Immunology 4
Specific immunity - molecules

- Immunoglobulines
- Complement
- MHC
- Cytokines

Molecules of specific immunity

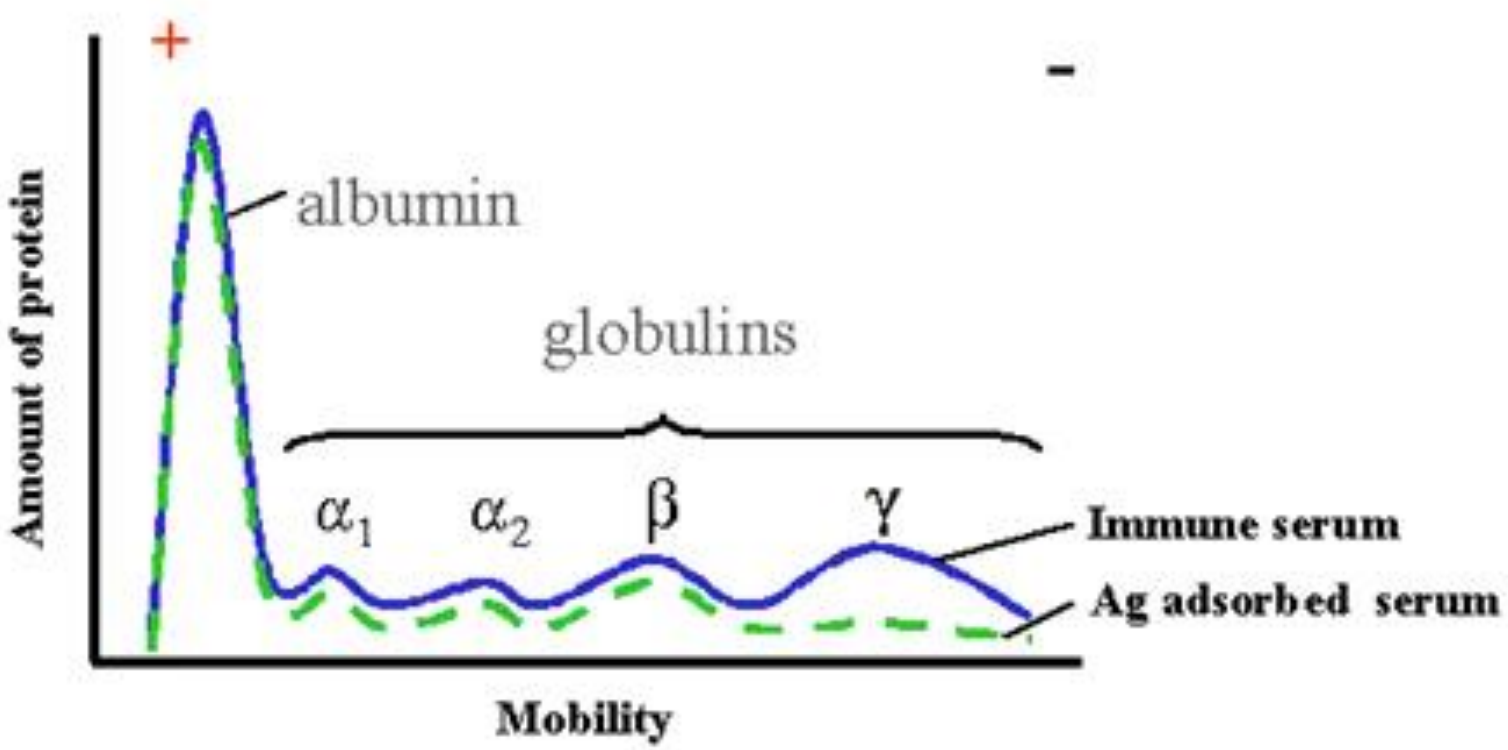
- some are common for nonspecific immunity, too
- others only for specific immunity (BCR, TCR = antigen specific receptors on B resp. T cells)
- T cells receptor **- TCR**, B cell receptor **- BCR**
- TCR bind specific antigen, bound on molecule **MHC**
- BCR bind specific antigen directly
- B cells synthetise **immunoglobulins**
- synthesis of **cytokines** after activation of T and B cells

Differences

- **Nonspecific immunity**
  - system prepared before exposition antigen
  - not differe quality and type of antigen
  - can be strenghten via cytokines after exposition to antigen
- **Specific immunity**
  - system prepared before exposition, but induced by exposition to antigen
  - lag phase
  - strenghten by antigen and in next exposition
  - slight differentiation of antigens

- ***Major signs of specific immunity are <u>memory</u> and <u>specificity</u>***

Electrophoresis of serum proteins



ANTIBODIES

Blood serum of immunised annimals contains specific molecules – antibodies, that bind antigen, that induced their production

Behring, Kitaso 19th century

Serum – liquid part after centrifugation of clotted blood
Plasma – liquid part after short centrifugation of not clotted blood

Serum containing antibodies against specific antigen – **antiserum**

Antibody – molecule of immunoglobuline with specificity for the epitope.

FUNCTION OF ANTIBODIES

- **specific** binding of antigen and neutralisation of its function
- this binding results in other reactions that lead to
- elimination of antigen (activation of complement, macrophages, opsonisation in phagocytosis).

Reaction of antigen and with antibody differs
according to the type of antigen
*corpuscular antigens – microbes, erytrocytes – *agglutination*
*solubil antigens – immunocomplexes in solution - *precipitation*

Immunoglobulins

- Synthetised by **B lymphocytes** (B cells)
- present in cytoplasma and on membrane **B bb**
- synthetised and secreted by **plasmatic cells**

Structure

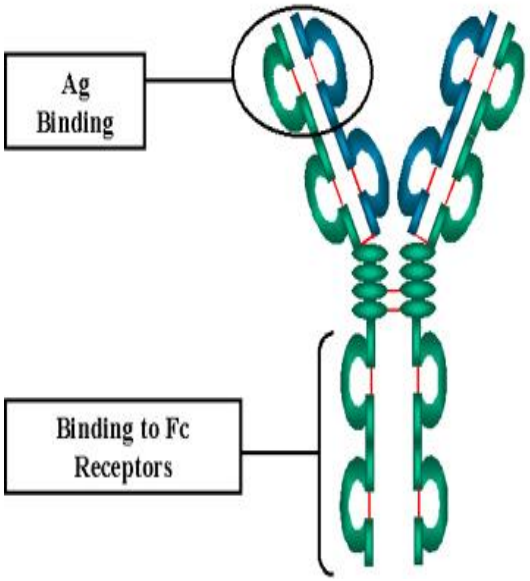
Isotypes

Structure of immunoglobulins

- 4 polypeptides
  – 2 identical light chains (LC)
  - 2 identical heavy chains (HC)

bound by disulfidic binding

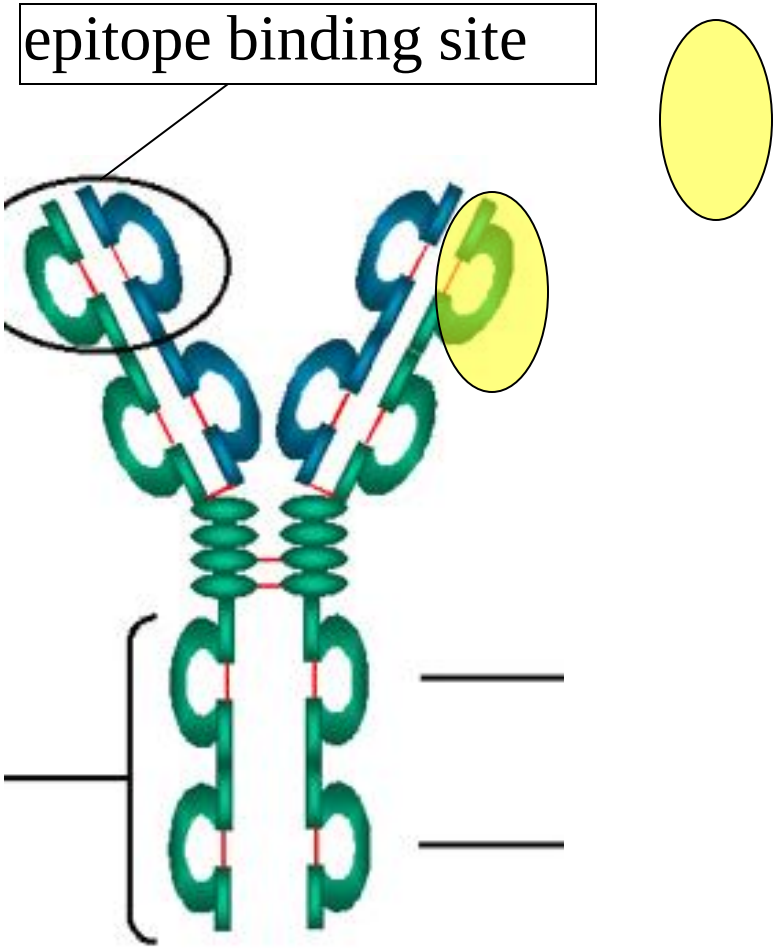
- NH end of one light chain and one heavy chain form the epitop binding site



Structure of immunoglobulins

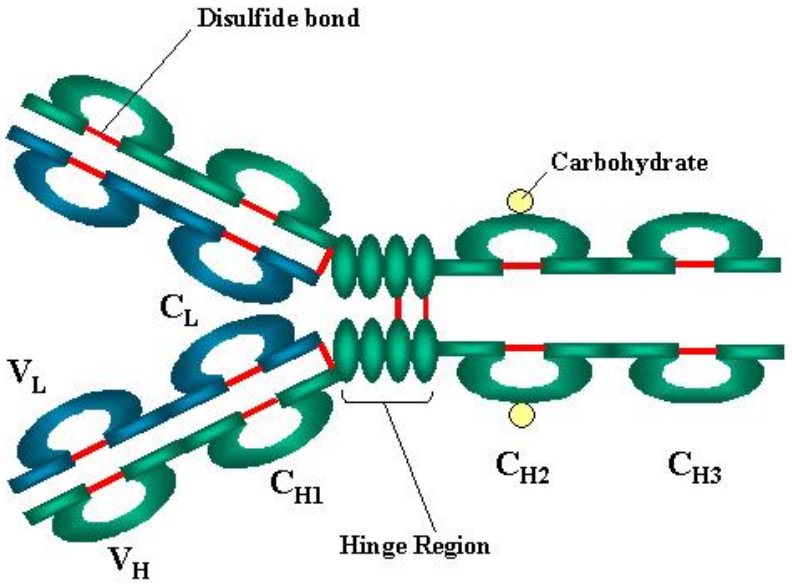
HC and LC are divided to identical domains

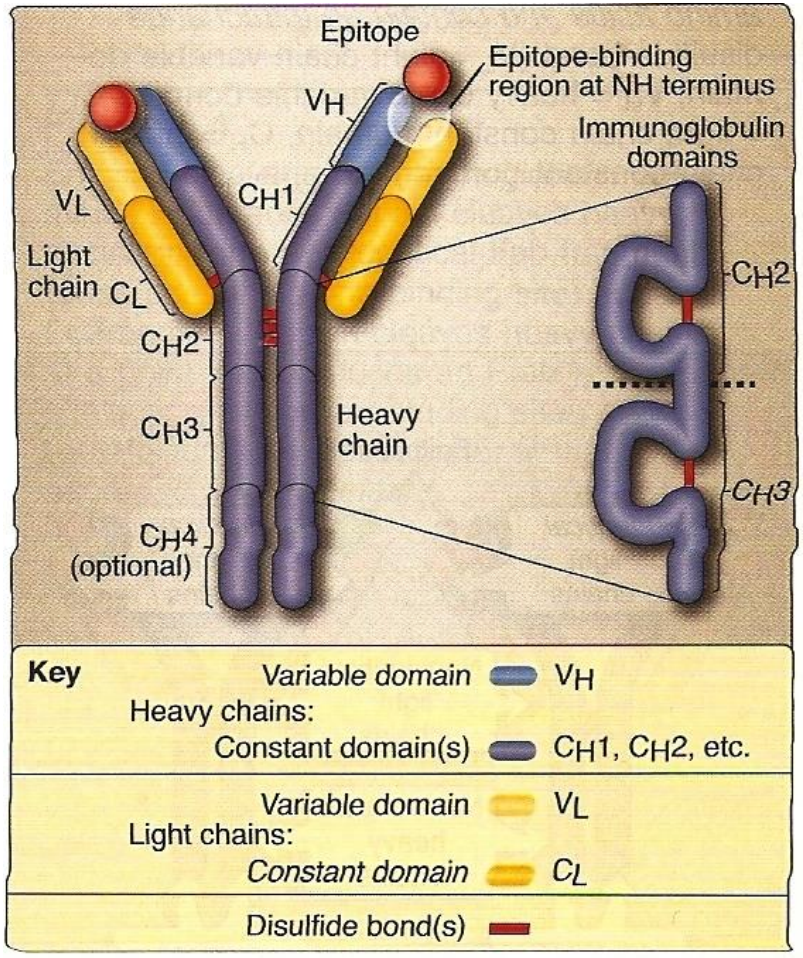
epitope binding site

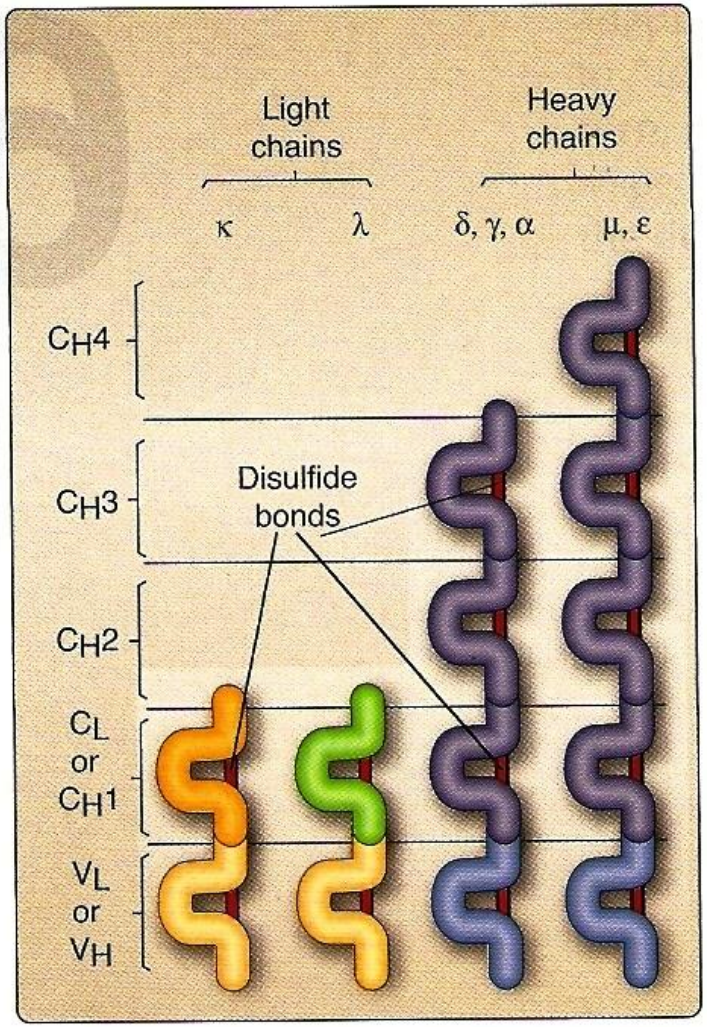


LC contains 2 domains
- 1 constant C_L
- 1 variable V_L

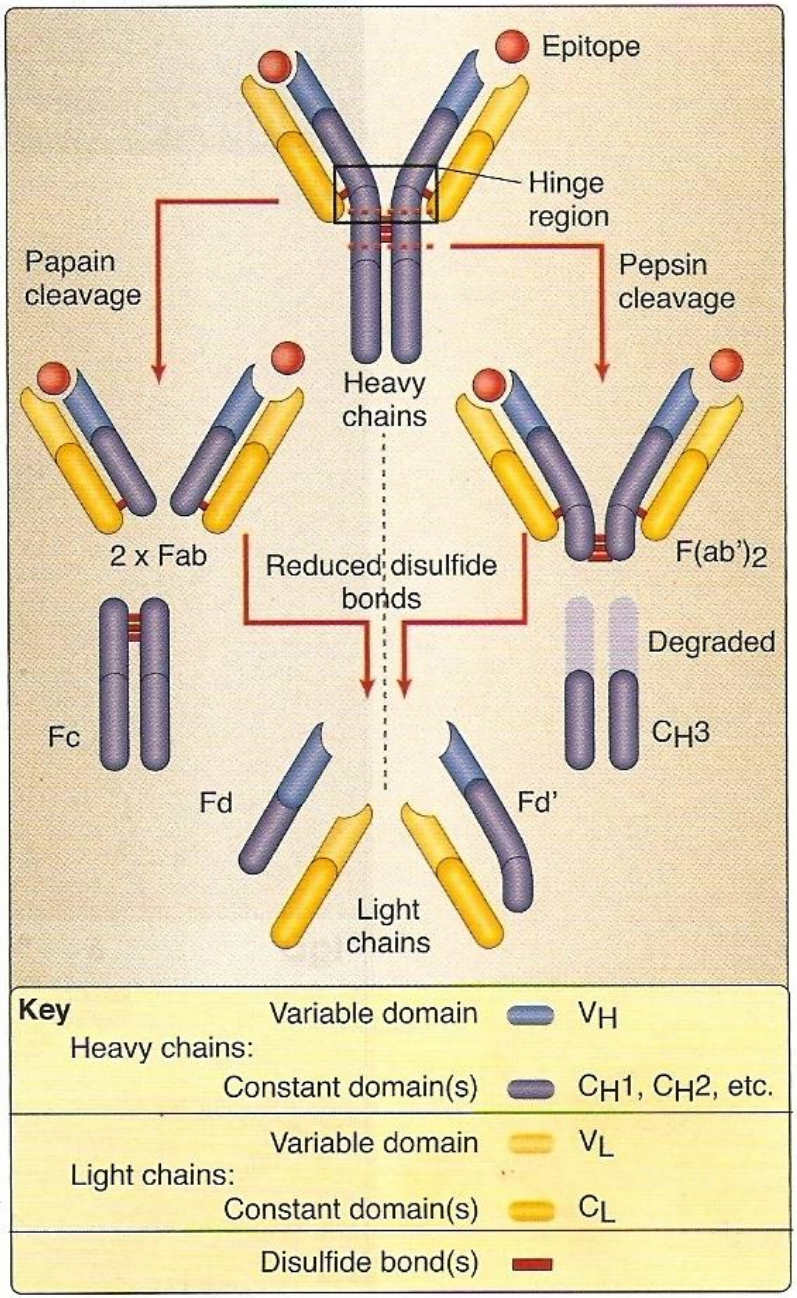
HC contains 4 or 5 domains
- 1 variable V_H
- 4 or 5 C_H




Epitope
Epitope-binding region at NH terminus
VH
Immunoglobulin domains
VL
CH1
Light chain
CL
CH2
CH3
Heavy chain
CH4 (optional)
CH2
CH3
Key
Variable domain
VH
Heavy chains:
Constant domain(s)
CH1, CH2, etc.
Variable domain
VL
Light chains:
Constant domain
CL
Disulfide bond(s)

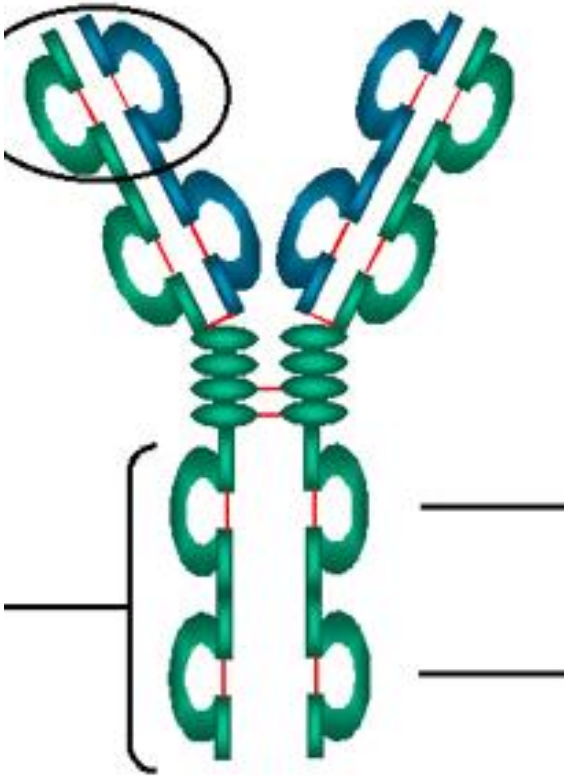

Light chains
Heavy chains
κ
λ
δ, γ, α
μ, ε
CH4
CH3
Disulfide bonds
CH2
CL or CH1
VL or VH

Papain, Pepsin fragmentation



Štructure of immunoglobulines

Light chain – kappa (κ) or lambda (λ) – one palsmatic cell produce only one type of light chain



Haevy chains – isotypes

– mi (μ) – IgM

– epsilon (ε)– IgE

- gama (γ) – IgG

- alfa (α) – IgA

- delta (δ) – IgD

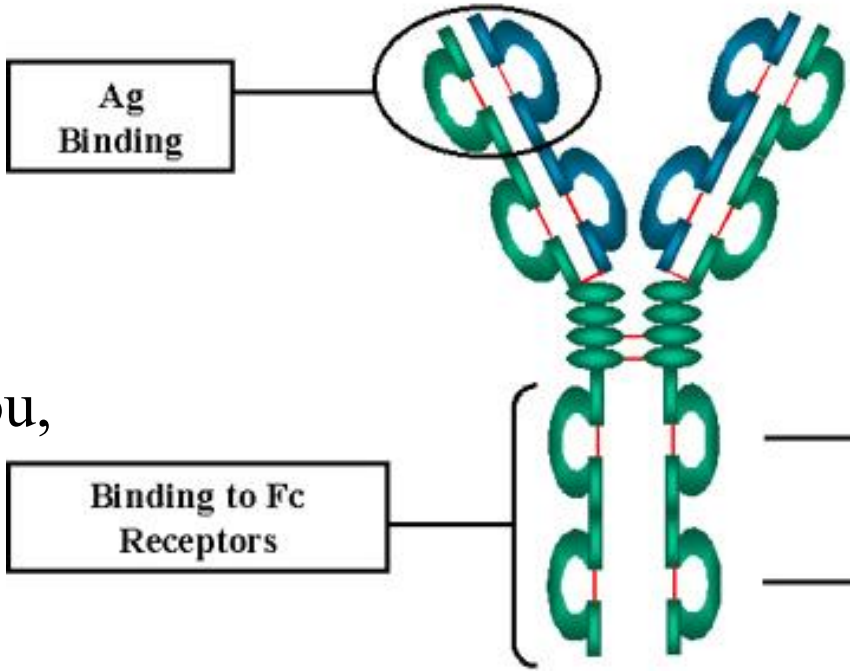
IgG

Existuje ako povrchový aj secernovaný monomér

2 γ + 2κ (alebo 2λ)

Existujú 4 podtypy ťažkých reťazcov a teda 4 IgG podtriedy:
IgG_1 IgG_2 IgG_3 IgG_4

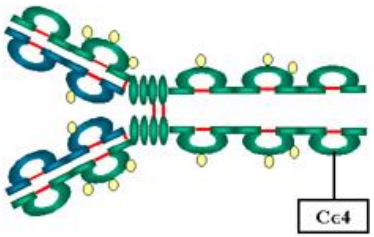
- Najviac v sére,
- aktivácia komplementu,
- opsonizácia,
- neutralizácia mikroorganizmov,
- ADCC,
- hypersenzitívne reakcie (II., III.,),
- prechod transplacentárnou bariérou,
- protektívne



Classes (isotype) of Ig

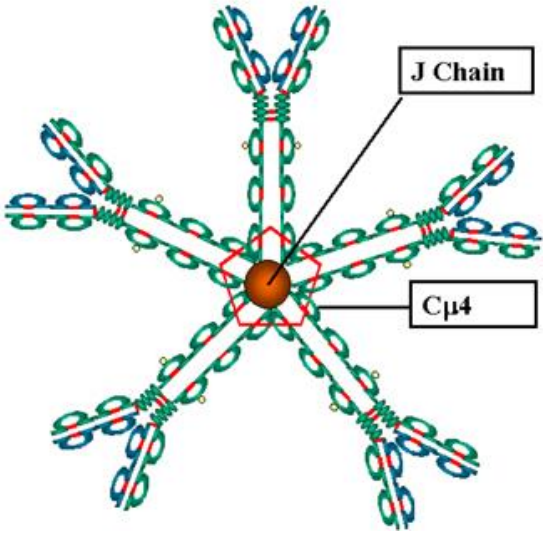
IgE

- Structure
  - Monomer
  - Extra domain (C_{H4})



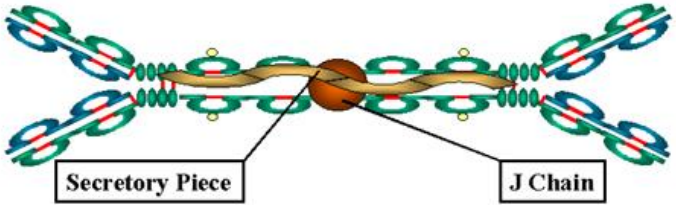
IgM

- Structure
  - Pentamer (19S)
  - Extra domain (C_{H4})
  - J chain



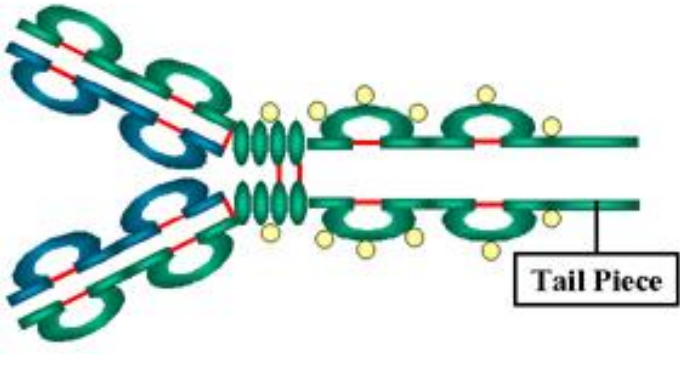
IgA

- Structure
  - Serum - monomer
  - Secretions (sIgA)
    - Dimer (11S)
    - J chain
    - Secretory component

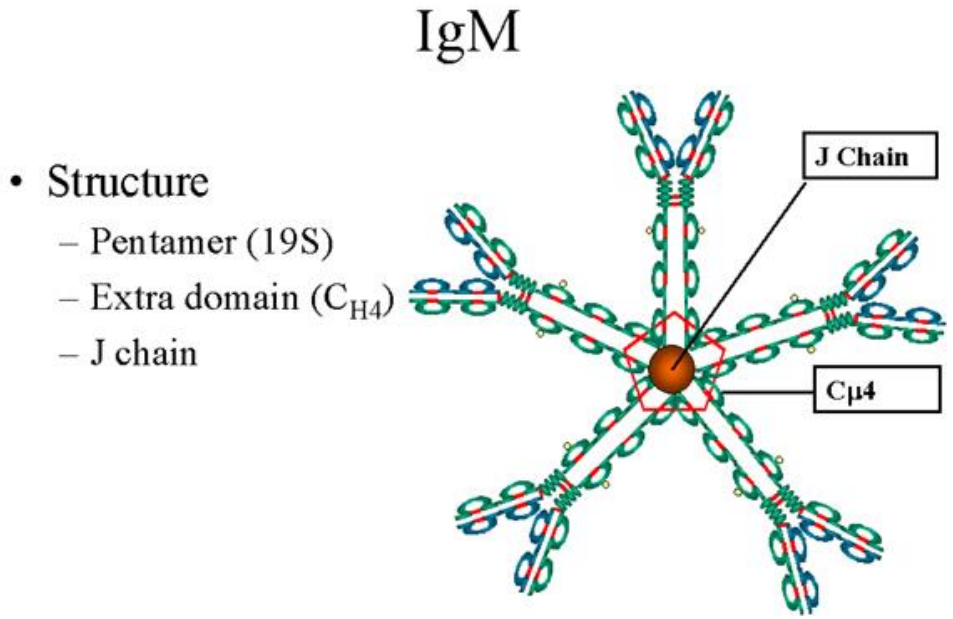


IgD

- Structure
  - Monomer
  - Tail piece



IgM

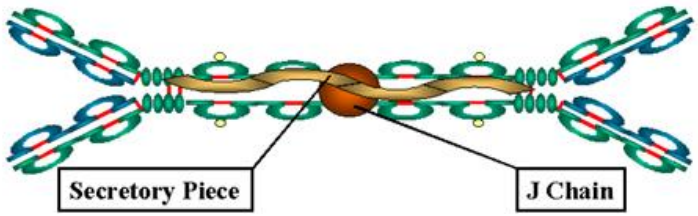


- bound as monomer on cell surface
- or in serum and liquids as pentamer with 10 H a 10 L chains bound in paire by disulfidic bindings and J chains.
- First synthetised after stimulation (in utero, too)
- Immobilisation of antigen
- activation of C´ by classical path

IgA

IgA

- Structure
  - Serum - monomer
  - Secretions (sIgA)
    - Dimer (11S)
    - J chain
    - Secretory component



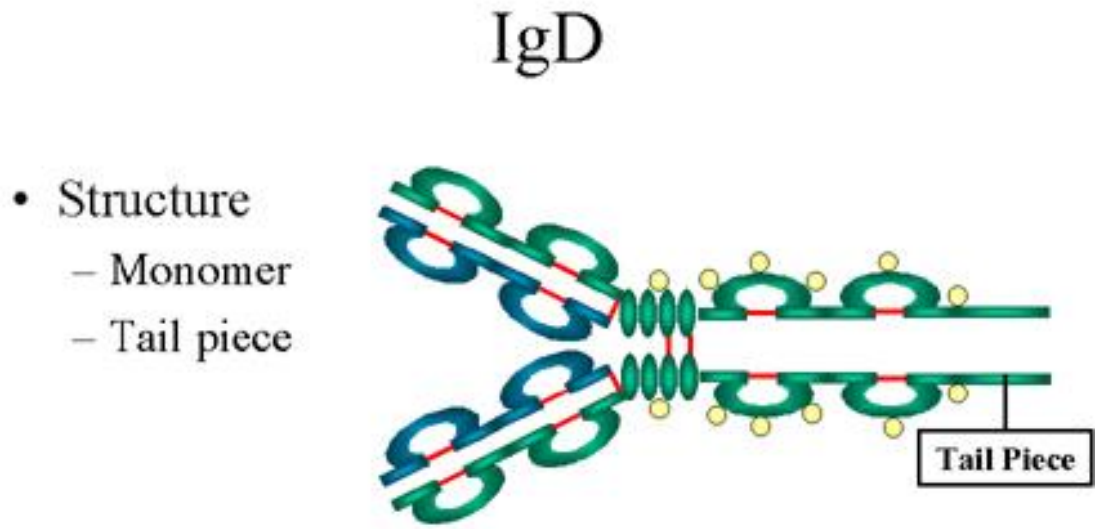
- monomer in seurm
- dimer

(2 monomers + J chain)

- mucuse – translocation through membrane bind on receptor that bind on dimer as SC (secretion component) – that gives resistence to ensymatic degradation
  - saliva, tears, mucous, breast milk, GIT secretion
- IgA_1 - serum and secretion up diaphragma
  IgA_2 – secretion epitelium, GIT, respiratory trakt
- Mostly produced

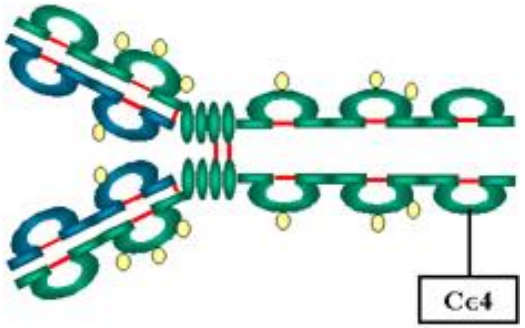
IgD

- Monomer
- Almost the only role is the role of receptor for antigen on the surface of B cell
- Other functions are not known
- Contain the intercelular part - tail piece



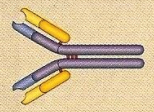
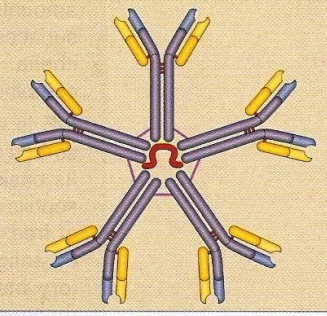
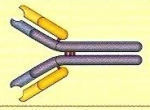
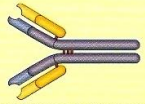
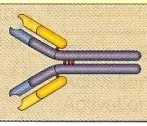
IgE

- Structure
  - Monomer
  - Extra domain (C_{H4})



IgE

- in low concentration in serum
- mostly on the surfaces of mastocytes, monocytes and eosinofils and basofils
- Specific receptors for Fc fragment of IgE molecule (FCεRI). Mastocytes are bound by antigens on Fab phragments that leads to degranulation and the release of histamin lead to alergic reactions.

Isotype	Heavy Chains[a]	Heavy Chain Subclass	Additional Chains	Formula[a]	Number of Monomers[b]	Subclass	Valence	MW[c]	Half Life (days)	Serum Level (mg/dl)	Serum Level Percent	Stick Figure
IgM	μ			$2\mu^d + 2\kappa$ or 2λ	1		2	180,000				
		μ	J chain	$5[2\mu + 2\kappa$ or $2\lambda] + J$	5	IgM	10	900,000	1	45–150[e]	5–8	
IgD	δ			$2\delta + 2\kappa$ or 2λ	1		2	180,000	2.8	3	<1	
IgG	γ			$2\gamma + 2\kappa$ or 2λ	1		2	150,000	23	720–1500[e]	75–85	
		γ_1		$2\gamma_1 + 2\kappa$ or 2λ	1	IgG1	2	150,000	23	430–1050		
		γ_2		$2\gamma_2 + 2\kappa$ or 2λ	1	IgG2	2	150,000	23	100–300		
		γ_3		$2\gamma_3 + 2\kappa$ or 2λ	1	IgG3	2	150,000	8	30–90		
		γ_4		$2\gamma_4 + 2\kappa$ or 2λ	1	IgG4	2	150,000	23	15–60		
IgA	α			$2\alpha + 2\kappa$ or 2λ	1			170,000	5.8	90–325	10–16	
		α_1		$2\alpha_1 + 2\kappa$ or 2λ	1 — serum	IgA1	2	170,000	5.8	80–290		
			J chain & SC[f]	$2[2\alpha_1 + 2\kappa$ or $2\lambda] + J + SC^f$	2 — external[g] upper body and GI	sIgA1	4	390,000	na	na		
		α_2		$2\alpha_2 + 2\kappa$ or 2λ	1 — serum	IgA2	2	170,000	5.8	10–35		
			J chain & SC[f]	$2[2\alpha_2 + 2\kappa$ or $2\lambda] + J + SC^f$	2 — external[g] GI	sIgA2	4	390,000	n.a.	n.a.		
IgE	ε			$2\varepsilon + 2\kappa$ or 2λ	1		2	190,000	2.5	0.03	<1	

B cell

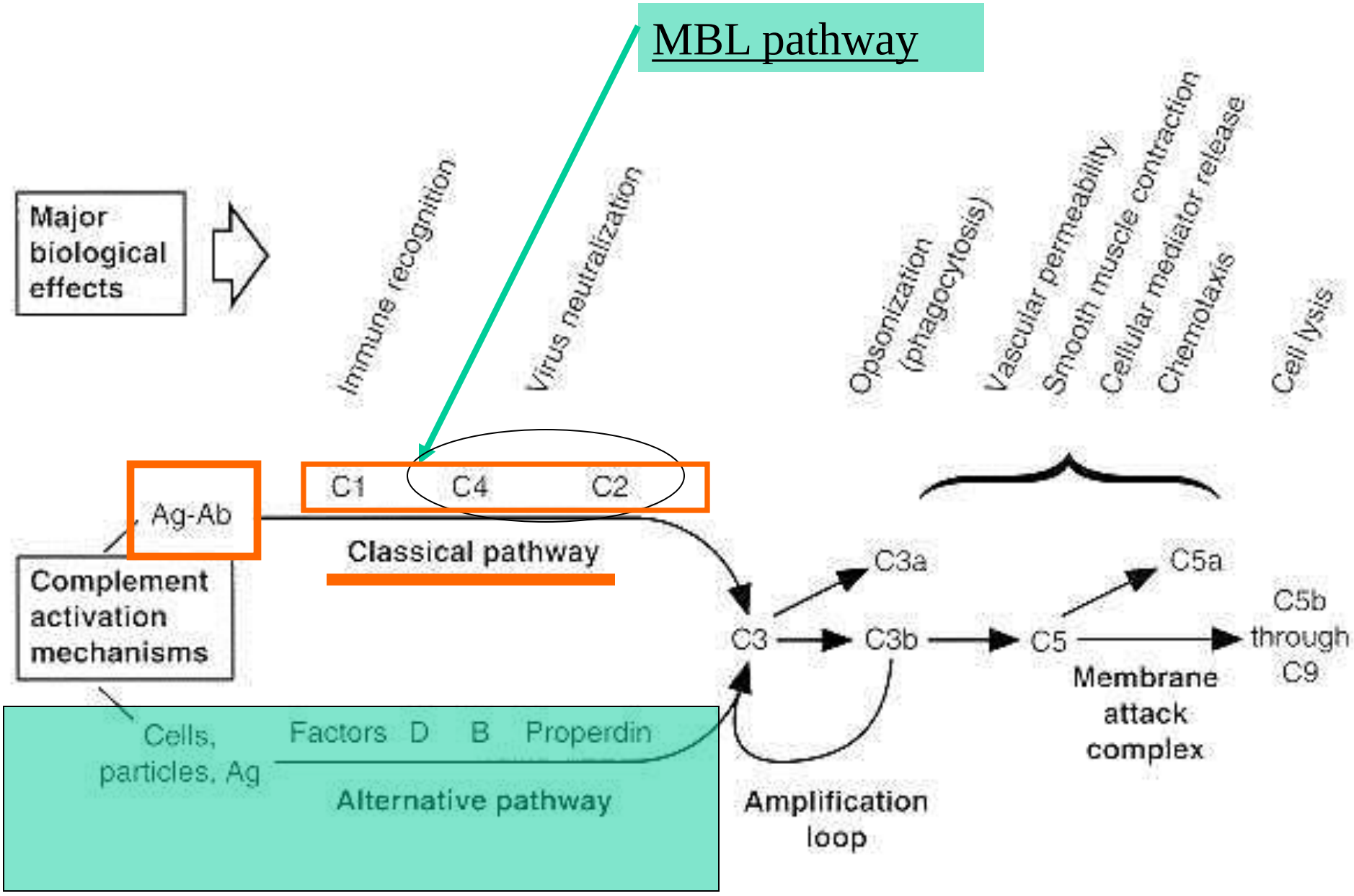
- produces immunoglobulines with light chain kappa or lambda
- produces only one isotype (heavy chain)
- nonstimulated B cell has on the surface the molecule of monomer of IgM or IgD as BCR
- After secretion to tissues the IgG, IgE will be monomer., IgM – as soluble pentamer, IgA - soluble dimer and monomer

Complement

- Consists of innactive circulating glycoproteins activated in cascade way after innitial stimulation

- 3 paths of stimulation:
  - c*lassic (antigen + antibody*)
  - alternative (microbes and their products)
  - MBL path

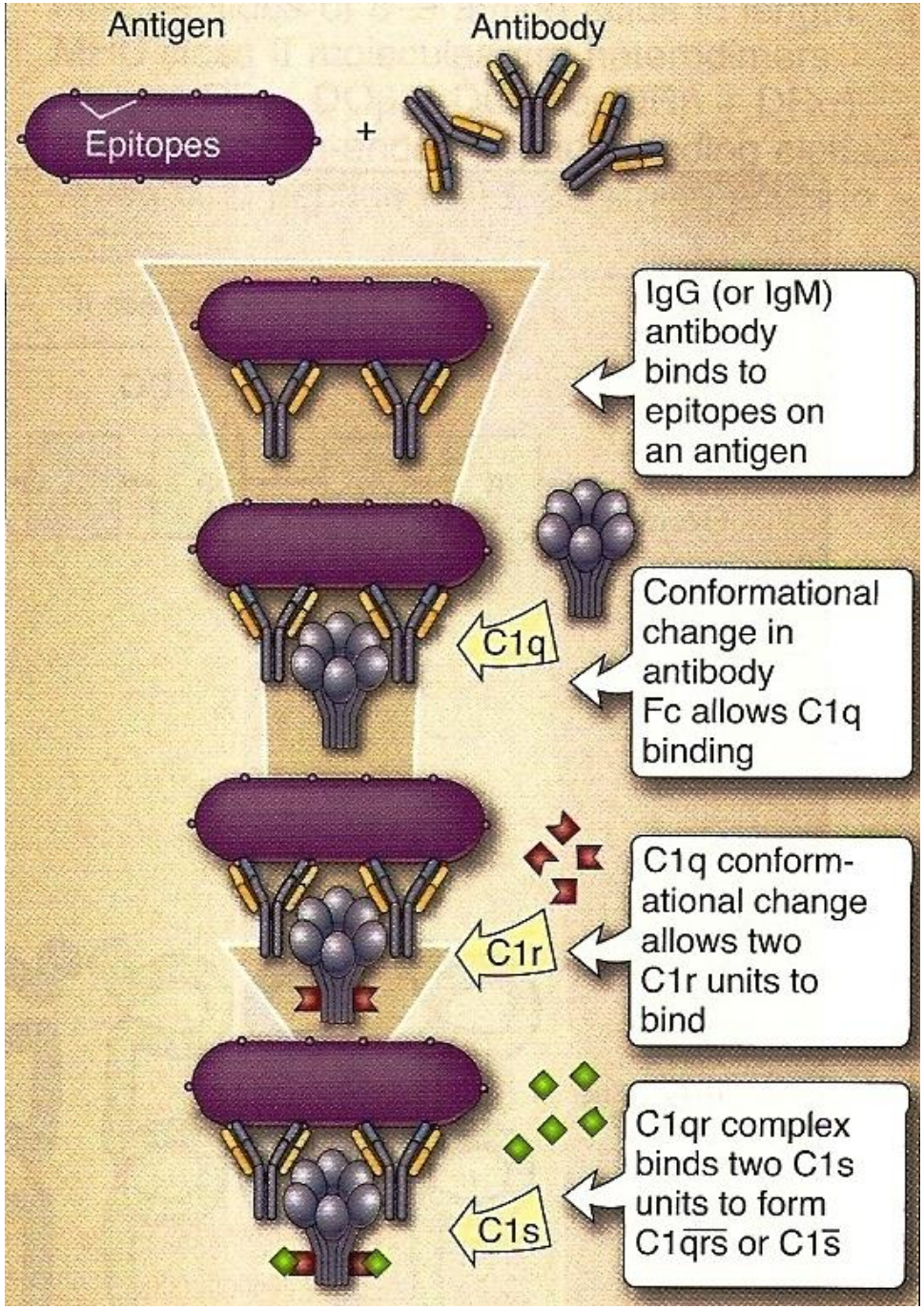
- Results in production of:
  MAC membrane attact complex
  it is bound on cell surface (of microbe) formation of disruptions in surface memgrane that leads to lysisof cell

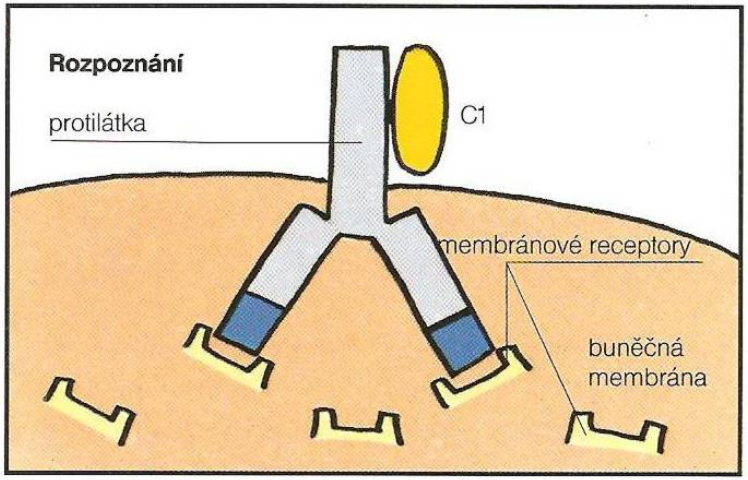

MBL pathway
Major biological effects
Immune recognition
Virus neutralization
Opsonization (phagocytosis)
Vascular permeability
Smooth muscle contraction
Cellular mediator release
Chemotaxis
Cell lysis
Ag-Ab
C1
C4
C2
Classical pathway
Complement activation mechanisms
C3a
C5a
C3
C3b
C5
C5b through C9
Membrane attack complex
Cells, particles, Ag
Factors D B Properdin
Alternative pathway
Amplification loop

Activation of classical pathway C′

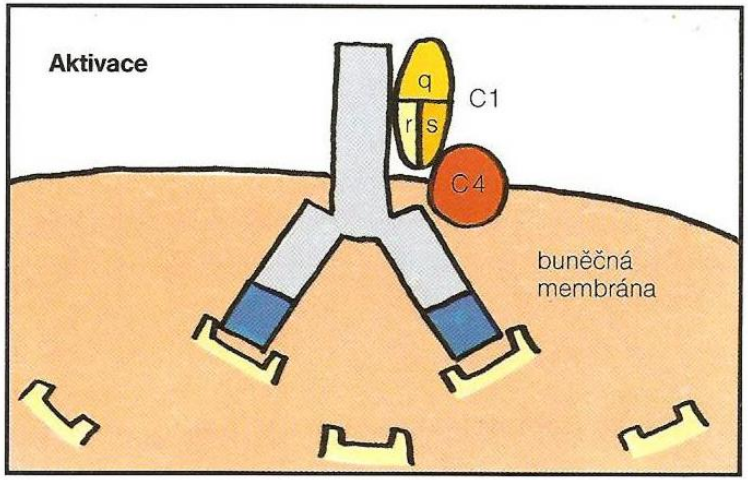
- Ag + Ab = production of MAC, opsonisation (C3b), production of anaphylatoxins (C3a, C5a, C4a)
- Activation C1:
  - IgM (IgG) + Ag = konformationnal change of Fc = binding of C1q on C_H2 (Fc fragment of Ig molecule).
  - Subsequently binding of C1r and C1s
  - $\overline{C1qrs}$ has ensymatic activity and disrupt C4, C2, C3 on fragments

$\overline{C4b2b3b}$, that is C5covertase – beggining of MAC

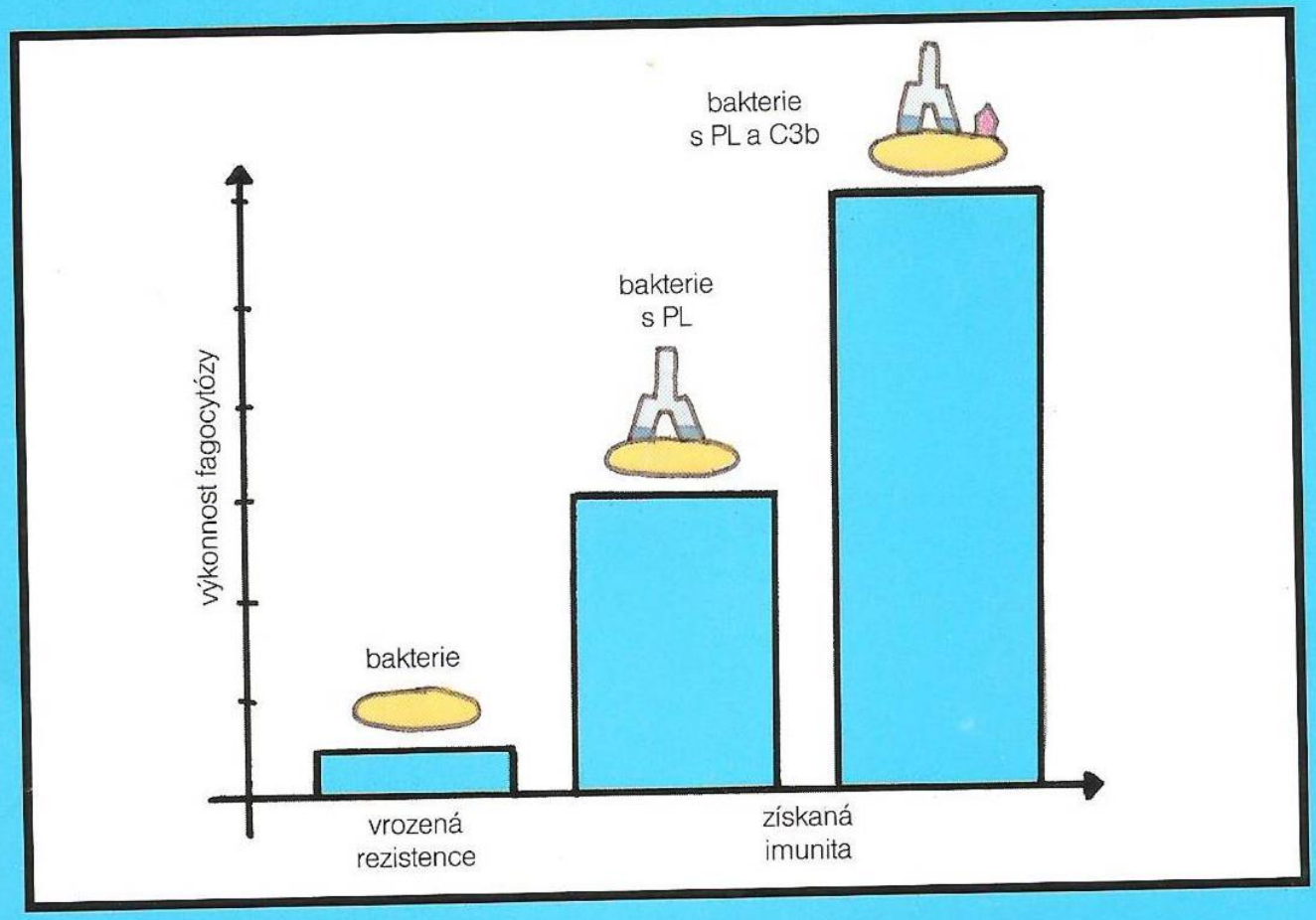

Antigen
Antibody
Epitopes
+
IgG (or IgM) antibody binds to epitopes on an antigen
C1q
Conformational change in antibody Fc allows C1q binding
C1r
C1q conform-ational change allows two C1r units to bind
C1s
C1qr complex binds two C1s units to form C1$\overline{qrs}$ or C1$\overline{s}$



Obr. 9: Když imunoglobulin (protilátka) rozpozná antigen a fixuje se na něj, může se připojit C1 k Fc dílu protilátky a stane se aktivním enzymem.



Obr. 10: C1 se skládá ze tří podjednotek. C1q komponenta zajišťuje fixaci na protilátce. Následovně je C4 navázana na buněčnou membránu.


bakterie
s PL a C3b
bakterie
s PL
bakterie
výkonnost fagocytózy
vrozená
rezistence
získaná
imunita

MHC – major histocompatibility complex

- Named also HLA complex
  = segment on chromosome 6 containning several **genes** needed for good function of immunity (6.7). These genes **encode** ensymes and structural **molecules** needed for activation and function of T and B cells. These are molecules of classes **I, II a III MHC** (resp.HLA).

MHC

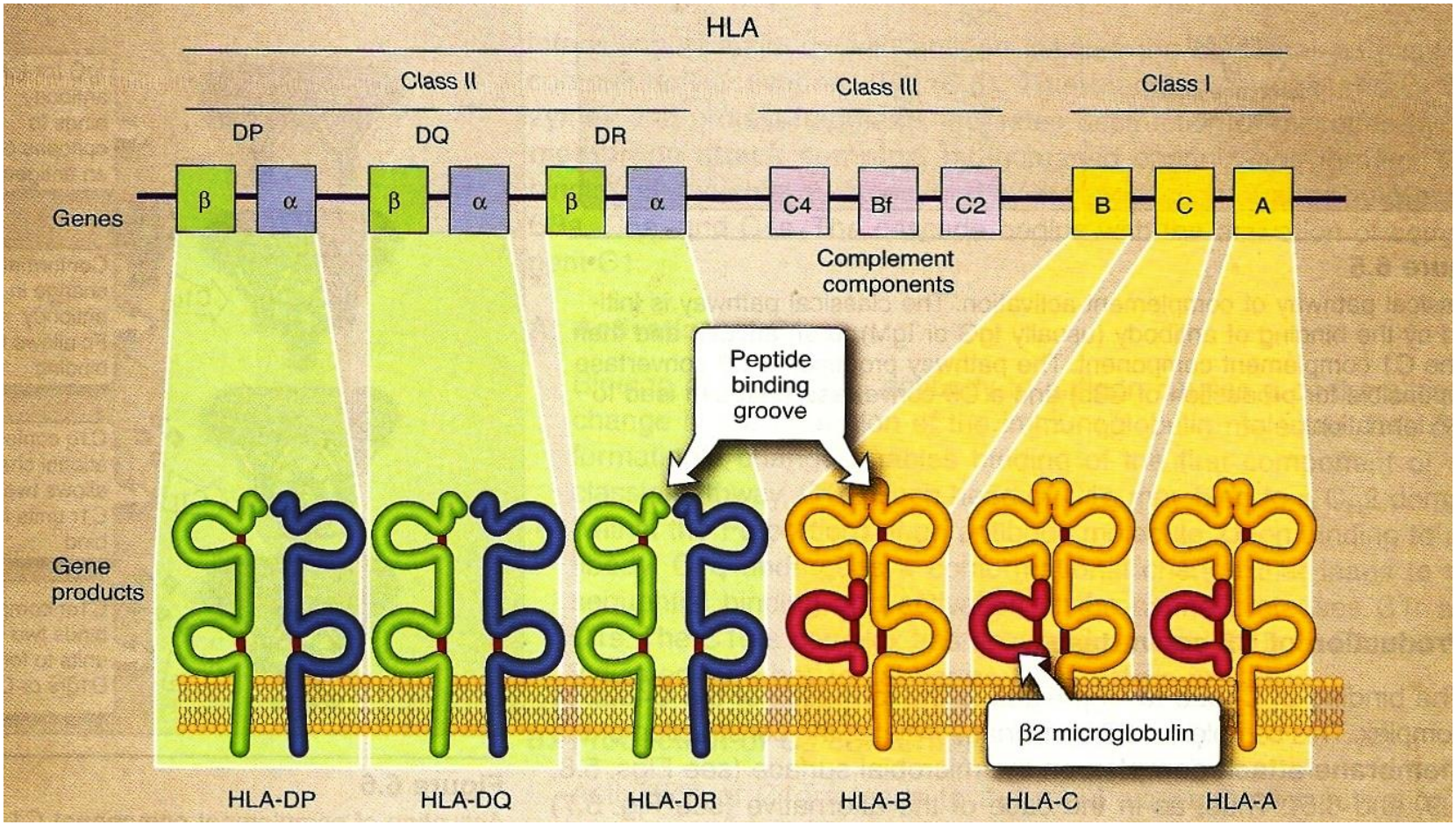
- Surface molecules
- MHC class I, II and III (C4,Bf C2 – part of complement)
- **MHC I -** 3 domaines – polypeptide chains and beta2 microglobulin

- presentation of endogenous antigens to T lymphocytes.
- present on all cells with nucleus

- **MHC II -** alfa a beta polypeptid chains

- presentation of exogennous antigens
- present on macrophages, B lymphocytes and some other antigen presenting cells - APC)

Genotype and fenotype

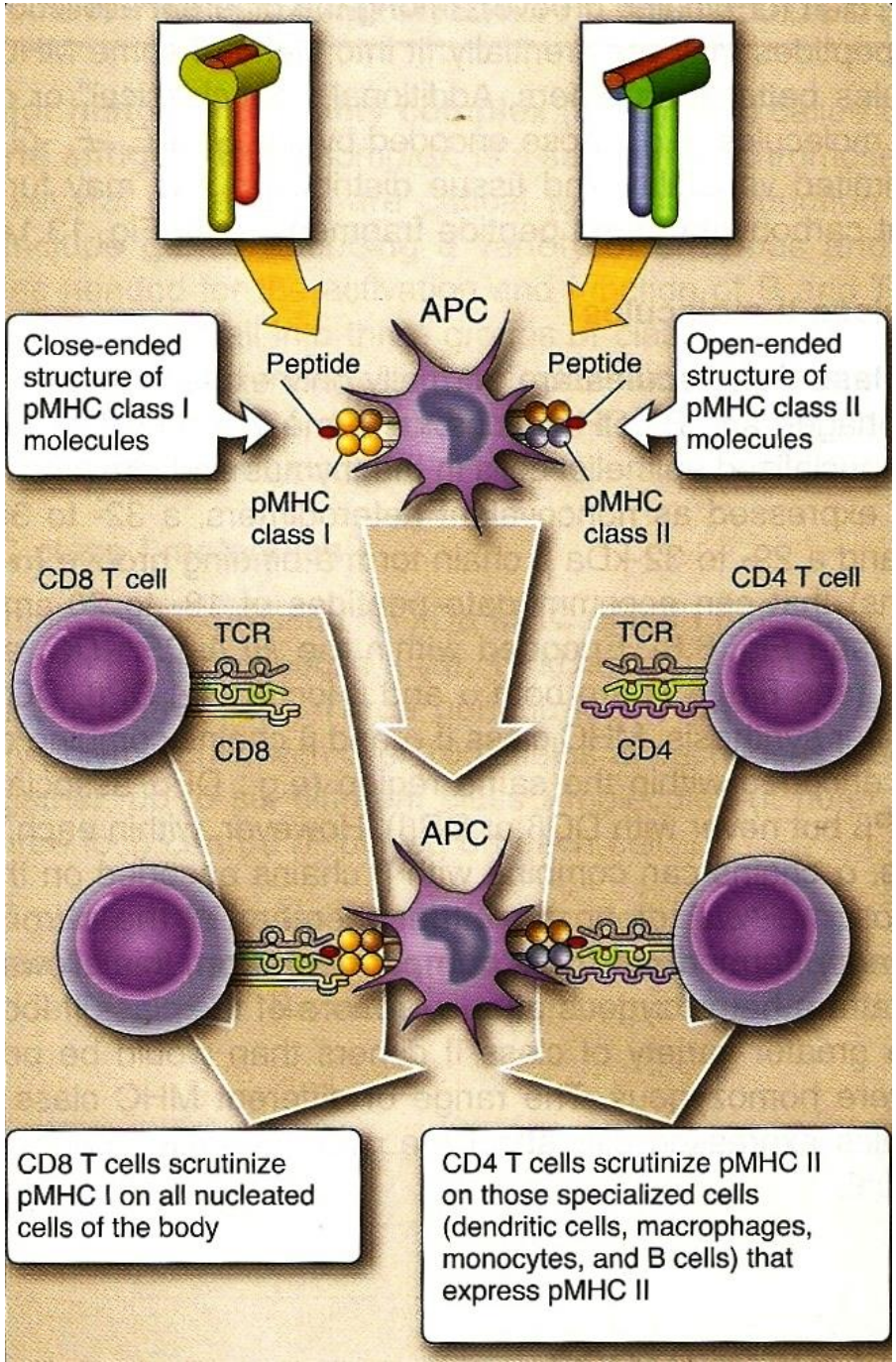


MHC I – on cells with nucleus

- polymorphisme HLA – A, - B, - C
  with more than 100 allels for each locus
- in heterosygots: one of 6 different types of molecules of MHC I (AA, AB, BB, BC,CC, AC) can be exprimed on individual cell
- MHC I forme cleft between α1- α2 domaines, that bind 8-9 aminoacides peptid.
- There are minor MHC Ib (HLA E, -F, -G, -H locuses)

MHC II – on APC cells

- dendritic, macrophage, B cells, some activated T cells, specialised epitelial cells in thymus and intestine.
- Heterodimer of α1 chain and β1chain
- Encoded in HLA-DP, -DQ, -DR segment of 6th chromosomes
- After synthesis α combines only with β chain of the same locus (DPα to DPβ) on the same (*cis*) or other (*trans*) member of chrmosome pair
- Enable a big variability of dimers of MHC II in heterosygots


APC
Close-ended structure of pMHC class I molecules
Peptide
Peptide
Open-ended structure of pMHC class II molecules
pMHC class I
pMHC class II
CD8 T cell
CD4 T cell
TCR
TCR
CD8
CD4
APC
CD8 T cells scrutinize pMHC I on all nucleated cells of the body
CD4 T cells scrutinize pMHC II on those specialized cells (dendritic cells, macrophages, monocytes, and B cells) that express pMHC II

T cell receptor, TCR

Antigen specific receptor on T cell

Heterodimer peptid pair: αβ, γδ

Differenciation of the type of heterodimer peptid pair is done early during development of T cell

Contain variable (Vα, Vβ., or Vγ, Vδ) and constant Cα, Cβ., or Cγ, Cδ) part of domains.(like in Ig molecule)

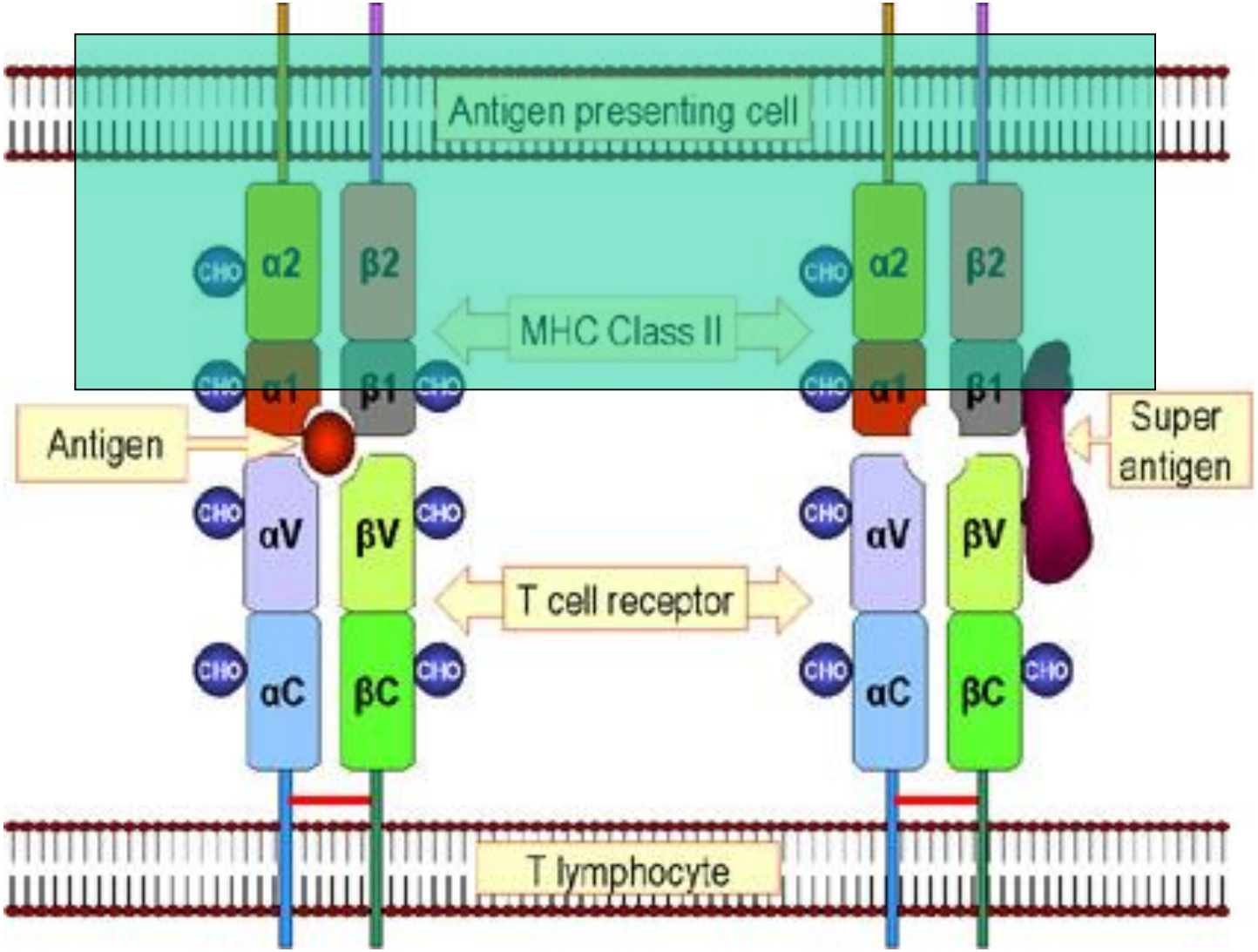
Each T cell has unique TCR (like in Ig on B cells)

TCR recognise antigen only in cleft of MHC (differ from Ig, that recognise soluble peptides)

T cell receptor - TCR

Responsible for diversity of antigen specificity (recognises) 10^{15} epitopes

- Interaction of antigen connected to *MHC II on APC – antigen presenting cell* – and TCR leads to transmission on signal via CD3 and activation of T cells for next reactions.


Antigen presenting cell
α2
β2
CHO
MHC Class II
α1
β1
Antigen
Super antigen
αV
βV
T cell receptor
αC
βC
T lymphocyte

Molecules of cell interaction

Interaction is performed:

- by direct intercellular **contacts**
- signals (**soluble molecules**) secerned, sent and received by others

Immunocompetent cells positively or negatively regulate their functions, migracte to specific places, make vital decisions.

Cytokines, Chemokines, Adhezsines, CD molecules, Signla transmission molecules

Cytokines

Biologically active substances released from specific cells influencing other cells (intercellular mediators, signals)

lymphocytes - lymphokines,

monocytes - monokines,

interleukines – communiction between leu

autocrine – influence *the same cells*,

paracrine – influence *narrow*, *by site cells*

Secreting is short-time and limited

Influence releasing and production of other cytokines – waterfall (cascade) activation and stimulation

Agonistic or antagonistic action

Cytokines acc. function

Mediators and regulators of <u>nonspecific immunity</u>

- TNF - tumor necrosis factor, interleukín -IL 1, IL 10, interferon gama IFN-gama

Mediators and regulators of <u>specific immunity</u>

-IL 2, IL 4, IL 5, IL 10, IFN gama

Stimulators of <u>hematopoesis</u>

- IL 3, colony stimulating factors CFS

Other cytokines, molecules

- Chemokines – chemoattraction based on chemical gradiet, chemotaxis
- Adhesive molecules – produce contact between cells (direct intercellular)
  – *integrines*, *selectines*, *addressines*– tissue tropismus
- CD – Cluster of differentiation molecules – on surfaces of different cells – indicators of functional capacity of cells

CD3 – transmission of signal throught cell membrane after activation of TCR

CD4 – on 2/3 T cells, Thelper, recognise antigen in MHC II

CD8 - on 1/3 mature T cells, T supressoric, T cytotoxic, recognise antigen in MHC I

Signal transmitting molecules

- Leu use surface receptors to patrol extracellulare space
- Enganement of receptor and ligand communicate signal in the cell throught the cytoplasmatic tail, that starts the cascade of chemical signals, that regulate transkription of genes in nucleus that is responsible for changes of cell activity.
- (example: molecules of JAK-STAT, Ras-MAP path)