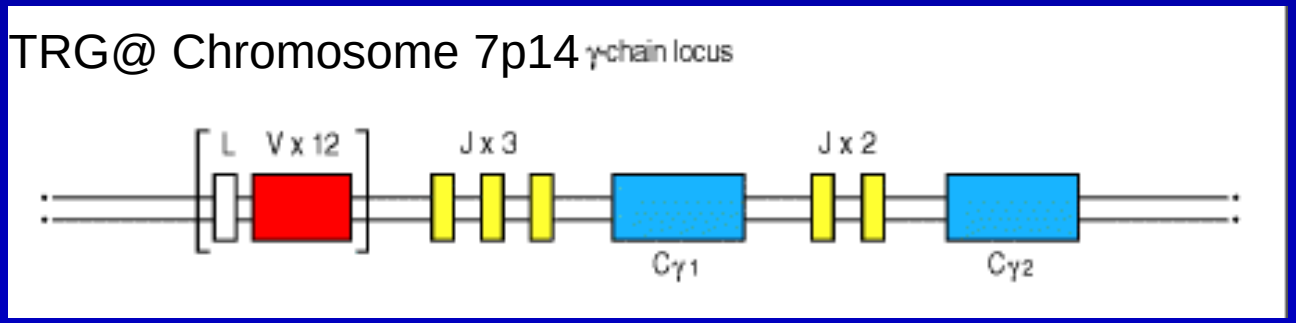


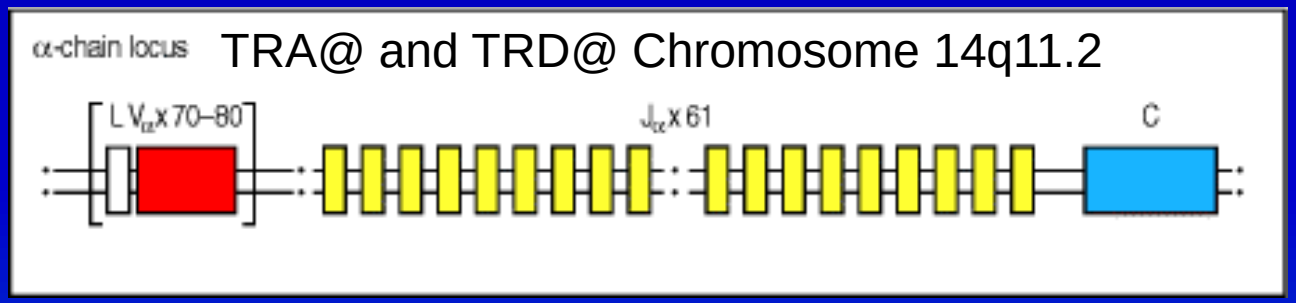
T Cell Receptor (TCR)

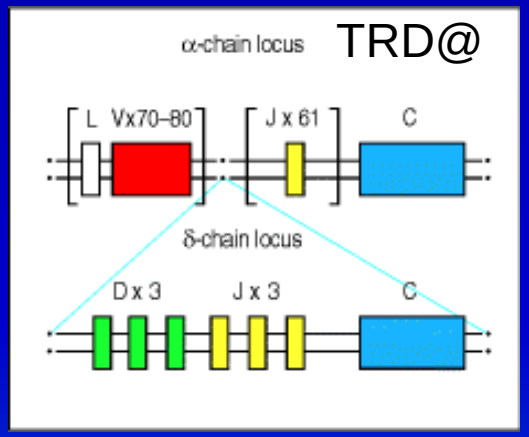


T Cell Receptor Genes

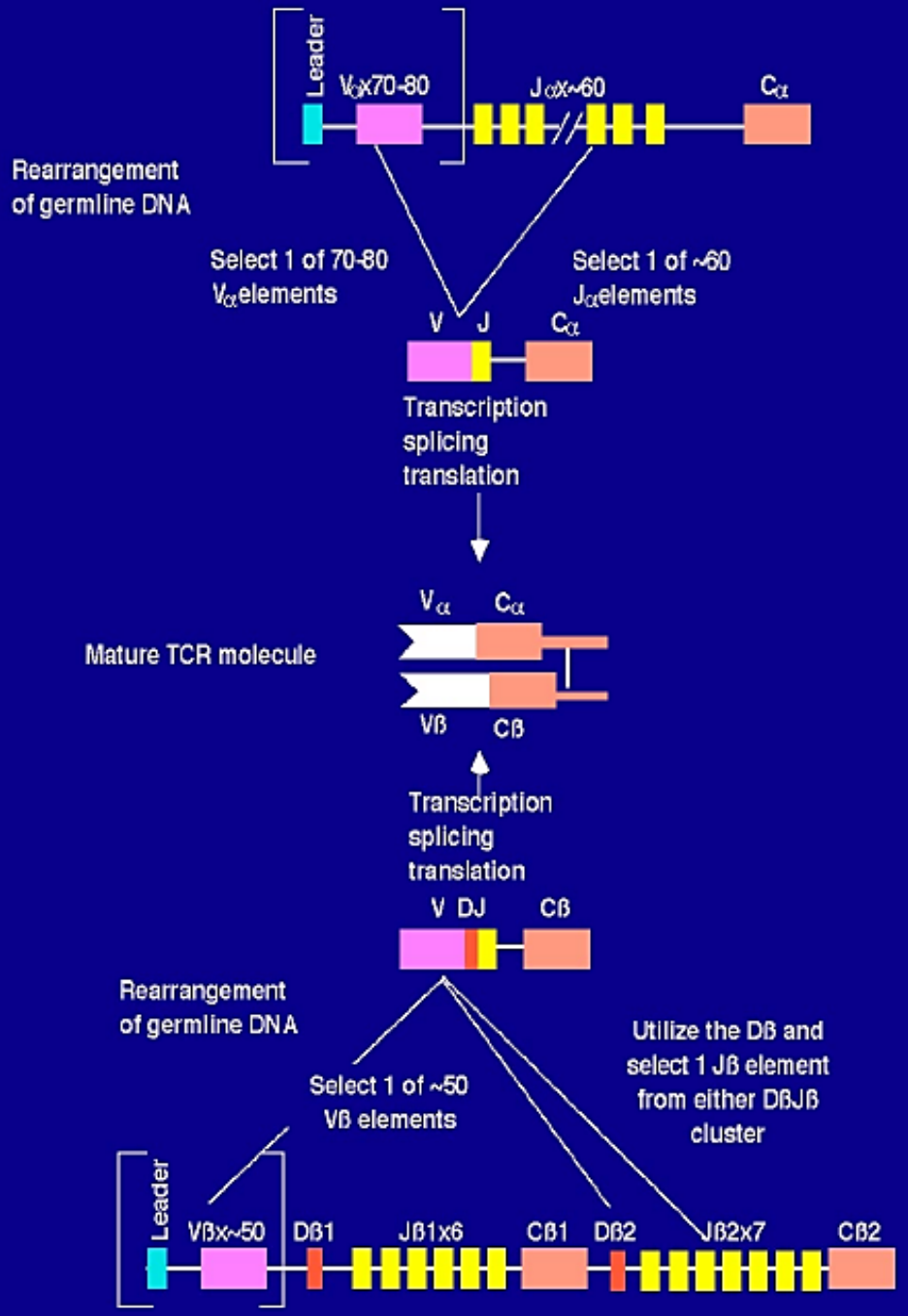




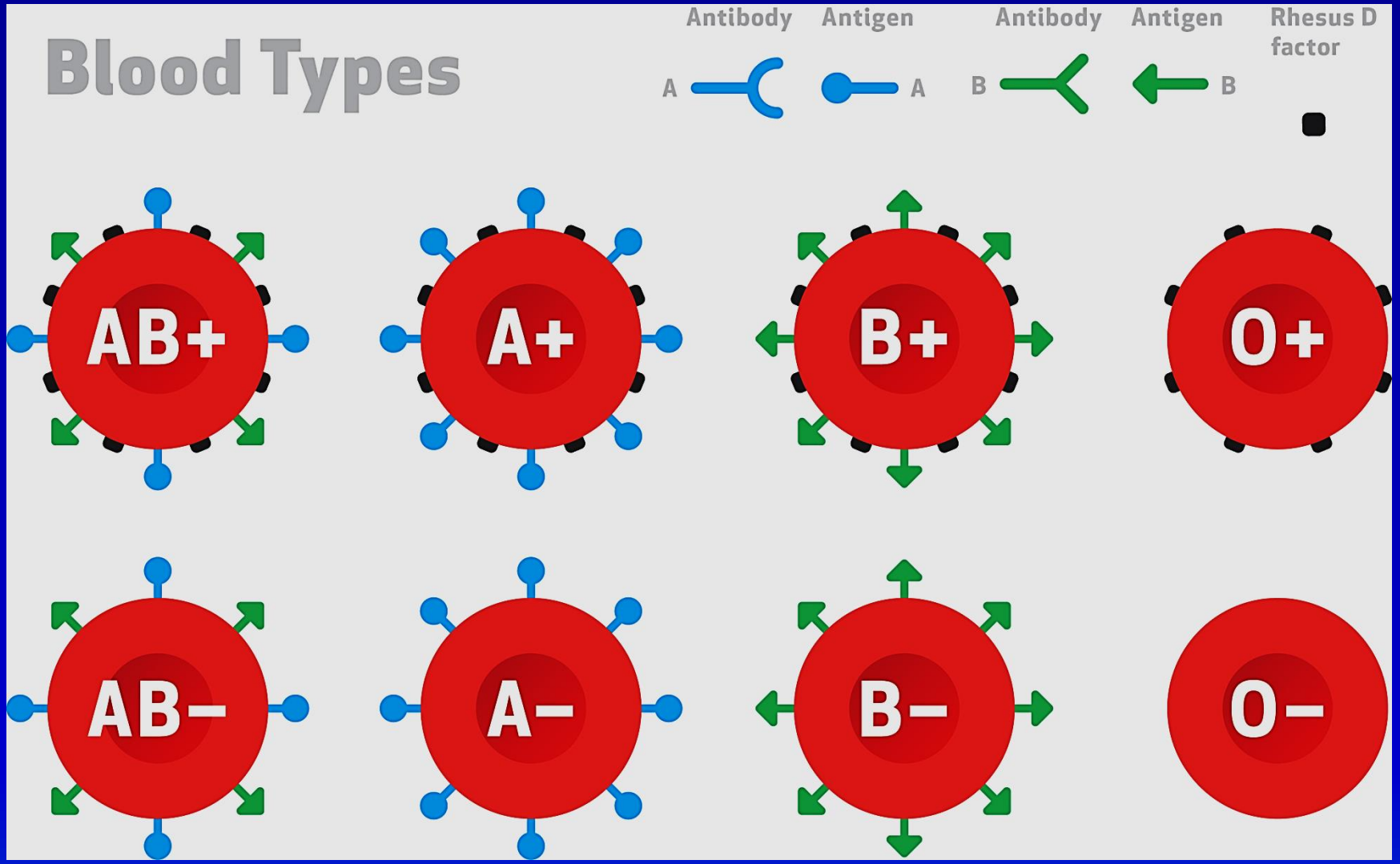




T Cell Receptor Gene Rearrangement Patterns

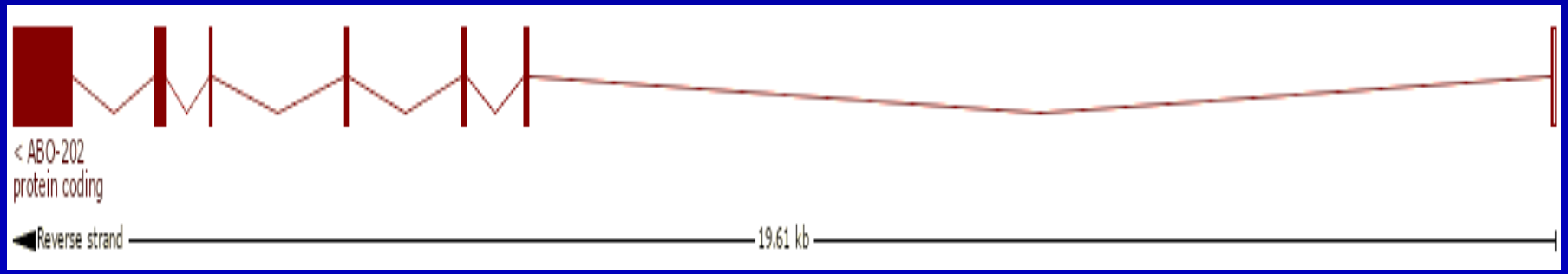


ABO Red Cell Antigen



ABO gene

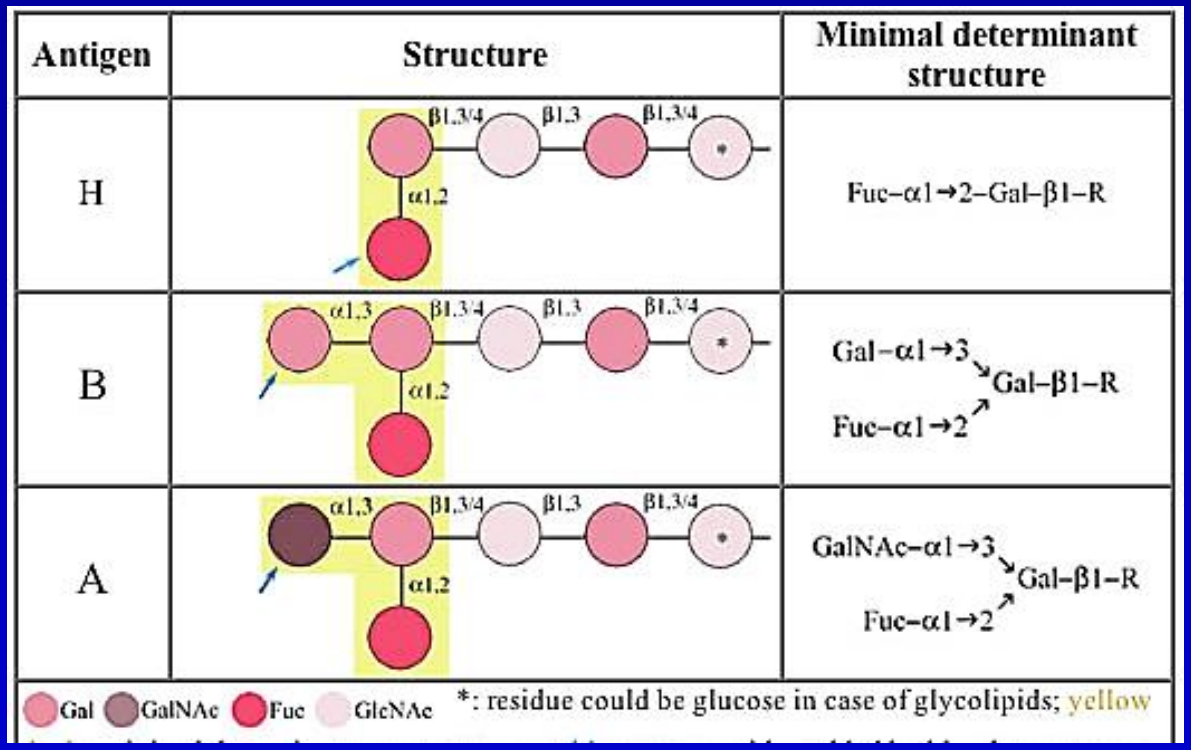
- **Chromosome: 9q34.2**
- **ABO gene is organized in 7 exons**
- **Spans over 19 kb**



- **Base pairs: 1,147**
- **Amino acids: 373**

ABO Alleles

- **O blood:** deletion of guanine 258 near the N-terminus of the protein which results in a frameshift and translation of an almost entirely different protein



- **A, B blood:**
  - The first of the seven **nucleotide substitutions which distinguish the A and B transferases, resides in coding exon 6**; exon 7 contains the other 6 nucleotide substitutions which result in four amino acid substitutions that differentiate the A and B transferases.
  - Among those, substitutions responsible for alterations at two sites (L266M and G268A) determine the A or B specificity of the enzyme (Yamamoto and Hakamori).

II. Clinical Immunogenetics

When To Suspect a Host Defense Defect?

- Frequent infections
  - Recurrent
  - Persistent, despite therapy
  - Severe
  - Caused by opportunistic pathogens
- Failure to thrive
- Family history

Immune Defects and Infections

Adaptive Immune System

- T cell or combined defect--viruses, fungi and bacteria; opportunistic pathogens
- B cell defect--bacterial; respiratory tract

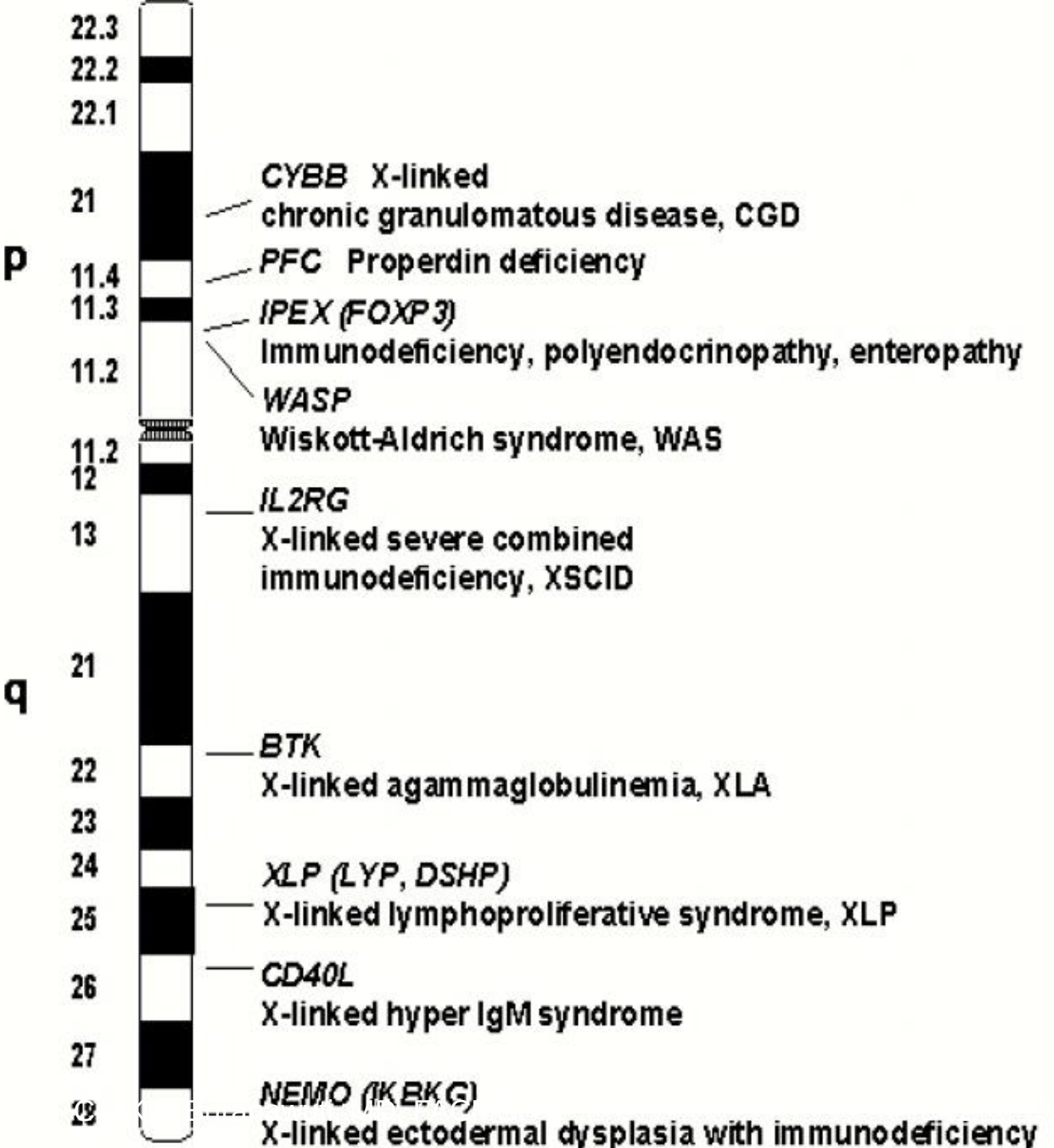
Innate Immune System

- NK cell defect--virus-infected cells (cancer cells)
- Granulocyte defect--staphylococci, other bacteria
- Macrophage activation defect--mycobacterial disease
- Complement defect (C5-9)--neisserial infections

Examples of Some Genetically Caused Immunodeficiency Syndromes

Immunodeficiency syndrome	Causative defect	Characteristic immune defect
Severe combined immunodeficiency (*scid*)	Several including a defect in the *Prkdc* DNA repair gene, as well as ADA or PNP deficiencies	No T or B lymphocytes
MHC class I deficiency	*Tap* gene mutations	No CD8 T cells
MHC class II deficiency	At least five, including a defect in the class II transactivator (*CIITA*) gene	No CD4 T cells
X-linked agamma-globulinemia	*Btk* tyrosine kinase gene	No B lymphocytes
X-linked hyper-IgM syndrome	Defective CD40 ligand (*Cd40l*) gene	No isotype switching from IgM
Phagocyte deficiencies	Multiple gene defects	Loss of phagocytic activity
Complement deficiencies	Multiple gene defects	Loss of complement activity

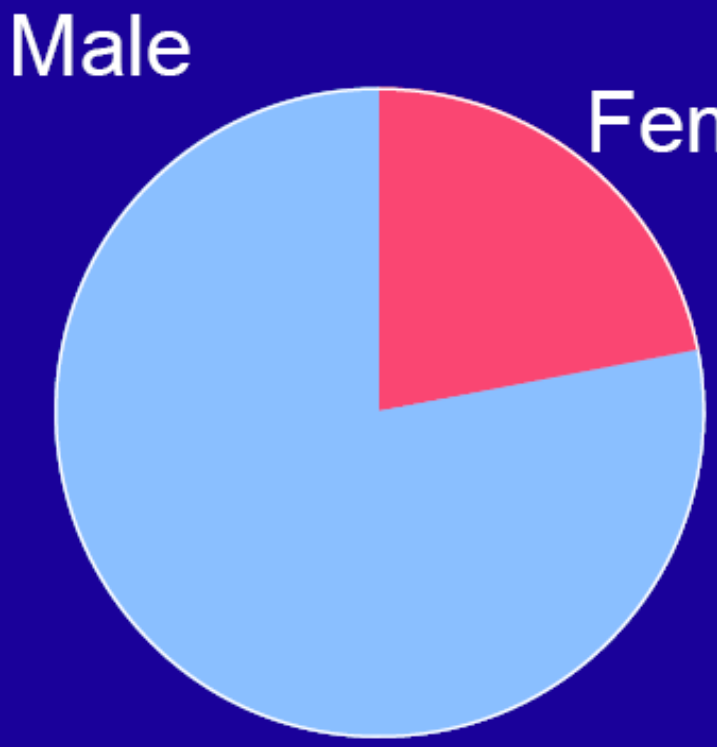
Nine X-Linked Primary Immune Diseases



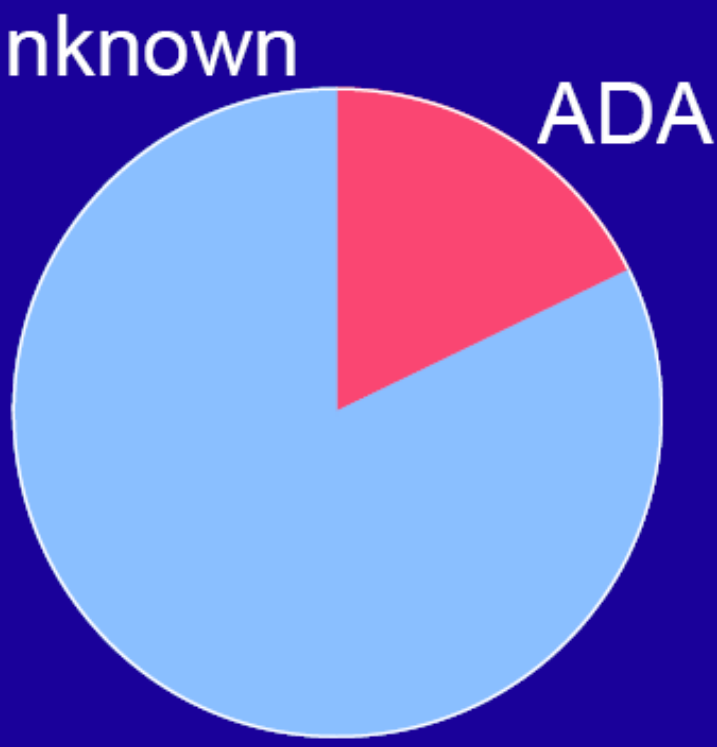
Severe Combined Immunodeficiency (SCID)

- Infections in first year
  - severe, persistent despite routine treatment
  - opportunistic pathogens
- Failure to thrive
- Few or absent T cells
  - poor proliferation to mitogens
- Non-functional or absent B cells
  - low Ig's; no specific antibody responses
- Fatal without immune reconstitution

SCID Genetic Analysis pre-1993



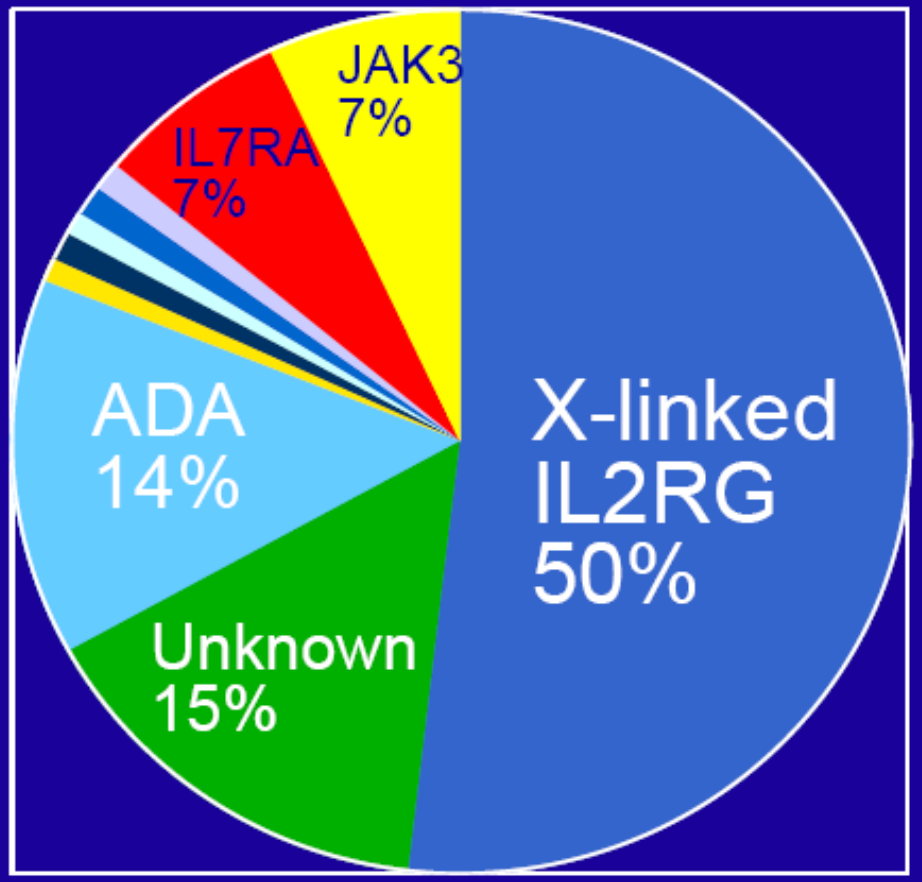
Most patients male; predominant X-linked gene



Adenosine deaminase defective in a minority of cases

SCID Genetic Analysis Today

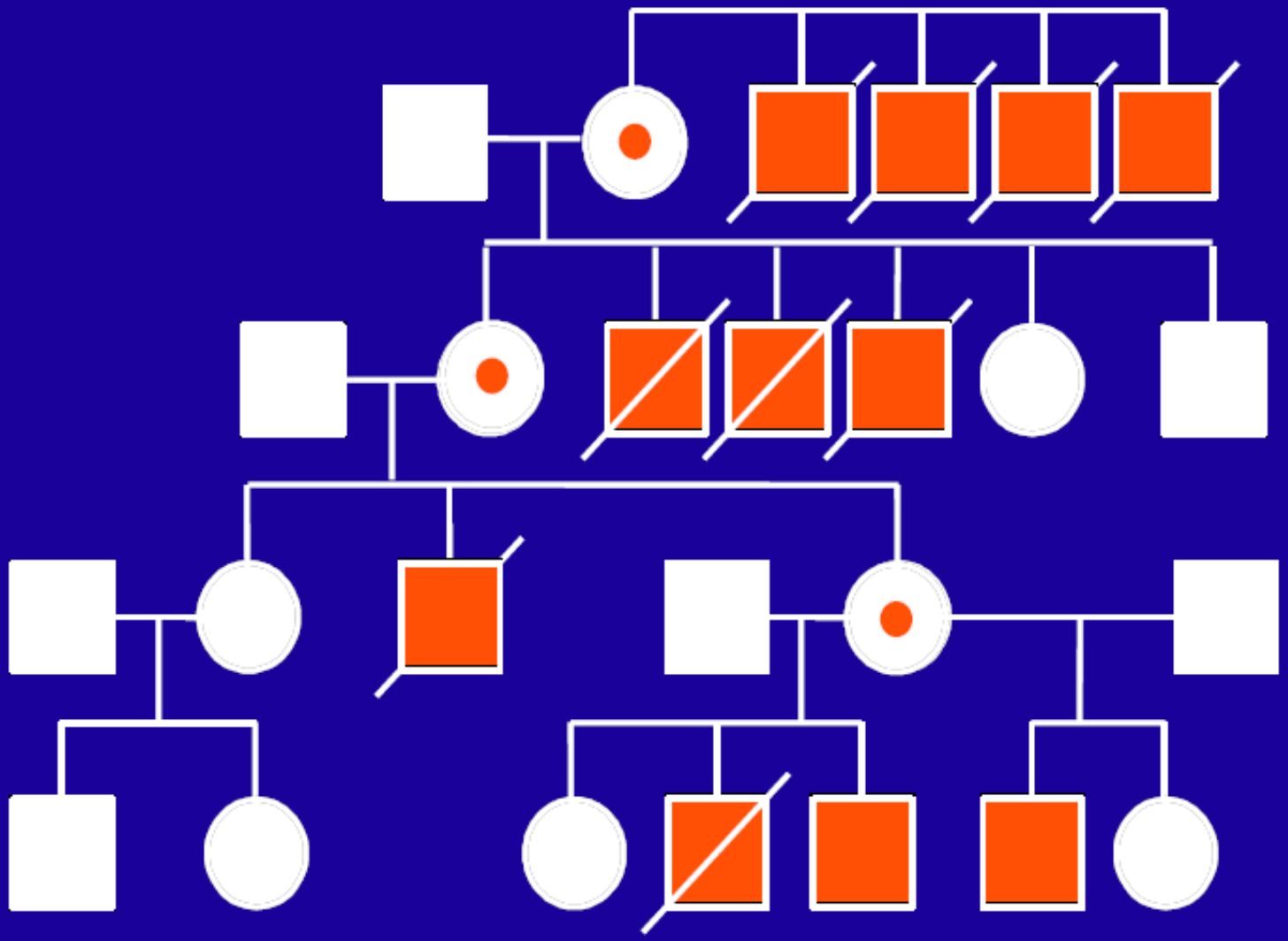
- X-linked SCID most common
- >10 genotypes known
- Specific gene defect can be found in 80%
- Clinical applications:
  - Carrier, prenatal dx
  - Gene therapy
  - Predict response to bone marrow transplant



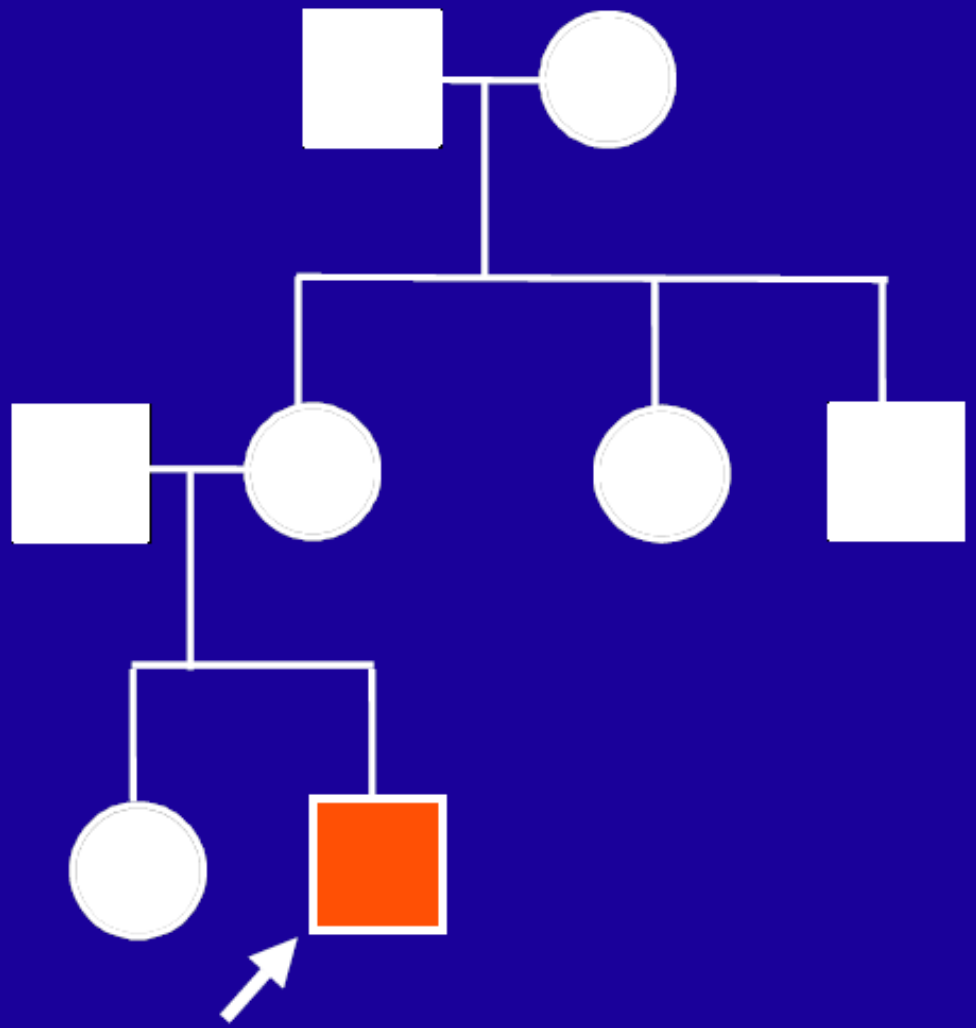
SCID Genes

			T	B	NK
• IL2RG	XL	50%	-	+	-
• ADA	AR	14%	-	-	-
• JAK3	AR	7%	-	+	-
• IL7RA	AR	7%	-	+	+
• RAG	AR	<7%	-	-	+
• ARTEMIS	AR	<5%	-	-	+
• CD45	AR	rare	-	+	+
• TCRD (CD3δ)	AR	rare	-	+	+
• FOXN1	AR	rare	-	+	+

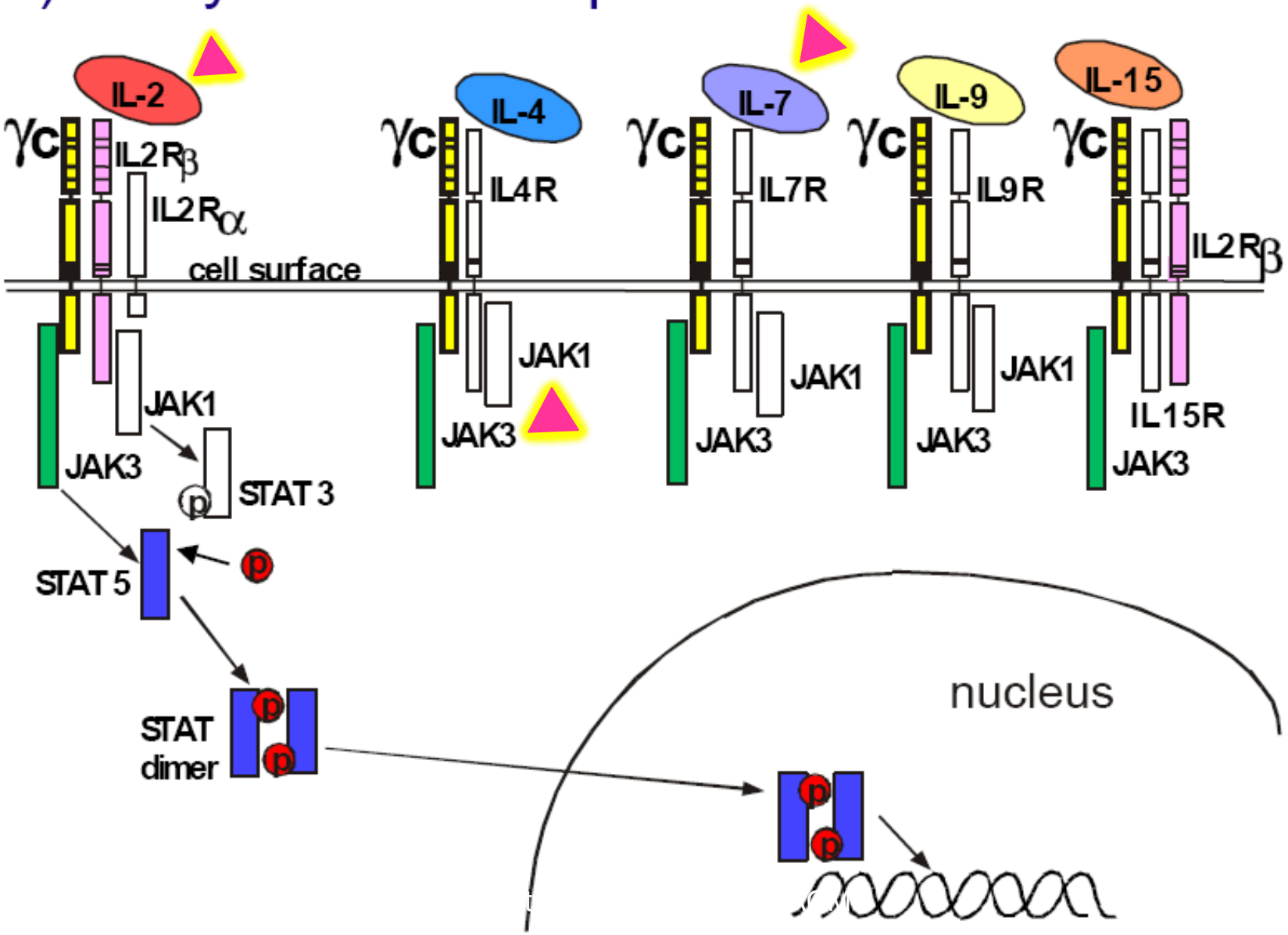
X-Linked Inheritance of SCID



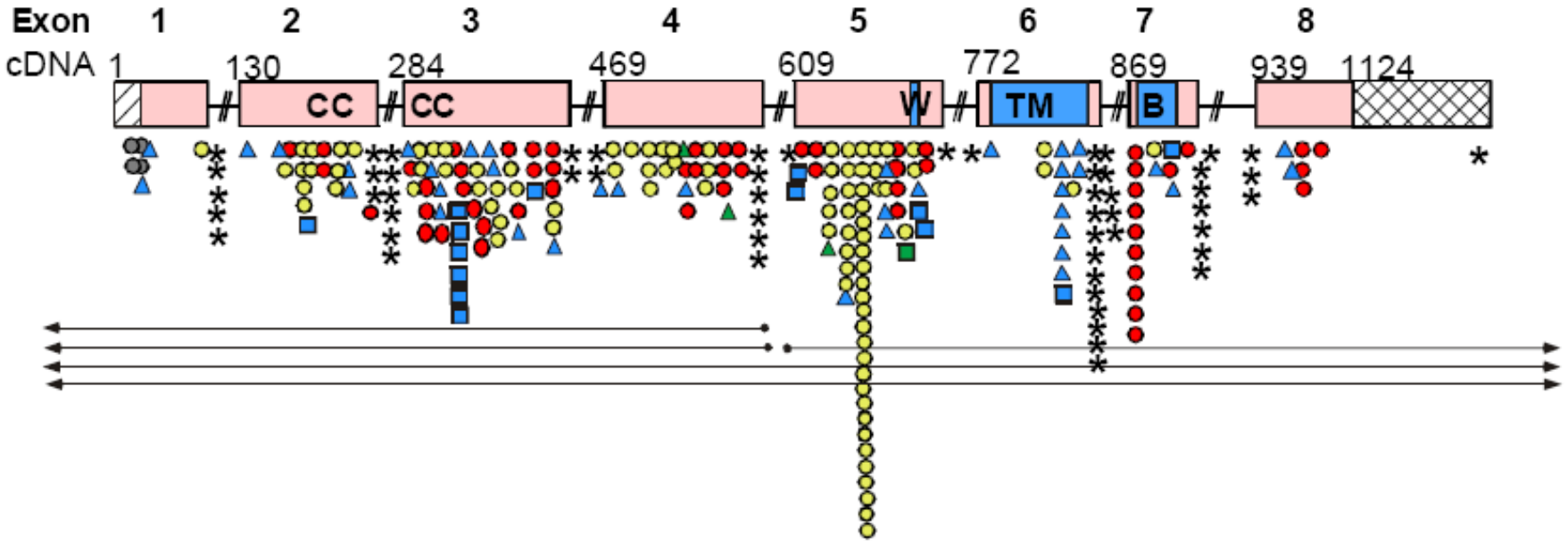
Most Common SCID Presentation: Sporadic Male



XSCID IL2RG Protein: Common Gamma Chain (gc) of Cytokine Receptors



IL2RG Mutations in XSCID



IL2 RG Domains

- signal sequence
- C conserved cysteine
- W WSXWS box
- TM transmembrane
- B box1-box2 domain
- 3' untranslated

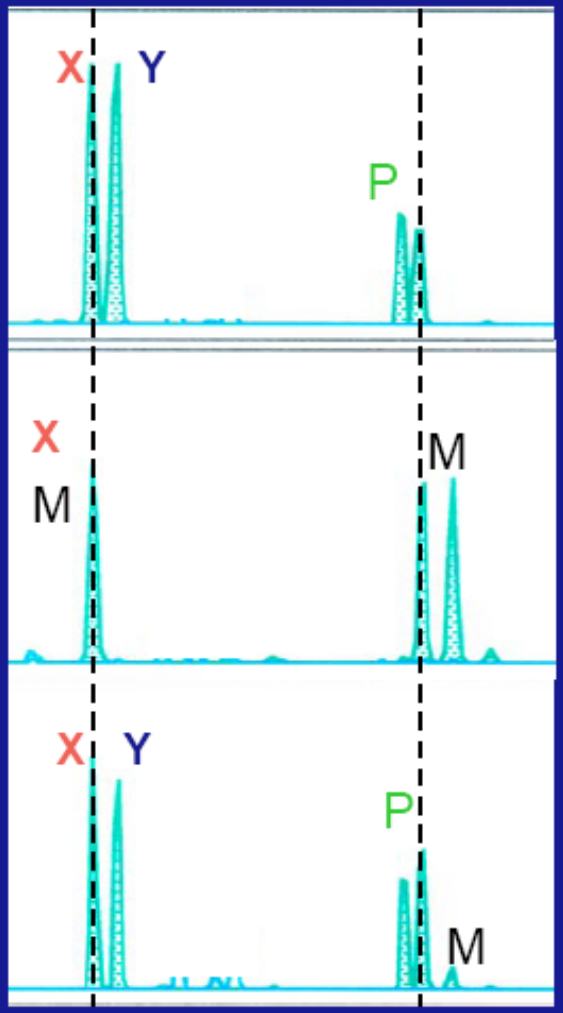
X-linked γc-SCID Mutations

- nonsense
- missense
- insertion, frame shift
- deletion, frame shift
- insertion, in frame
- deletion, in frame
- RNA processing
- large deletion
- translation mutations

IL2RGbase, Puck et al.

Limitations of Bone Marrow Transplant (BMT) Treatment for XSCID

- Most patients lack HLA-matched sibling
- Graft vs. host disease
- Persistent immune defects
  - T cell loss
  - Non-functional host B cells

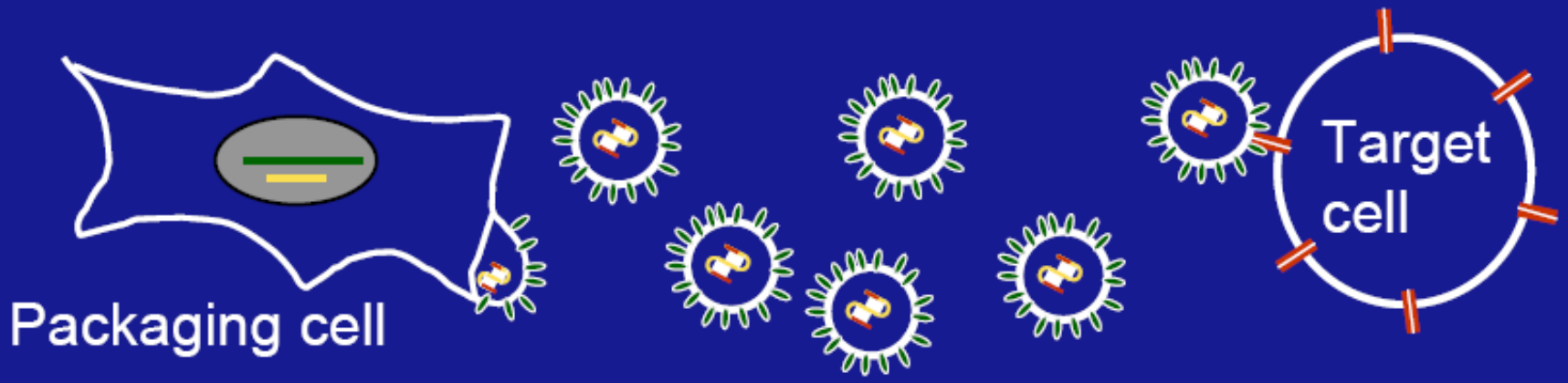


Integrating Retrovirus Vectors:

Backbone

Maloney murine leukemia virus
Lentivirus

Envelope	Receptor	Host range
Amphotropic	PiT-2	mouse, dog, primate, human
Gibbon Ape Leukemia Virus	PiT-1	human, primate, dog
Feline Leukemia Virus	Neutral aa	human, primate, dog



XSCID: A Good Pilot Disease for Gene Therapy

- BMT does not cure all patients
- Hematopoietic stem cells, HSC, can be removed, transduced and re-infused
- γc expressed in all blood lineages, not tightly regulated
- No immune elimination of corrected cells
- *In vivo* selective advantage for lymphocytes expressing normal γc protein
- Success in mouse model

Gene Therapy for the Treatment of Primary Immune Deficiencies. Kuo CY, Kohn DB
Curr Allergy Asthma Rep. 2016 May;16(5):39.

Gene Therapy of Human Severe Combined Immunodeficiency (SCID)-X1 Disease
Cavazzana-Calvo M, Hacein-Bey S, deSaint Basile G, et al.Science 288 (Apr):669-672, 2000.

Gene Therapy for X-Linked Severe Combined Immunodeficiency: Where Do We Stand?
Cavazzana M, Six E, Lagresle-Peyrou C, André-Schmutz I
Hum Gene Ther. 2016 Feb;27(2):108-16.

Sustained Correction of X-Linked Severe Combined Immunodeficiency by ex Vivo Gene Therapy
Hacein-Bey-Abina S, Le Deist F, Carlier F, et al.New Engl J Med (Apr 18):1185-1192, 2002.

Adverse Events in French XSCID Gene Therapy Trial

Of 10 infants treated, 9 had excellent immune restoration.

Two developed leukemia 27-30 mo. after treatment.

Pt #4 (age 1 mo): expansion of T cell clone

- Gene therapy vector in reverse orientation intron 1 of *LMO2*, a T-ALL associated transcription factor.
- Other factors: family history of childhood cancer, chickenpox, ch 6:13 translocation.
- Chemotherapy, then allogeneic BMT.

Pt #5 (age 3 mo): expansion of at ≥ 2 separate T cell clones

- Vector 5' to *LMO2* in forward orientation
- Chemotherapy

Agammaglobulinemia

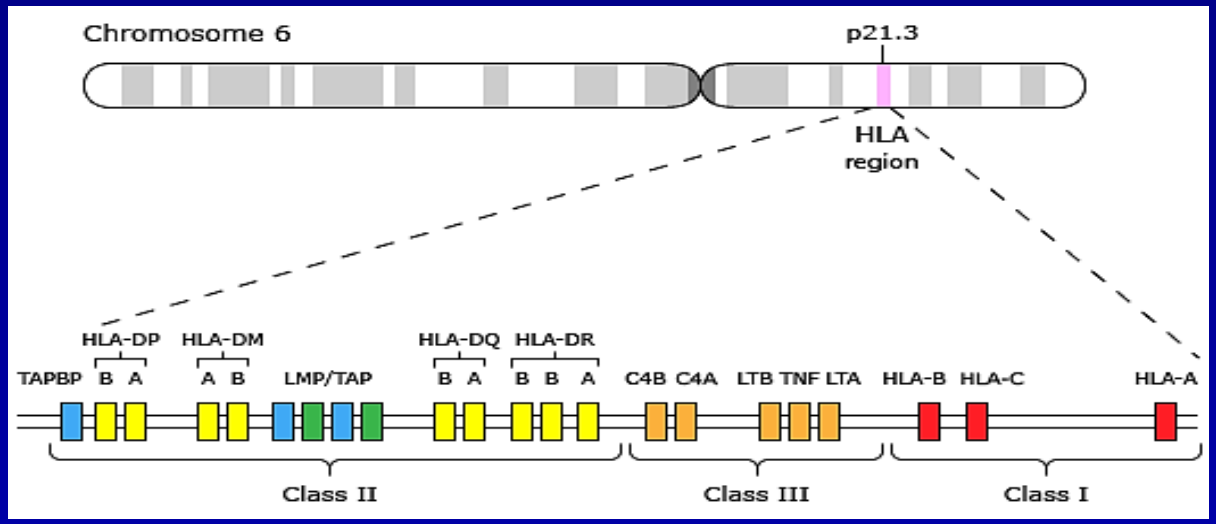
- Recurrent infections starting in infancy
  - Otitis, pneumonia
  - Pyogenic bacteria
- Few or absent B cells; bone marrow contains pre-B cells
- No circulating antibodies

Agammaglobulinemia Genotypes

- X-Linked (XLA)
  - >80% of cases
  - Males
  - Defect in BTK, B cell tyrosine kinase
- Autosomal Recessive
  - Males and females
  - μ heavy chain immunoglobulin gene
  - λ5 surrogate light chain gene
  - Igα component of B cell antigen receptor
  - BLNK scaffold adaptor protein for B cell signaling

X-Linked Hyper IgM Syndrome, CD40 Ligand Deficiency

- Failure of isotype switching; very low IgG, IgA, IgE
- CD40L expressed on activated T cells is essential for B cell activation; T cell defects and neutropenia are also found
- Recurrent bacterial respiratory infections, also PCP pneumonia and hepatobiliary disease
- Autosomal recessive forms of failure to isotype switch have been defined
- Treatment:
  - Early, aggressive management of infections
  - Prophylactic antibiotics
  - Bone marrow transplantation if HLA identical sibling exists



Thank You & Good Luck !