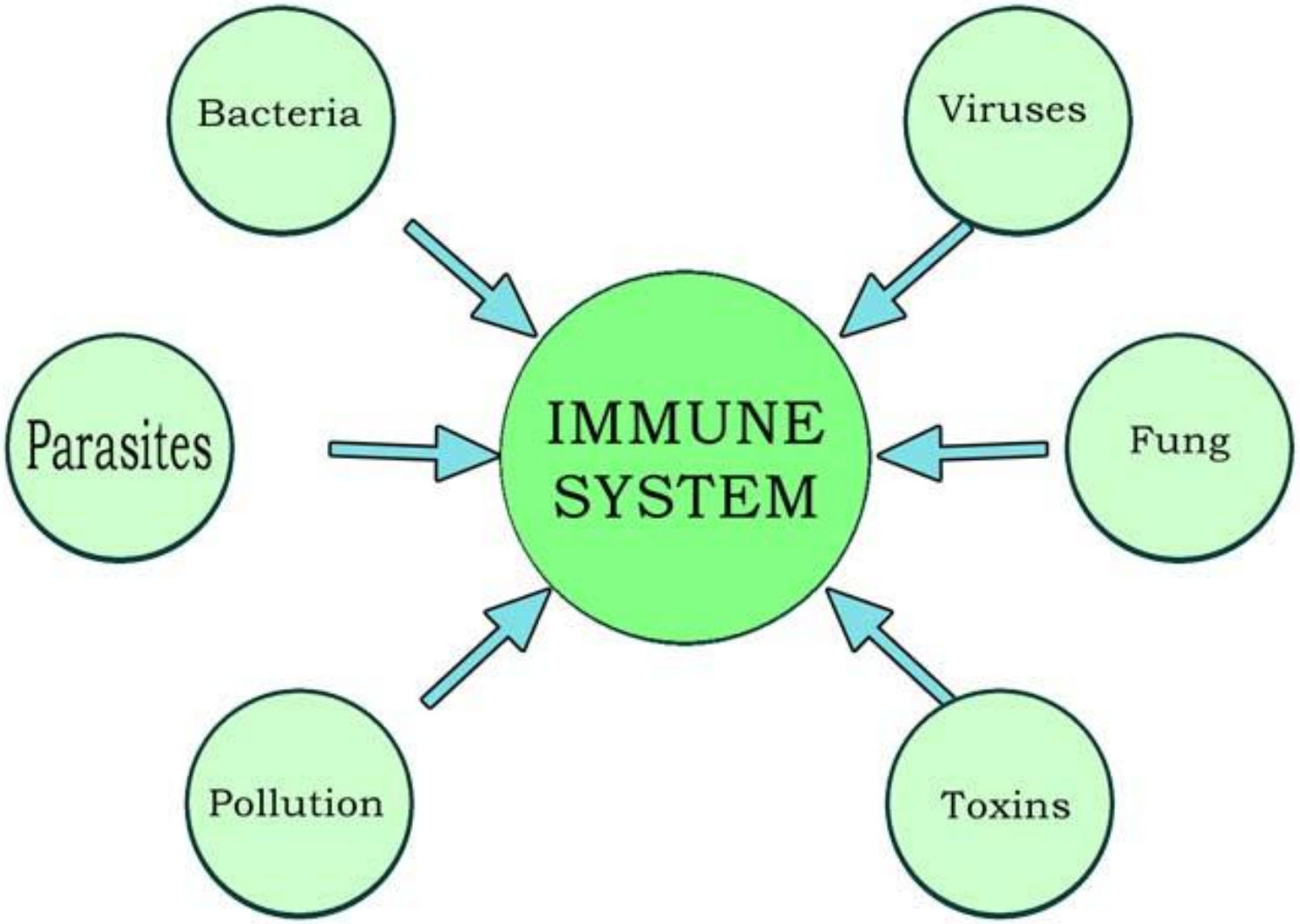
1ª PARTE: INTRODUÇÃO AO SISTEMA IMUNE

2a Parte: Genética Molecular das Imunoglobulinas

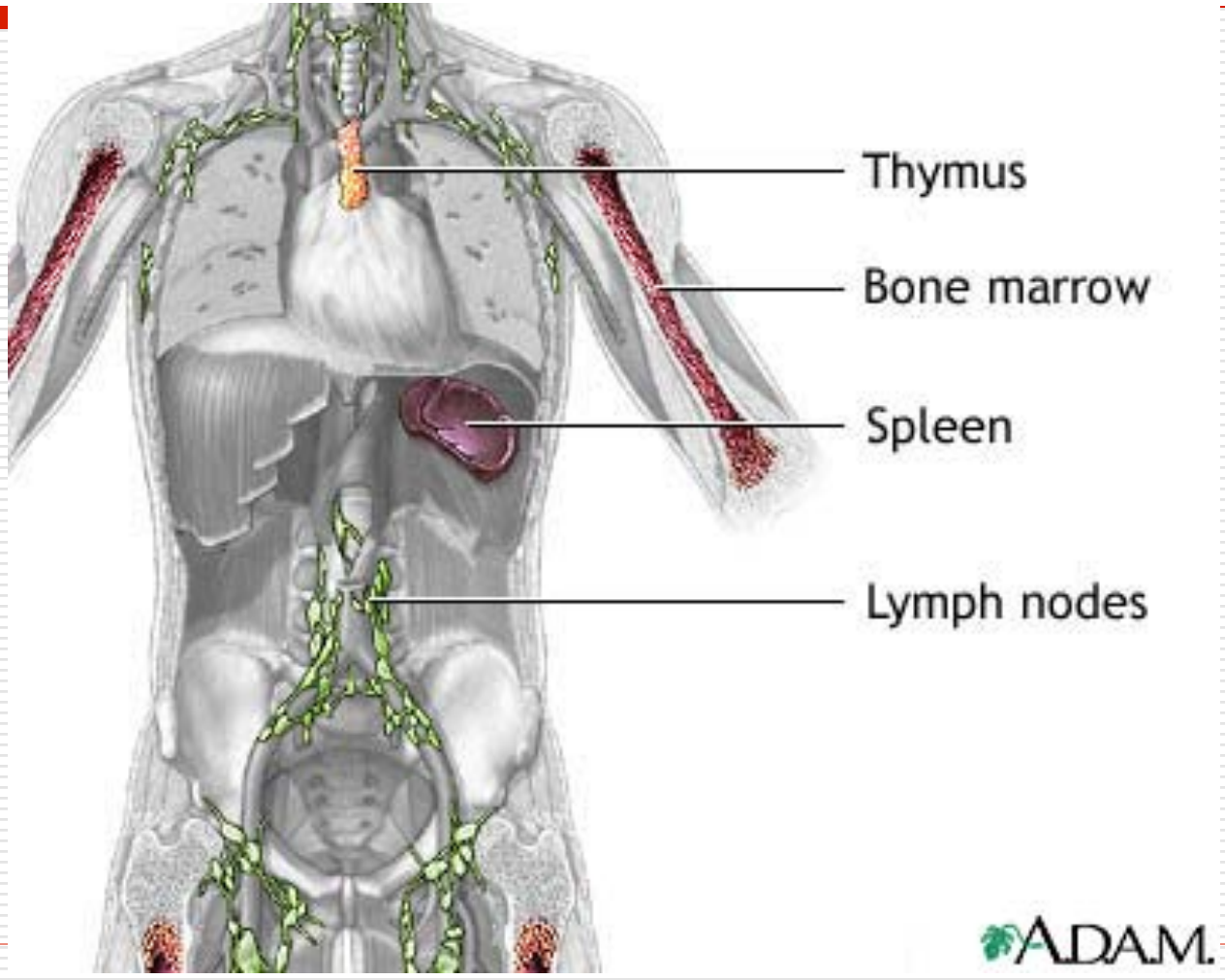
IMGT Education
http://www.imgt.org/IMGTeducation/Tutorials/IGandBcells/_PT/Genetica/

Marie-Paule Lefranc e Gérard Lefranc
(Université de Montpellier II, France)

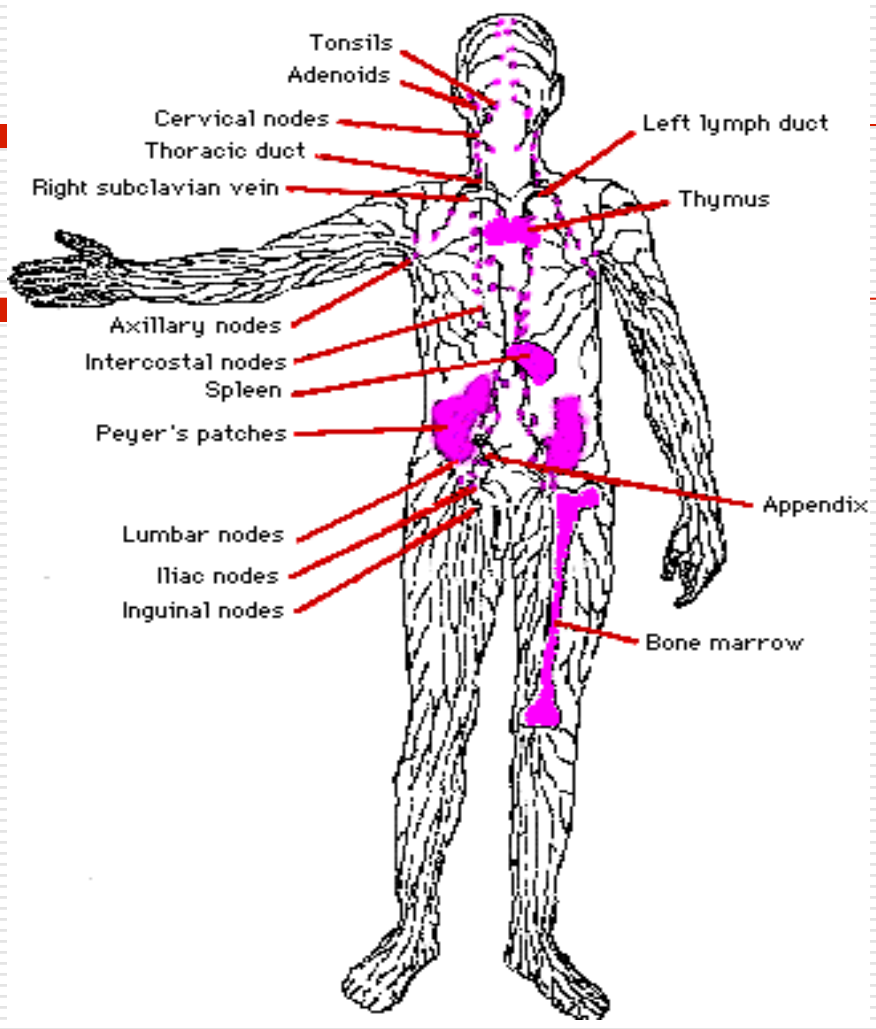
Tradução de Geraldo Passos (Universidade de São Paulo)


Bacteria
Viruses
Parasites
IMMUNE SYSTEM
Fung
Pollution
Toxins

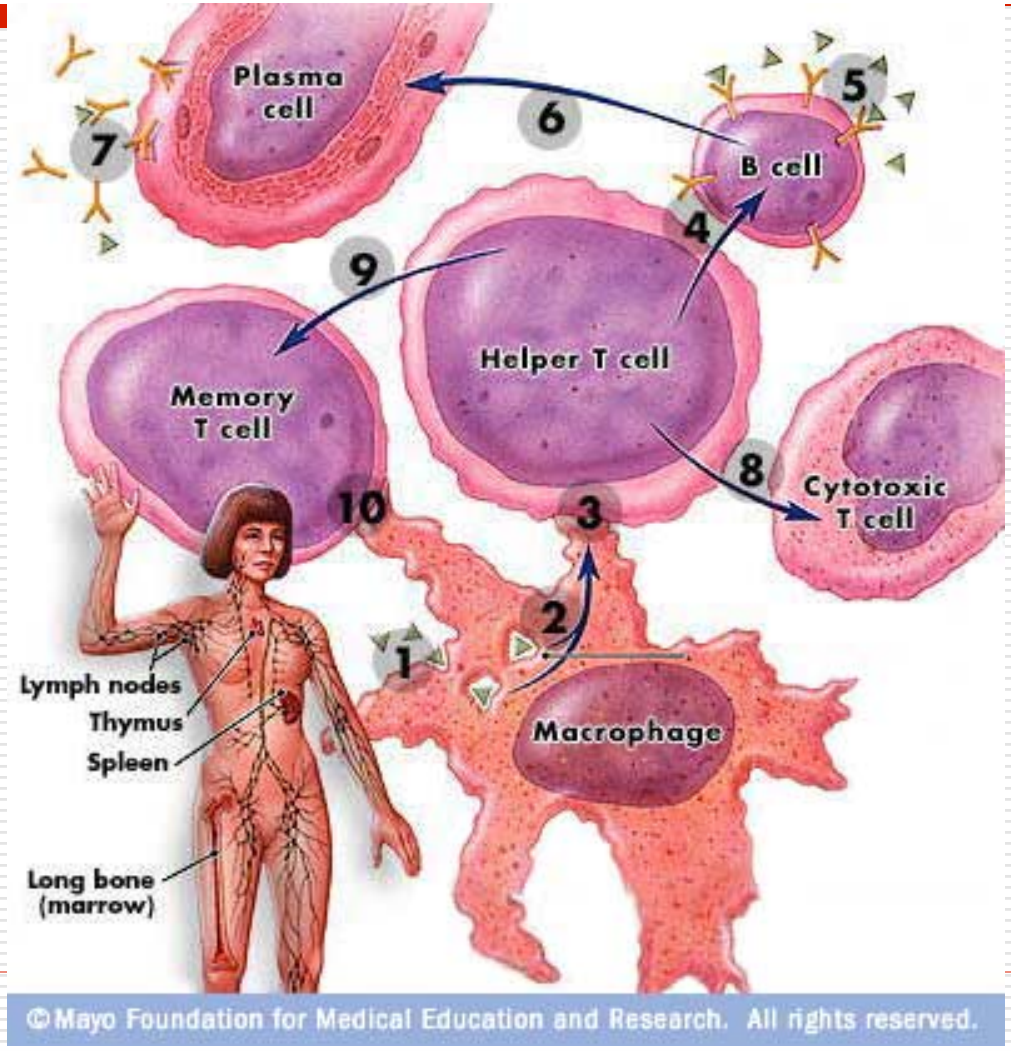
Anatomia do sistema imune

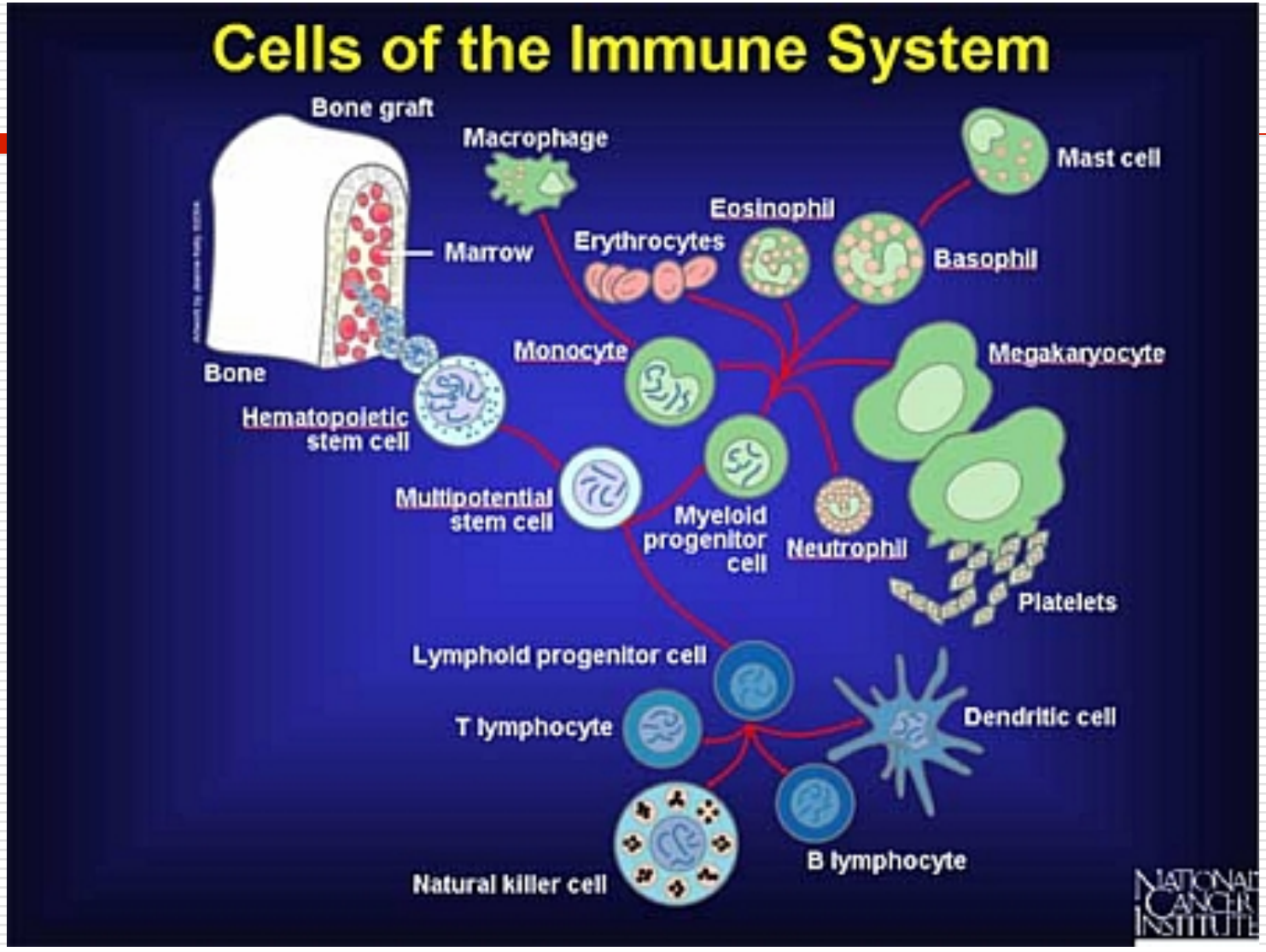


Anatomia do Sistema Imune

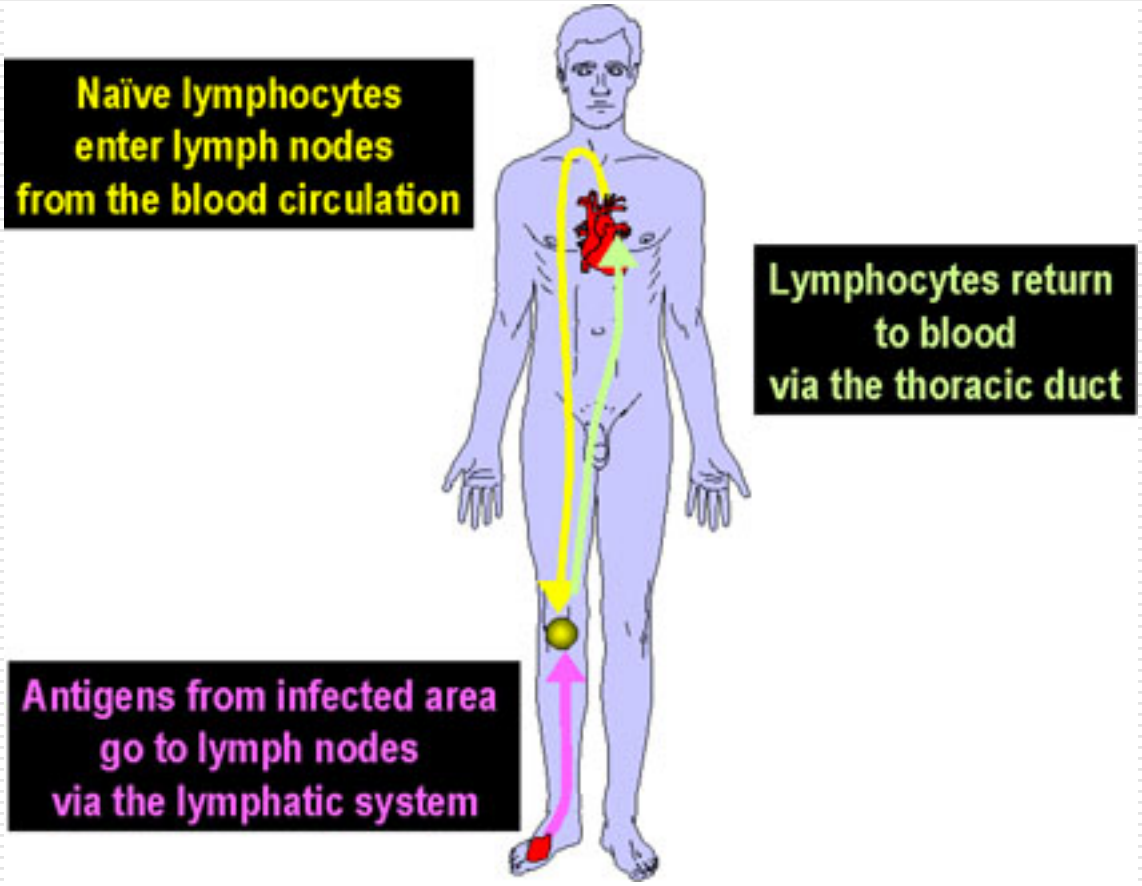


Principais células efetoras do sistema imune

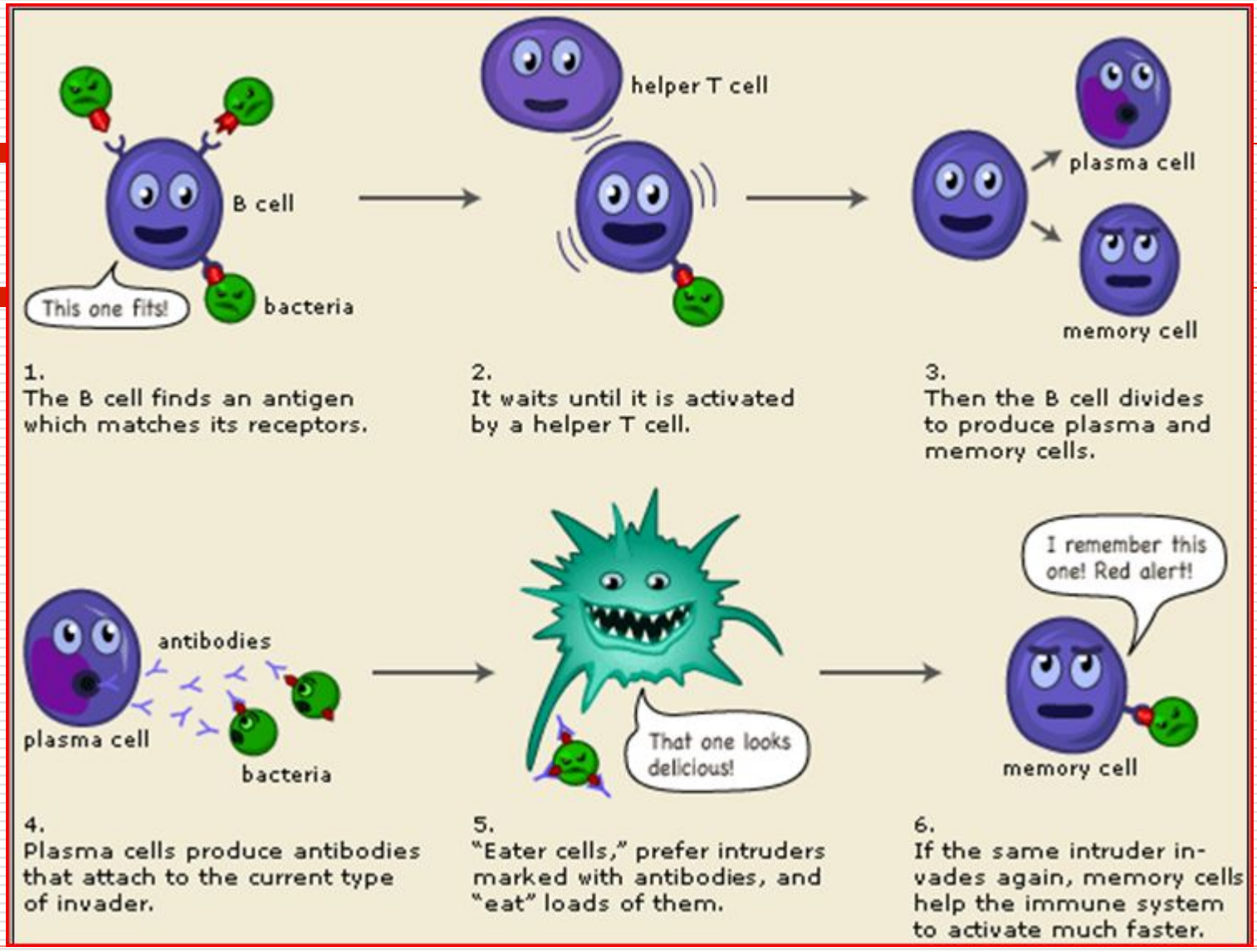



Cells of the Immune System
Bone graft
Macrophage
Mast cell
Eosinophil
Erythrocytes
Basophil
Marrow
Monocyte
Megakaryocyte
Bone
Hematopoietic stem cell
Multipotential stem cell
Myeloid progenitor cell
Neutrophil
Platelets
Lymphoid progenitor cell
T lymphocyte
Dendritic cell
B lymphocyte
Natural killer cell

Movimentação dos antígenos e das células do sistema imune no corpo

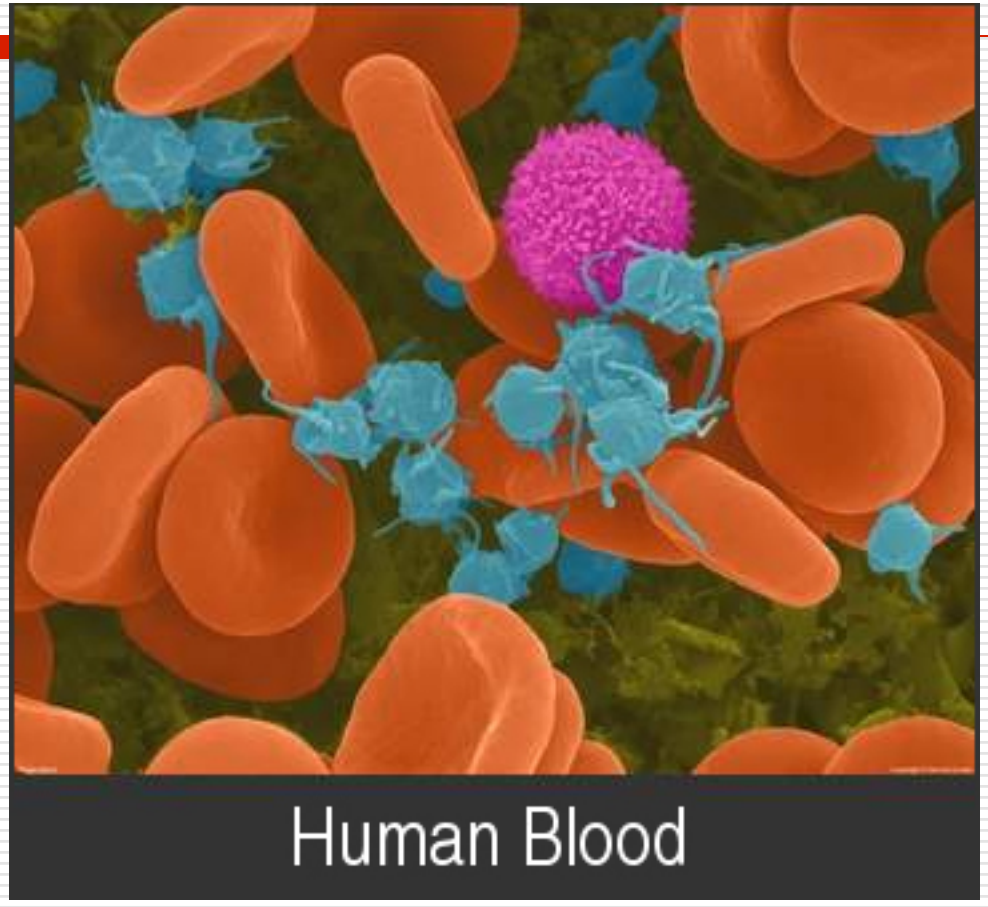


Ativação das células B

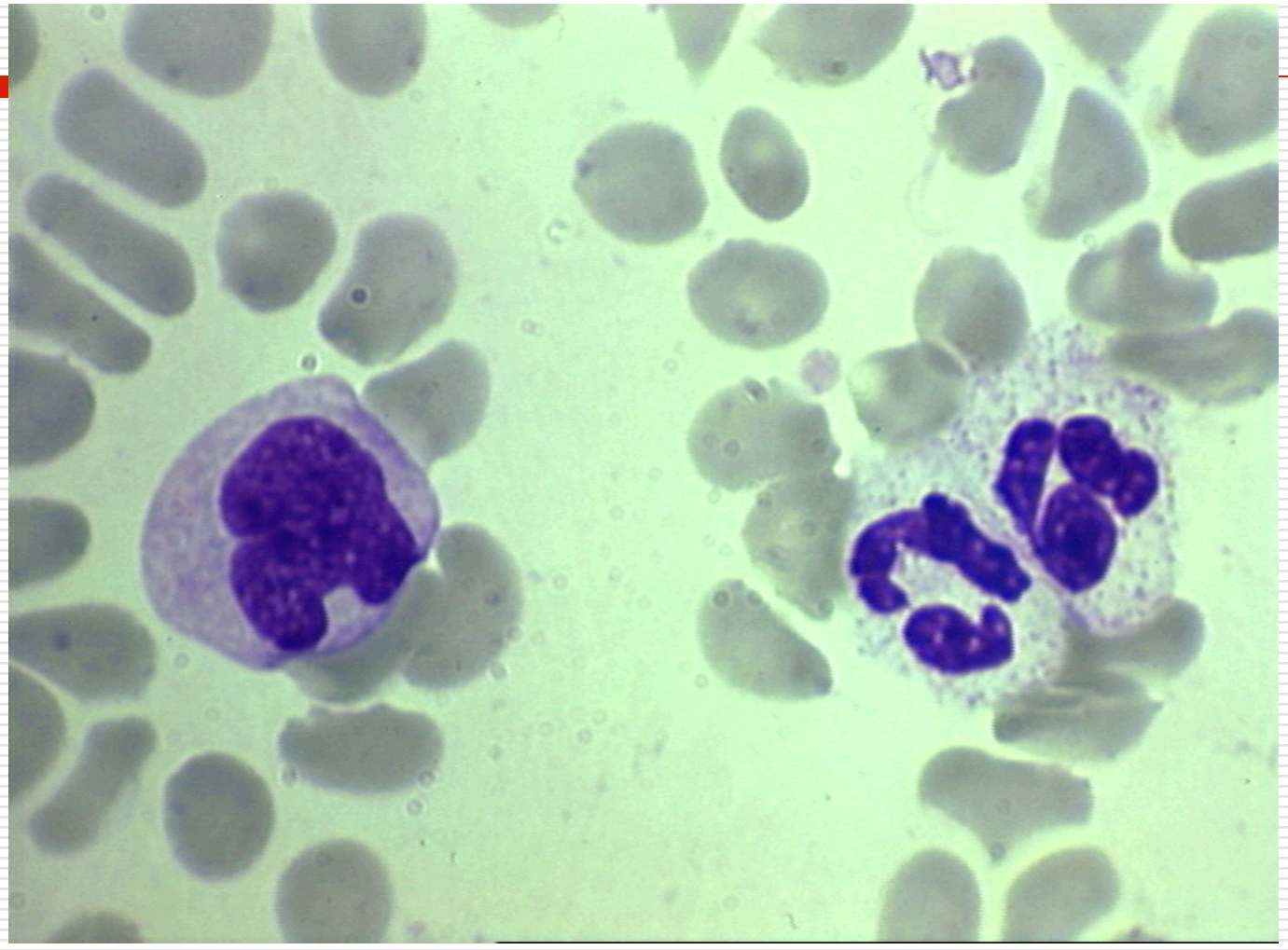


smabiology.blogspot.com

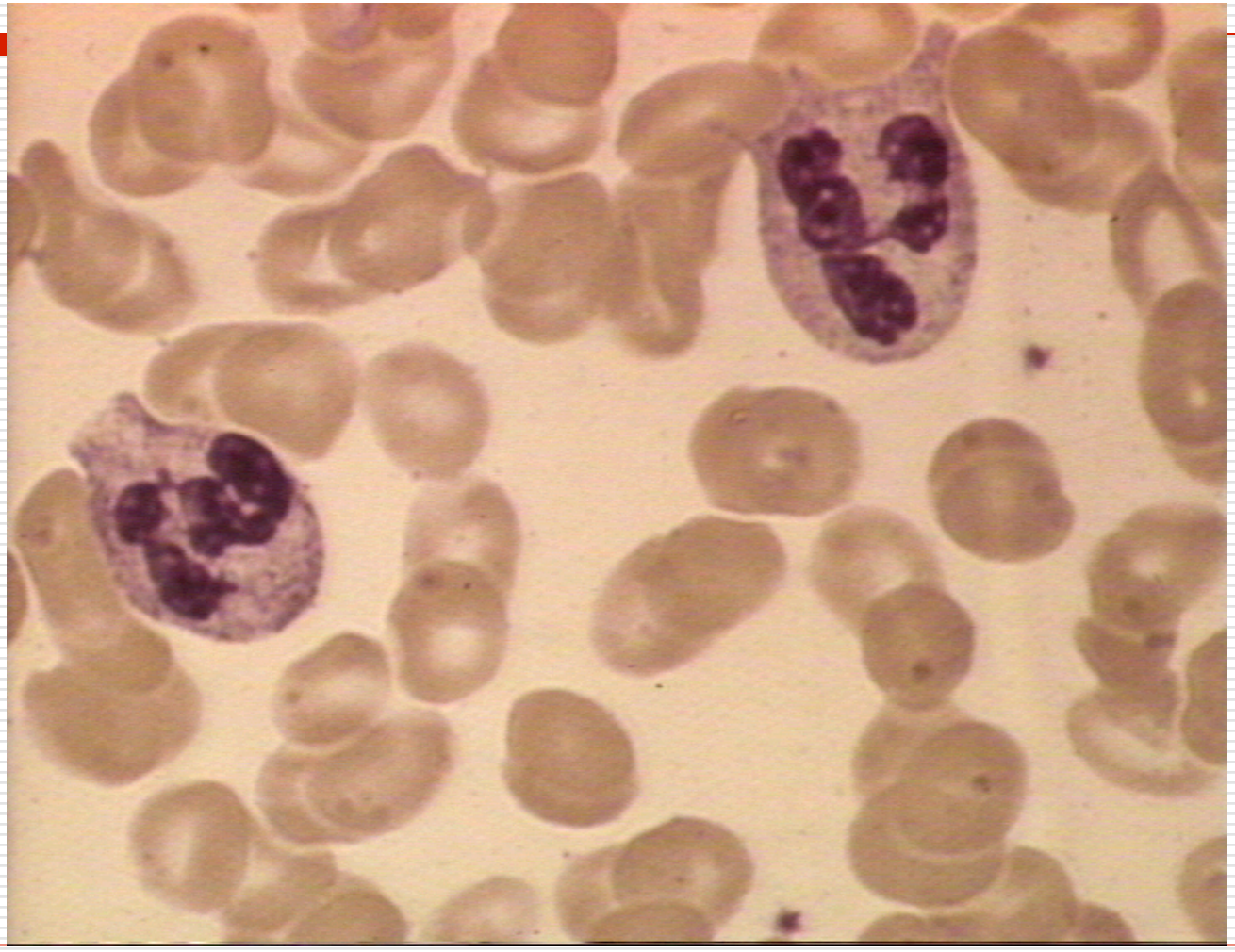
Sangue humano mostrando as hemácias e os leucócitos



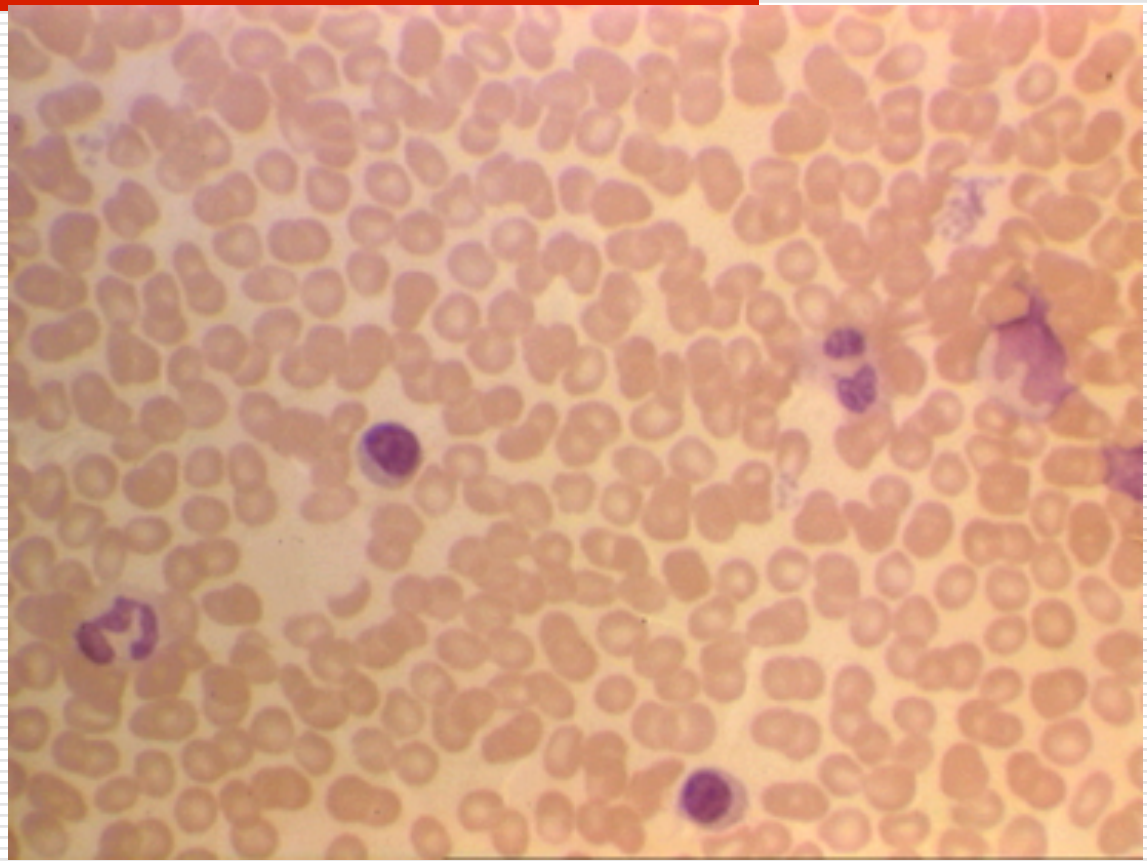
Monócitos



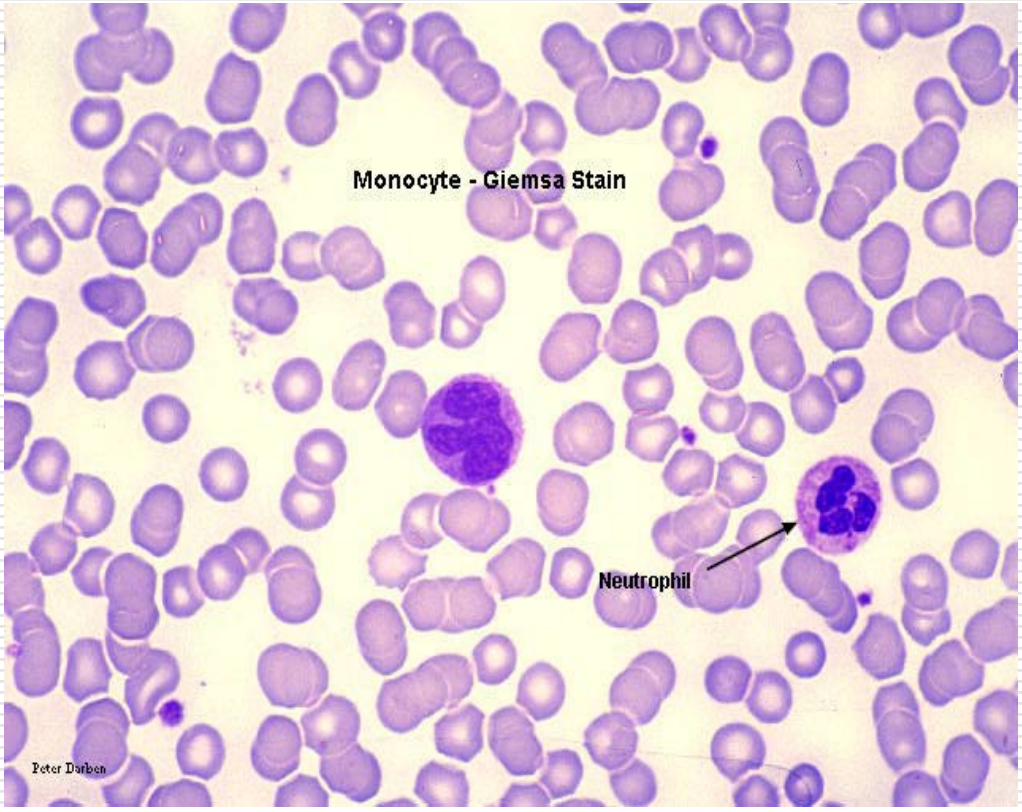
Neutrófilos



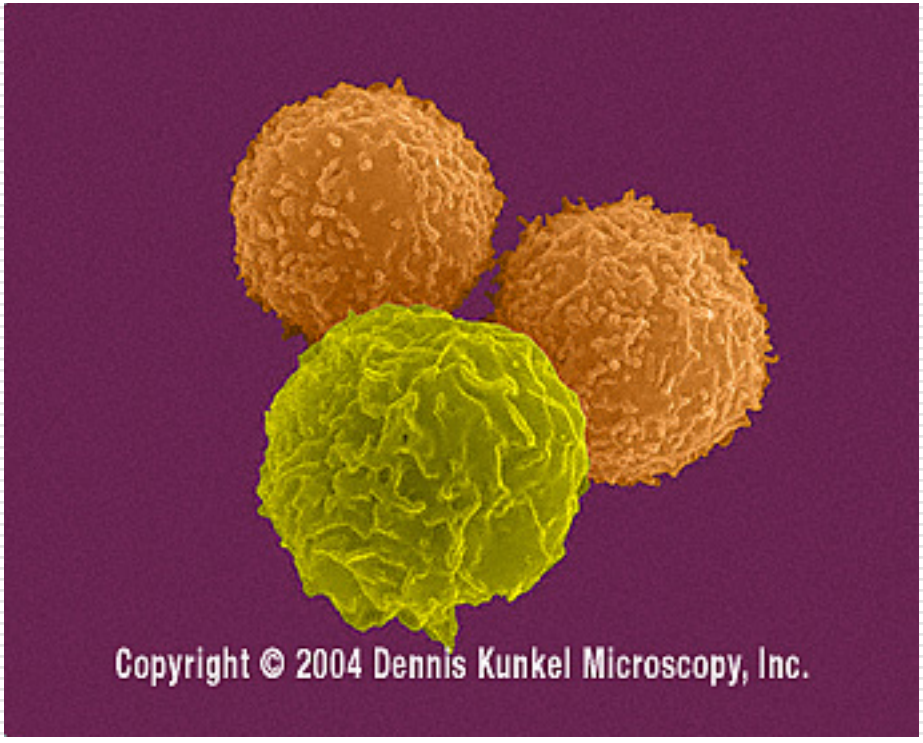
Linfócitos



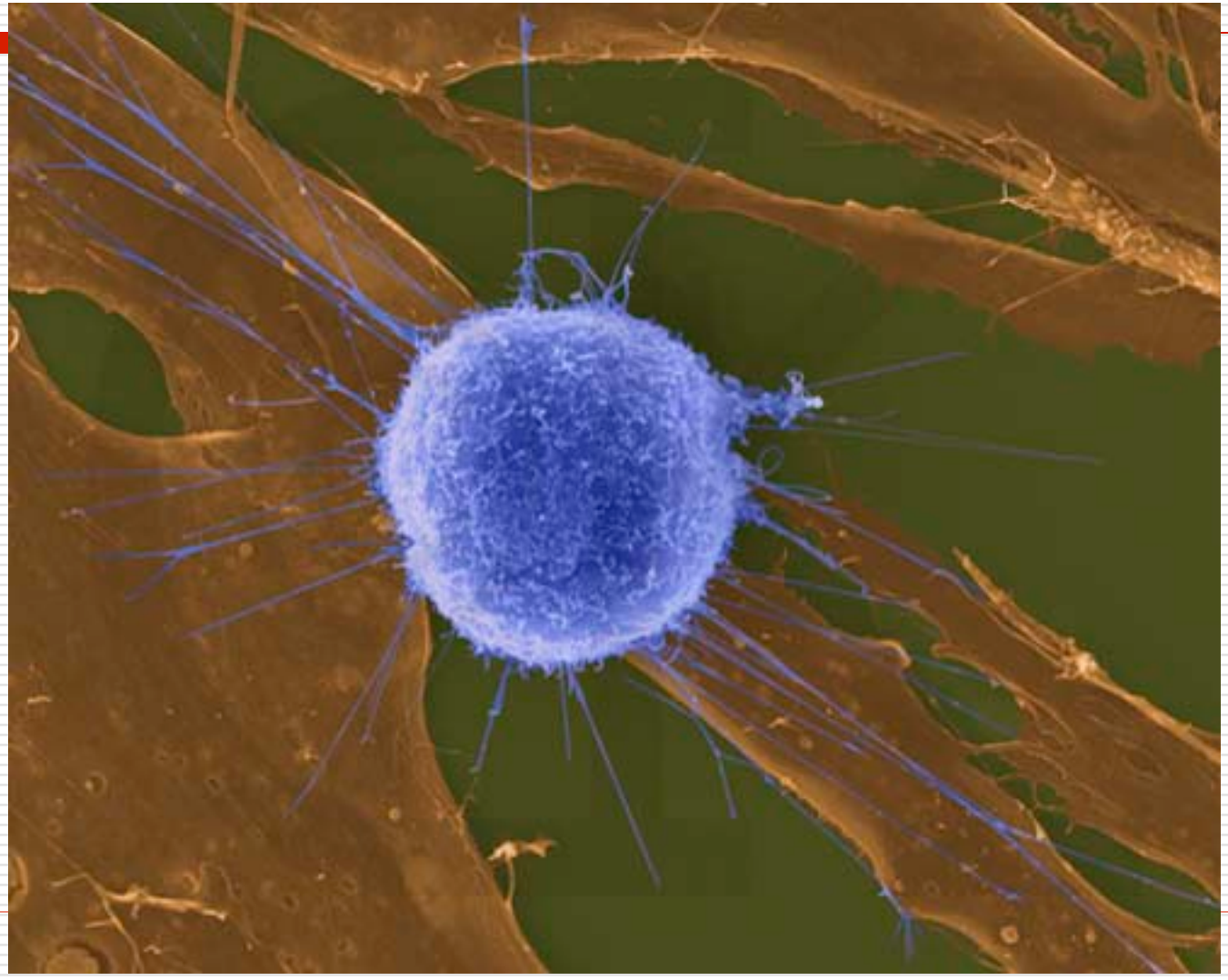
Monócitos e Neutrófilos



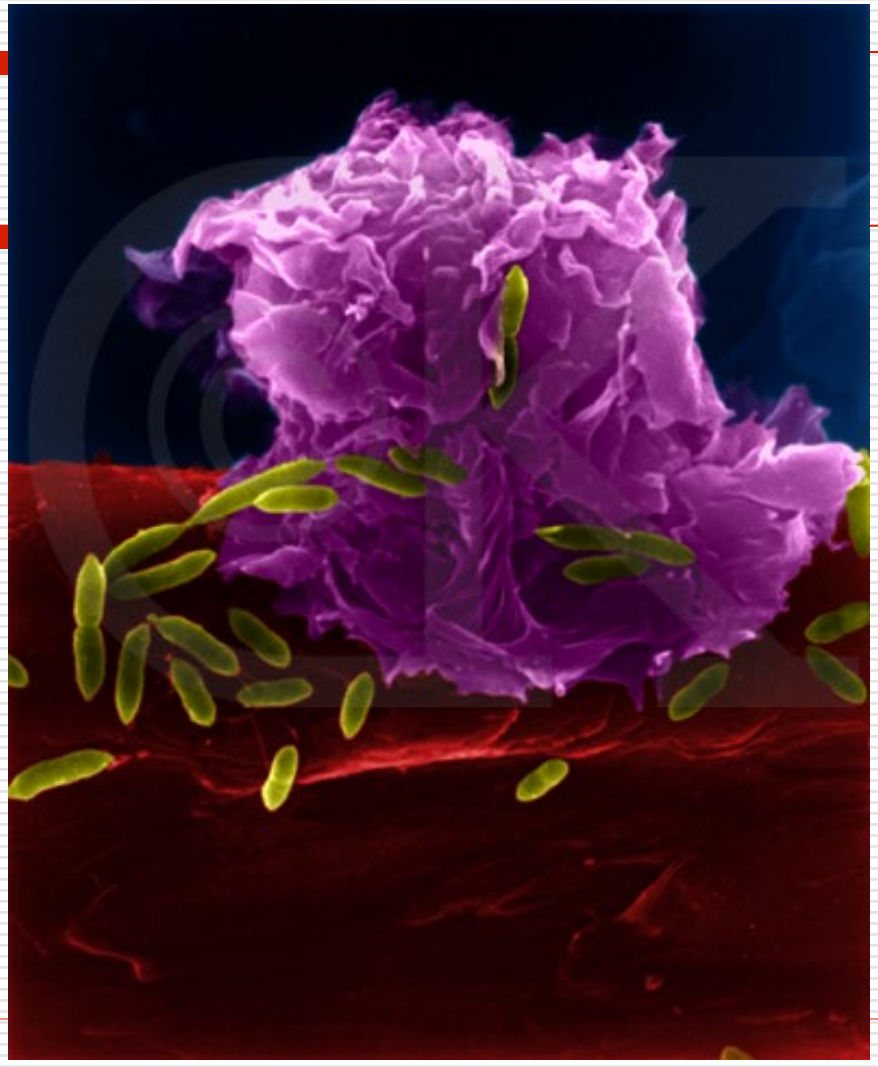
Linfócitos T e Neutrófilos



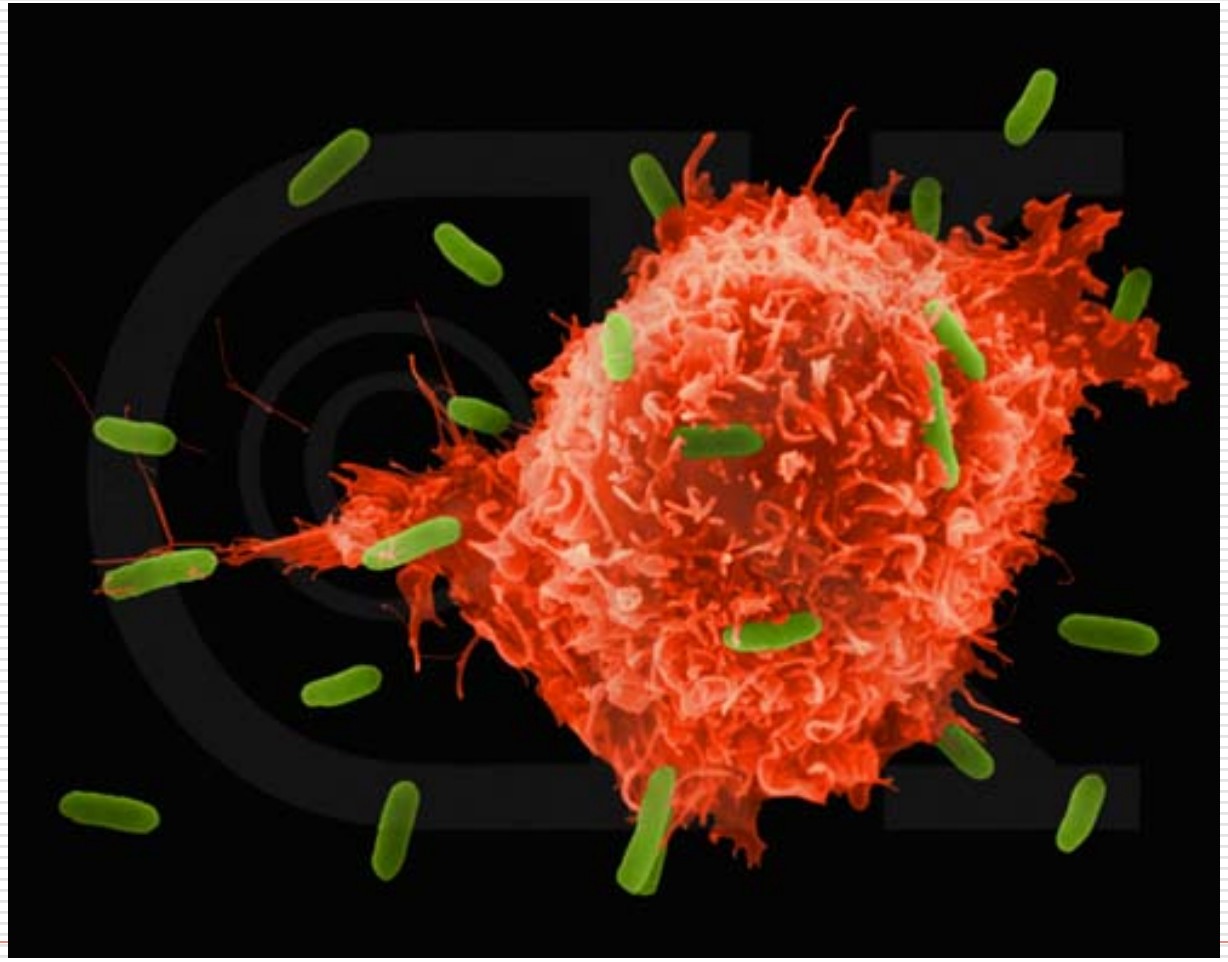
Linfócito T



Macrófago Alveolar

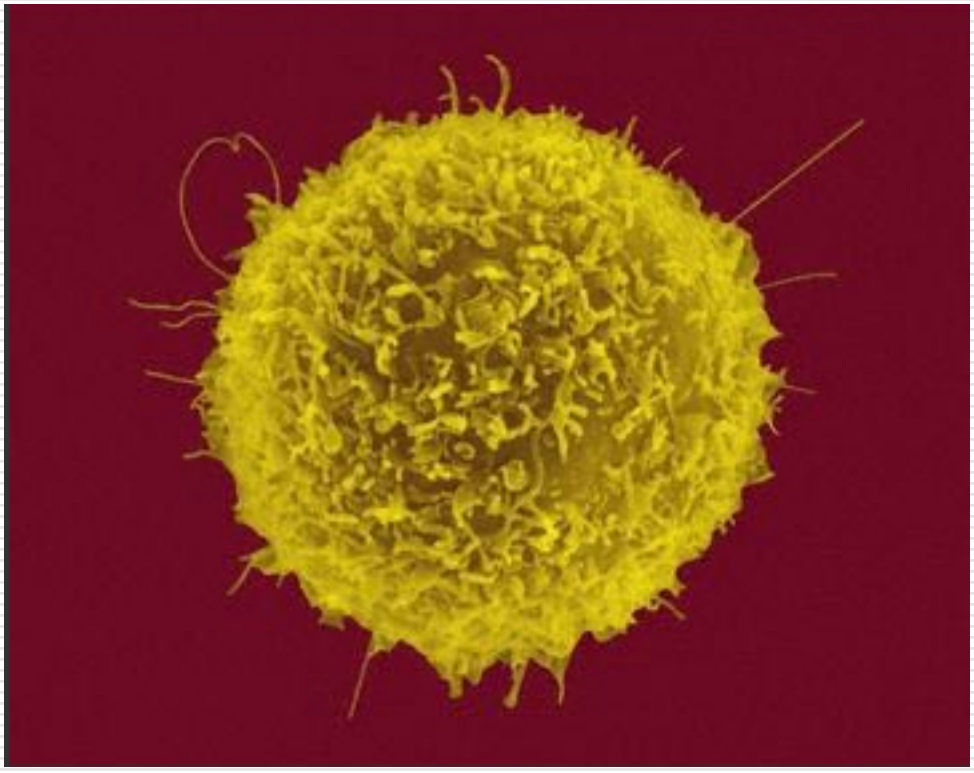


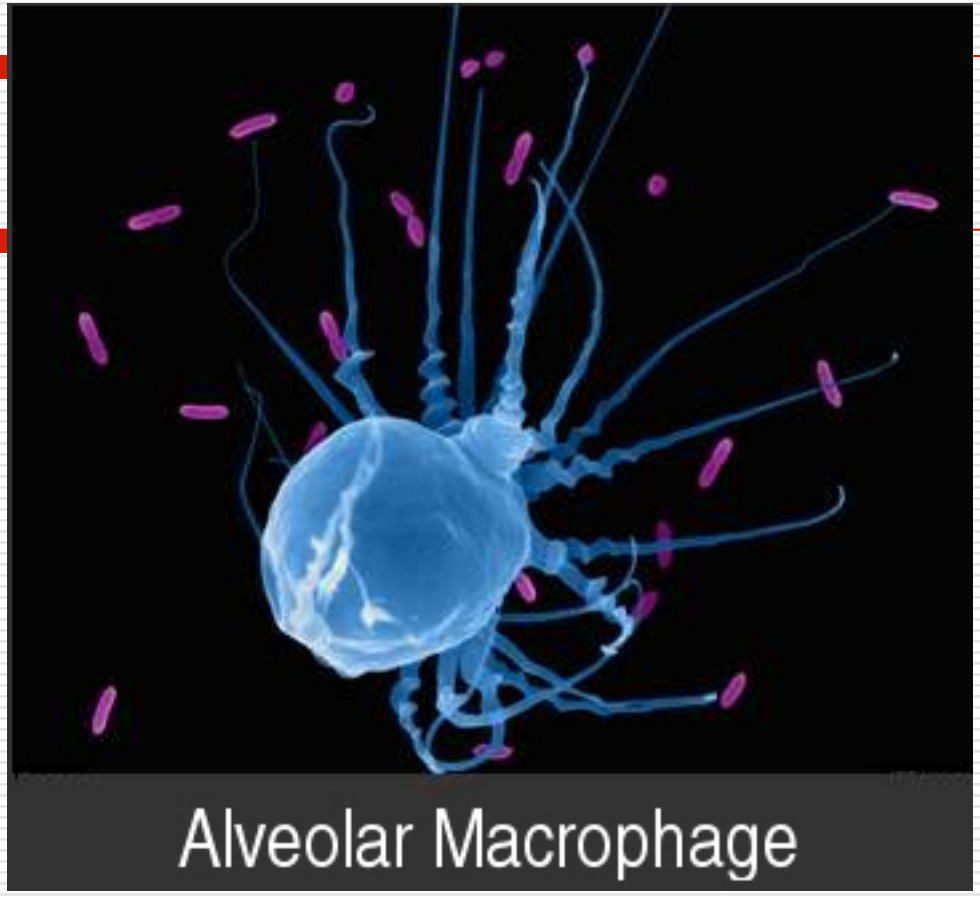
Macrófago fagocitando *E. coli*



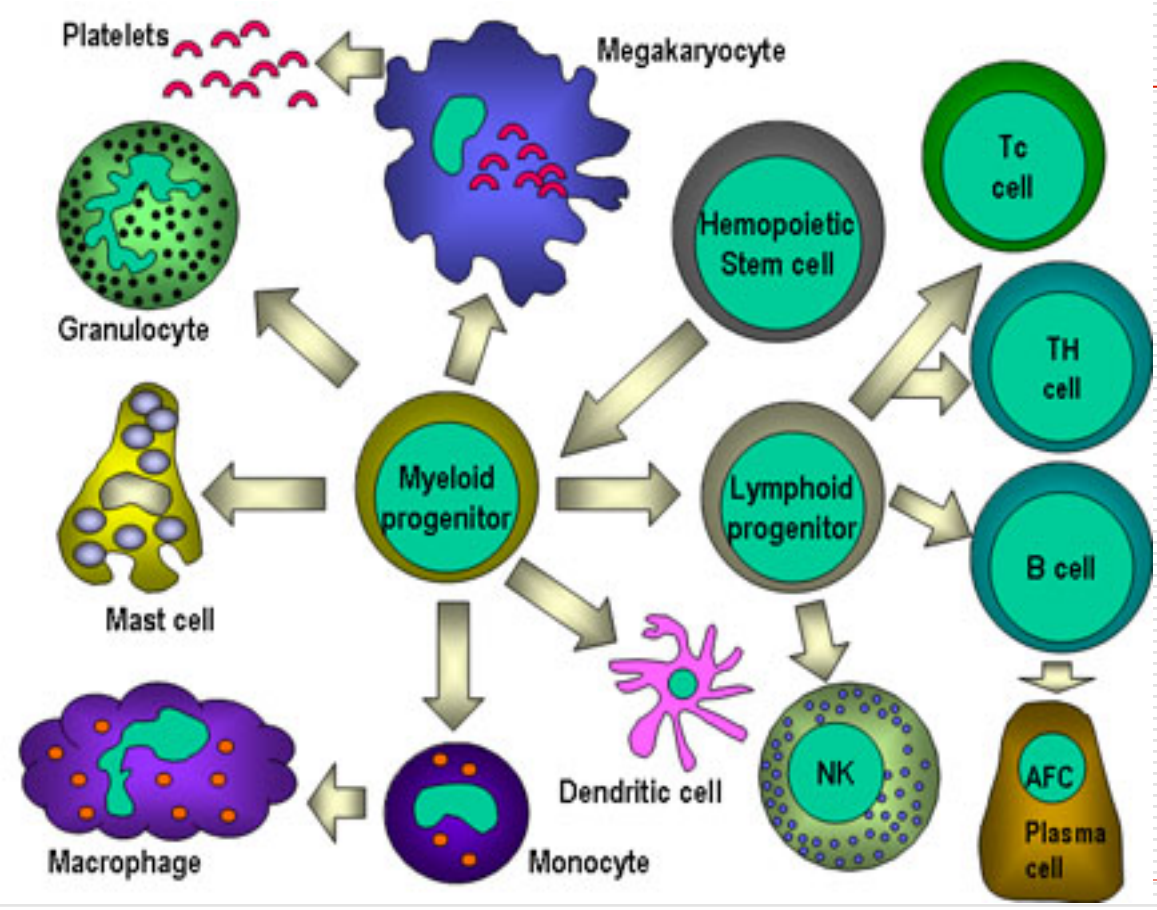
Linfócito B (Secreta Anticorpos)

Linfócito T (Resposta Celular)

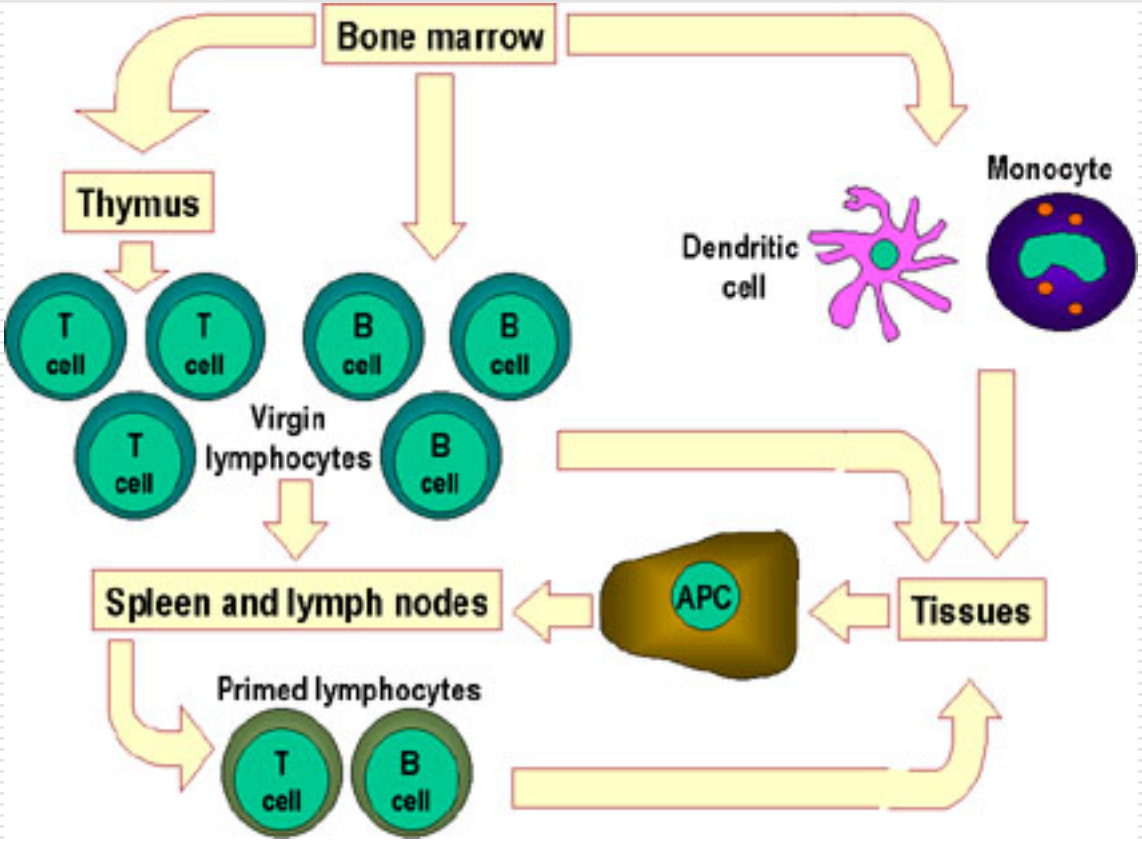



Alveolar Macrophage

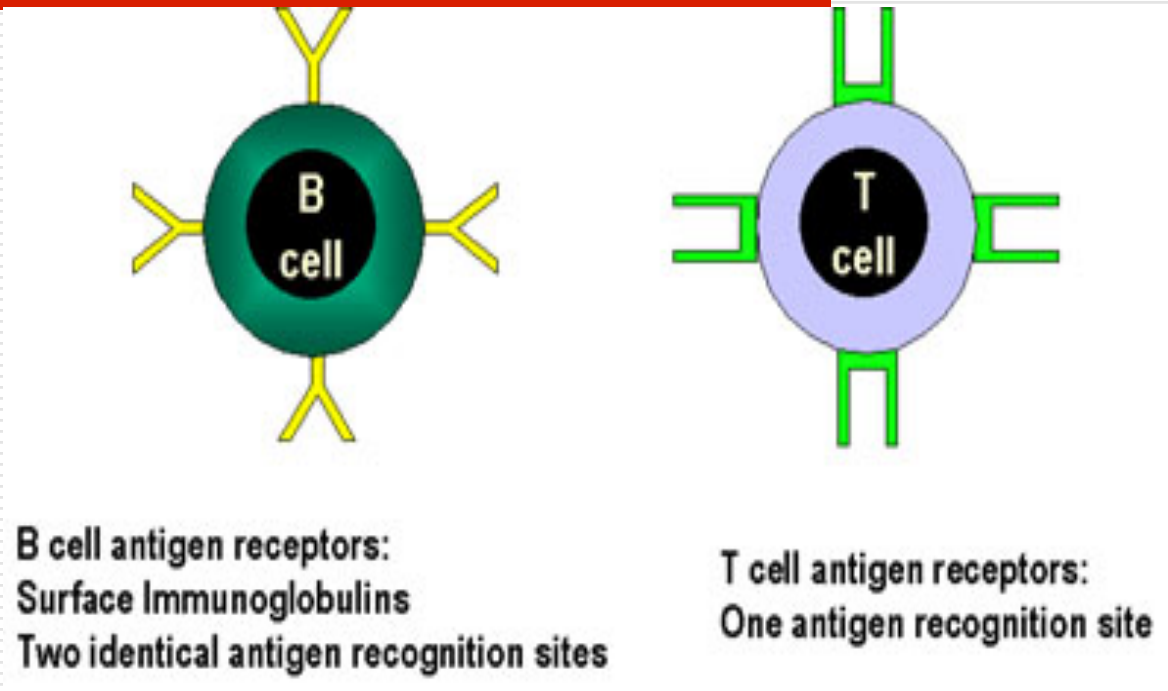
Geração das células mielóides e linfóides



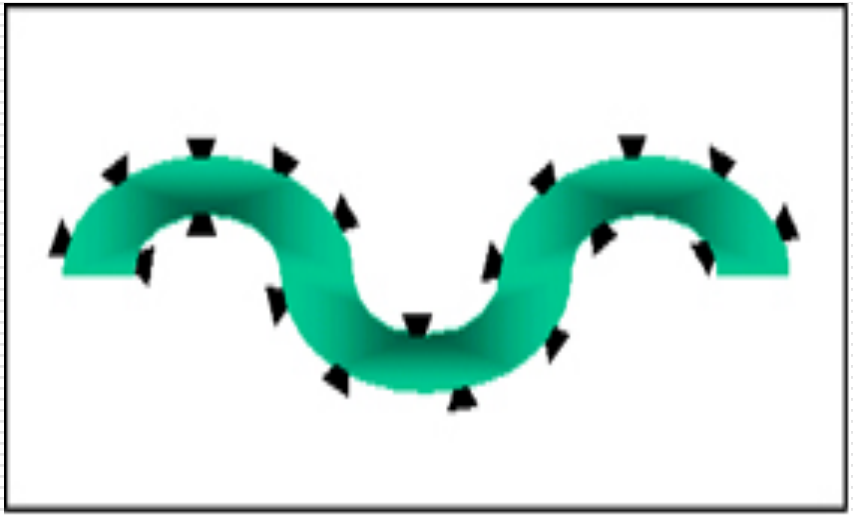
Destino das células mielóides e linfóides



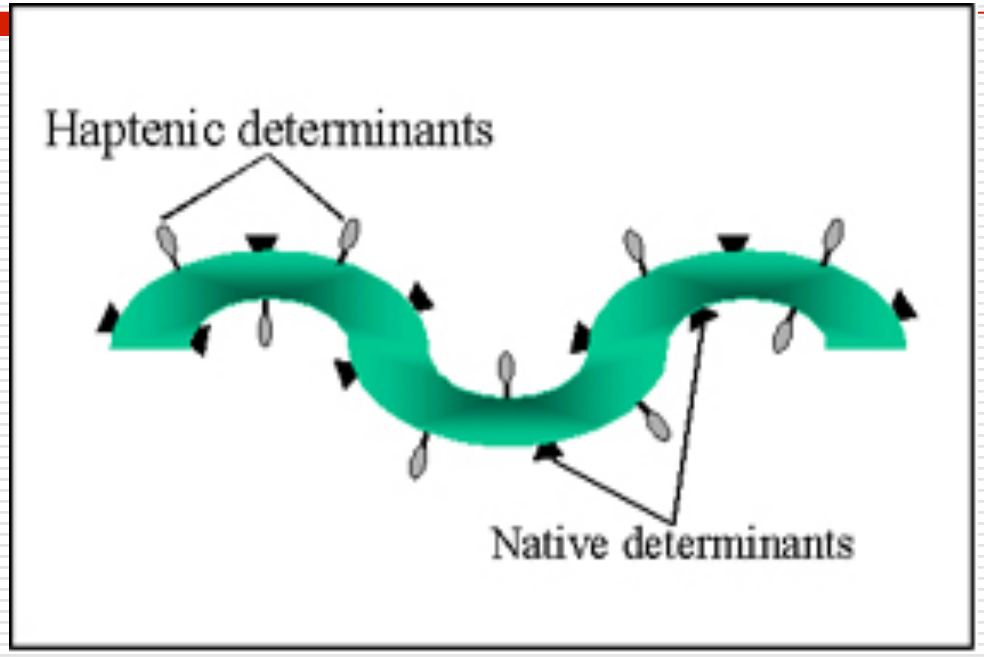
Linfócitos B e linfócitos T



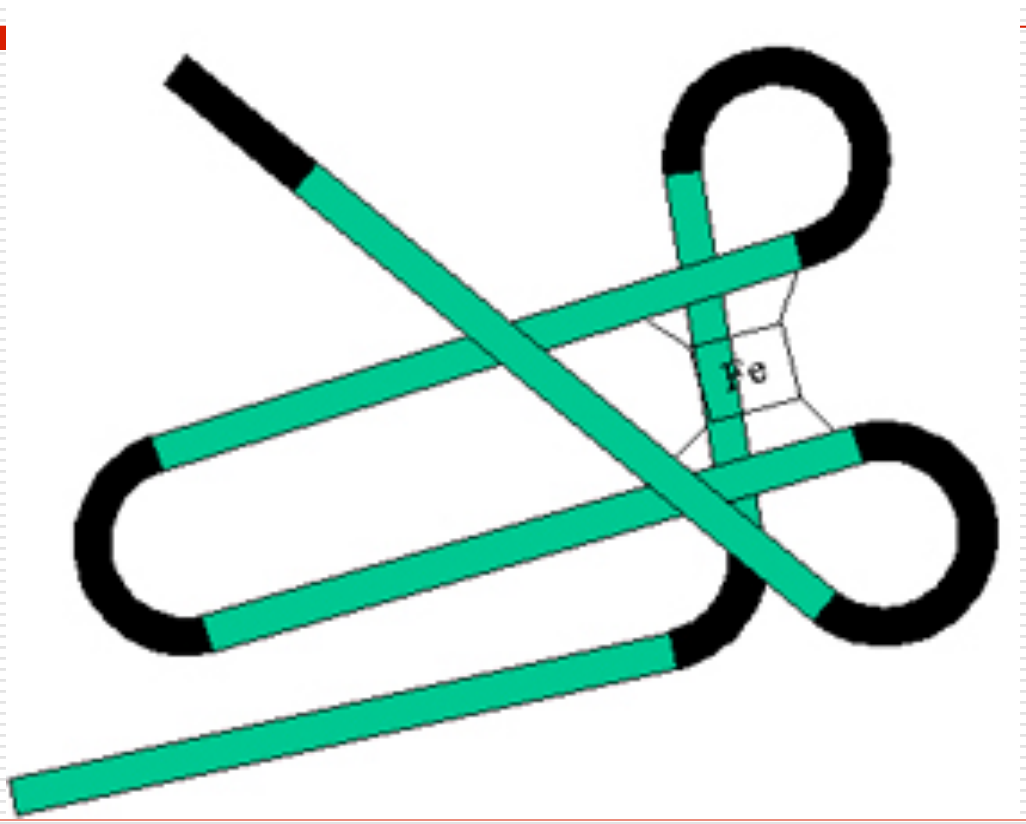
Conformação dos antígenos



Determinantes antigênicos

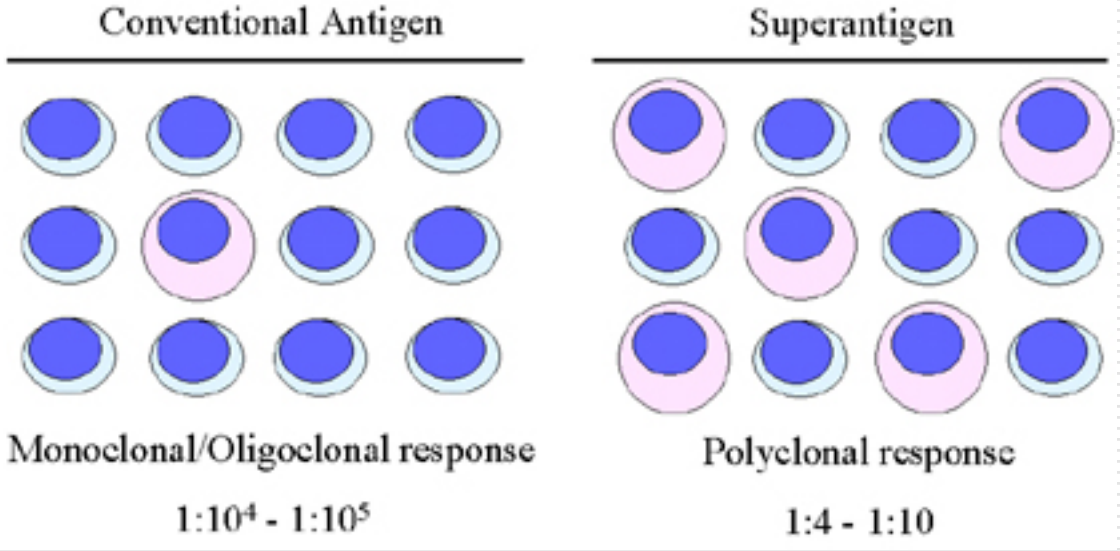


Determinantes antigênicos

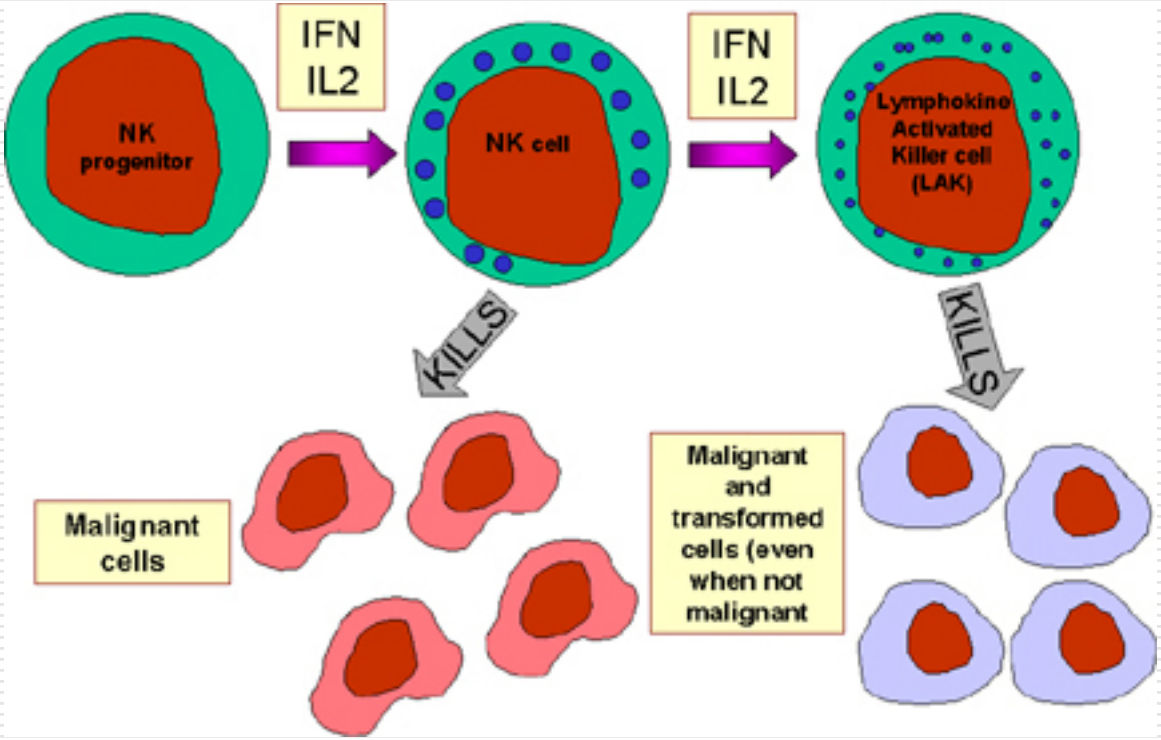


Superantigens

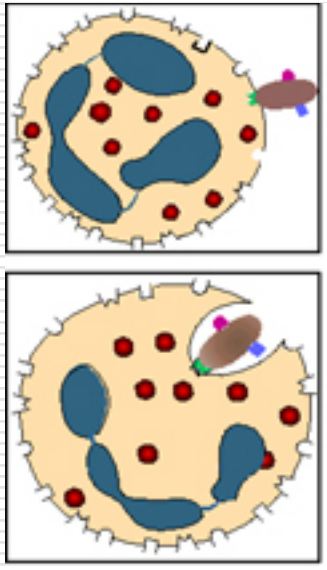
- Definition



Resposta imune inata [células NK (natural killer)]



Resposta inata: geração de oxigênio livre reativo



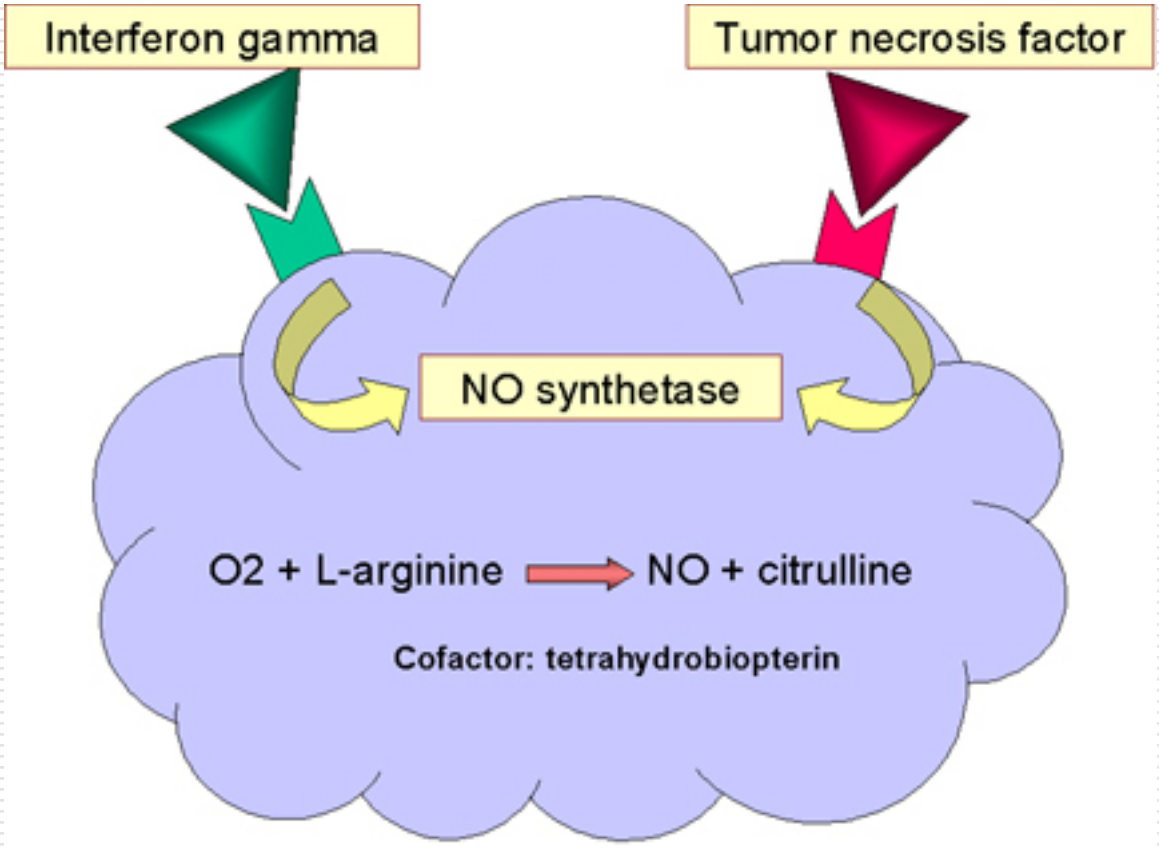
Glucose +$NADP^+$ → (G-6-P-dehydrogenase) Pentose-P + NADPH

$NADPH + O_2$ → (Cytochrome $_{b558}$) $NADP^+ + O_2^-$

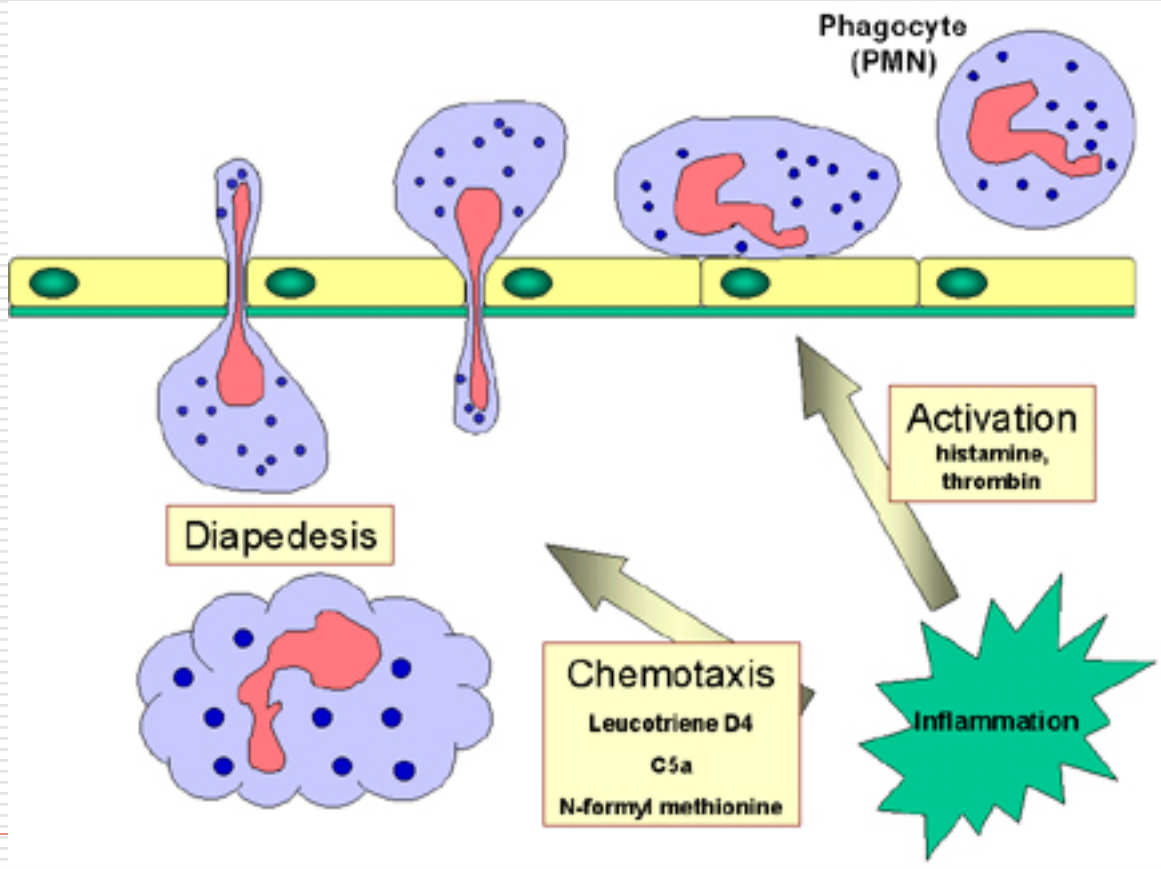
$2O_2^- + 2H^+$ → (Superoxide dismutase) $H_2O_2 + {}^1O_2$

$2O_2^- + H_2O_2$ → $.OH + OH^- + {}^1O_2$

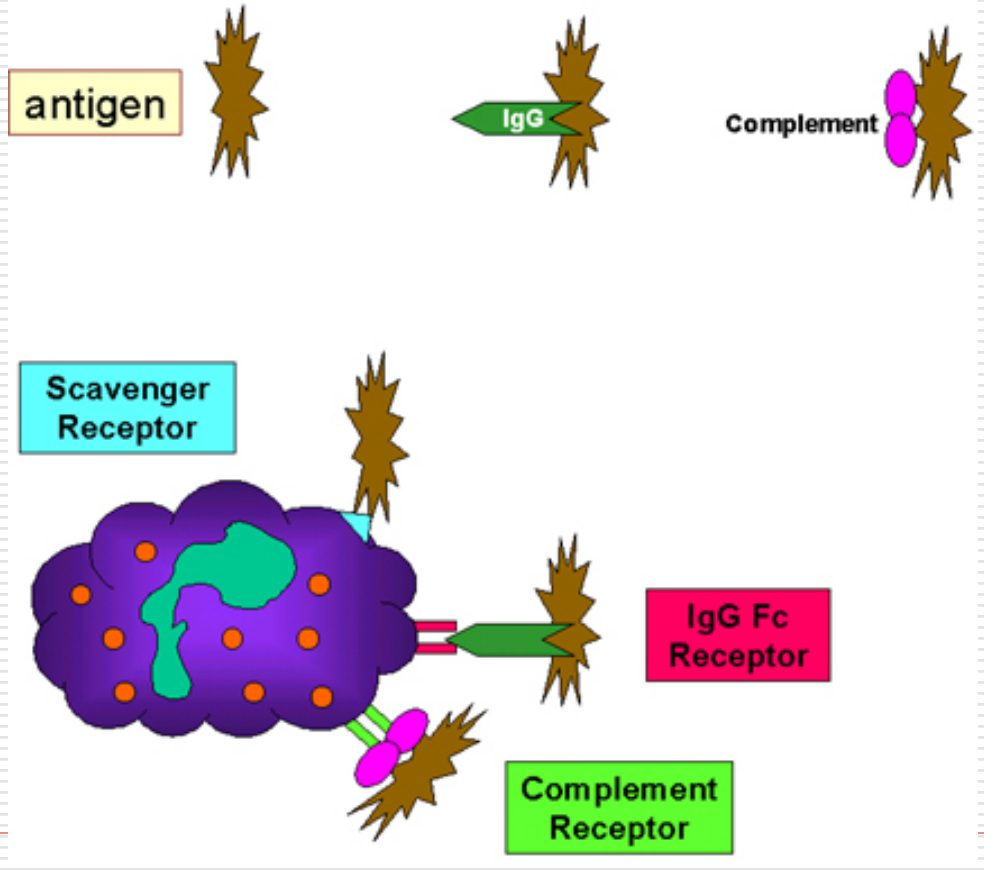
Resposta inata: geração de NO



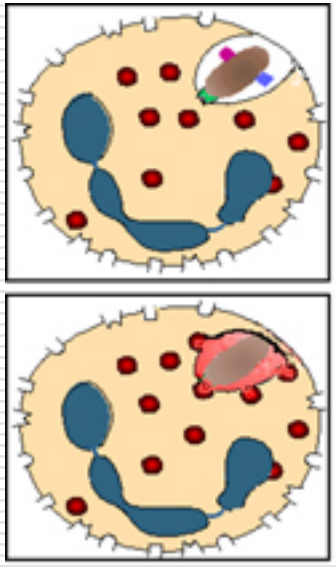
Resposta inata: migração de um fagócito (macrófago) para o local da inflamação



Resposta inata: sistema complemento



Resposta inata: geração de H_2O_2 e oxigênio livre reativo



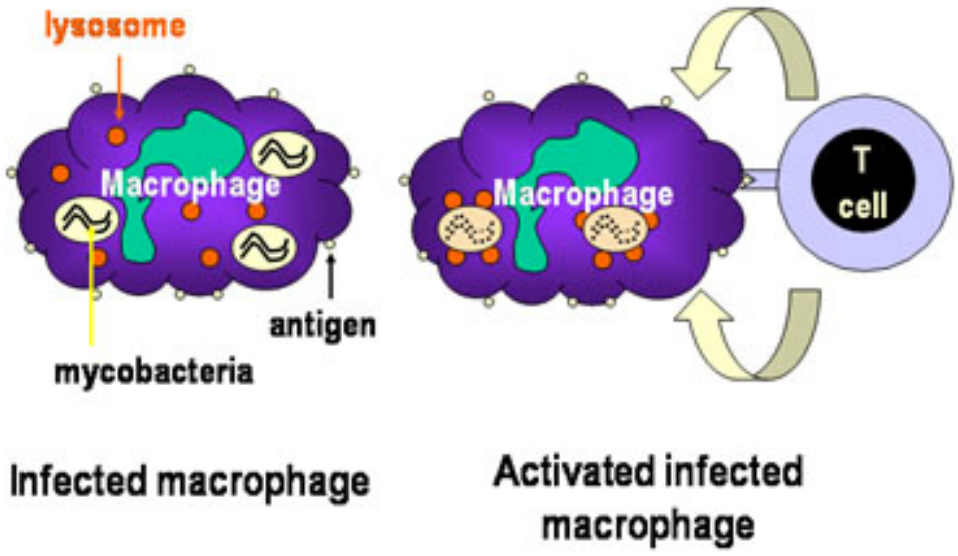
$$H_2O_2 + Cl^- \xrightarrow[\text{myeloperoxidase}]{} OCl^- + H_2O$$

$$OCL^- + H_2O \rightarrow {}^1O_2 + Cl^- + H_2O$$

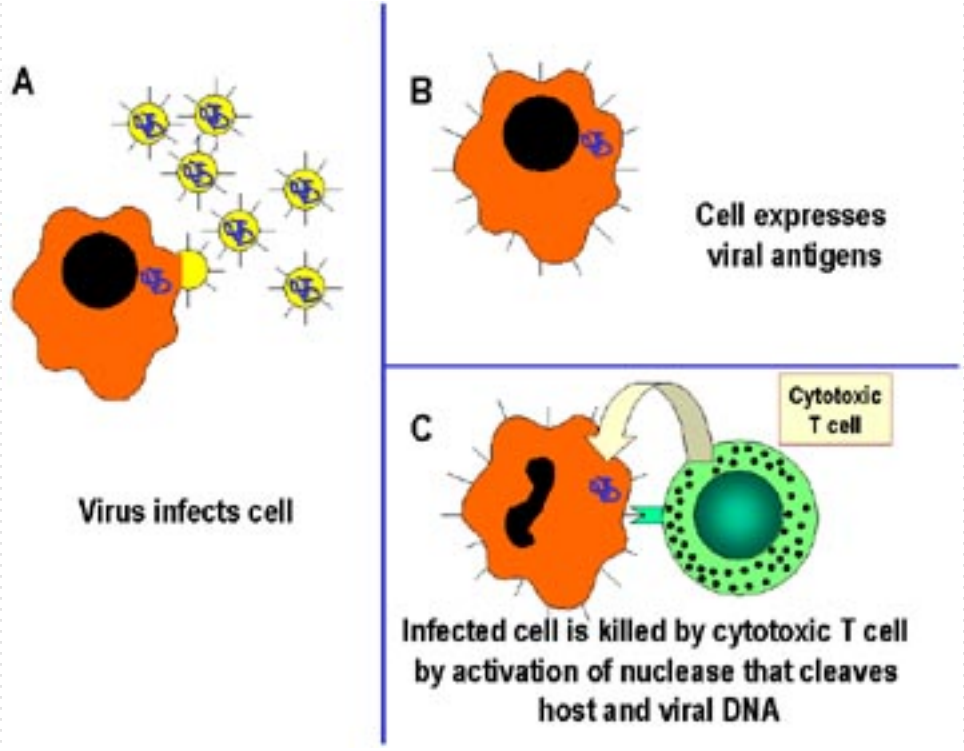
$$2O_2^{\cdot-} + 2H^+ \xrightarrow[\text{Superoxide dismutase}]{} H_2O_2 + {}^1O_2$$

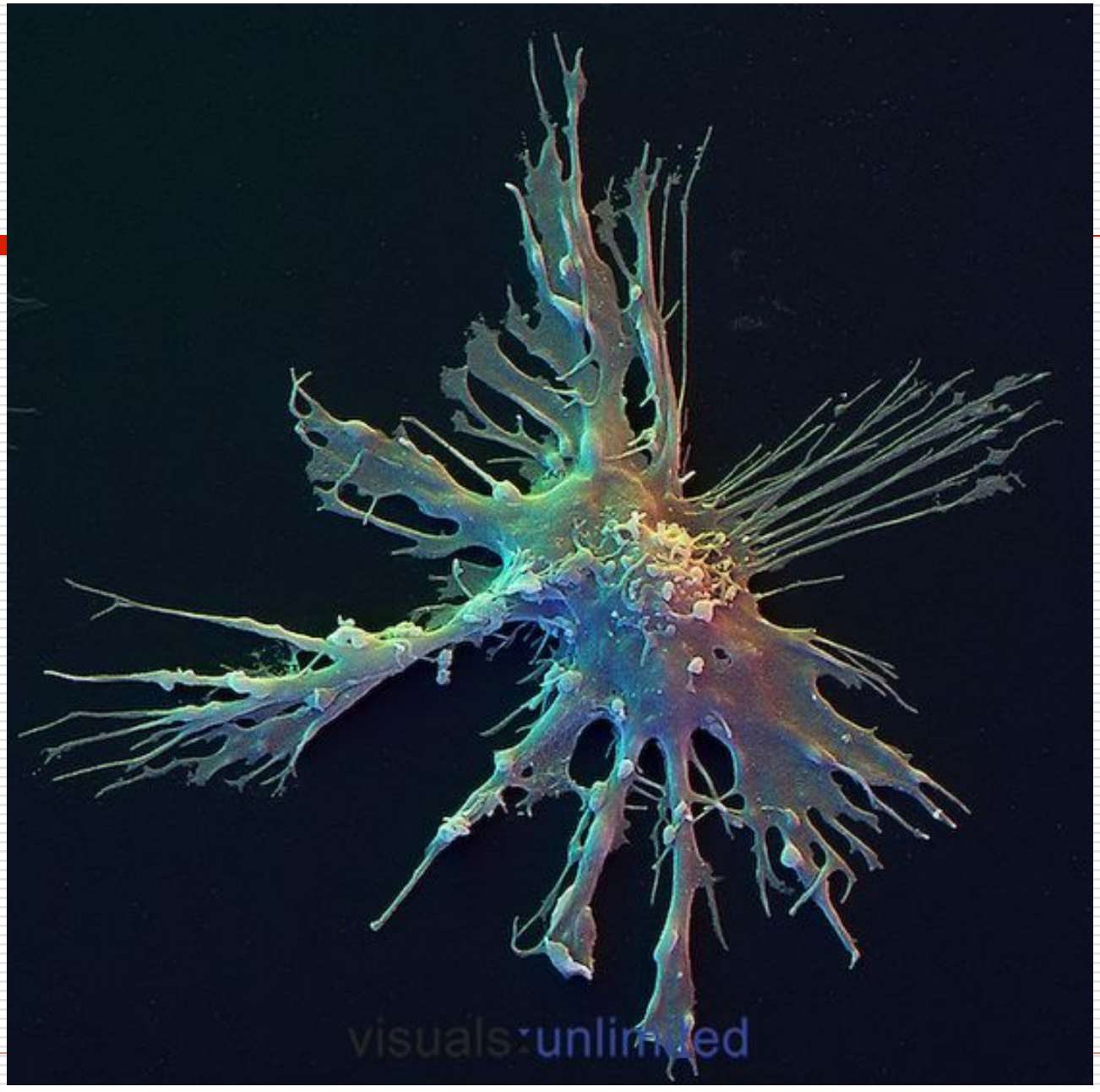
$$2\,H_2O_2 \xrightarrow[\text{catalase}]{} H_2O + O_2$$

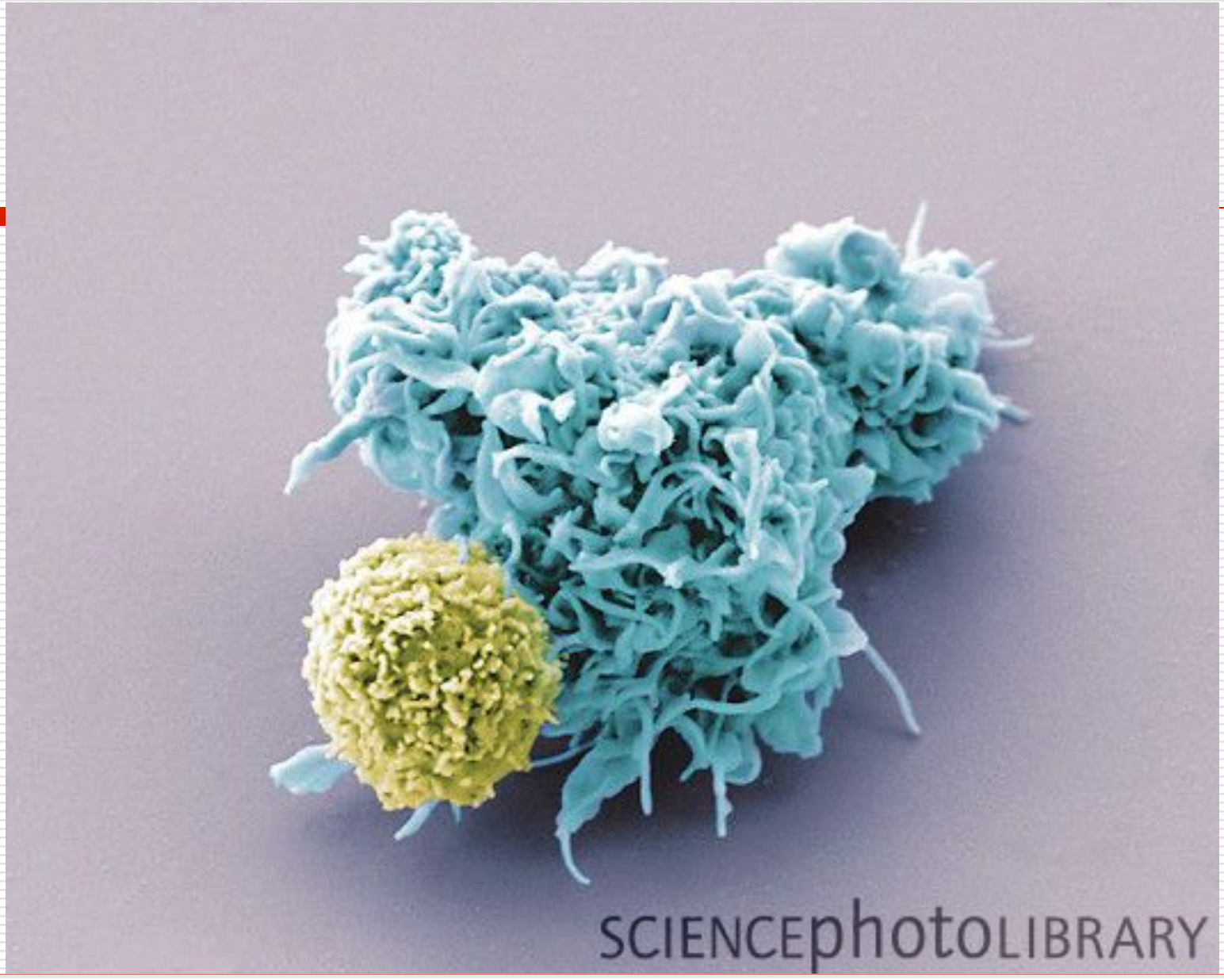
Apresentação de antígenos
Macrófagos → linfócitos T
(Interação entre resposta inata e adaptativa)



Resposta adaptativa (Linfócitos T citotóxicos)




visualsunlimited


SCIENCEphotoLIBRARY

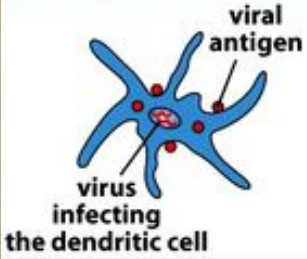
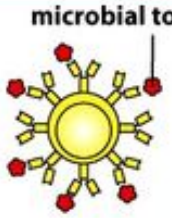
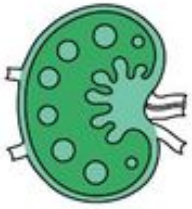
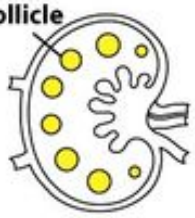
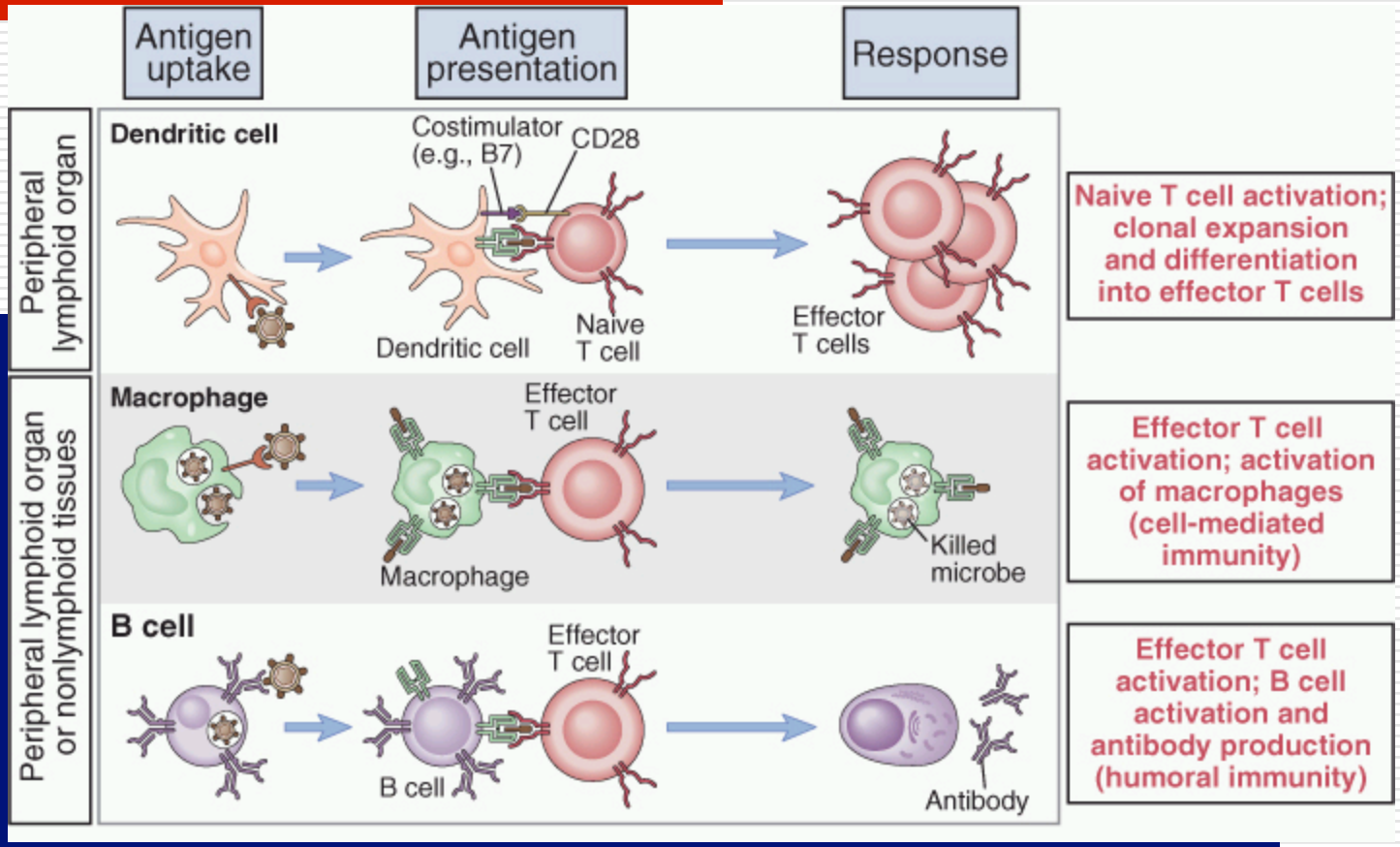
	Professional antigen-presenting cells		
	Dendritic cell	**Macrophage**	**B cell**
Cell type			
Location in lymph node			
Antigen uptake	+++ Macropinocytosis and phagocytosis by tissue dendritic cells Viral infection	Phagocytosis +++	Antigen-specific receptor (Ig) ++++
MHC expression	Low on tissue dendritic cells High on dendritic cells in lymphoid tissues	Inducible by bacteria and cytokines – to +++	Constitutive Increases on activation +++ to ++++
Co-stimulator delivery	Constitutive by mature, nonphagocytic lymphoid dendritic cells ++++	Inducible – to +++	Inducible – to +++
Antigen presented	Peptides Viral antigens Allergens	Particulate antigens Intracellular and extracellular pathogens	Soluble antigens Toxins Viruses
Location	Ubiquitous throughout the body	Lymphoid tissue Connective tissue Body cavities	Lymphoid tissue Peripheral blood

Figure 8.11 The Immune System, 3ed. (© Garland Science 2009)

Antigen presenting cells

Most important population of APC

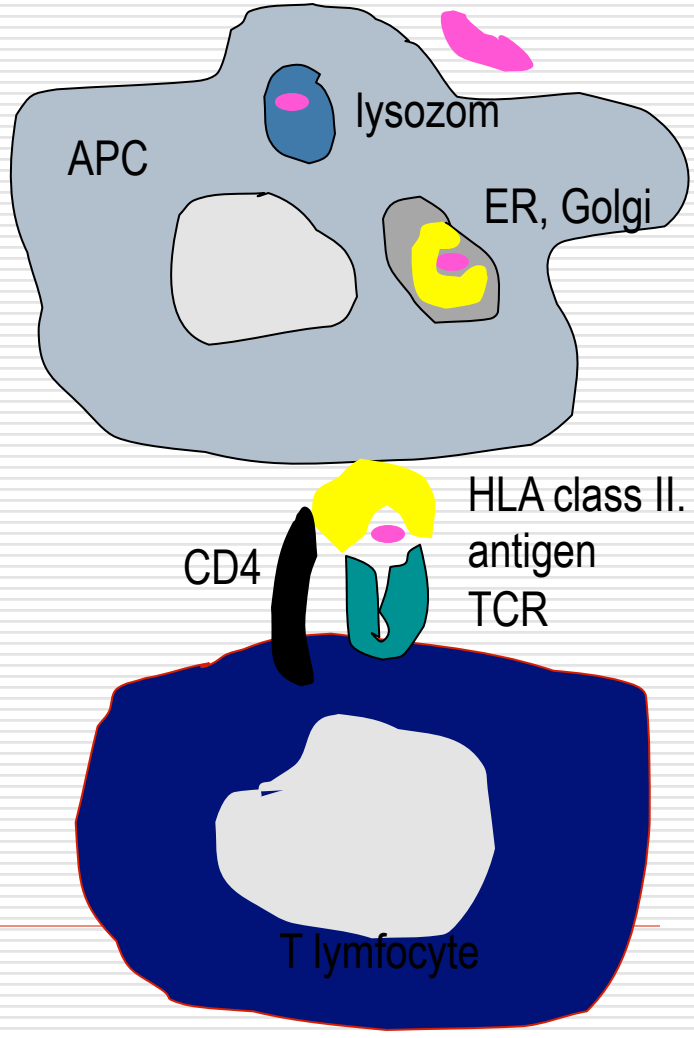


Other population of APC

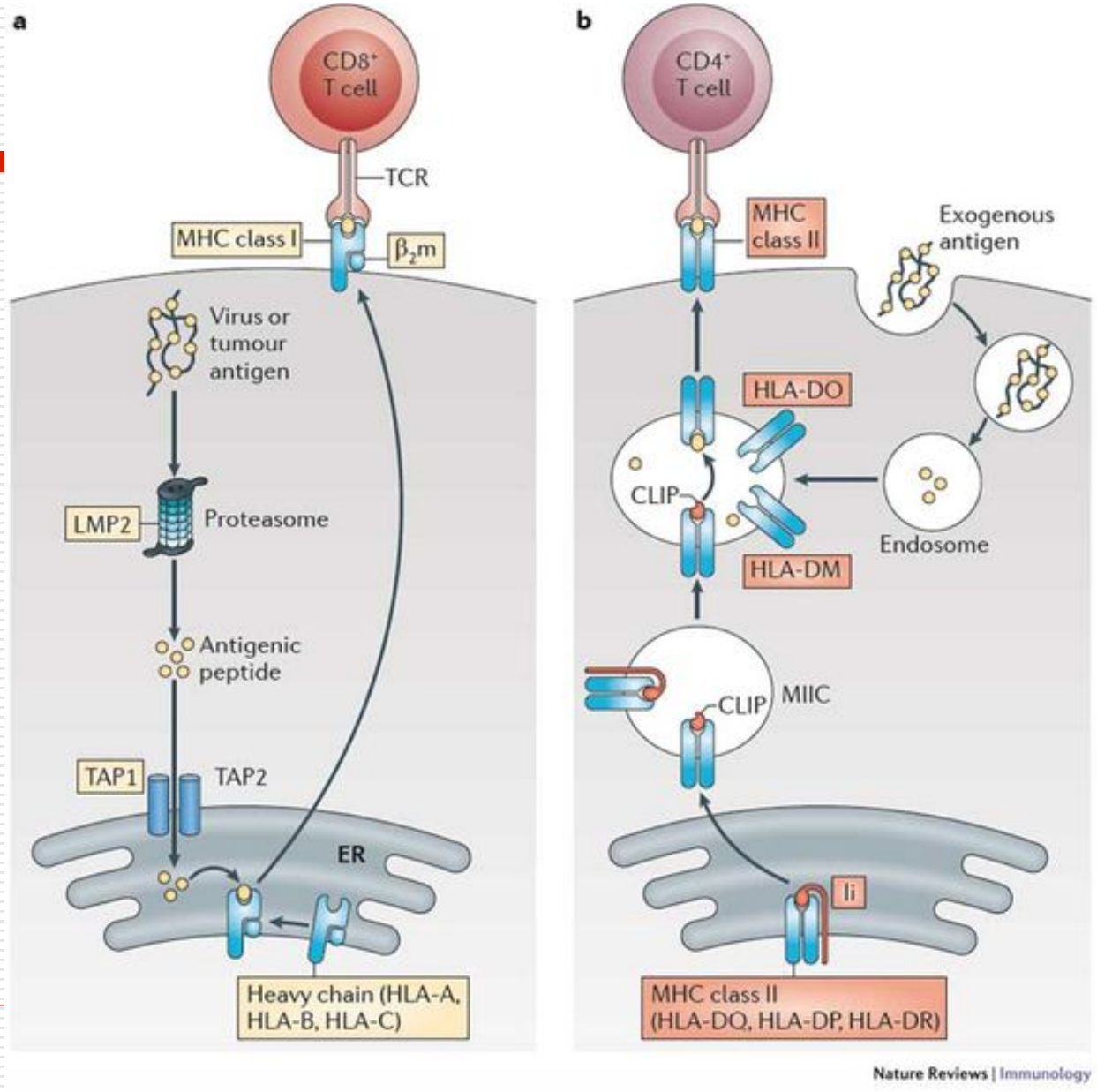
- Vascular endothelial cells: function as APC is **inducible**
- Various epithelial and mesenchymal cells: **inducible**

Function of MHC

- Recognition of antigen by T cells is necessary for induction of the immune response.
  - exogenous antigen presentation



MHC de classe I e MHC de classe II



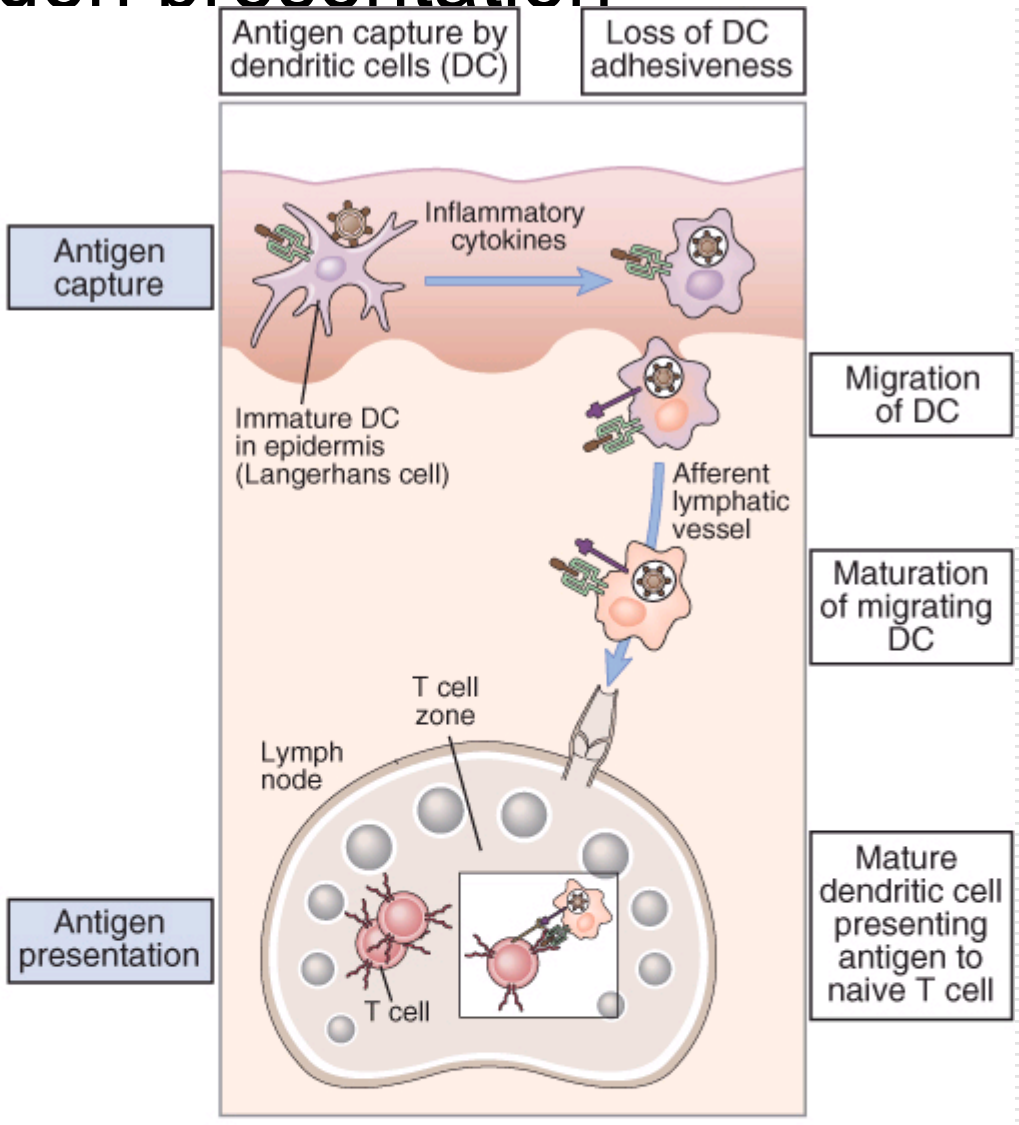
Dendritic cells in antigen presentation

Dendritic cells

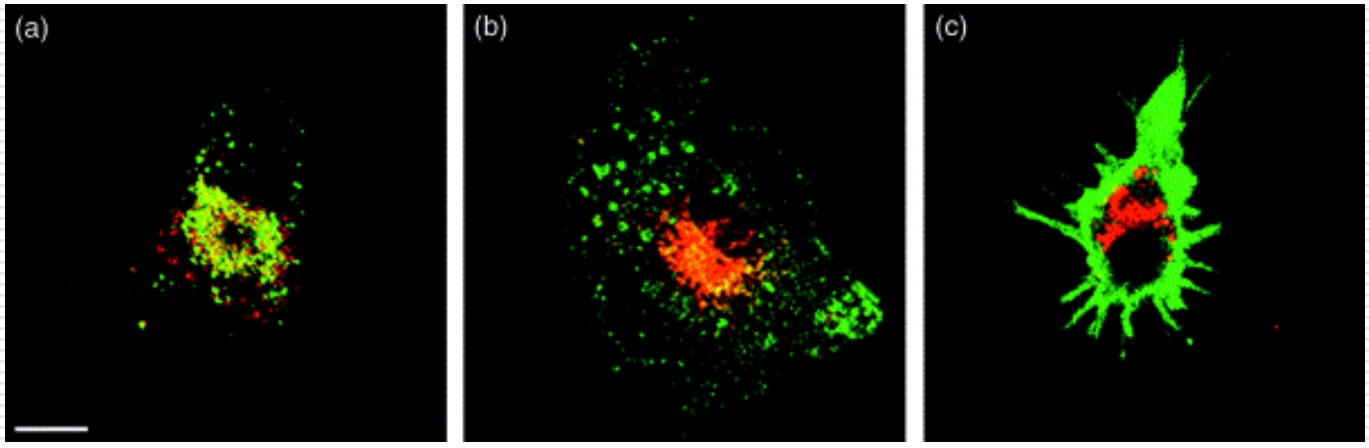
- most effective population in T cell activation
- used as immunoterapeutic tools in cancer vaccines

Immature DC: capture antigens in periphery

Mature DC: activation of T lymphocytes in lymphatic nodes



Antigen Presentation and Dendritic Cells



Immature (a), maturing (b), and mature (c) dendritic cells stained for MHC class II (green) and lysosomal marker Lamp-1 (red).

From Mellman et al., TICB 8: 231 (1998)

Exogenous antigens

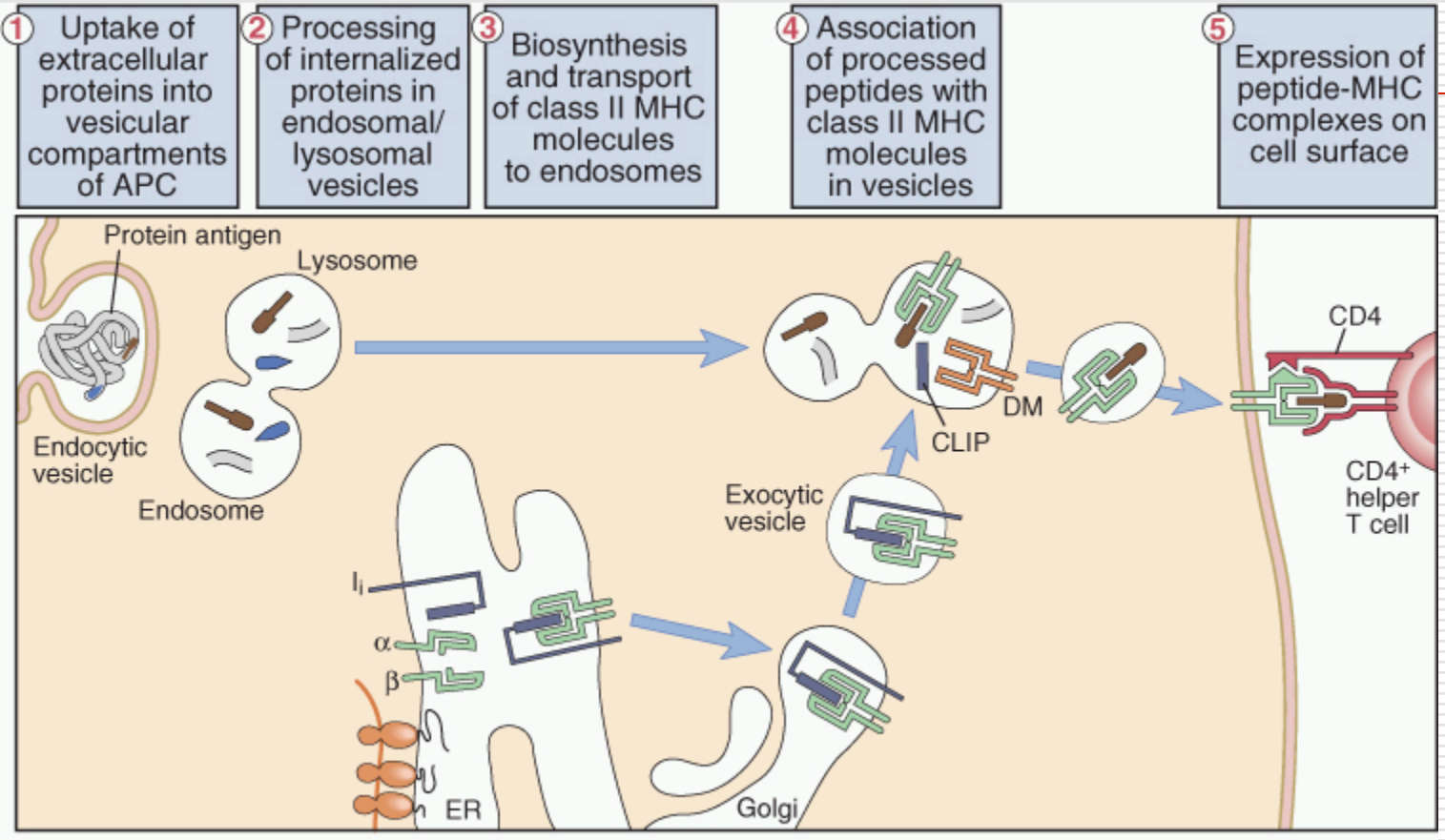
- Exogenous antigens (inhaled, ingested, or injected) are taken up by "professional" antigen-presenting cells
- These include:

  phagocytic cells like macrophages and dendritic cells

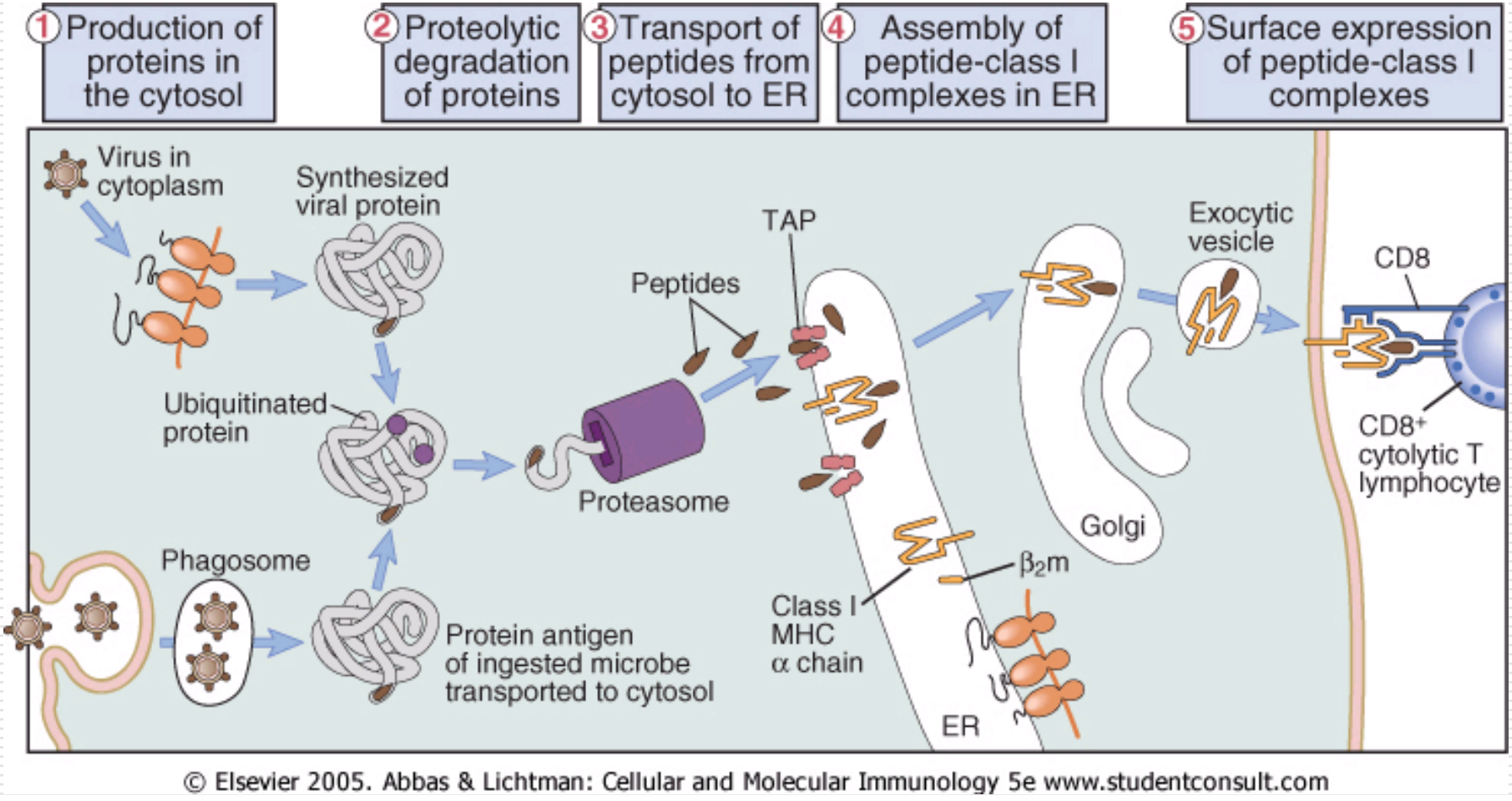
  B lymphocytes which are responsible

  for producing antibodies against the antigen.
- All these cells express HLA class II. molecules

Exogenous antigen processing

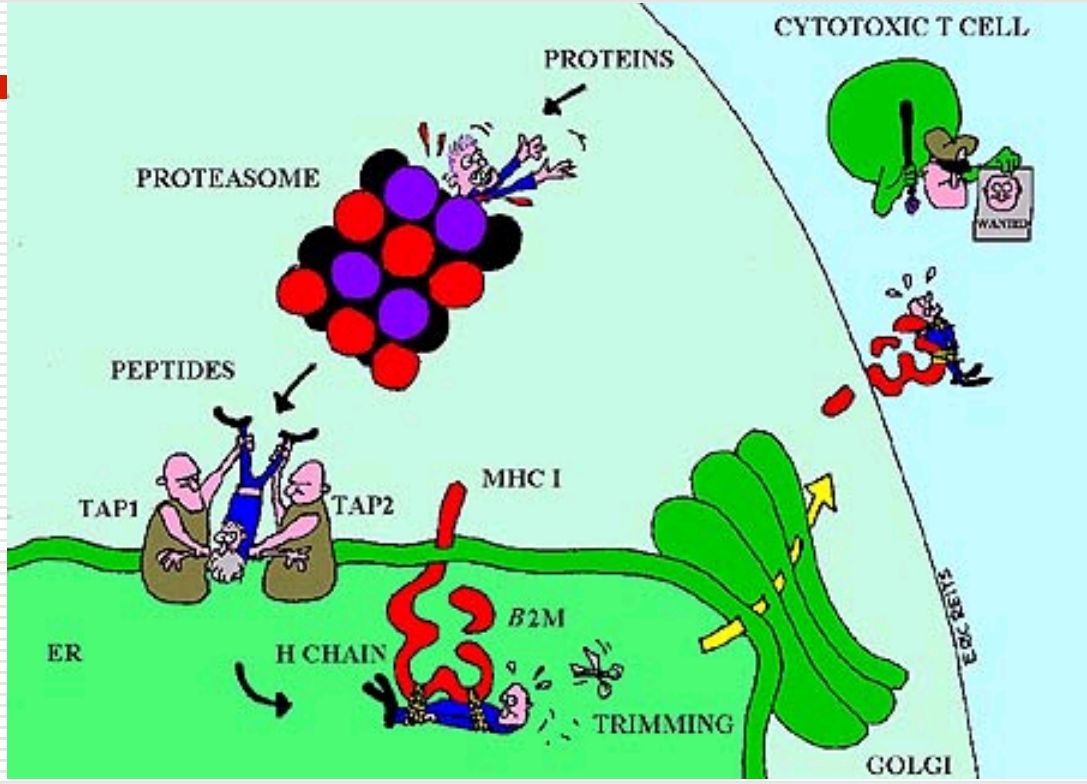


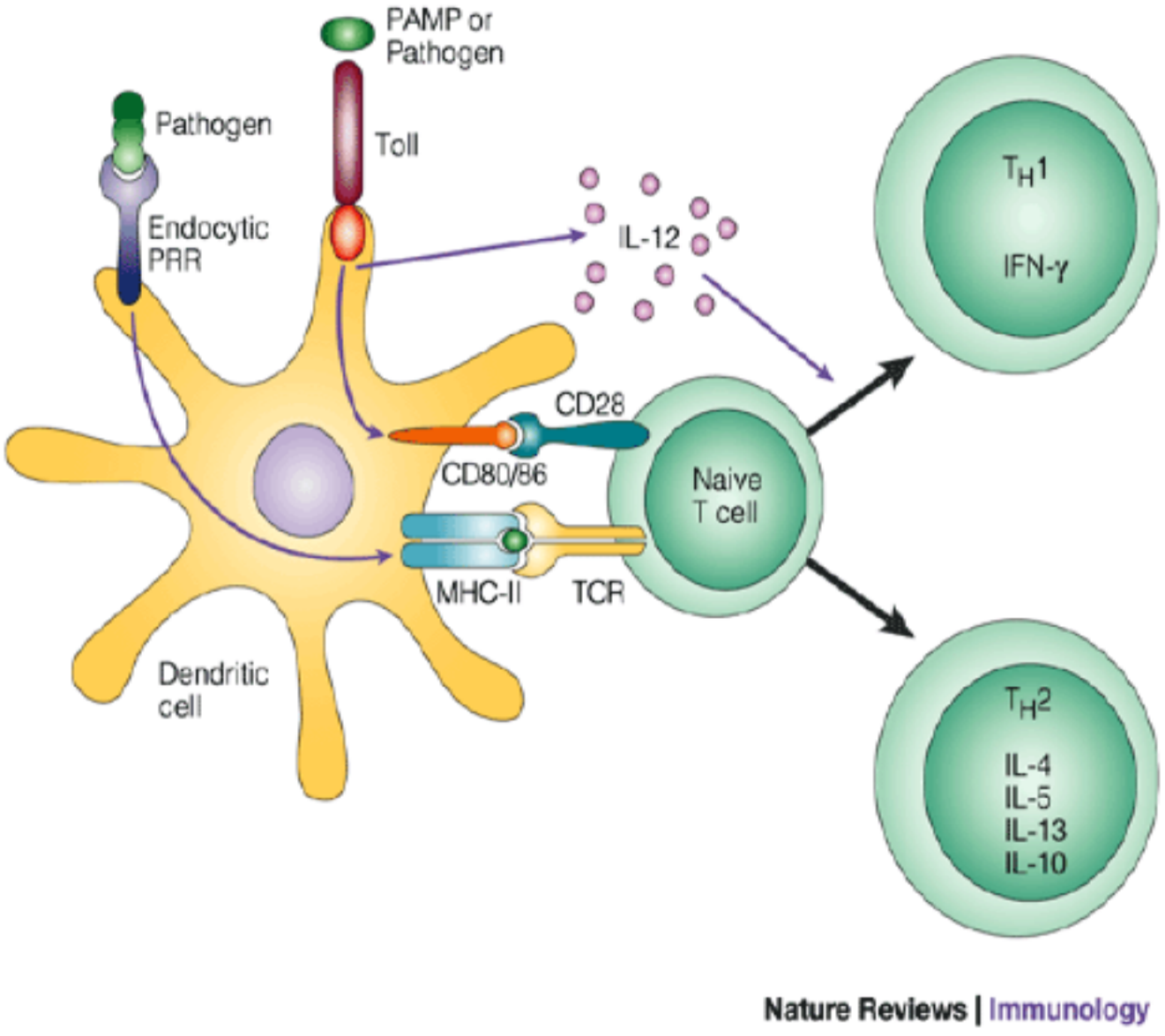
Endogenous antigen processing

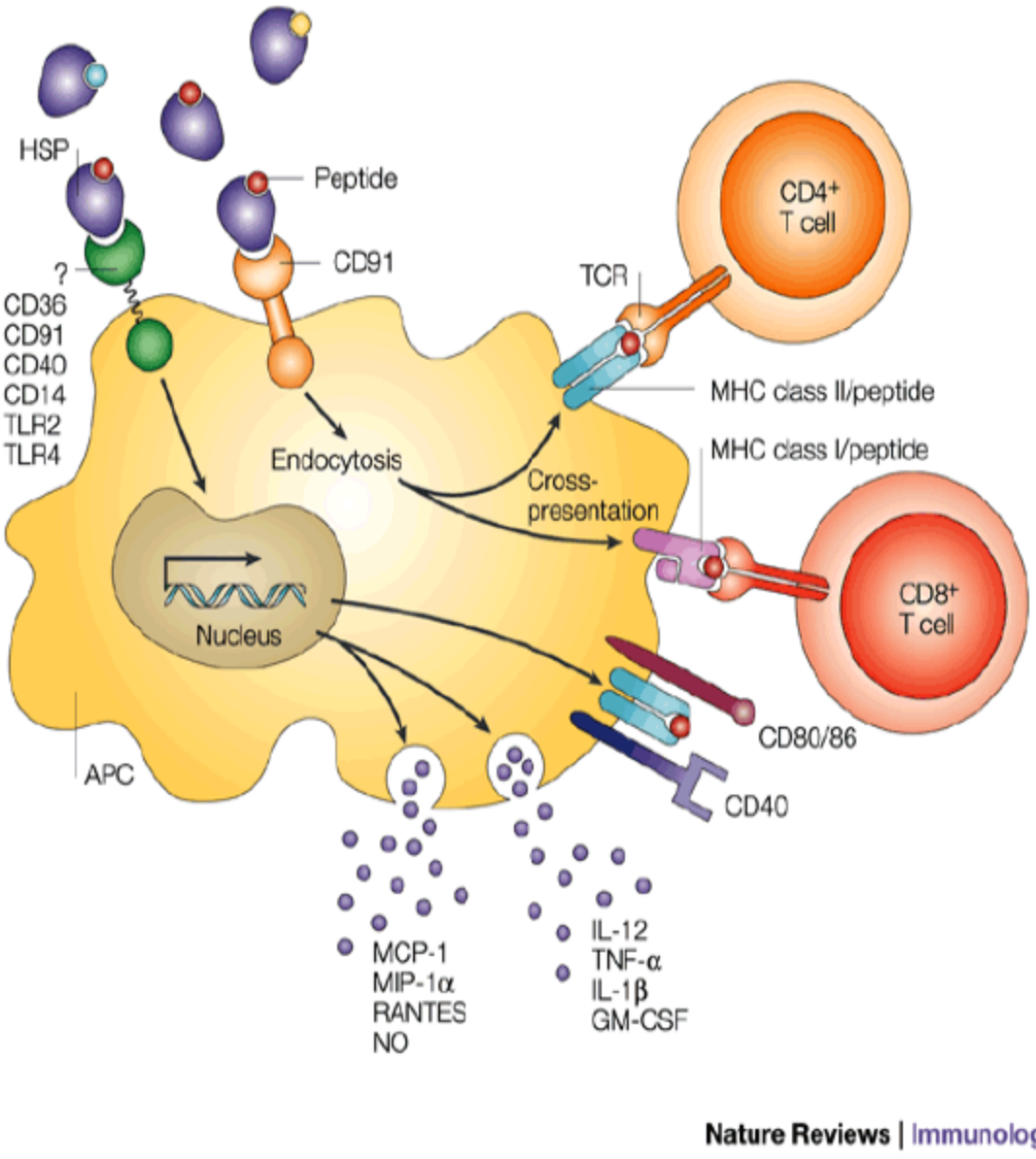


TAP (transporter associated with antigen presentation)

- Transport associated protein - TAP is responsible for the peptide transport from cytoplasm to ER.
- Proteins are degraded to peptide in proteasome.
- The peptides are picked up by TAP proteins and transported from the cytosol into the RER where they assemble with
  - the transmembrane polypeptide and beta-2 microglobulin.
  - this trimolecular complex then moves through the Golgi apparatus and is inserted in the plasma membrane

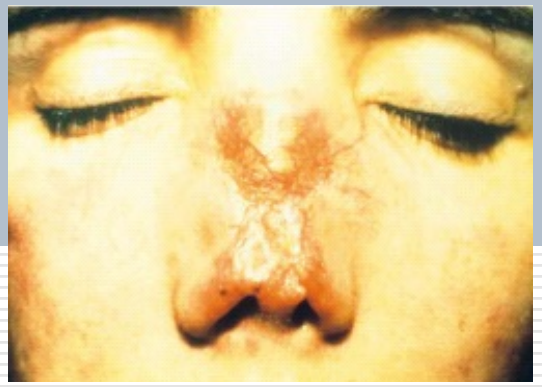



PAMP or
Pathogen
Pathogen
Toll
Endocytic
PRR
IL-12
T_H1
IFN-γ
CD28
CD80/86
Naive
T cell
MHC-II
TCR
Dendritic
cell
T_H2
IL-4
IL-5
IL-13
IL-10
Nature Reviews | Immunology


HSP
Peptide
?
CD36
CD91
CD40
CD14
TLR2
TLR4
CD91
TCR
CD4+
T cell
MHC class II/peptide
MHC class I/peptide
Endocytosis
Cross-
presentation
Nucleus
CD8+
T cell
CD80/86
CD40
APC
MCP-1
MIP-1α
RANTES
NO
IL-12
TNF-α
IL-1β
GM-CSF
Nature Reviews | Immunology

Imunodeficiences - MHC defect

- Bare lymphocyte syndrome:
  mutation in genes regulating class II MHC transcription
  - reduced number of CD4+ T cells in periphery
  - defective activation of CD4+ T cells
  - fatal, treatment: BM transplantation

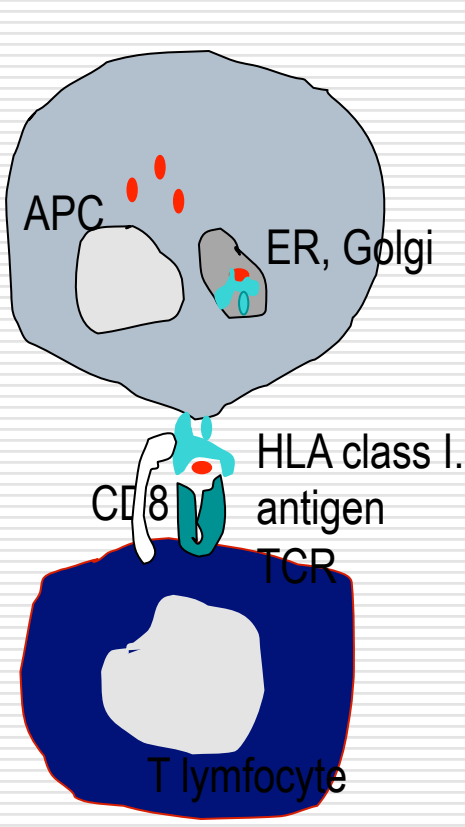


- Class I MHC deficiencies:
  - decreased number of CD4+ T cells in periphery
  - caused by TAP1, TAP2
  - patients suffer from respiratory tract bacterial infection

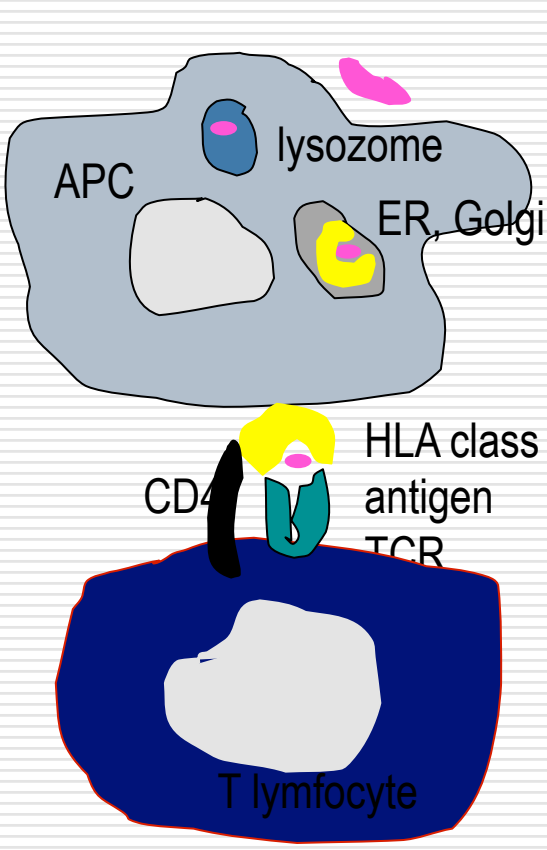
HLA-associated diseases

	HLA	Patients	Controls
Ankylosing spondylitis	*B27*	90%	9%
Type 1 diabetes	*DR3*	52%	23%
	DR4	74%	24%
	DR3 or *DR4*	93%	43%
Multiple sclerosis	*DR2*	86%	33%
Rheumatoid arthritis	*DR4*	81%	24%
Narcolepsy	*DR2*	>95%	33%

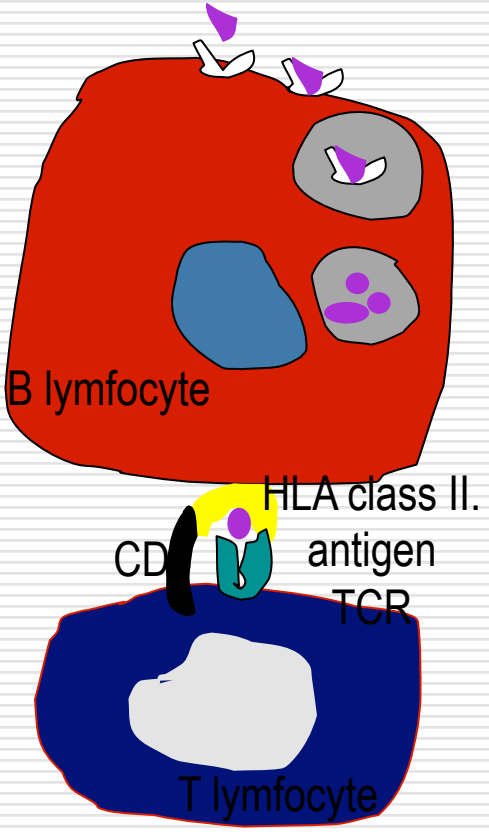
Summary - antigen presentation pathways



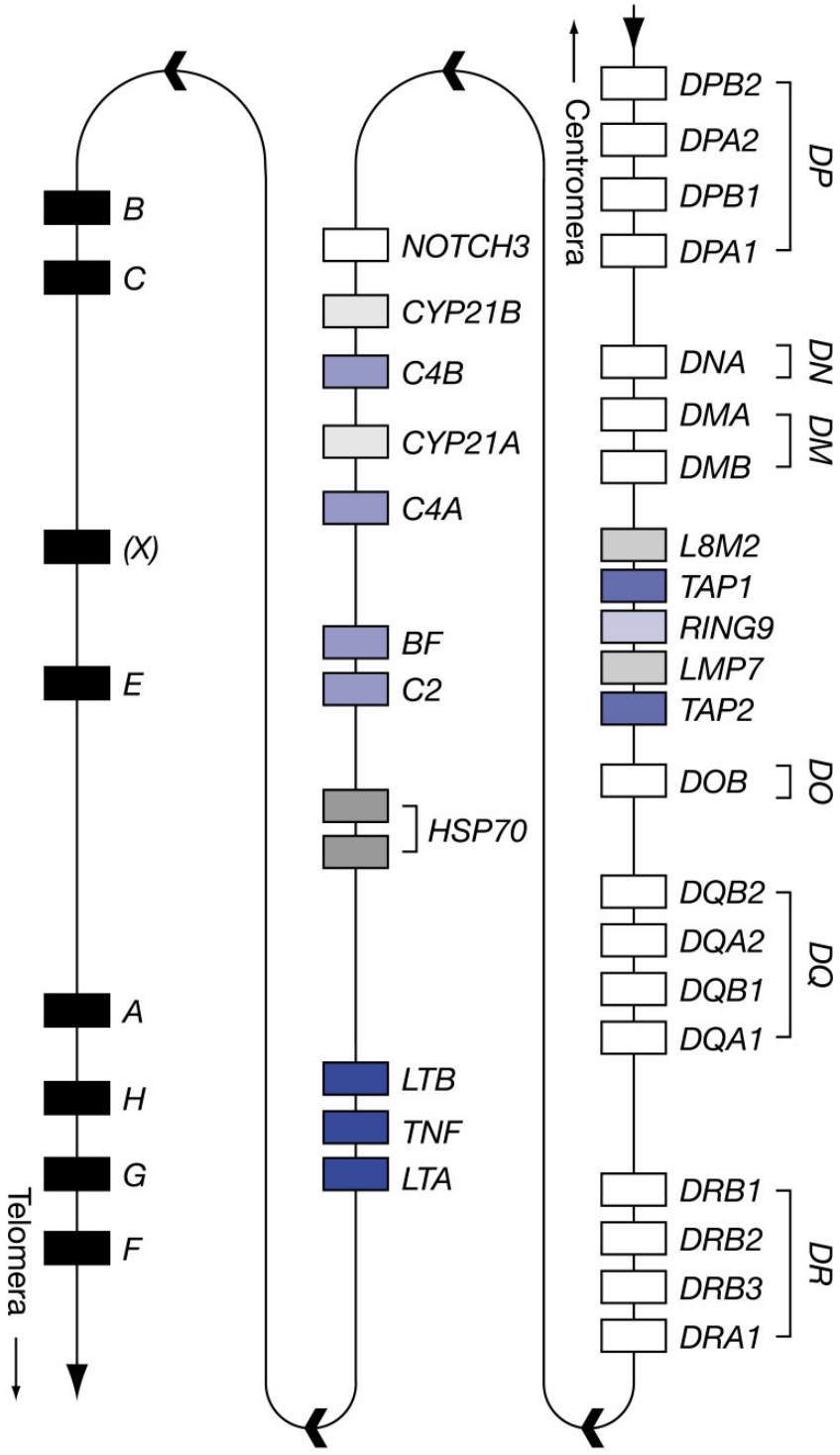
endogenous
cell destruction



exogenous
immune response



B lymfocytes
antibody production


B
C
(X)
E
A
H
G
F
Telomera
NOTCH3
CYP21B
C4B
CYP21A
C4A
BF
C2
HSP70
LTB
TNF
LTA
Centromera
DPB2
DPA2
DPB1
DPA1
DP
DNA
DN
DMA
DMB
DM
L8M2
TAP1
RING9
LMP7
TAP2
DOB
DO
DQB2
DQA2
DQB1
DQA1
DQ
DRB1
DRB2
DRB3
DRA1
DR

POLYMORPHISM OF MHC PROTEINS

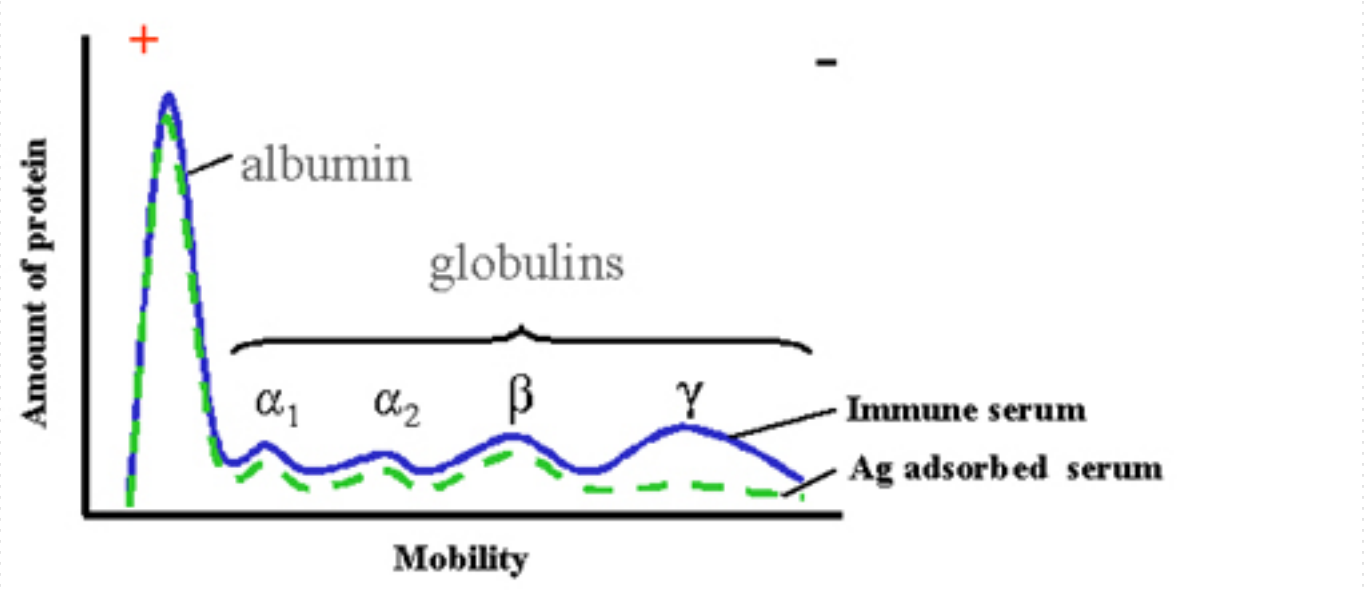
DRα 1	HLA-A >280
DRβ >400	HLA-B >500
DQβ >50	HLA-C >130

Variability is in the amino acid residues in the peptide binding site!

MHC gp	Motif
HLA-11	xxAspxxxxxTyr
HLA-A2,1	xLeuxxxxxxLeu(Val)
HLA-A11	xValxxxxxxLys
HLA-A24	xTyrxxxxxxLeu(Phe)
HLA-B7	xProArgxxxxxLeu
HLA-B27	xArgxxxxxxLys(Arg)

Disease	HLA	Relative risk*
Ankylosing spondyloarthritis	B27	87.4
Uveitis	B27	10
Goodpasture syndrome	DR2	15.9
Multiple sclerosis	DR2	4.8
Graves-Basedow disease	DR3	3.7
Systemic lupus erythematodes	DR3	5.8
Myasthenia gravis	DR3	2.5
Pemphigus	DR4	14.4
Rheumatoid arthritis	DR4	4.2
Hashimoto thyreoiditis	DR5	3.2

Proteínas do plasma sanguíneo

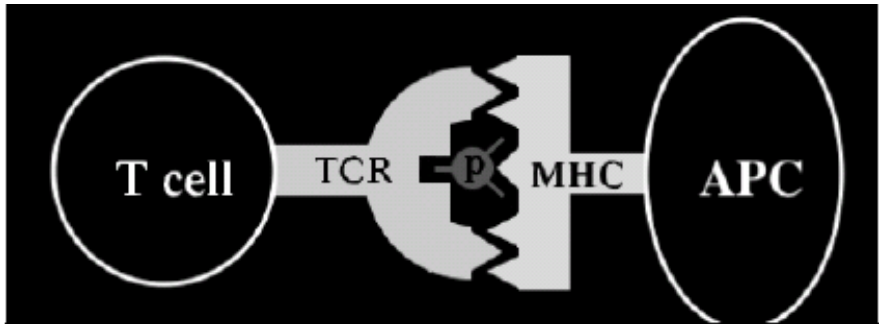


MHC in adaptive immunity

	Innate	Adaptive
Characteristics		
Specificity	For structures shared by groups of related microbes	For antigens of microbes and for nonmicrobial antigens
Diversity	Limited; germline-encoded	Very large; receptors are produced by somatic recombination of gene segments
Memory	None	Yes
Nonreactivity to self	Yes	Yes
Components		
Physical and chemical barriers	Skin, mucosal epithelia; antimicrobial chemicals	Lymphocytes in epithelia; antibodies secreted at epithelial surfaces
Blood proteins	Complement	Antibodies
Cells	Phagocytes (macrophages, neutrophils), natural killer cells	T and B Lymphocytes

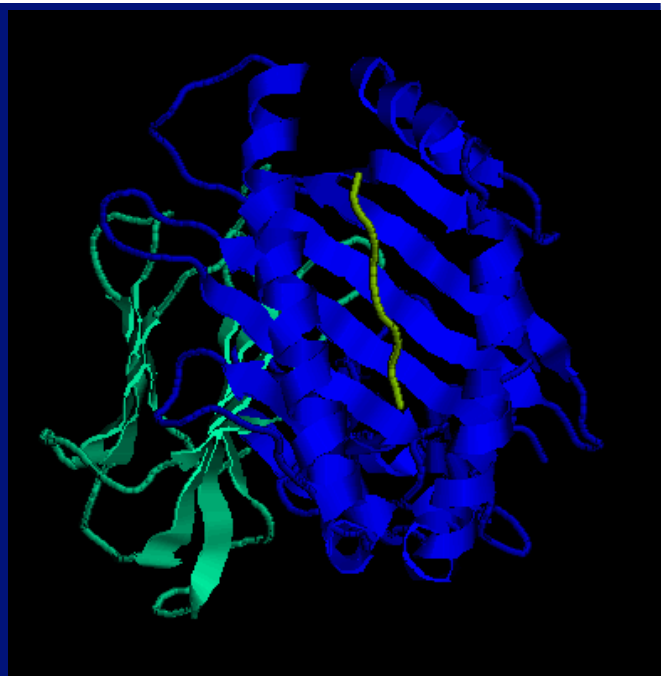
T cells recognise cell-associated antigens displayed on

MHC = major histocompatibility complex



Outline

- Adaptive immunity, role of MHC (HLA)
- discovery of HLA genes
- structure of HLA genes and molecules
- polymorphism of HLA molecules
- nomenclature of HLA system
- HLA association with disease
- antigen presentation



MHC GLYCOPROTEINS

CENTRAL MOLECULES OF IMMUNITY

MHC gp I – EXPRESSION NA ALL CELLS

MHC gp II – EXPRESSION ON APC

FUNCTION – "EXHIBIT" ON CELL SURFACE SAMPLES OF FRAGMENTS OF ENDOGENOUS (MHC gp I) RESP. EXOGENOUS (MHC gp II) PROTEINS.

THESE COMPLEXES ARE THEN RECOGNIZED BY T-LYMPHOCYTES (T_h, T_c)

HLA – MHC: basic facts

- Two groupes of MHC genes:

  structurally and functionally distinct

  class I recognition by CD8+ T cells

  class II recognition by CD4+ T cells

- HLA molecules are responsible for the compatibility of the tissues of genetically different individuals and for the rejection of transplant

- MHC genes are codominantly expressed in each individual
- monozygotic twins have the same histocompatibility molecules on their cells
- MHC genes are the most polymorphic genes present in the genome!

  (Up to 250 alleles identified for some loci)

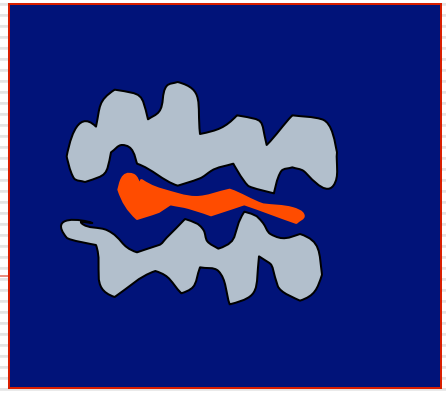
MHC expression

Class I

On all nucleated cells (no MHC on red blood cells, weak expression on cells in CNS)

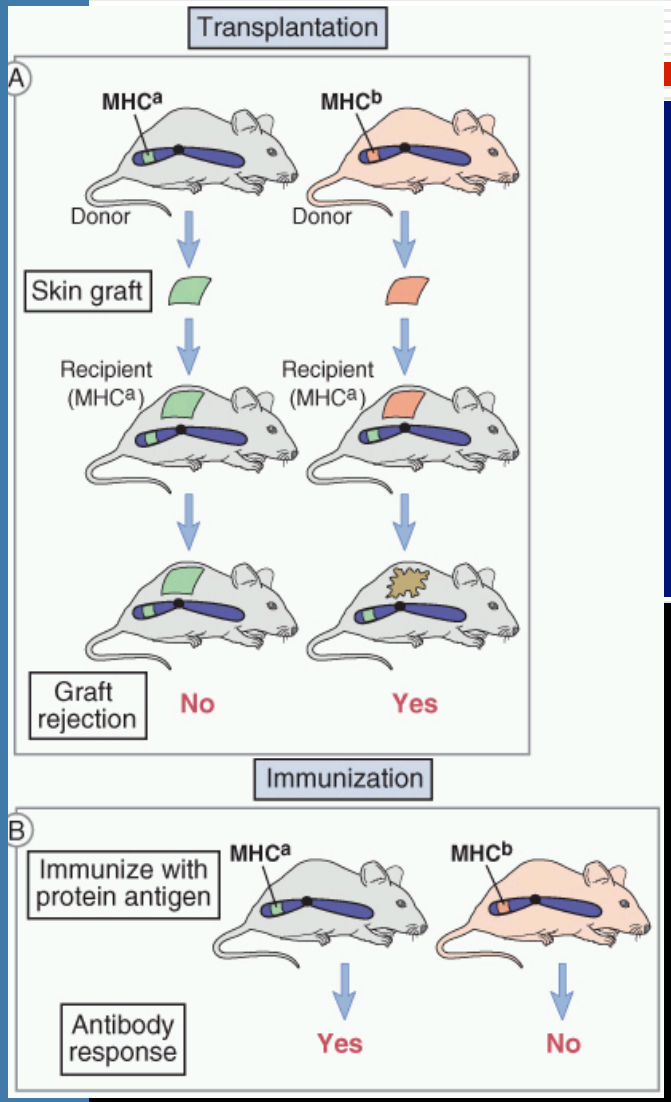
Class II

Found on **antigen presenting cells**



HUMAN		MOUSE	
MHC I	HLA-A, -B, -C	MHC I	H-2K, D, L
Ib	E, F, G CD1)	Ib	Qa, TL, H-2M3 (CD1)
MHC II	DR, DQ, DP	MHC II	I-A, I-E
	(DM)		(DM)

Discovery of Human MHC



- Recognition of a graft as self or foreign is an inherited trait

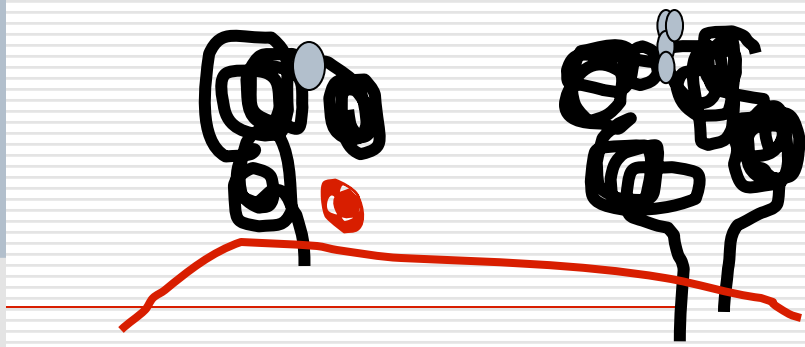
- histocompatibility genes: differences between self and foreign were attributed to their genetic polymorphisms
- Mouse study: identification of MHC locus
- Human MHC

In study with transplanted patients discovered „human leukocyte antigens“ HLAs

HLA-A, HLA-B, HLA-C (**class I MHC genes)**

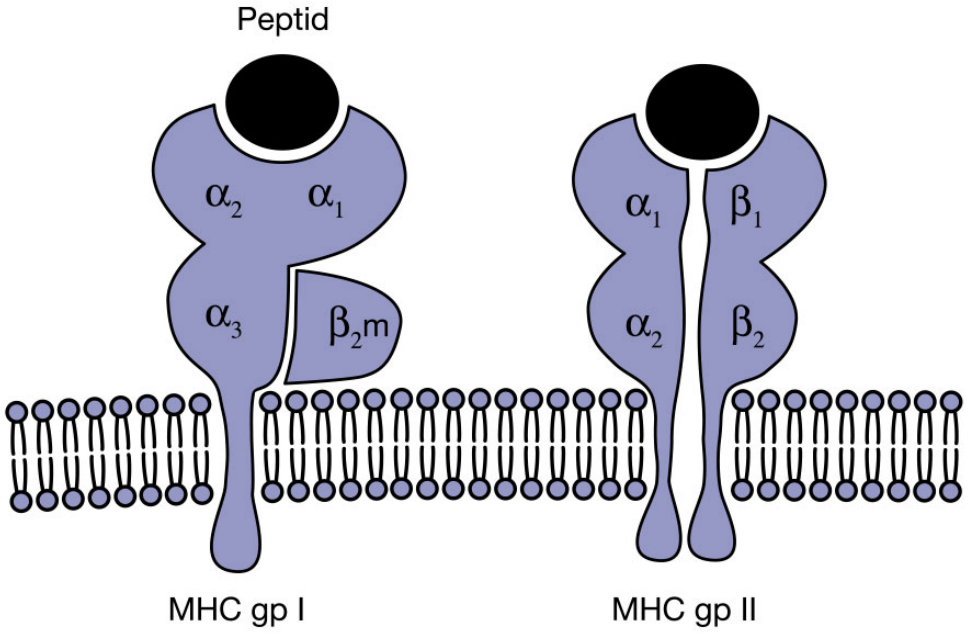
In study of mixed leukocyte reaction identified HLA-DR, HLA-DP, HLA-DQ (**class II MHC genes**

structure of HLA molecules

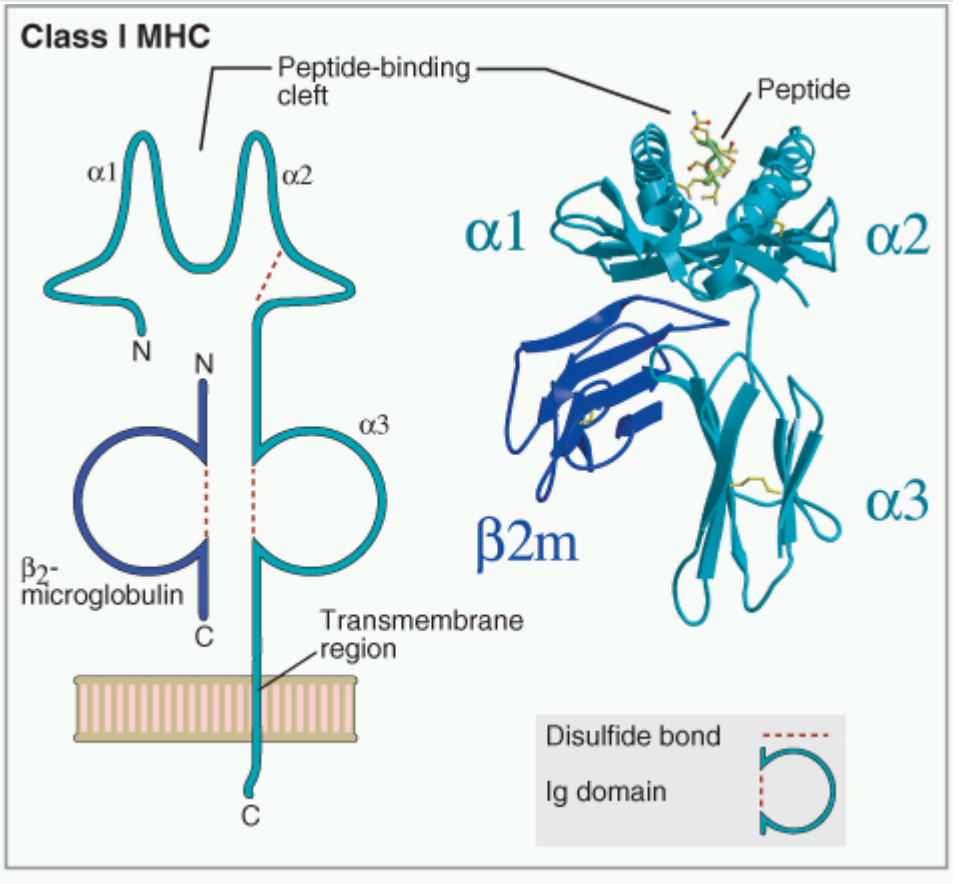


- glycoproteins, heterodimers (two chains)
- Structure of HLA molecules of both classes enables antigen binding and contact with T cell receptors. Extracellulary located peptide binding cleft
- polymorphic (predominantly in the cleft).
- Nonpolymorphic part of the molecule contains binding sites for the T cell molecules CD4 and CD8

(a)



HLA class I. molecules



1. Heavy chain

α1, α2 domain: polymorphic sites

α3 domain: binding of CD8

2. β-2 microglobulin

3. peptide

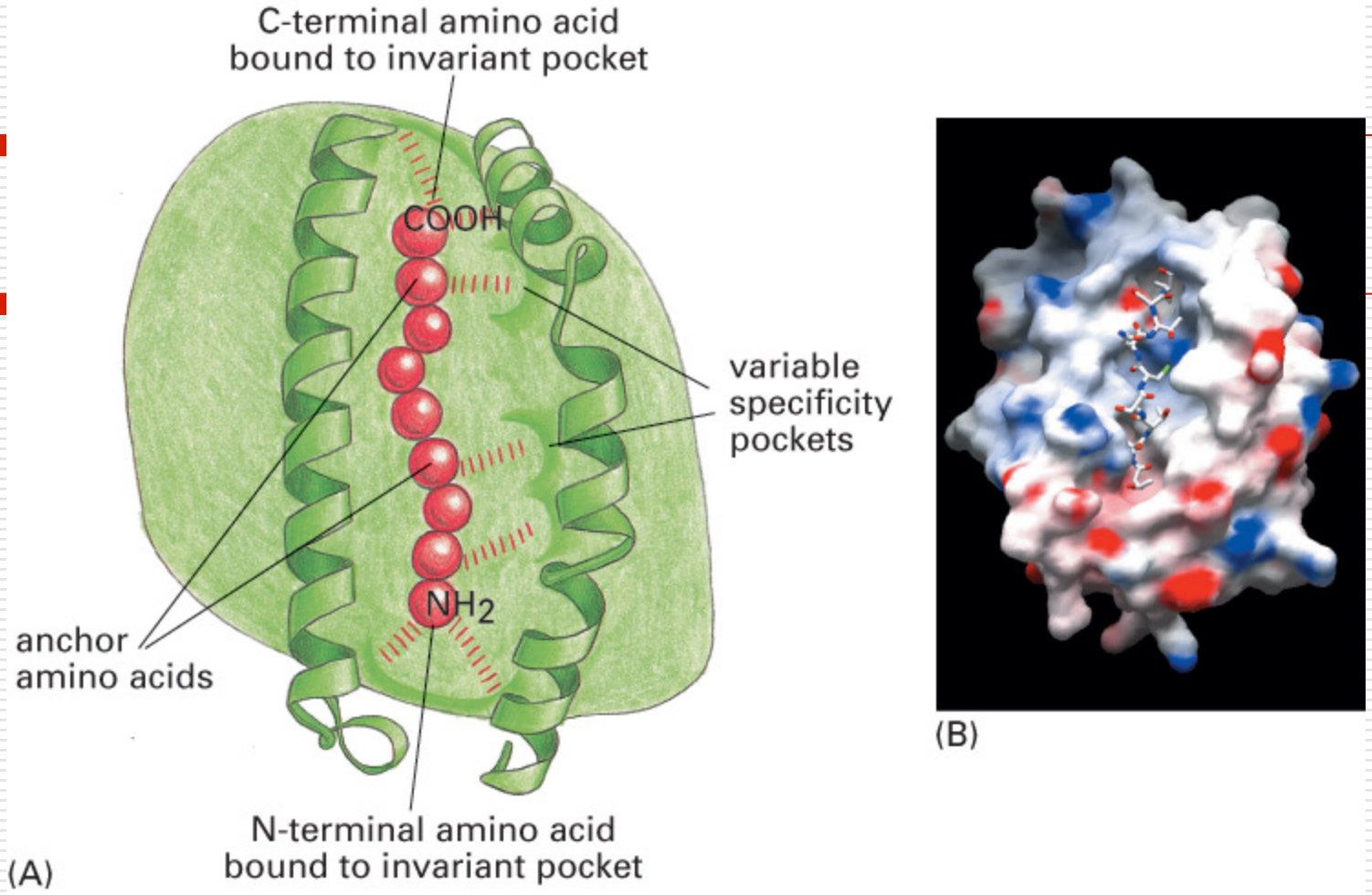


Figure 24–52. Molecular Biology of the Cell, 4th Edition.

Structure of HLA class II. molecules

1. α chain

α1: polymorphic sites

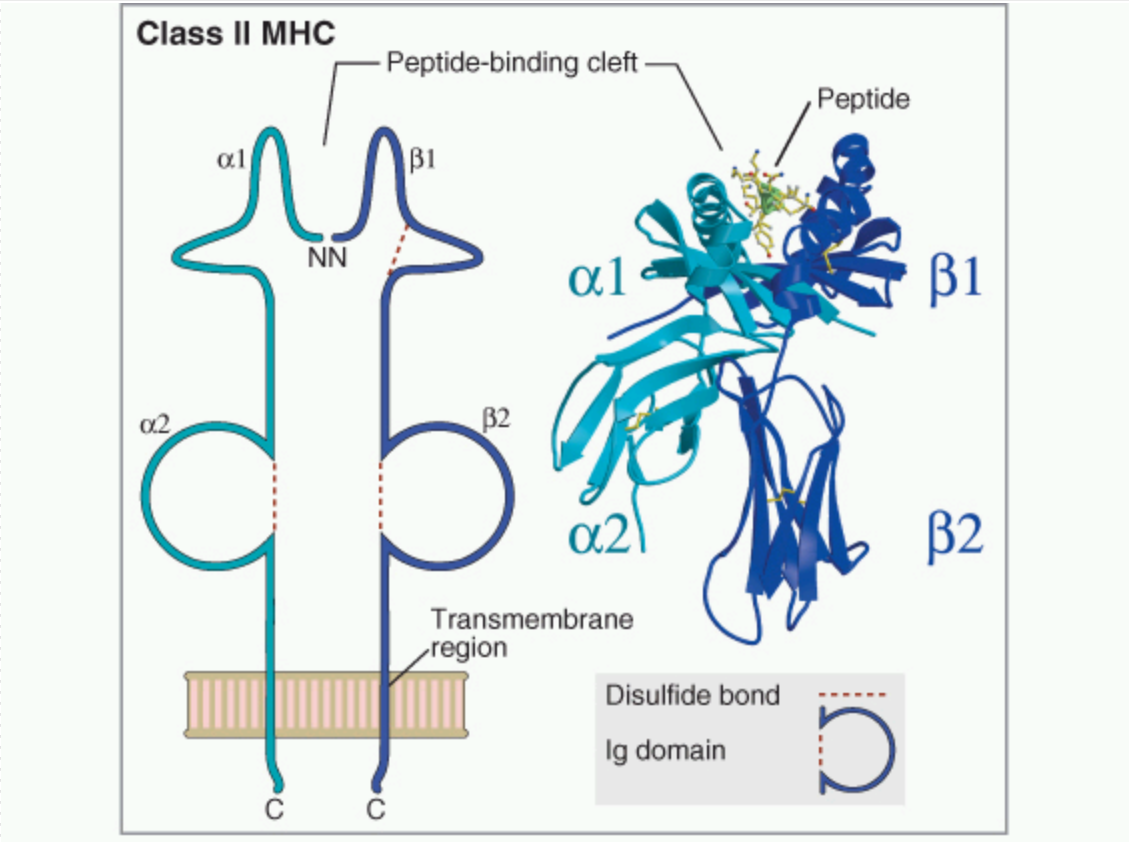
α2: binding of CD4

2. β chain

β1: polymorphic sites

β2: binding of CD4

3. peptide



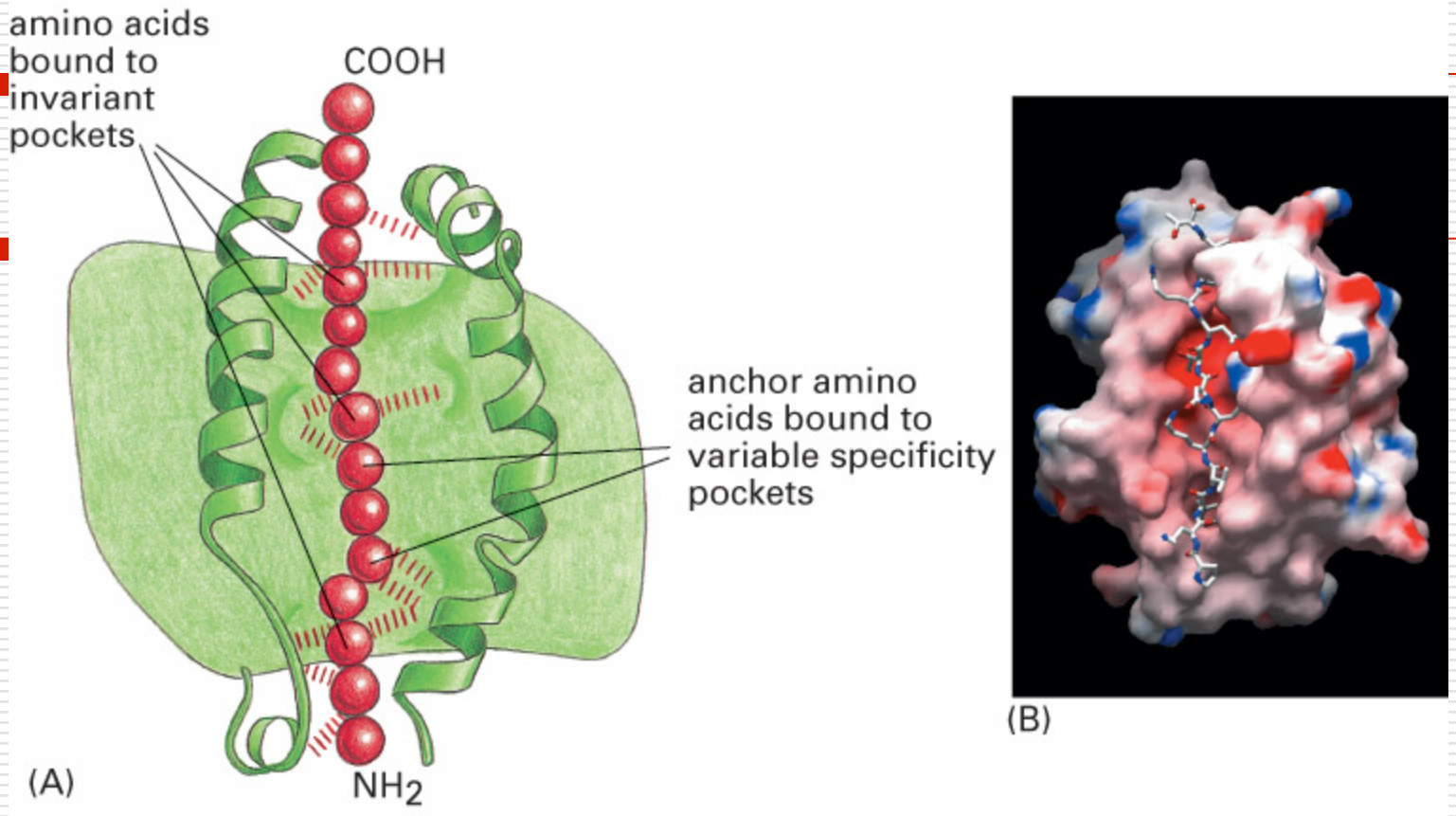


Figure 24–53. Molecular Biology of the Cell, 4th Edition.

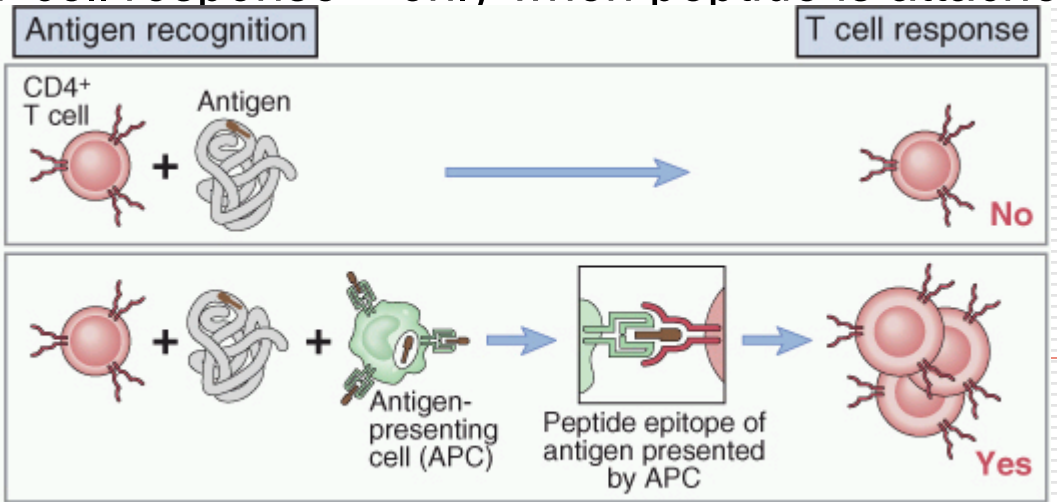
HLA and antigens

- Most T lymphocytes recognize only peptides
- T cells are specific for amino acid sequences of peptides - TCR
- Intracellular antigens are presented in connection with HLA class I. - CD8+ T cells recognition
- Extracellular antigens are presented in connection with HLA class II.

  – CD4+ T cells recognition

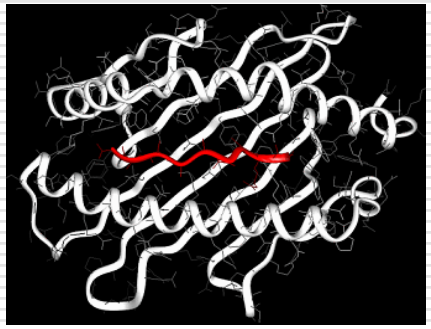
Experiment:

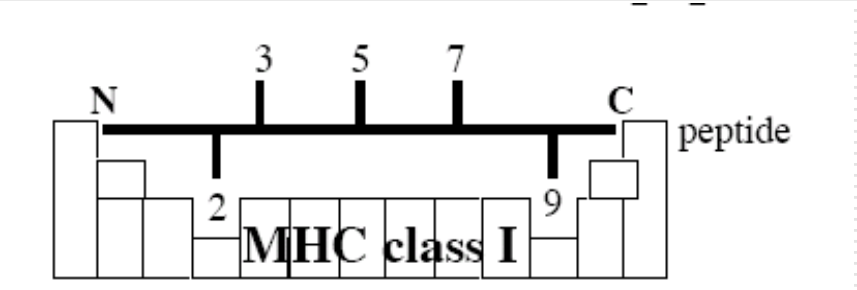
- T cell response – only when peptide is attached to APC



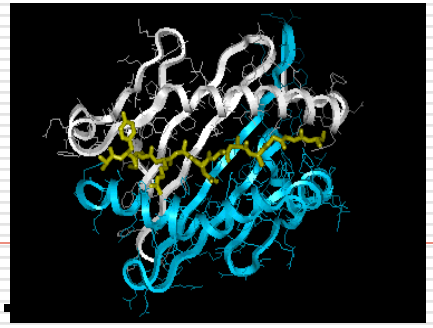
HLA and peptides

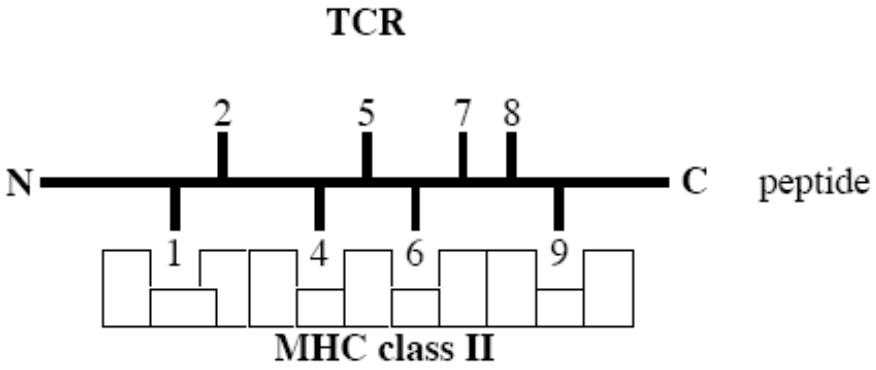
- antigenic peptides in the binding sites of HLA molecules
- One MHC - many peptides sharing structural features can bind
- Interaction has a very slow on- and off-rate (very stable)
- class I.

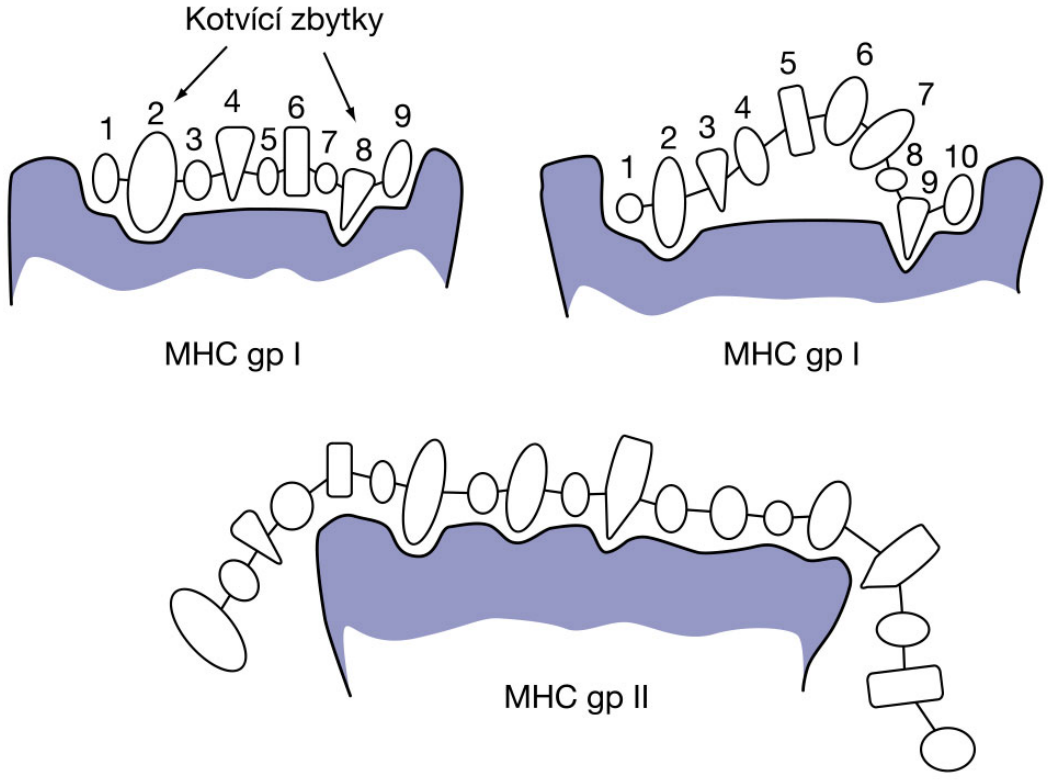




- class II.



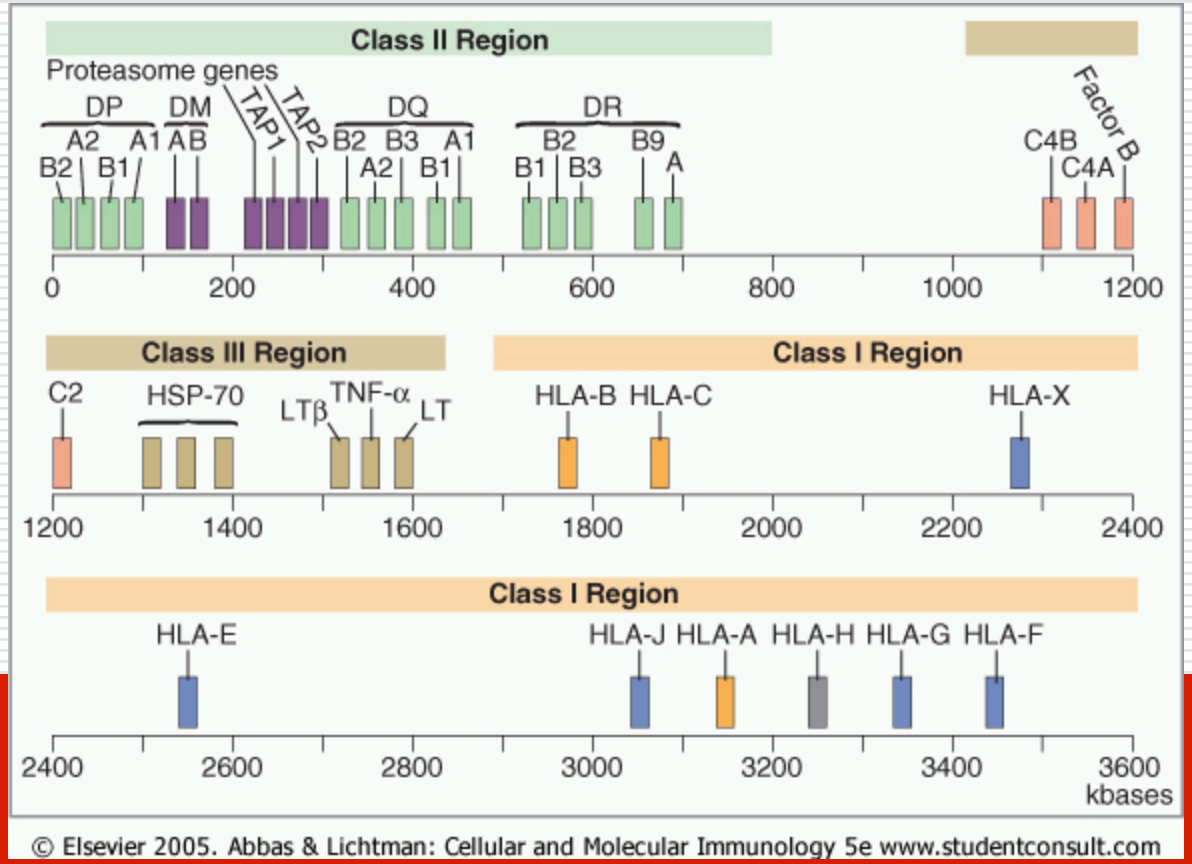



Kotvící zbytky
1
2
3
4
5
6
7
8
9
MHC gp I
1
2
3
4
5
6
7
8
9
10
MHC gp I
MHC gp II

HLA genes

MHC locus

- On chromosome 6
- HLA class III. are soluble molecules as complement, TNF, HSP
- Many other proteins involved in antigen presentation



HLA nomenclature

Nomenclature: The genetic "unit" of the HLA system is the allele, with each defined by its own DNA nucleotide sequence

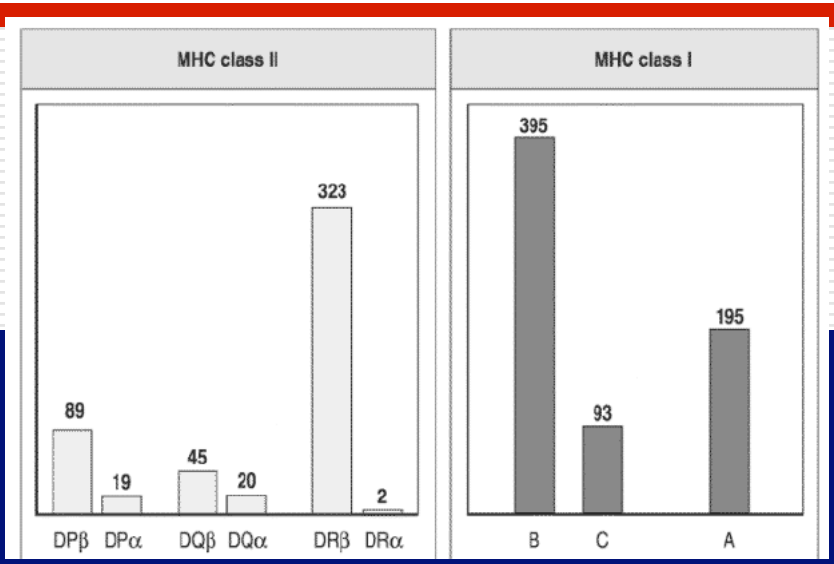
Allele	E.g. HLA-B*0801	}	"Specificity"
	*0802		HLA-B8
	…		
	*0821		
	*2701	}	
	*2702		
	*2703		HLA-B27
	…		
	*2725		

But to make things "simpler", alleles can be grouped in families, e.g. HLA-B*27

"specificity", is an old nomenclature used when human alloantibodies were used to first detect HLA serologic "specificities" or "antigens"

HLA polymorphism

- most polymorhic structures from all known systems.



- human study: 1000 donors, HLA-A, B genotyping
  - Over half the group had a combination that was unique.
  - Another 111 donors had a set of these molecules that they shared with only one other person in the group.
  - The most frequent phenotype (HLA-A1, HLA-A3, HLA-B7, and HLA-B8) was found in 11 donors.

EXTRAORDINARY POLYMORPHISM OF MHC PROTEINS:

HUNDREDS OF ALLELIC FORMS

IMPORTANT FOR BETTER PROTECTION OF BOTH AN INDIVIDUAL AND POPULATION

(COMPLICATION – TRANSPLANTATIONS)

HLA polymorphism – why?

Pathogen driven mechanisms

- Pathogens tend to escape
- Heterozygotes have advantage
- Frequency-dependent selection: the individual with the rarest allele has the best chance to survive an infection

cheetahs (low polymorphism): extremly susceptible to infectious diseases

vertebrate species can detect MHC genotype by smell!

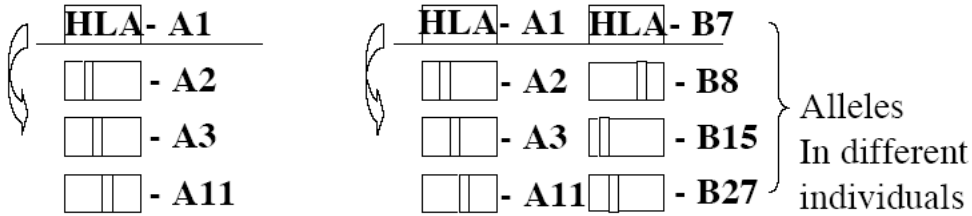
Diversity of MHC class I and II genes

Diversity of MHC class I and II genes

Arises from two mechanisms:

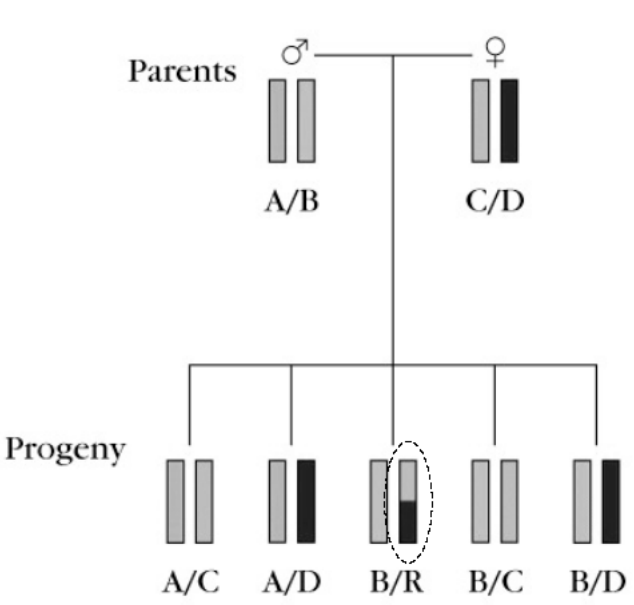
Duplication of a gene locus in an individual resulting in multiple loci, *polygeny*

Development of multiple alleles at a locus among individuals in the species, *polyallelism*



HLA haplotypes in a typical family

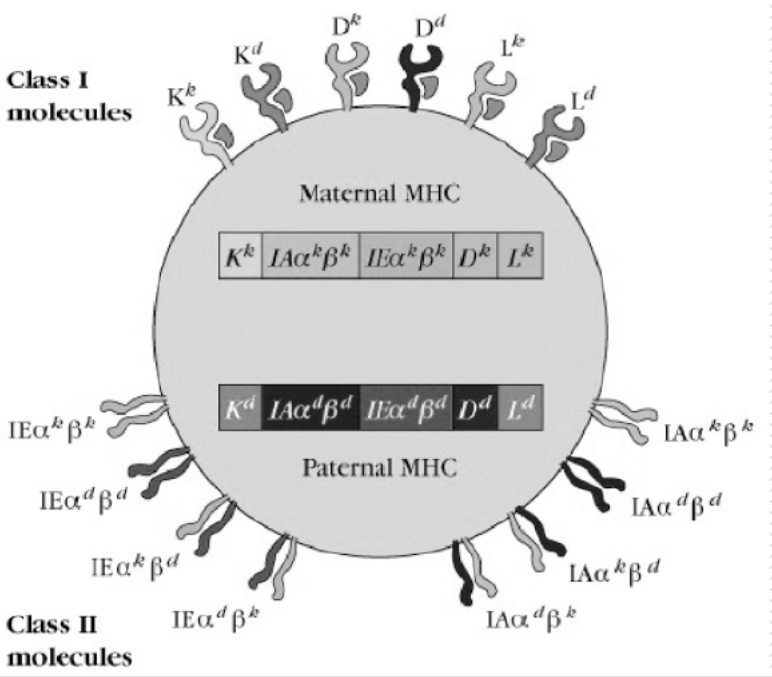
- Haplotype is combination of allelic forms of HLA molecules on one chromosome.
- We inherit 3 types of heavy chains for HLA class I. molecules from each parent .
- Everybody expresses 6 different types of HLA class I. molecules unless honmozygous status for some of the types was inherited.



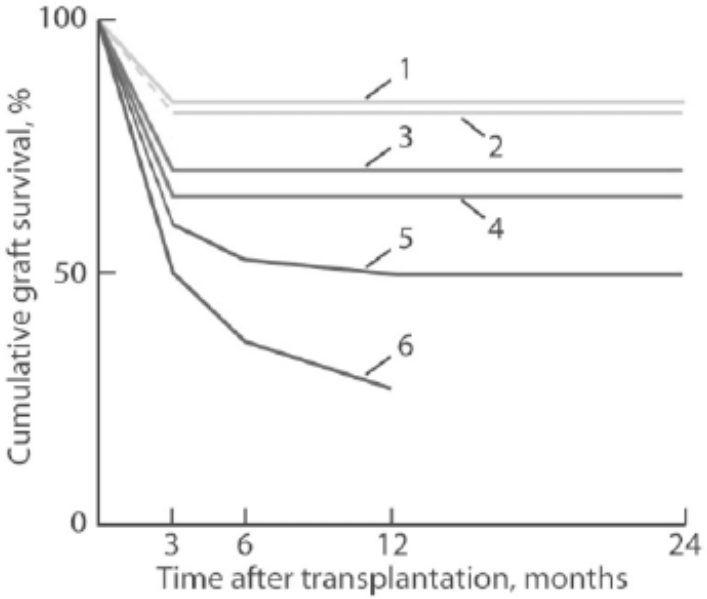
Meiotic recombination scrambles HLA haplotypes

Haplotypes	HLA Alleles					
	A	B	C	DR	DQ	DP
	1	7	w3	2	1	1
B	2	8	w2	3	2	2
C	3	44	w4	4	1	3
D	11	35	w1	7	3	4
R	3	44	w4	7	3	4

Co-dominant expression of MHC alleles



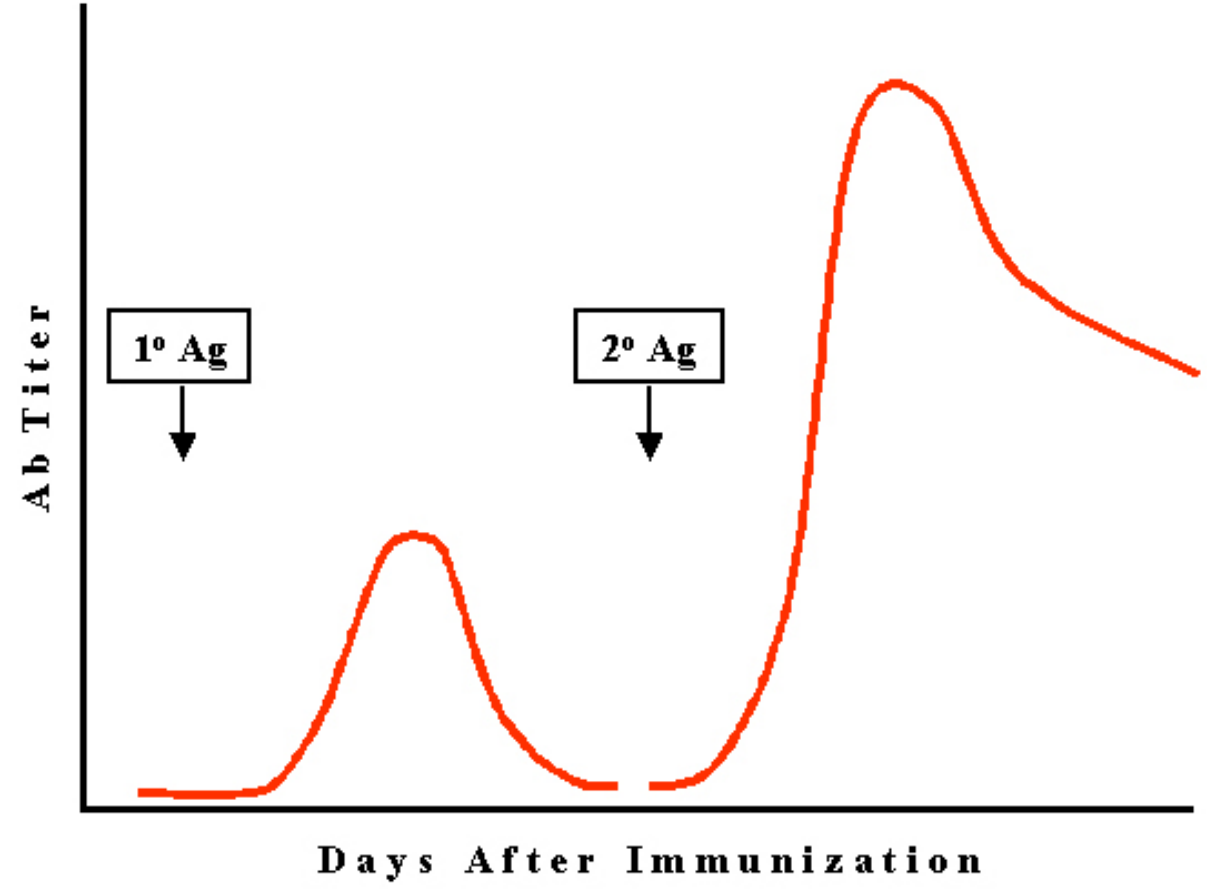
MHC control transplantat survival



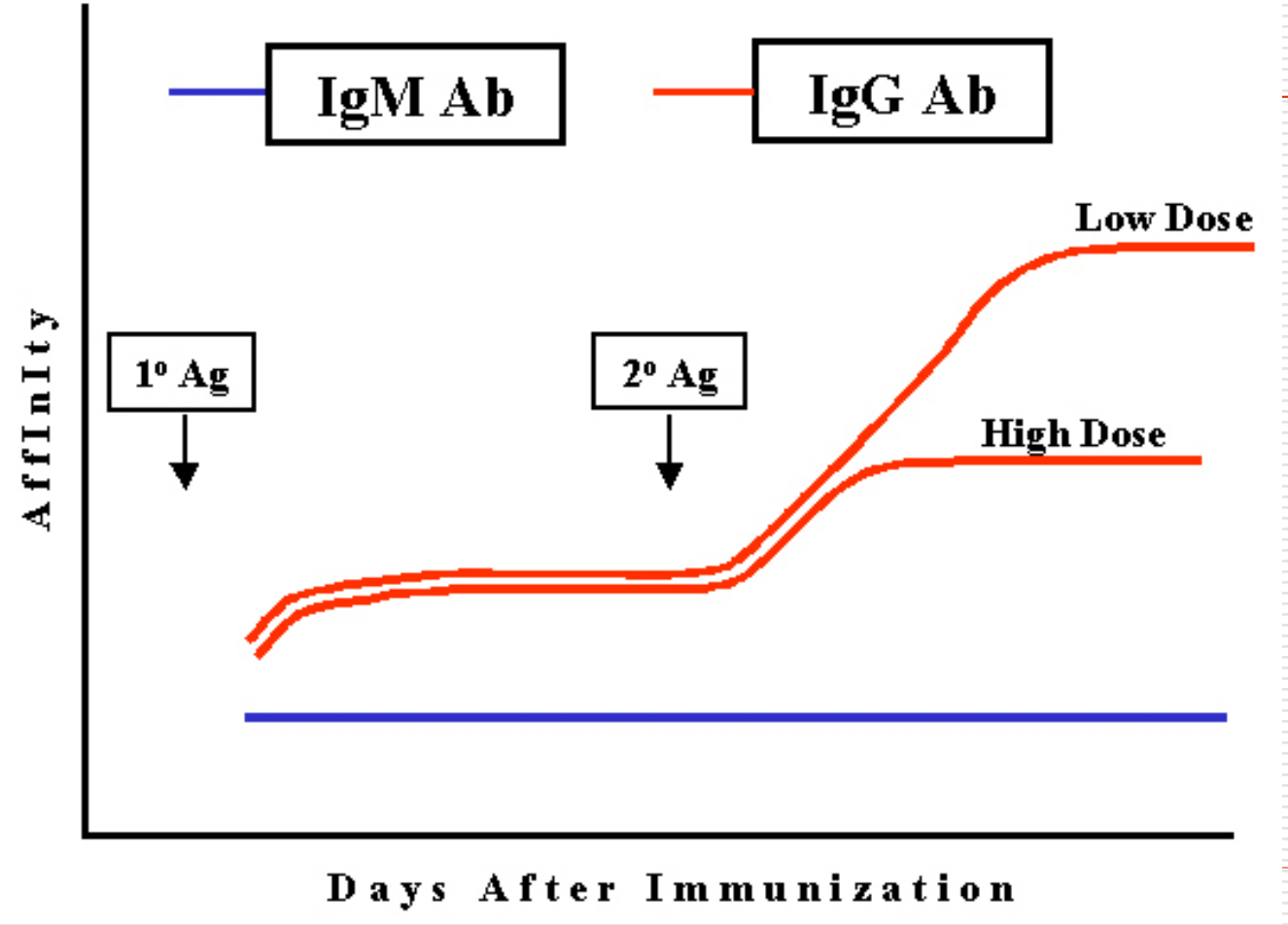
Curve no.	HLA mismatches (no.) Class I	Class II
1	0	0
2	1 or 2	0
3	3 or 4	0
4	0	1 or 2
5	1 or 2	1 or 2
6	3 or 4	1 or 2

- A graft is compatible only if there is a complete match at all MHC alleles, i.e. a two haplotype match for all MHC loci

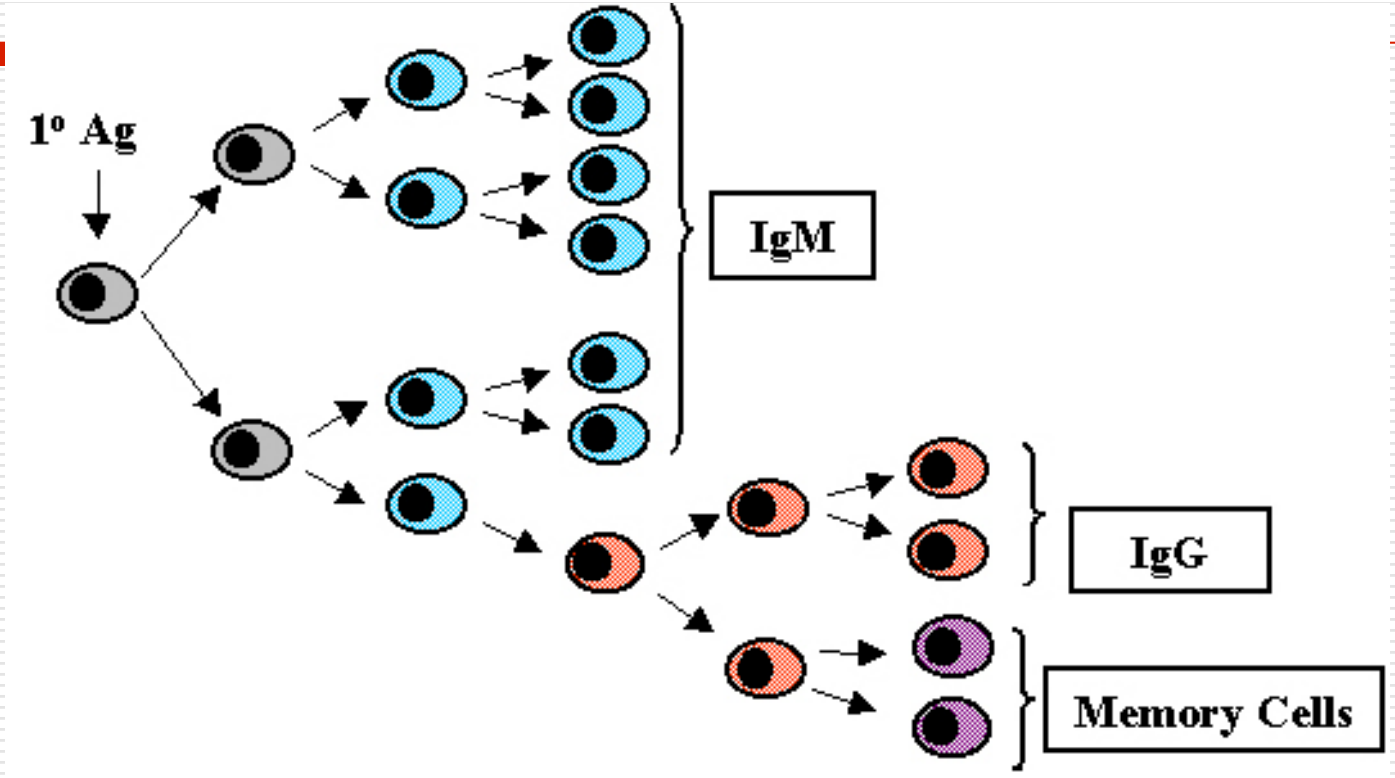
Sistema imune adaptativo:
Resposta primária e secundária



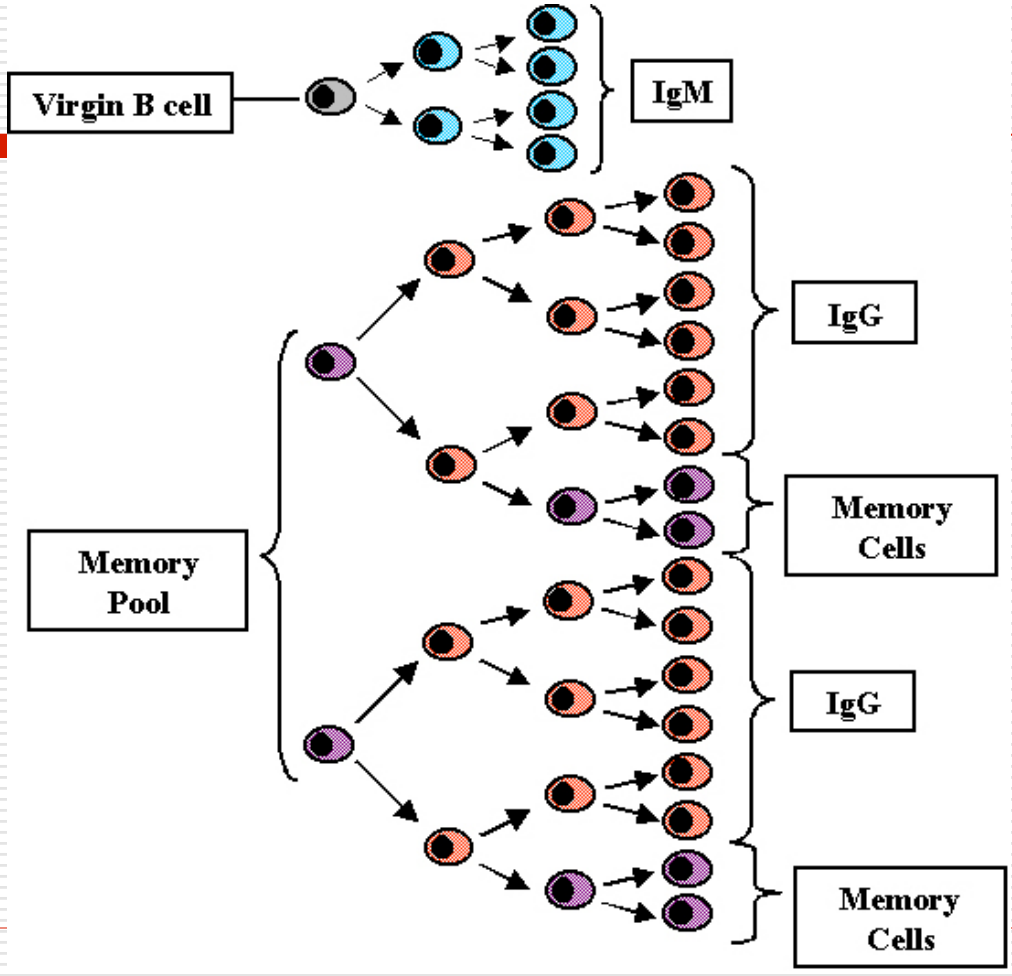
Troca da expressão de classes dos anticorpos
Resposta primária → IgM
Resposta secundária → IgG



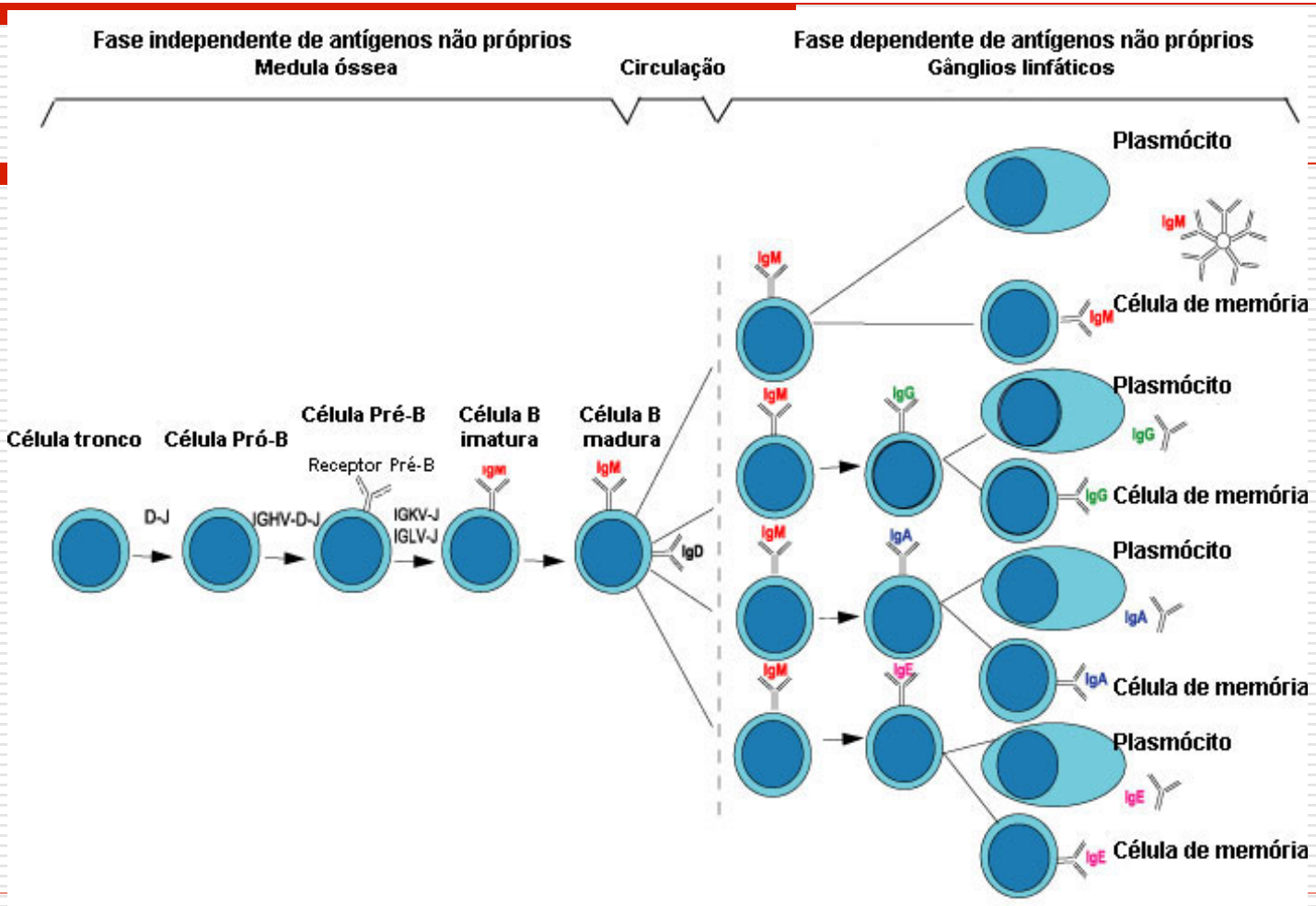
Expansão Clonal



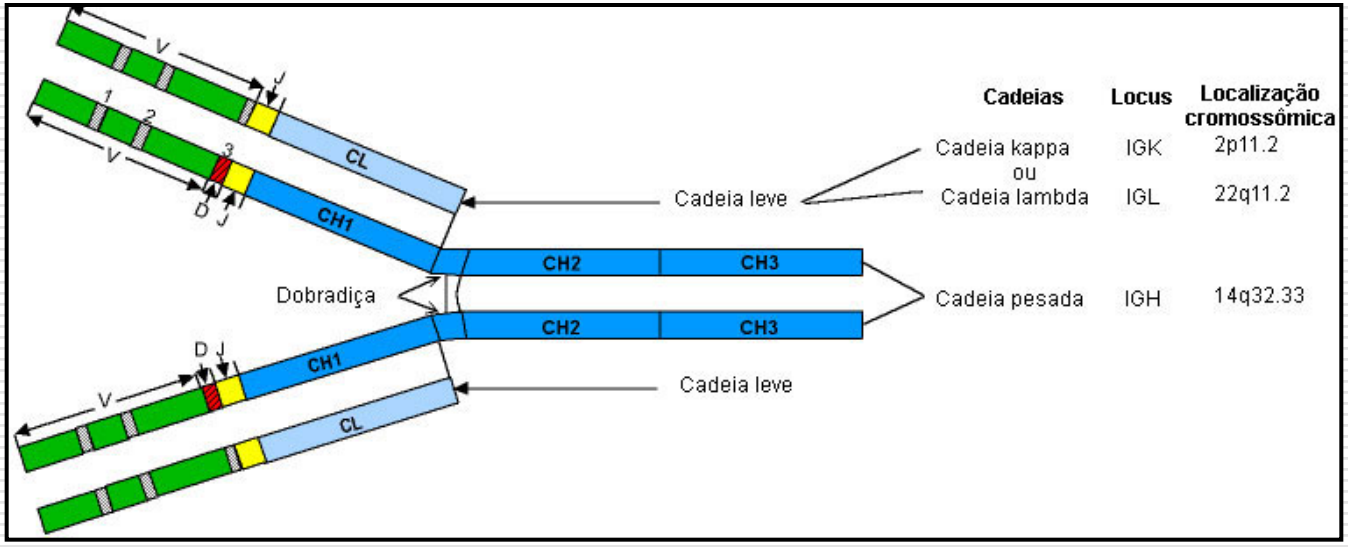
Expansão clonal e células de memória



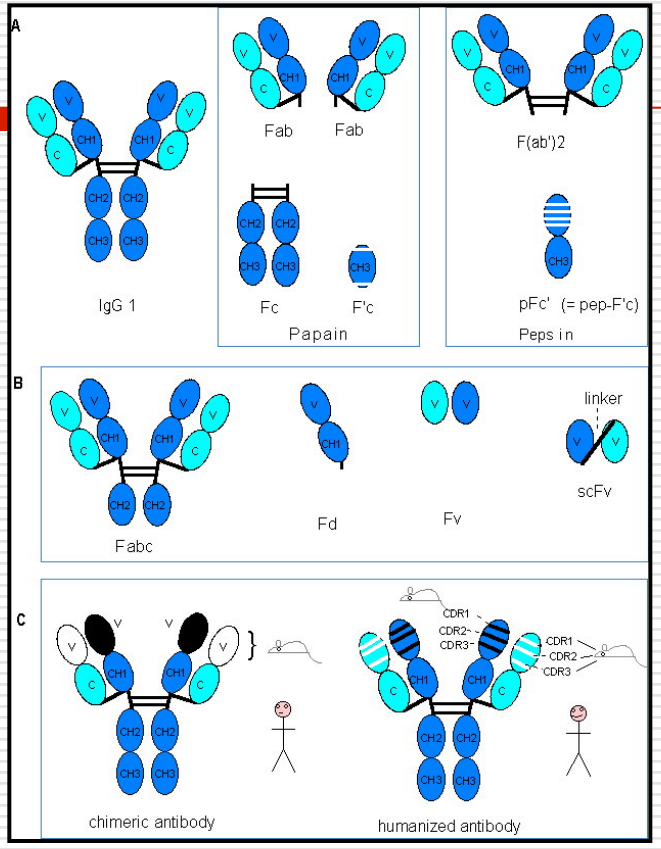
Maturação dos linfócitos B e secreção dos anticorpos



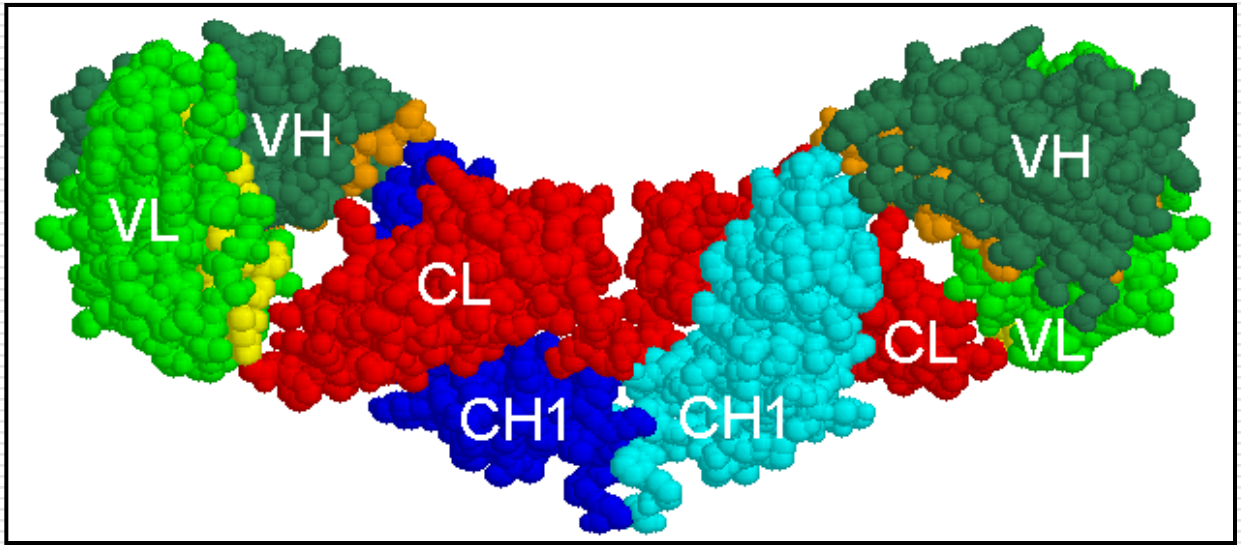
Esquema da estrutura de uma molécula de imunoglobulina (Ig)



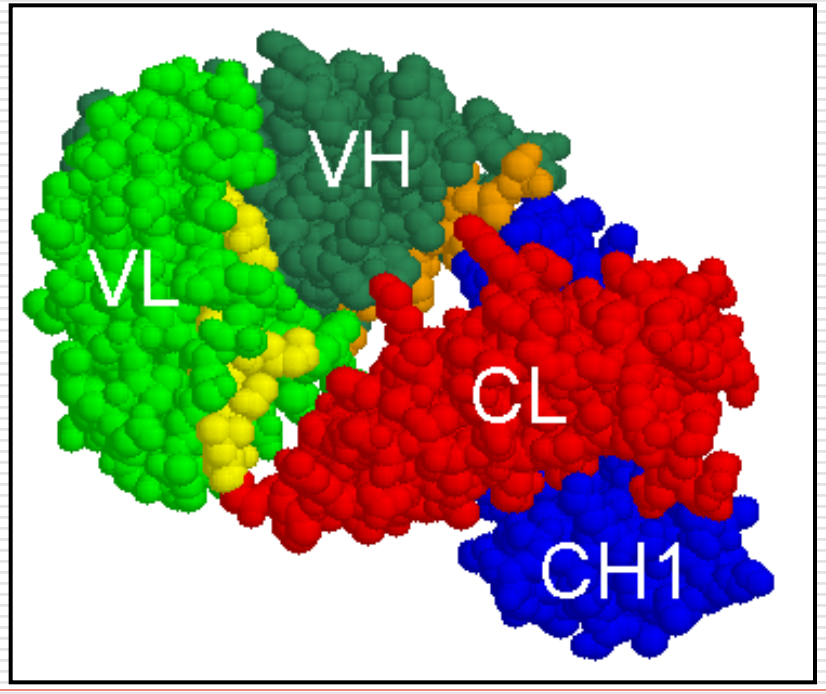
Imunoglobulinas: organização em domínios



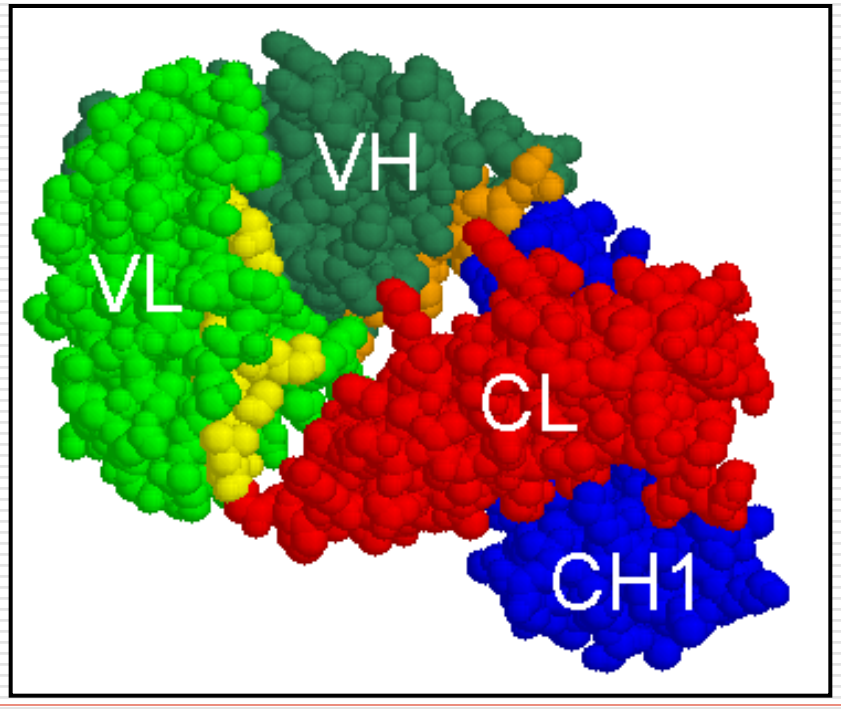
Resultado da digestão de uma Ig com pepsina



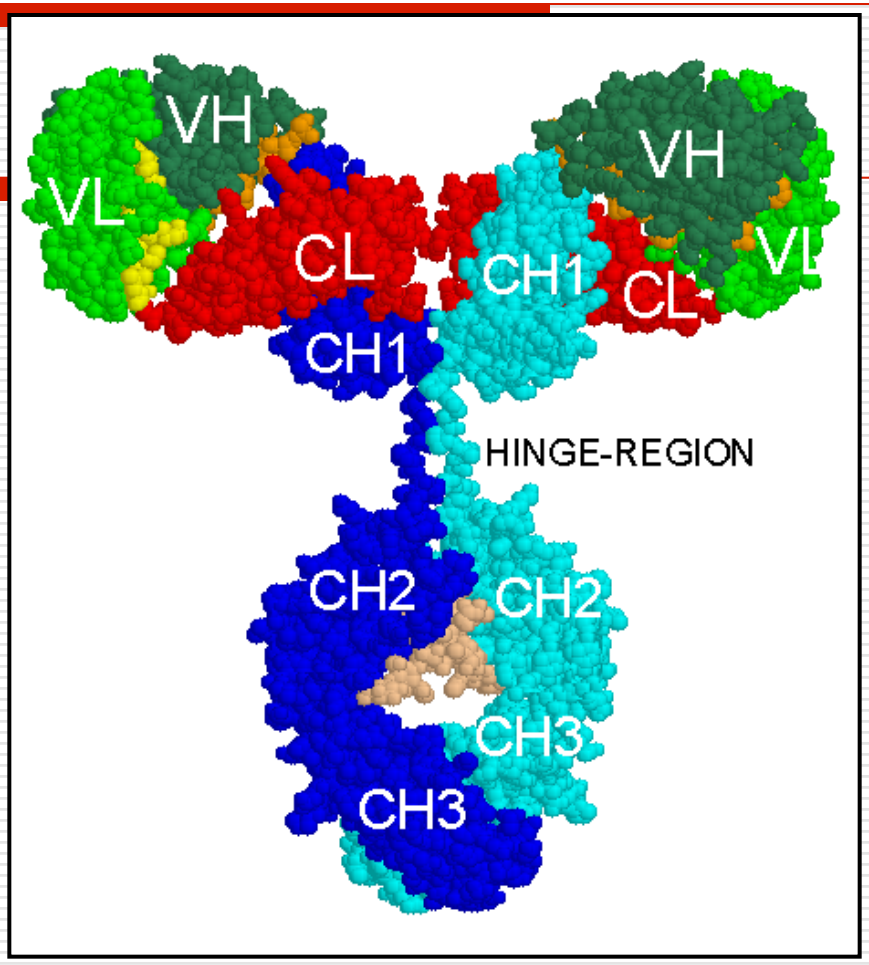
Resultado da digestão de uma Ig com papaína



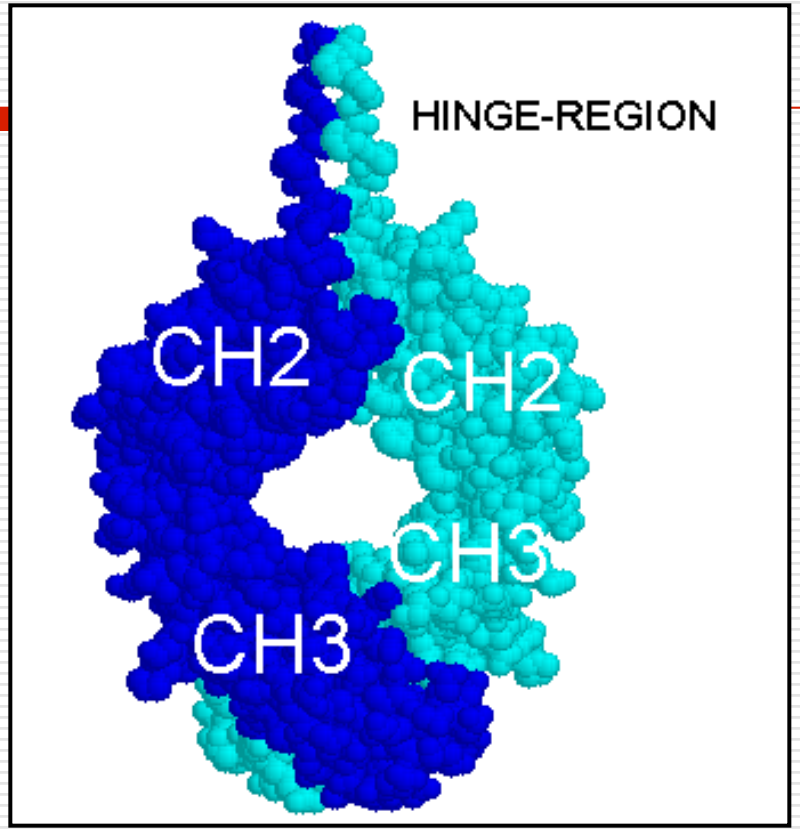
Fragmento Fab’



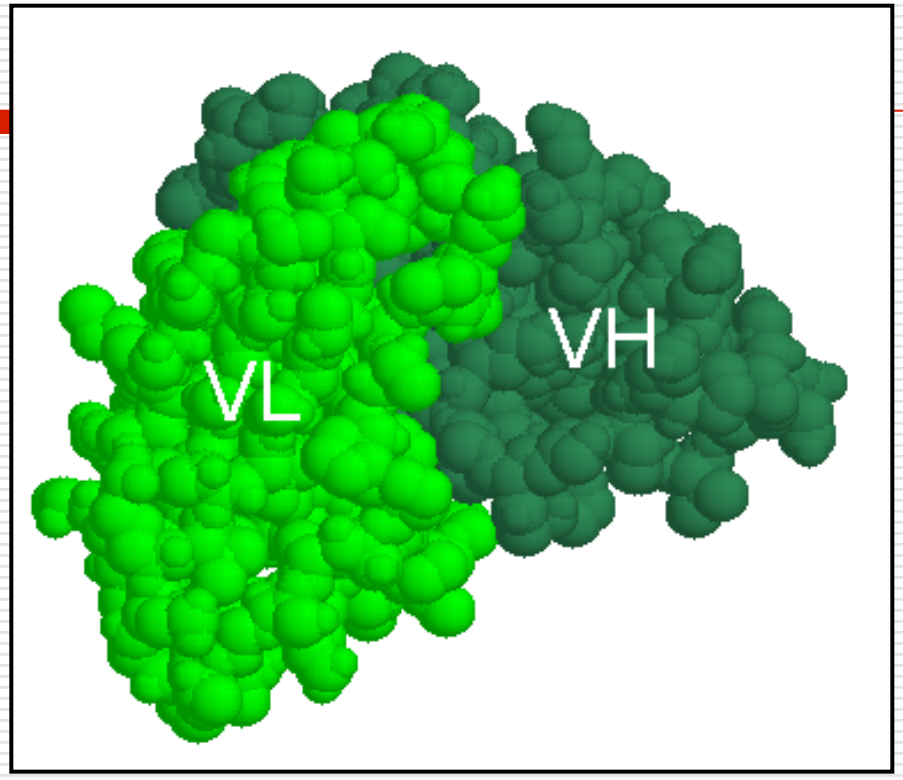
Estrutura tridimensional de uma molécula de Ig



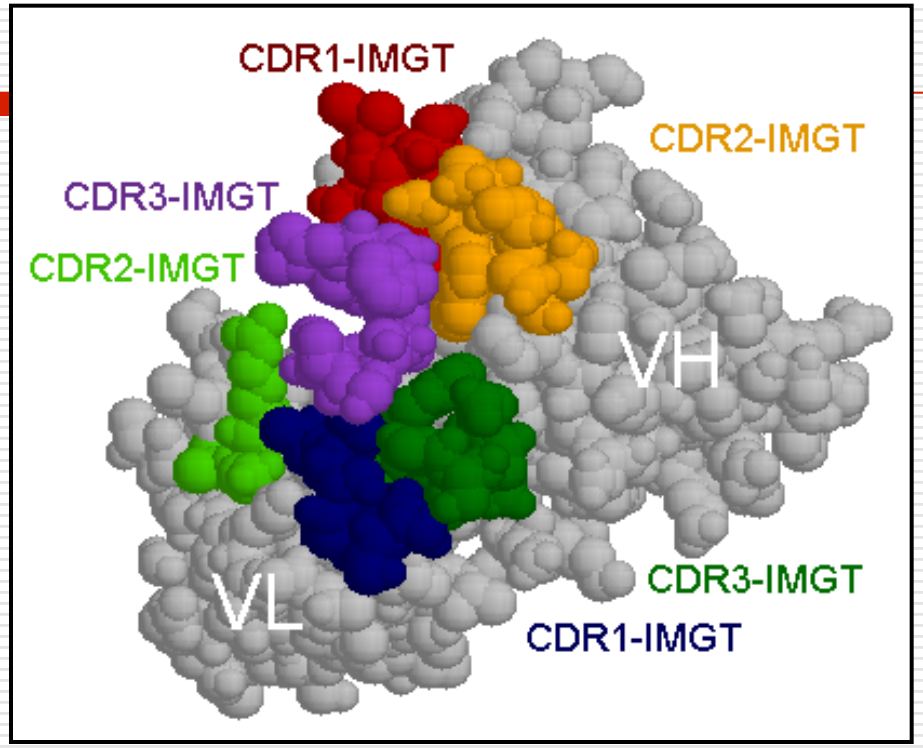
Fragmento Fc de uma imunoglobulina



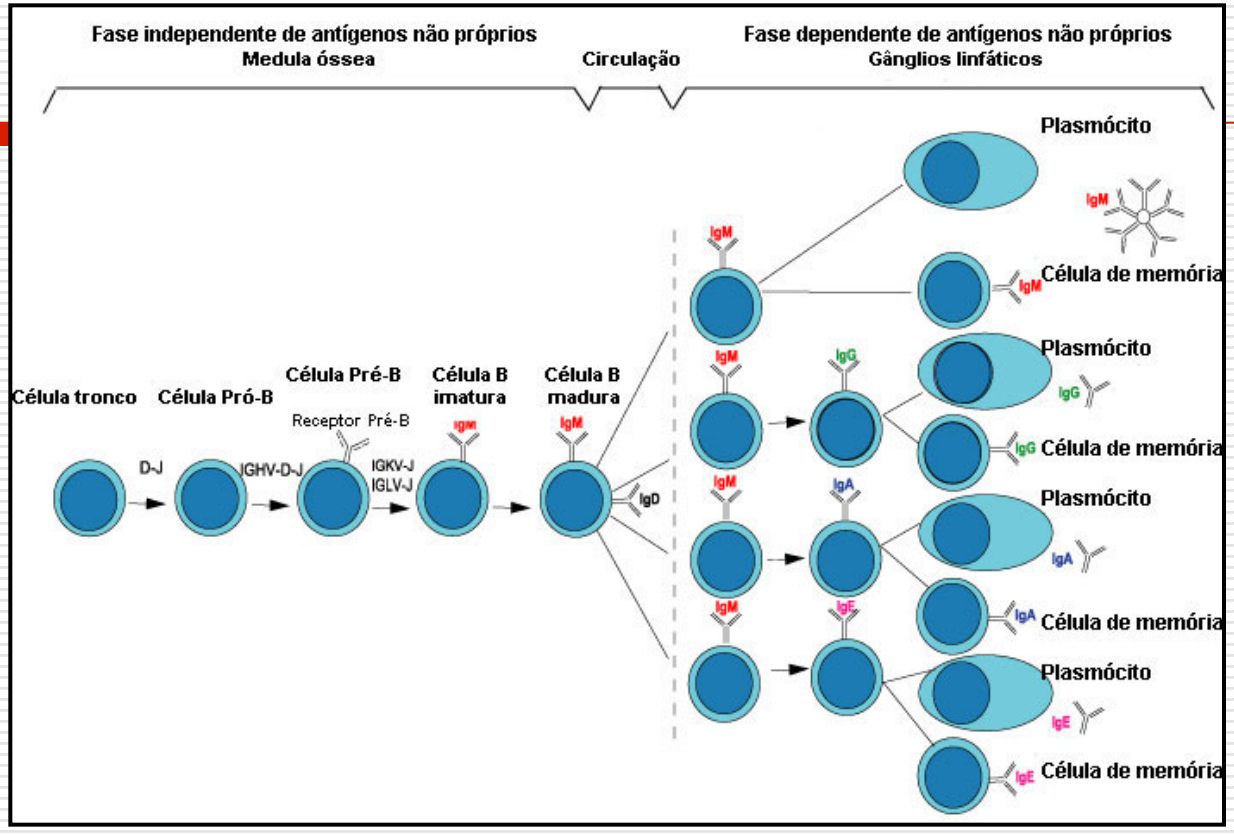
Fragmento Fv de uma imunoglobulina



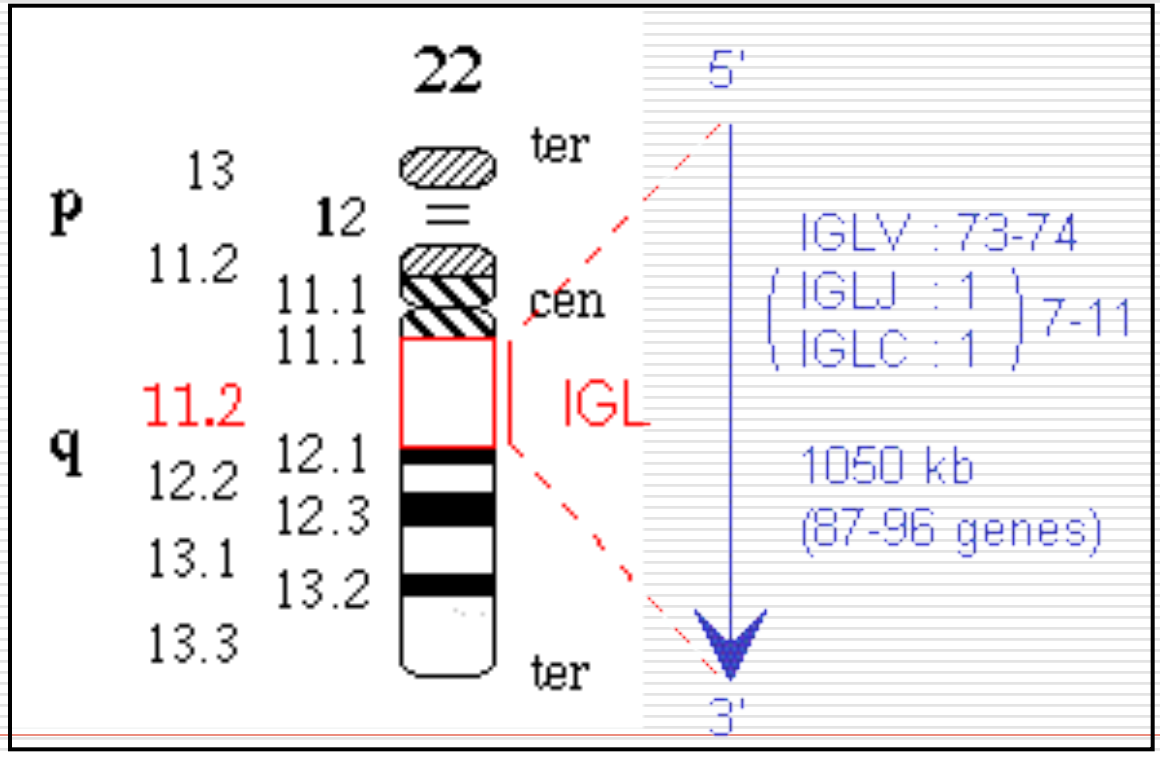
Fragmento Fv com CDRs



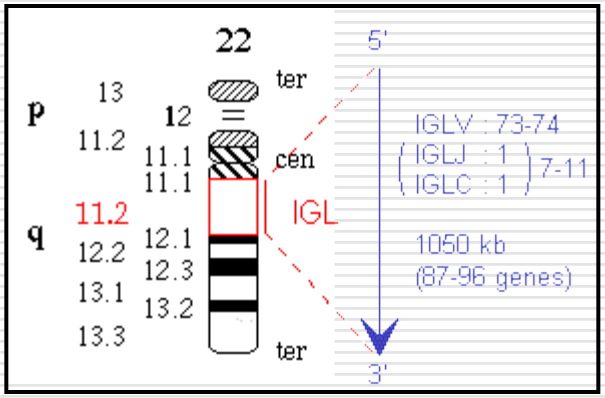
Diferenciação dos linfócitos B e a síntese dos anticorpos

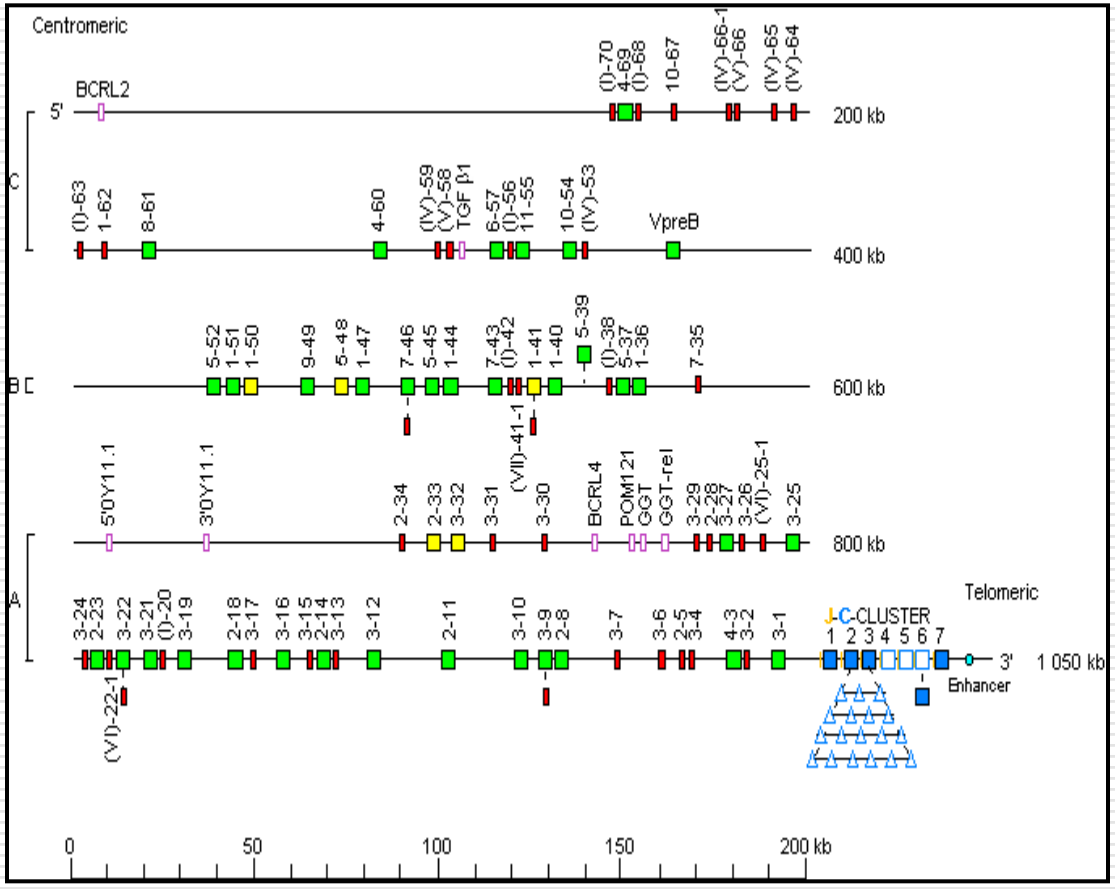


Localização cromossômica da cadeia leve lambda em 22q11.2

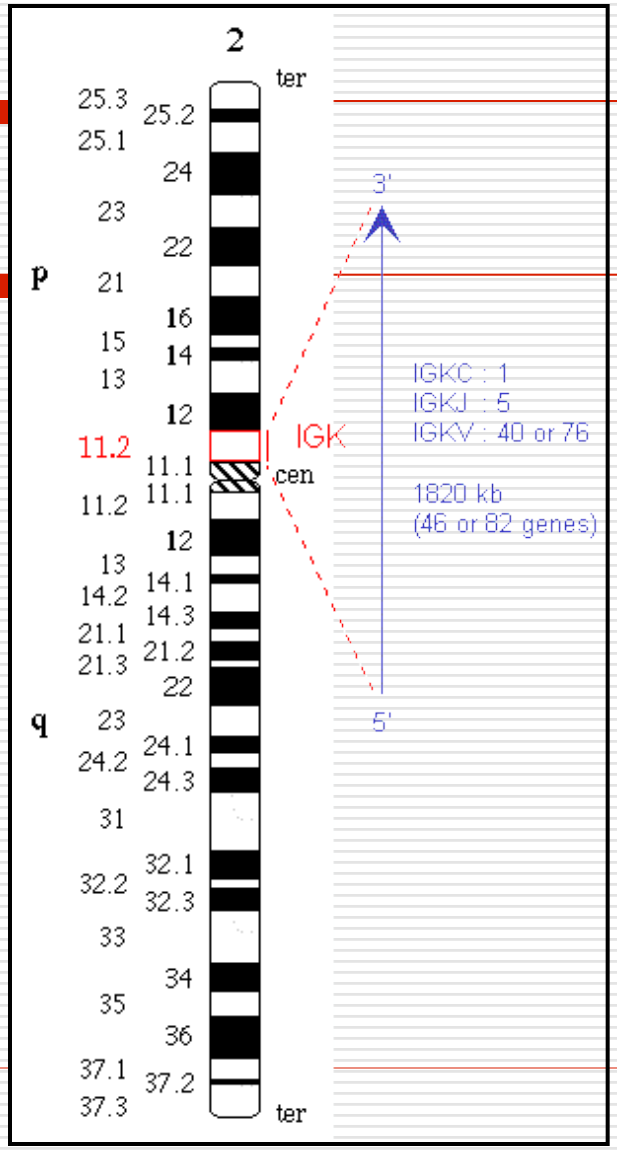


Locus Lambda Humano (22q11.2)

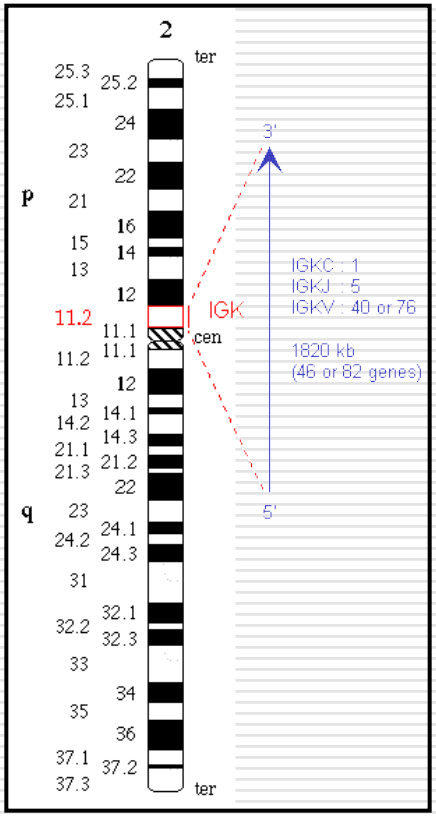


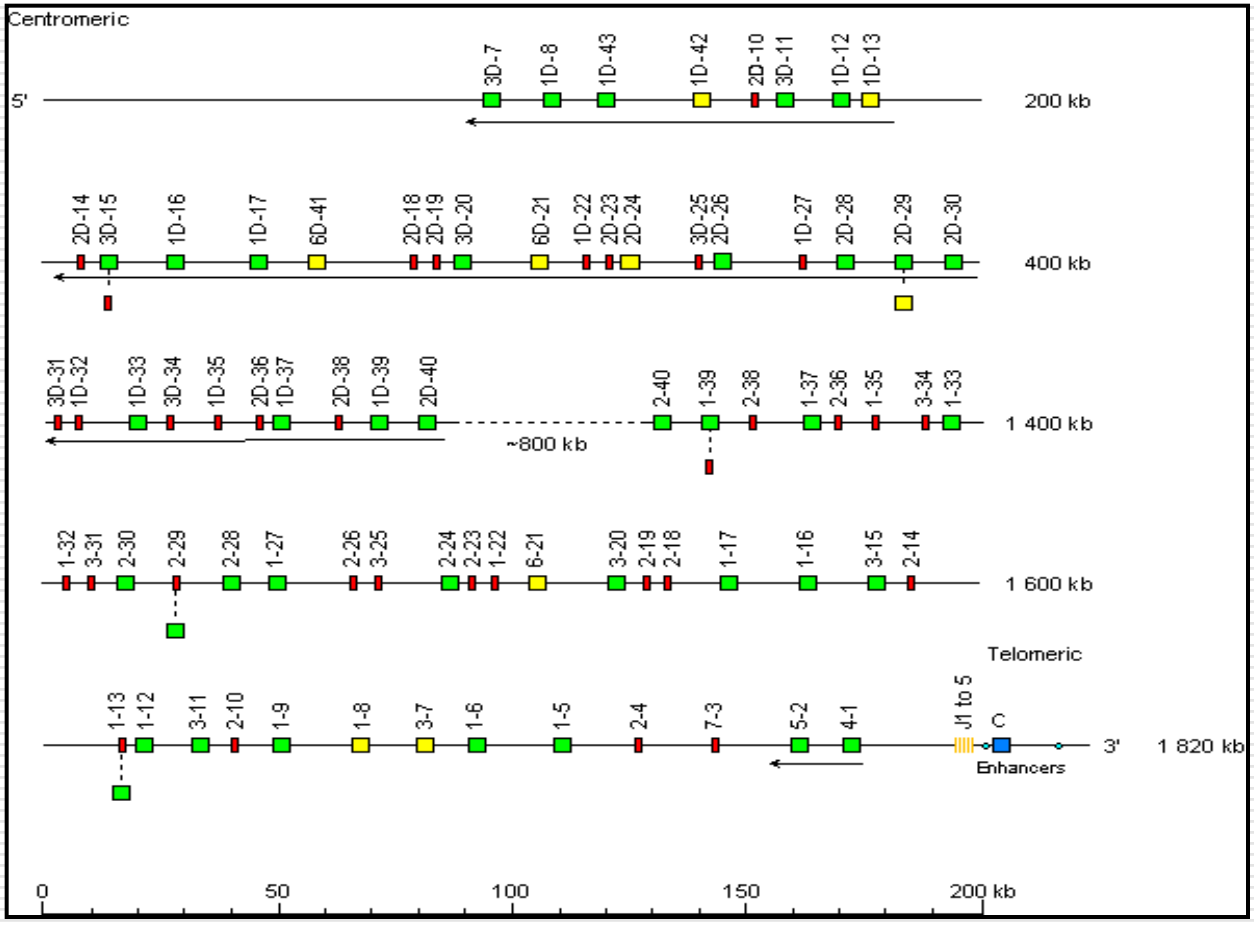


Localização cromossômica da cadeia leve kappa em 2p11.2

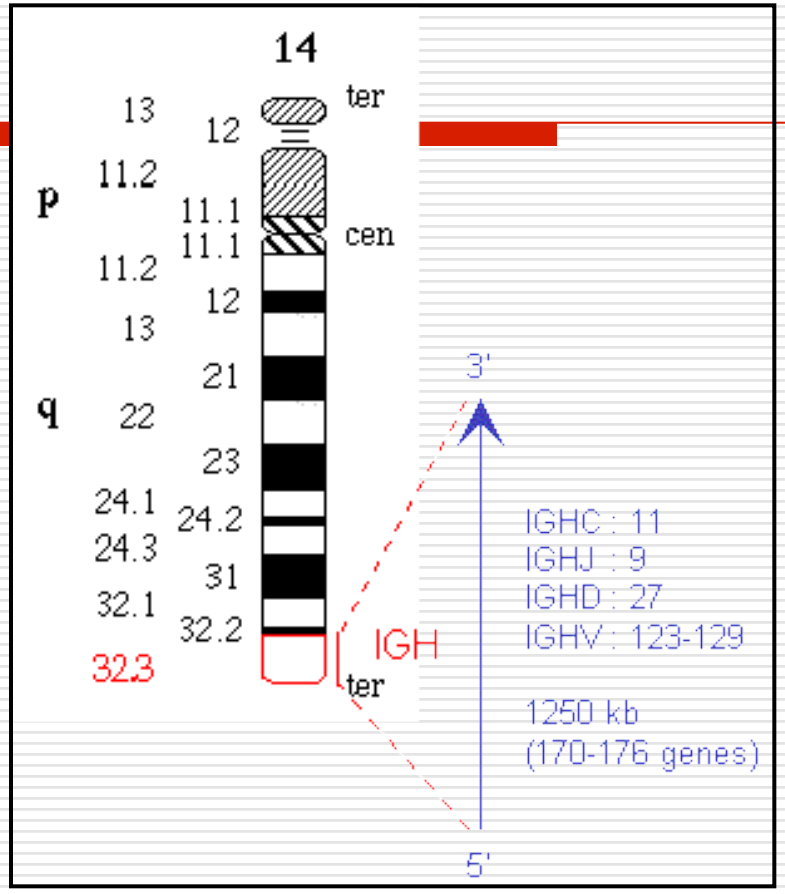


Locus kappa humano (2p11.2)

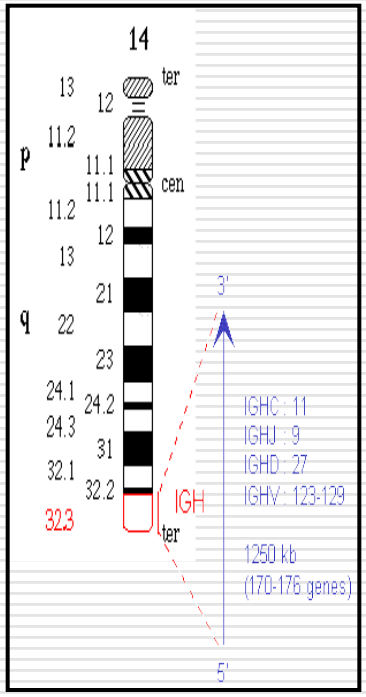


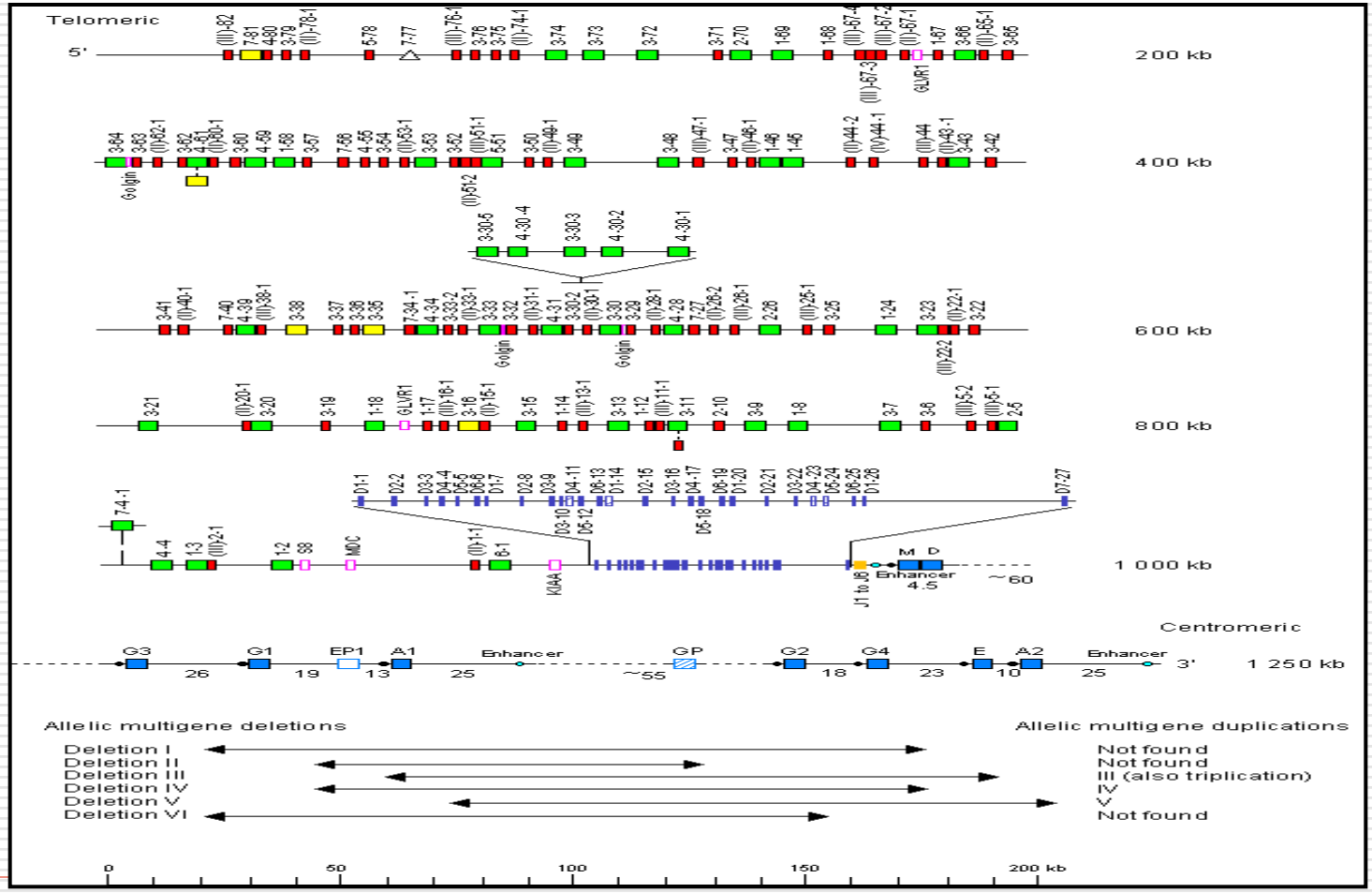


Localização cromossômica da cadeia pesada (IGH) em 14q32



Locus humano da cadeia pesada (14q32.33)



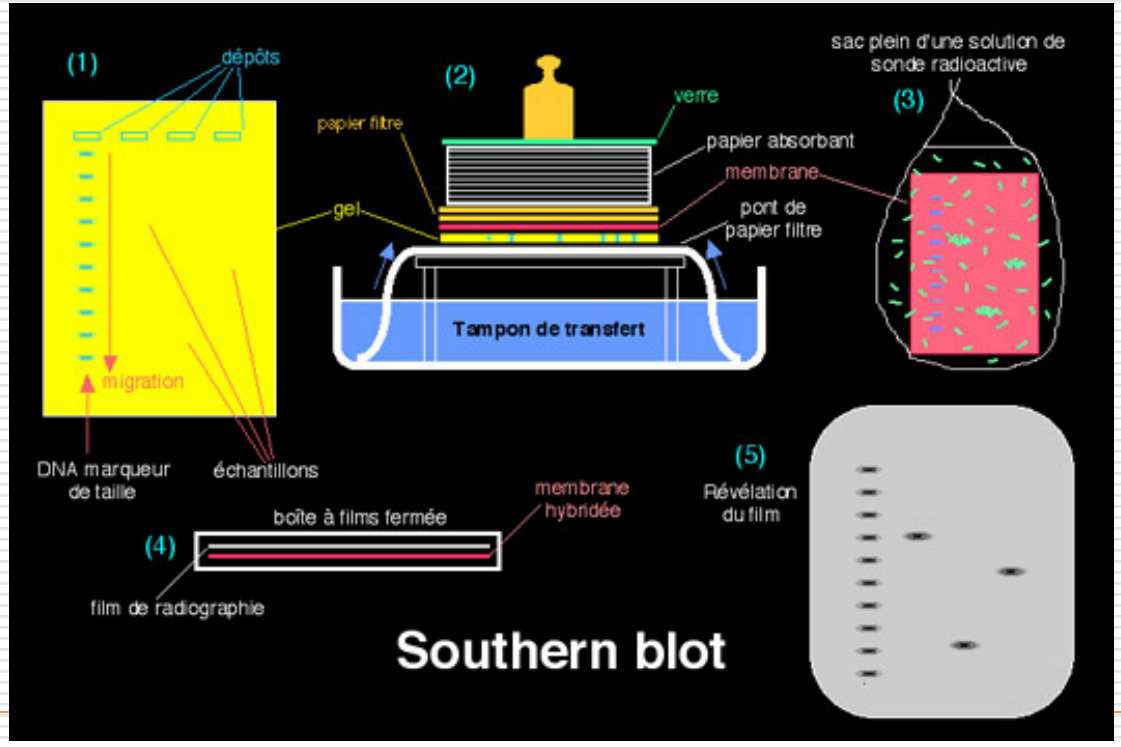


Número de segmentos gênicos de Igs

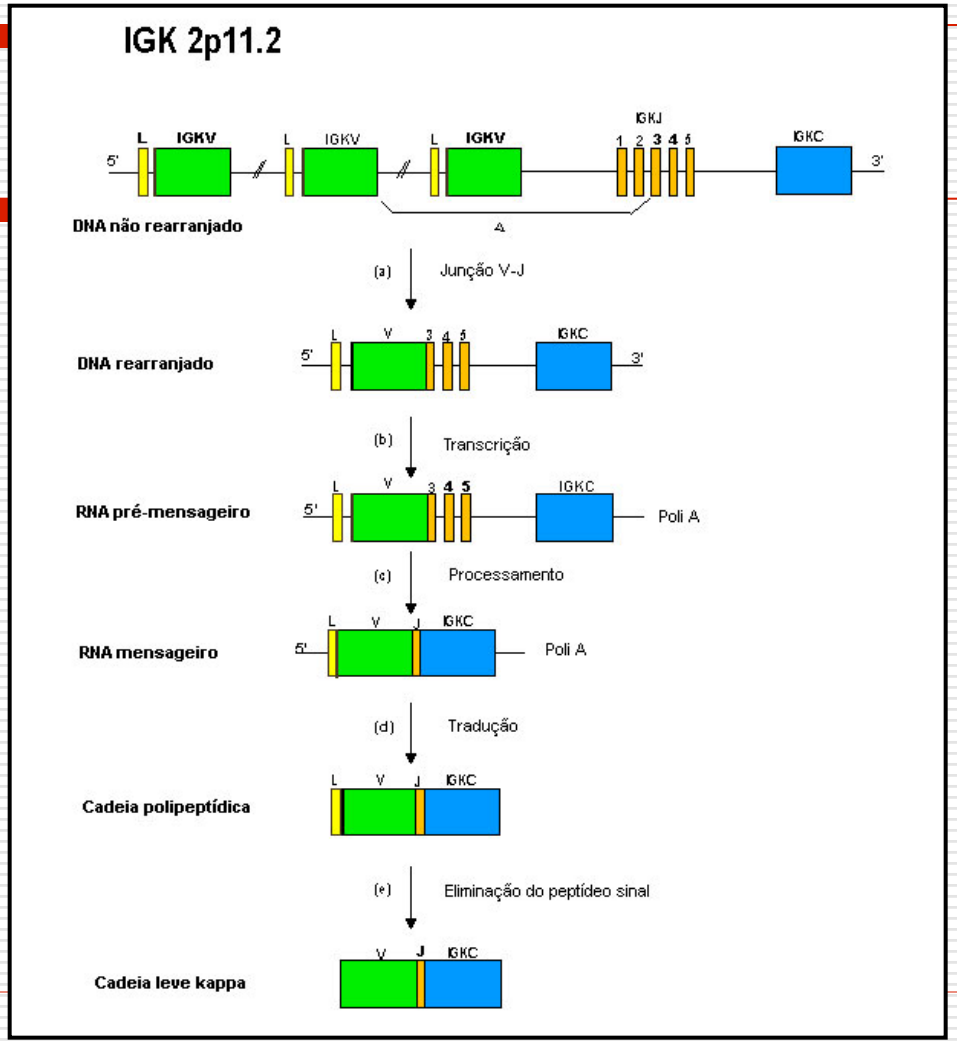
Figure 2.15

	Number of gene segments		
Segment	Light chains		Heavy chain
	κ	λ	H
Variable (V)	40	30	65
Diversity (D)	0	0	27
Joining (J)	5	4	6

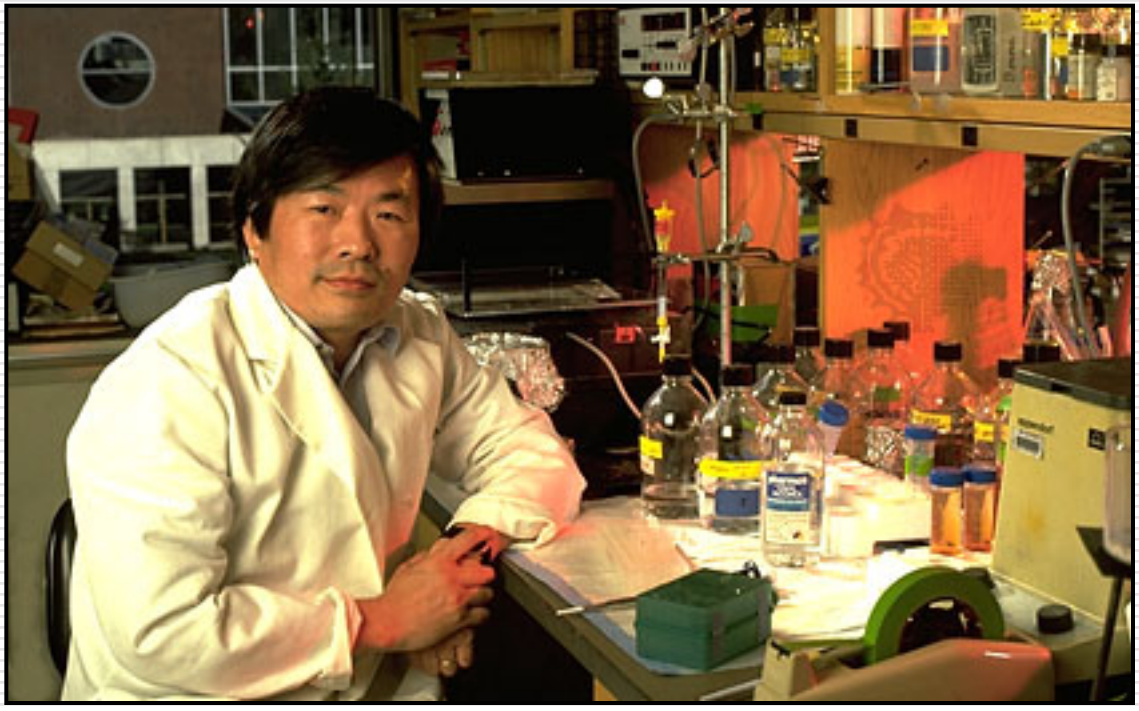
A técnica de hibridação molecular "Southern-blot" usada para evidenciar a recombinação V(D)J



Rearranjo V(D)J do DNA, transcrição e tradução da cadeia leve tipo kappa



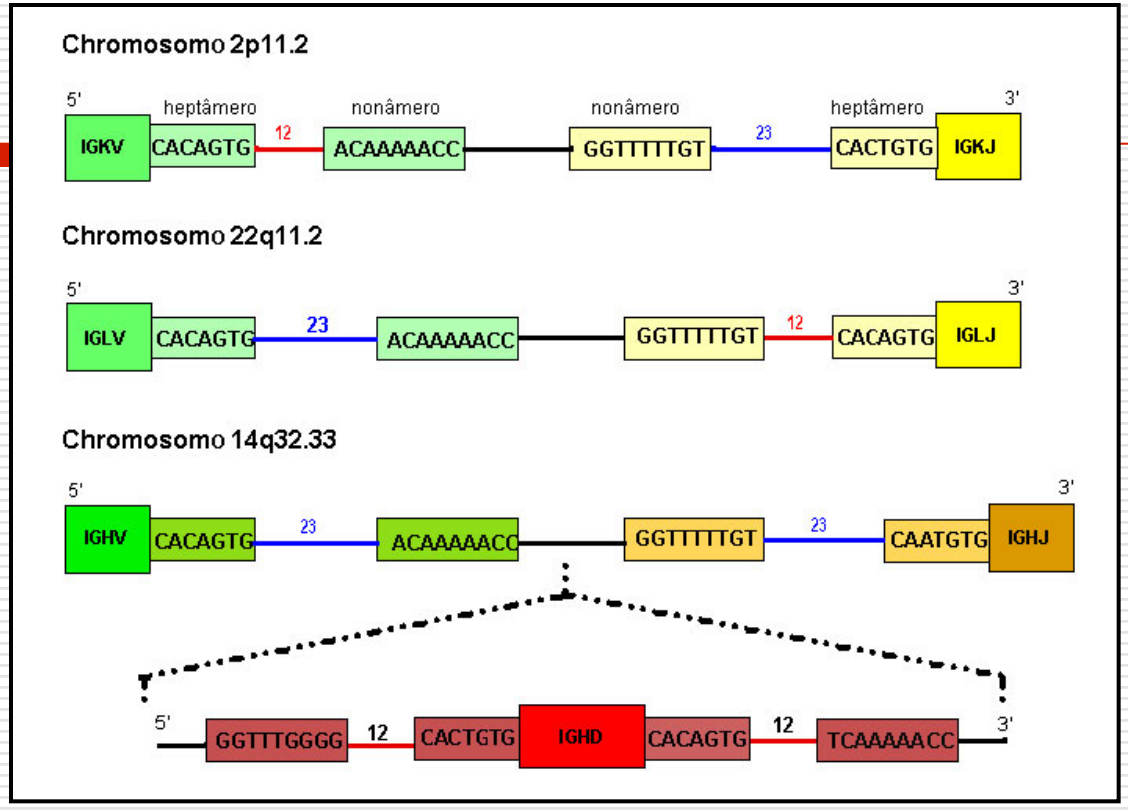
Susumu Tonegawa
Demonstrou a recombinação V(D)J nos genes de Igs



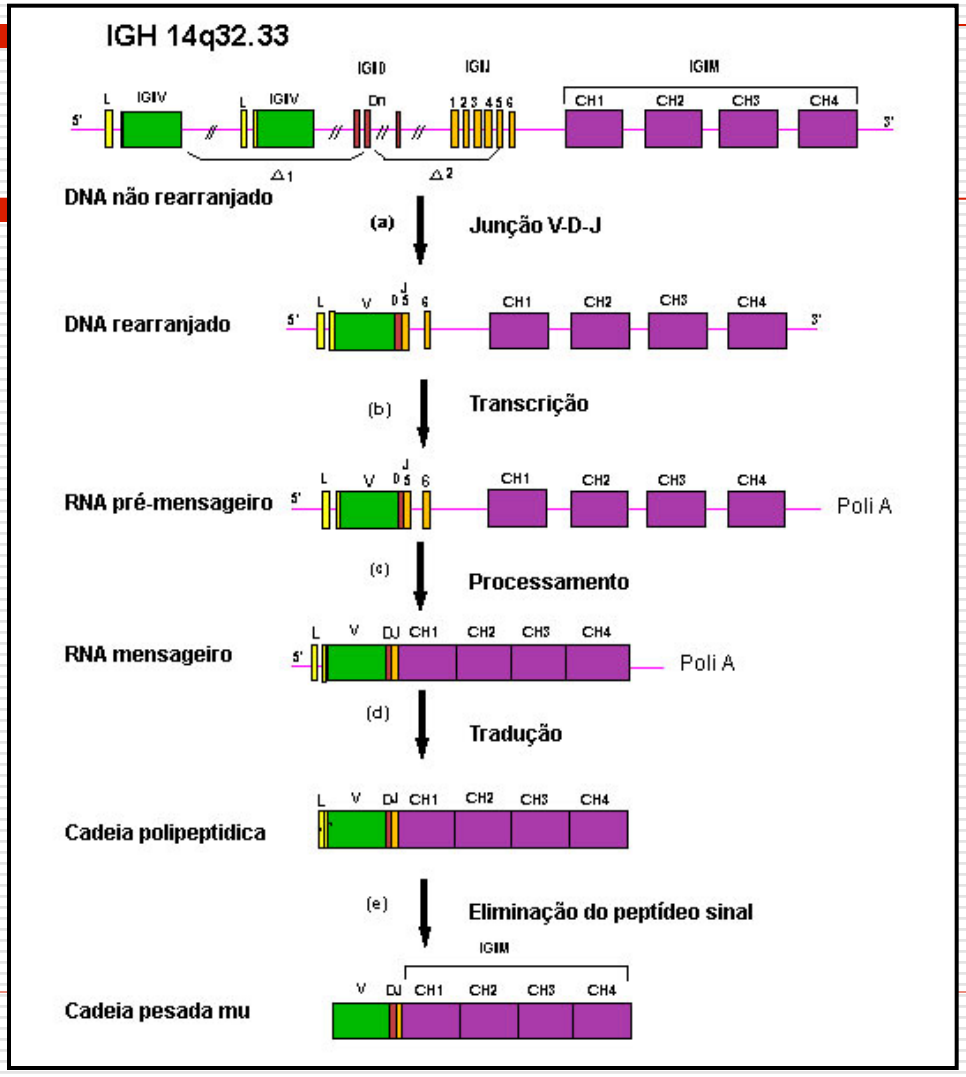
1987

2015

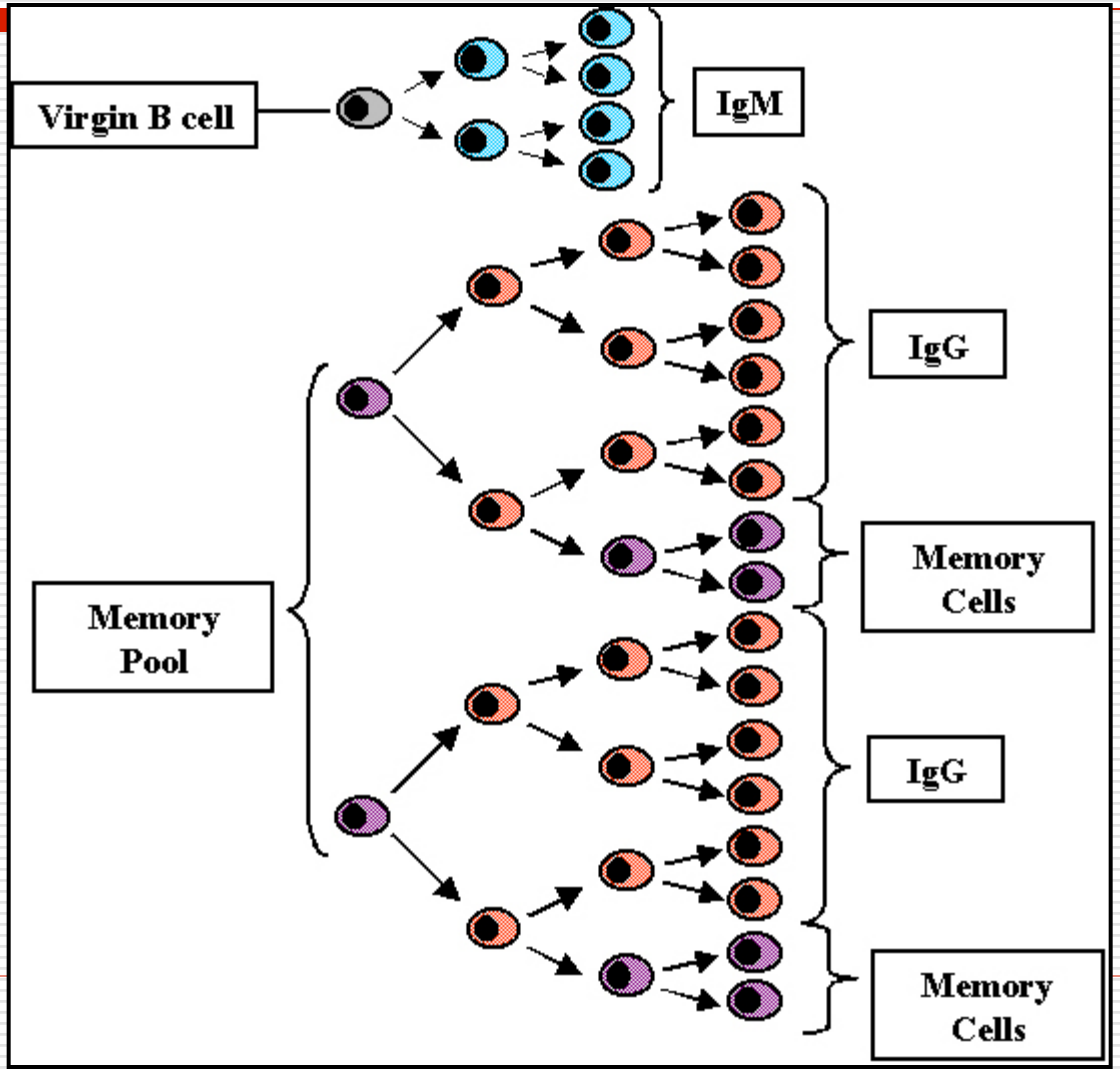
Sinais de junção



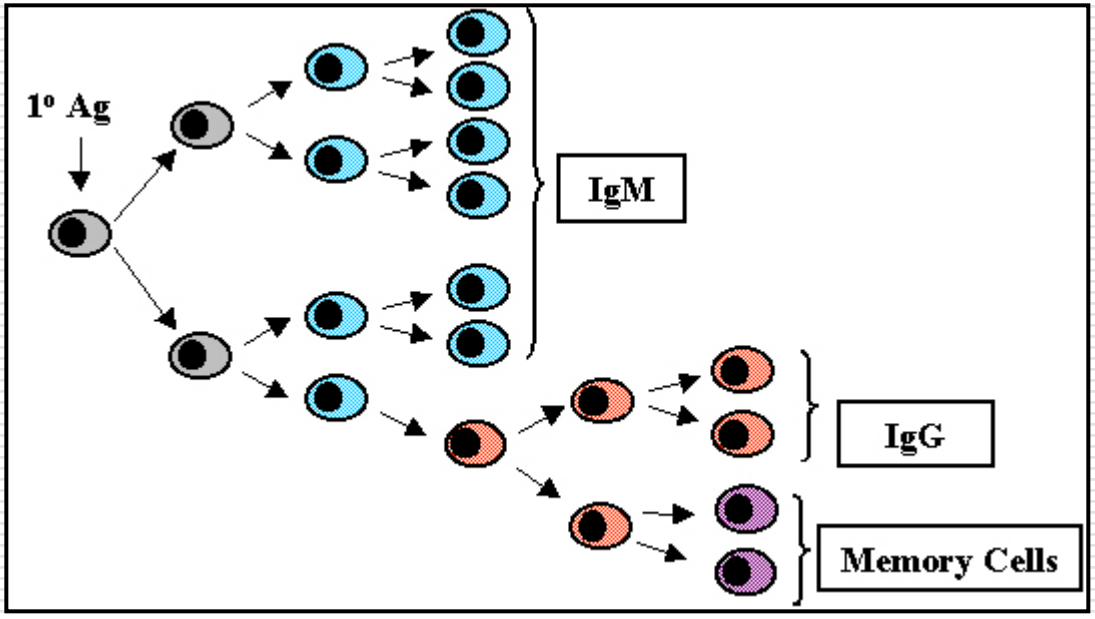
Síntese da cadeia pesada μ (Mû) (IgM)



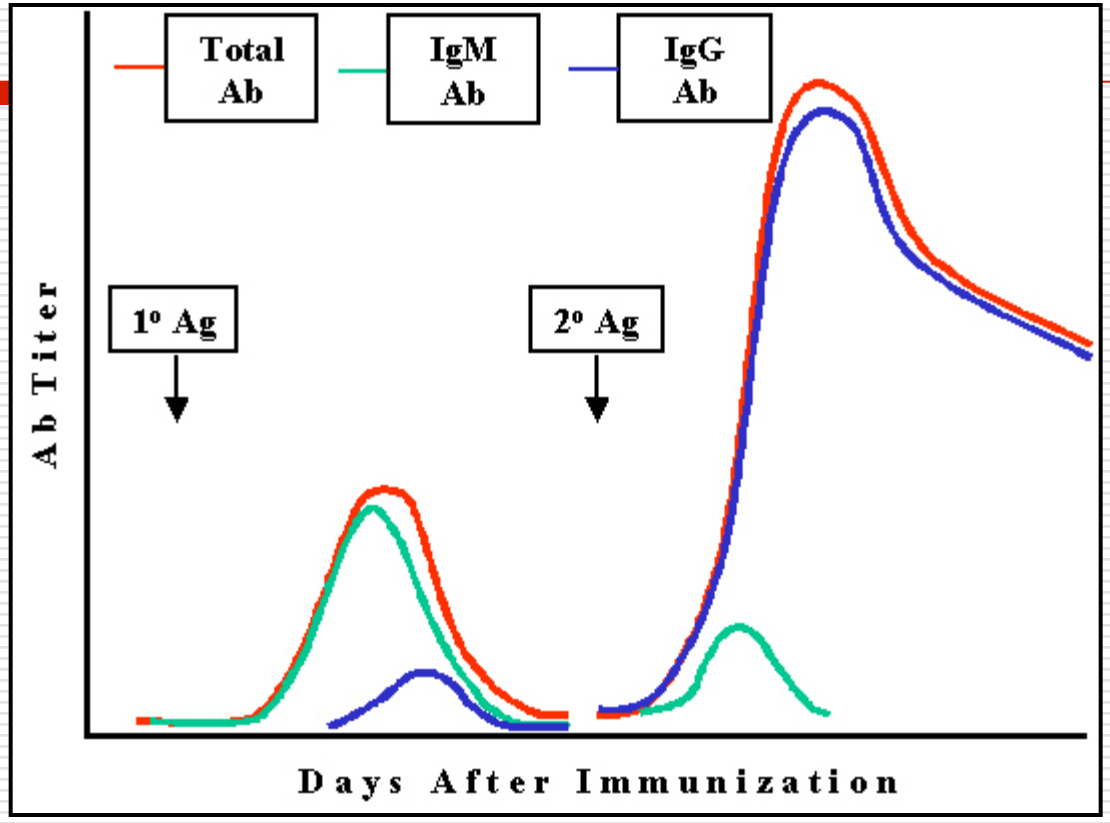
Geração de células de memória



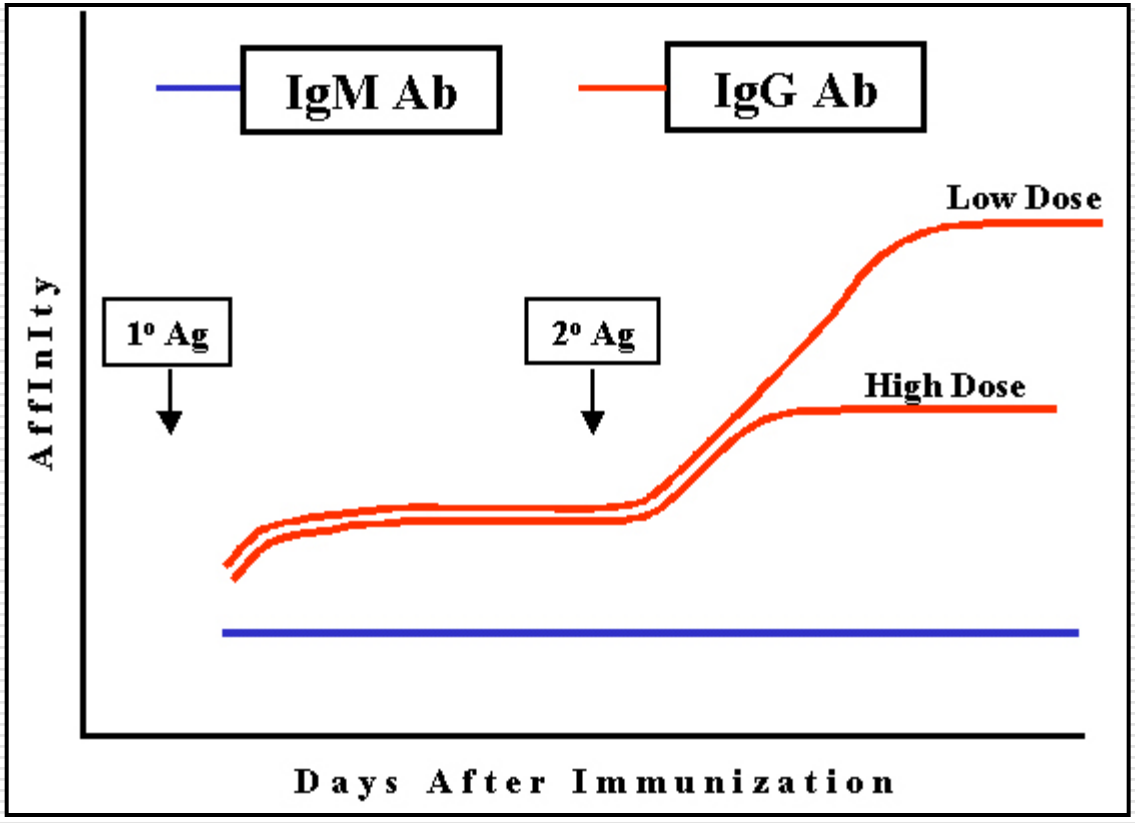
Expansão clonal durante a imunização



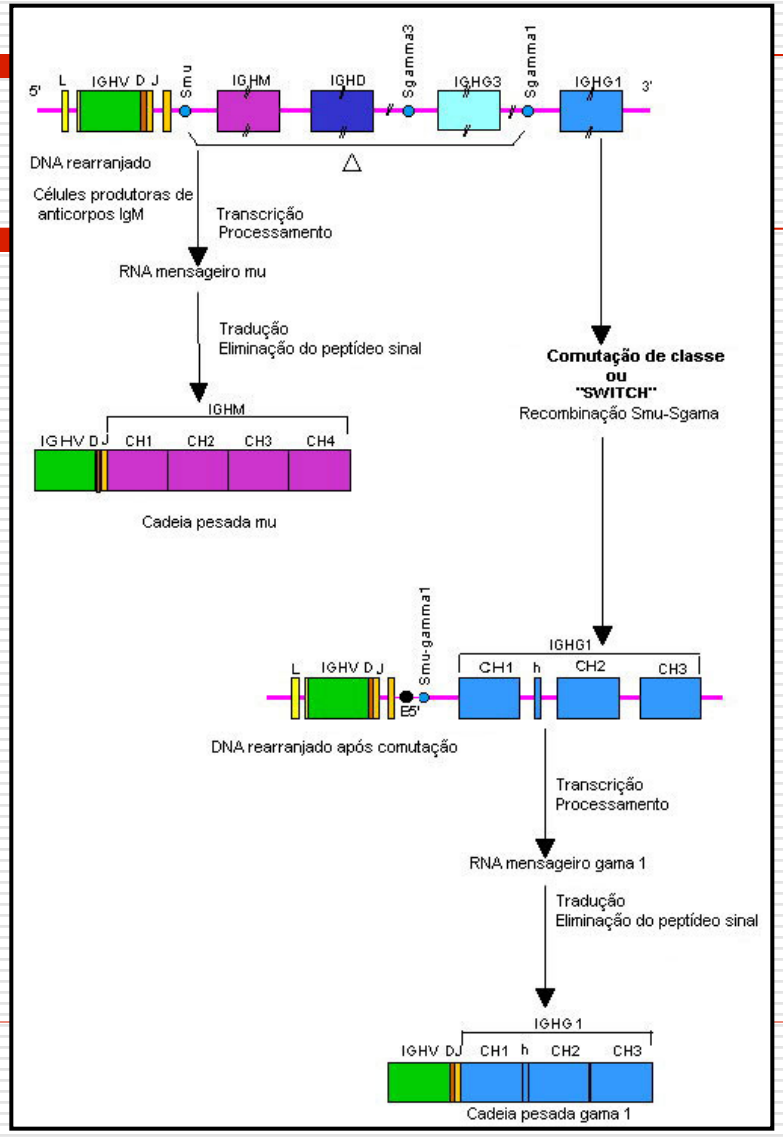
Resposta primária (IgM > IgG) e resposta secundária (IgG > IgM)



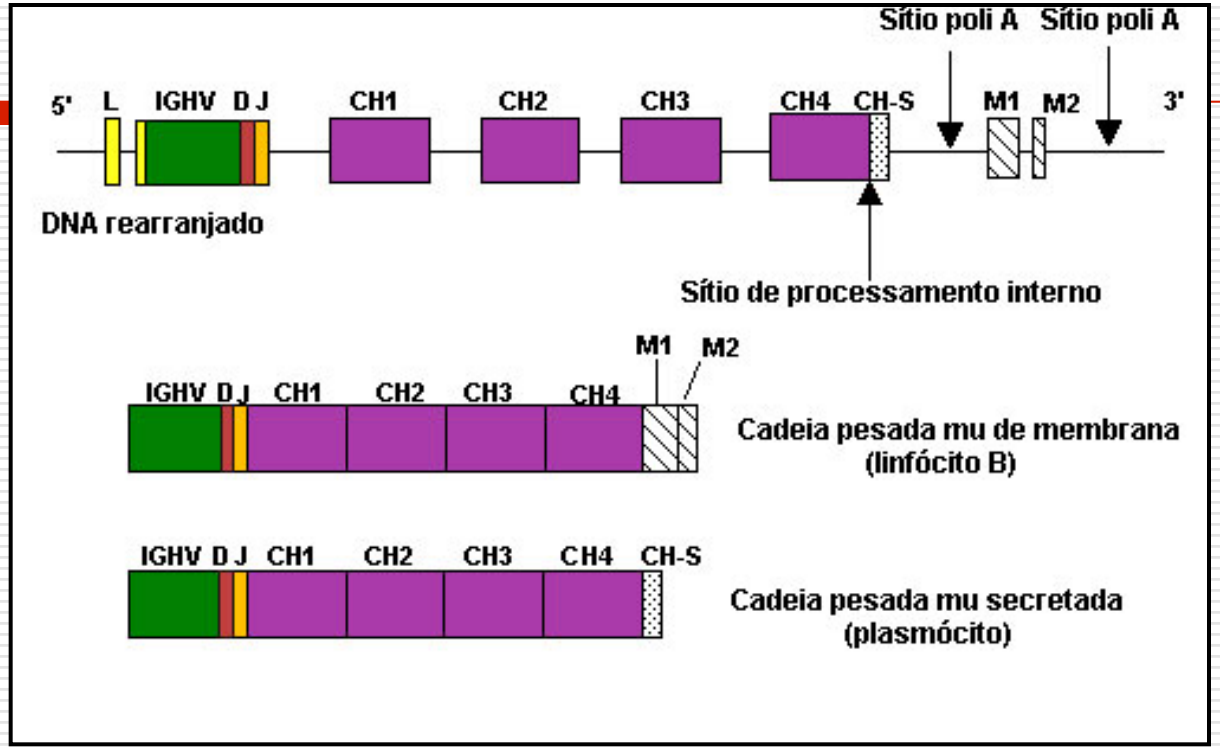
Afinidade antigênica dos anticorpos de resposta secundária



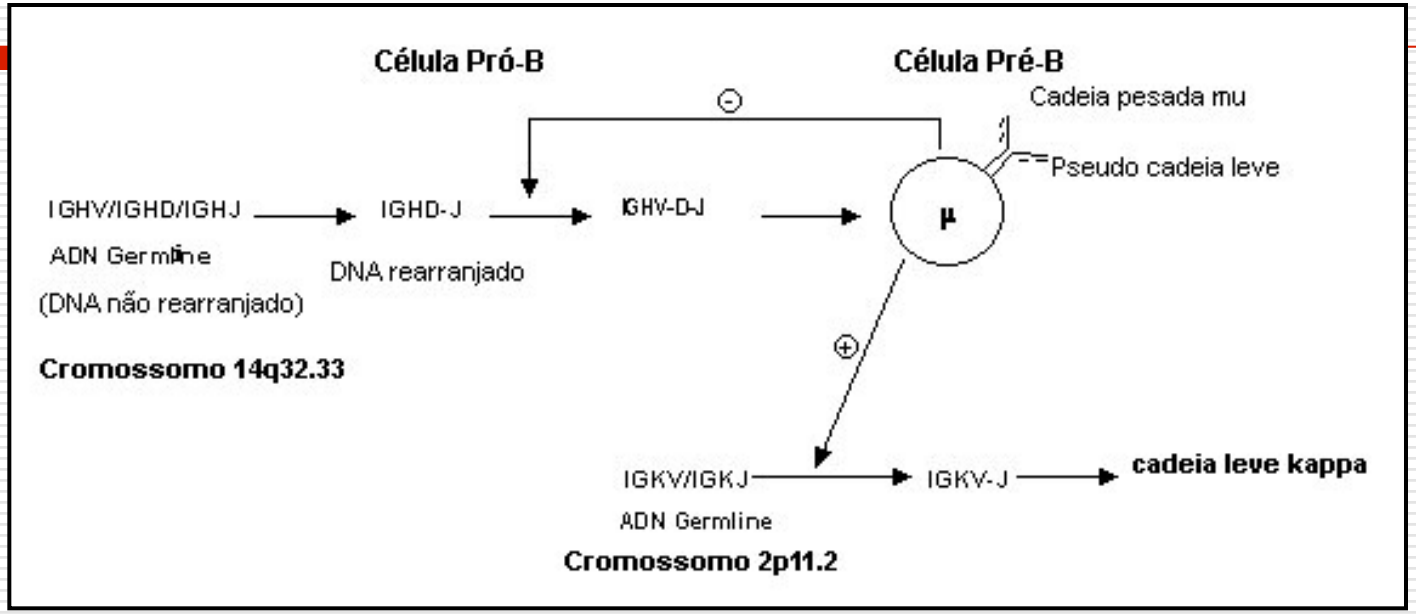
Comutação (controle da expressão) de classes de Igs



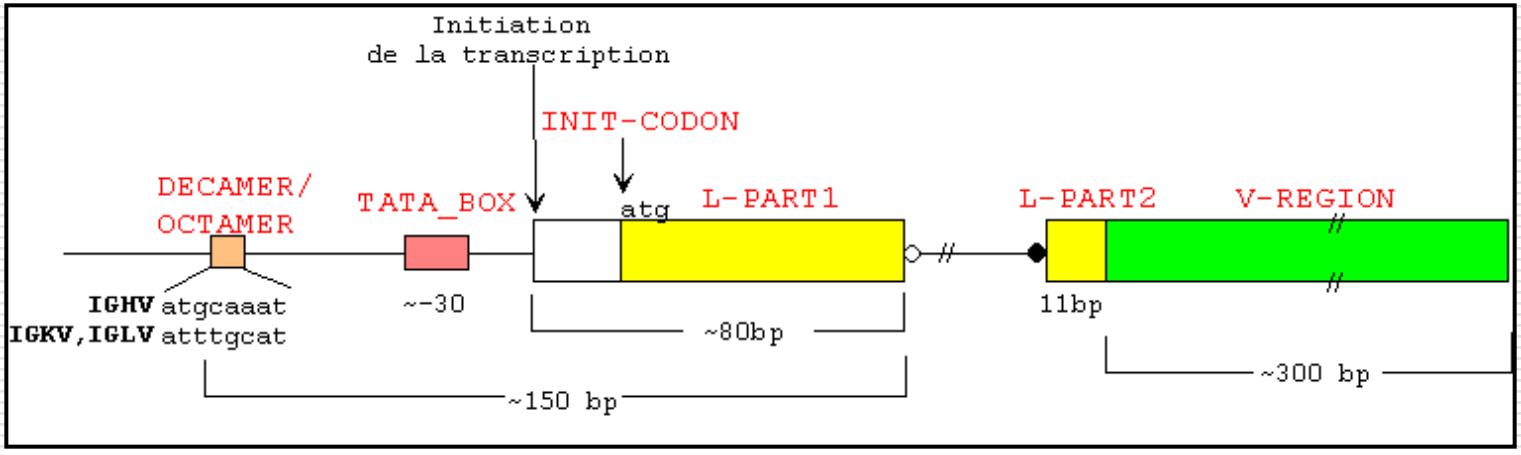
Comutação (controle da expressão) de IgM de membrana e IgM secretada



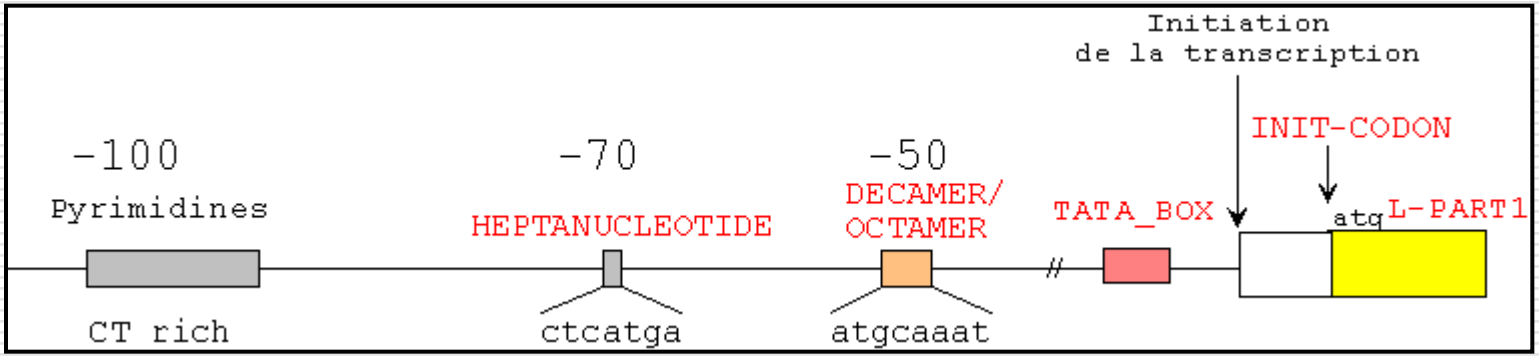
Rearranjos dos genes IgG e Igk



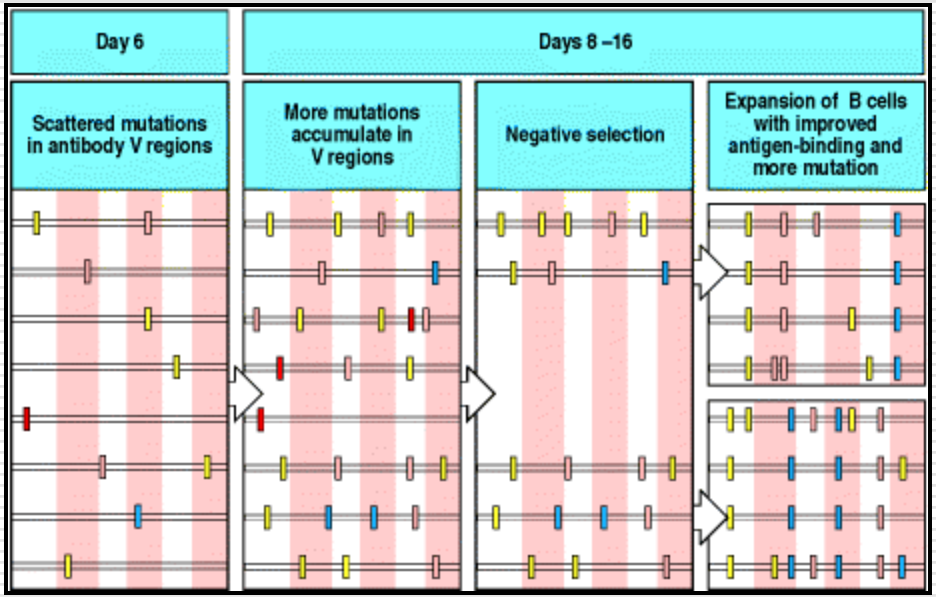
Regulação da transcrição das Igs



Seqüências promotoras nos loci de Igs



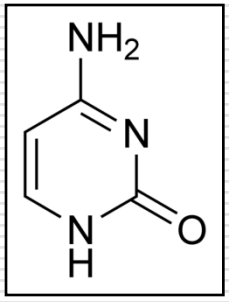
Mutações somáticas no DNA das regiões FR e CDR aumentam a diversidade anticórpica



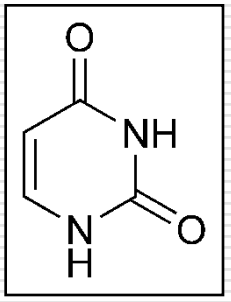
Mecanismos possíveis para Activation Induced Deaminase (AID):

Mecanismos possíveis para AID iniciar SHM:

- AID deamina citidina no DNA. Citidinas localizadas em pontos "hotspot" são preferencialmente deaminadas (segmentos WRCY W=adenosina ou timidina, R=purina, C=citidina, Y=pirimidina, ou o inverso RGYW G=guanidina). O mismatch resultante U:G (U= uridina) fica então sujeito a um dos destinos:
- O mismatch U:G é replicado criando 2 tipos de fitas, uma das quais permanece imutada, e a outra sofre a mutação tipo transição C => T. (U é análoga a T no DNA e é tratada comom tal na replicação).
- A uridina poderá ser excisada pela uracil DNA glicosilase (UDG) resultando num sítio apurínico/ apirimídico. Se replicado, resultará na incorporação ao acaso de uma das 4 bases i.e. A, G, C or T. Alternativamente, este sítio apurínico/apirimídico poderá ser reparado pela maquinaria de reparo de DNA.
- O mismatch U:G poderá ser reconhecido pela maquinaria DNA mismatch repair (MMR), o qual gera "gaps" no DNA e introduz mutações.



Citidina



Uridina

GOD = Generation Of Diversity

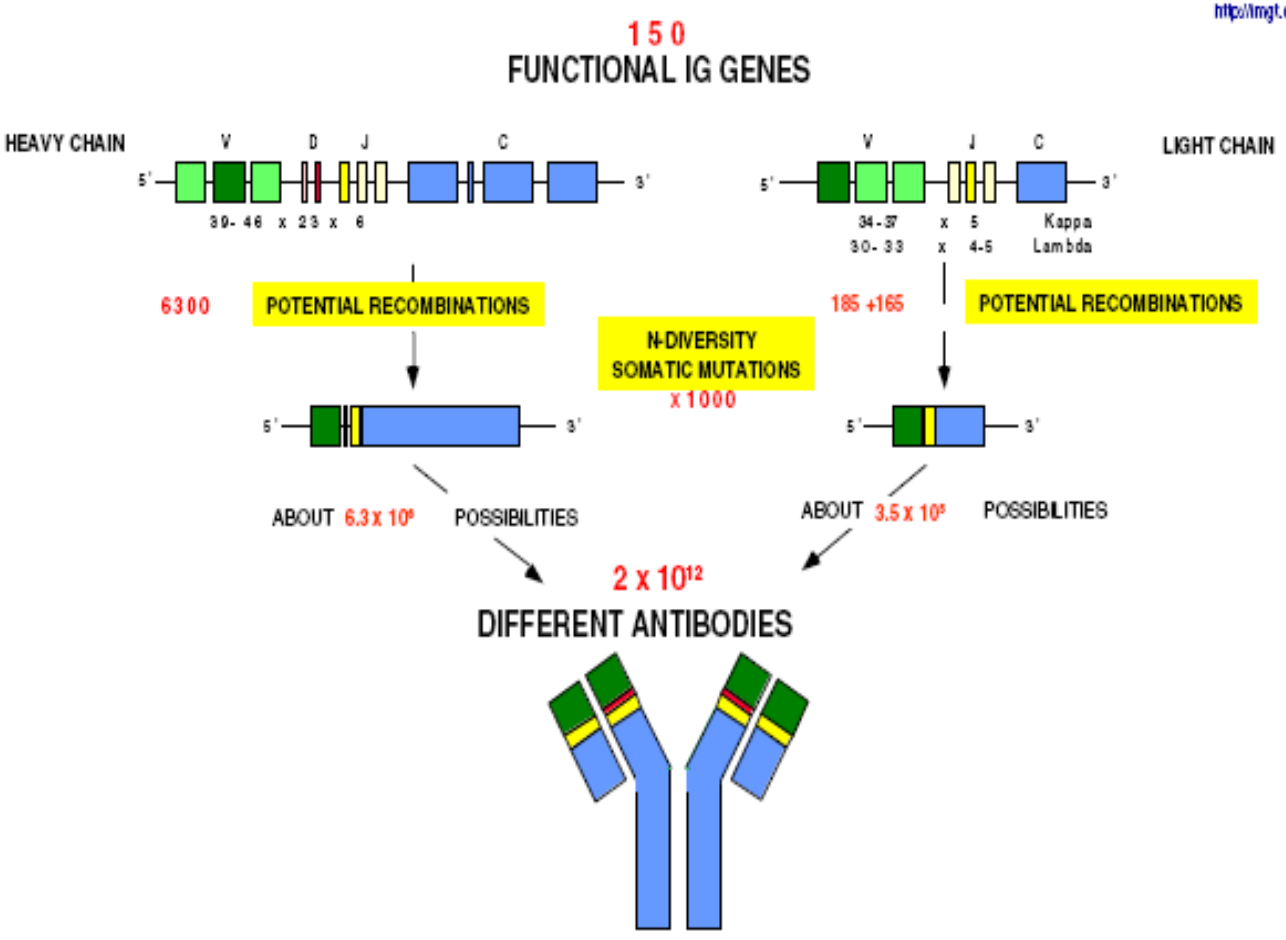
- Três mecanismos para GOD:
- Recombinação V(D)J

  (Recombinases RAG)
- Adição de nucleotídeos N

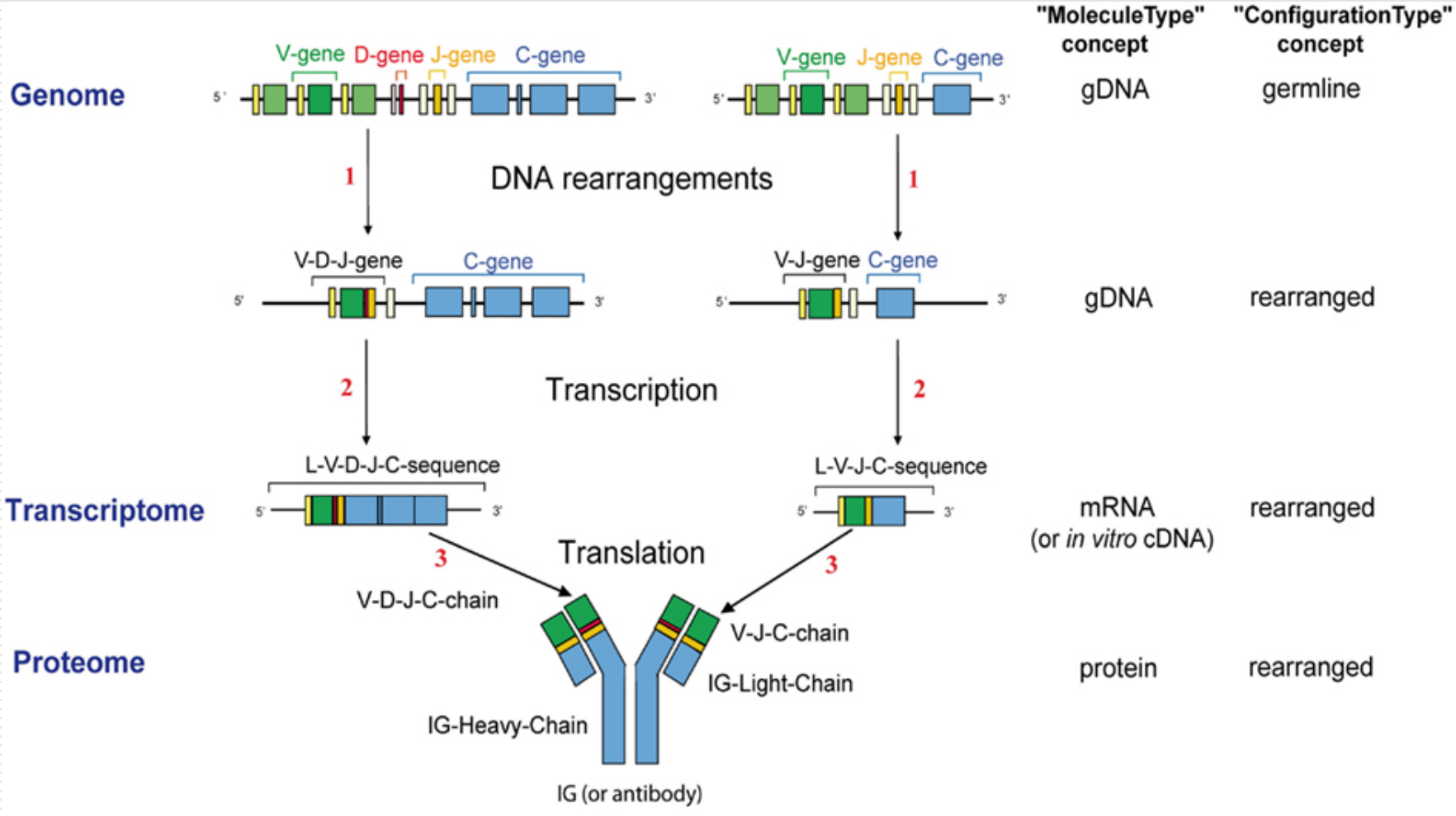
  (TdT = Terminal deoxinucleotidil Transferase)
- Mutações somáticas (regiões V)

  (AID = Activation-induced deaminase)

Immunoglobulin (IG) synthesis



The immunoglobulin genome, transcriptome and proteome



Front. Genet., 23 May 2012 | doi: 10.3389/fgene.2012.00079