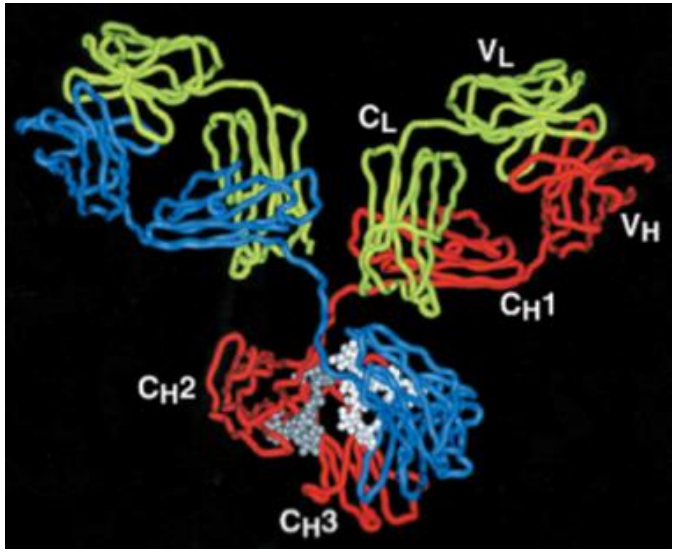


Anticorpos: Estrutura e Função
Antígenos
Reações entre Antígenos e Anticorpos
Aplicações

Profa. Isabel Santos
imsantos@fmrp.usp.br
r. 153267

A Natureza dos Anticorpos

Pesquisadores injetavam coelhos com diferentes antígenos

proteínas solúveis, bactérias, hemácias heterólogas....

Obtinham:

- Precipitinas
- Lisinas
- Aglutininas
- Opsoninas

Perguntas

Estruturas diferentes para cada função?

X

Teoria Unitária?

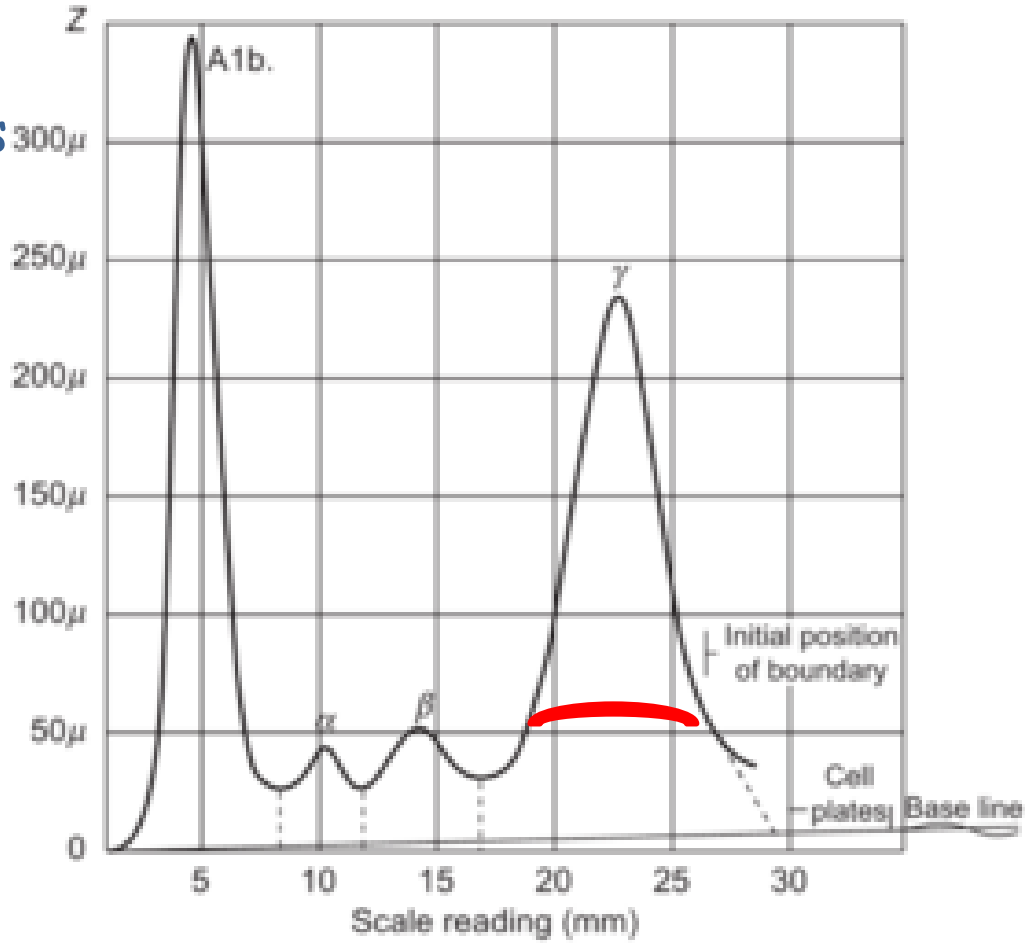


FIGURE 11.2 Electrophoretic patterns of serum from a rabbit injected with egg albumin containing ovalbumin-specific antibodies. Serum could be separated into four fractions based on electrophoretic mobility: albumin, alpha globulins, beta globulins, and gamma globulins. *From Tiselius and Kabat (1939).*

Desenvolvimento do Modelo da Imunoglobulina de Quatro Cadeias

Rodney Porter tratou anticorpos com a **enzima papaína** e obteve **três fragmentos**, dois dos quais se ligavam a antígenos, cada um contendo um único sítio de ligação, e o outro fragmento se cristalizou

George Edelman tratou anticorpos com agente redutor que **quebra pontes de dissulfeto** e obteve indícios de que os anticorpos continham ao menos dois tipos de cadeia

Nisonoff tratou tratou anticorpos com a **enzima pepsina** e obteve um fragmento com dois sítios de ligação com antígenos e vários peptídeos pequenos

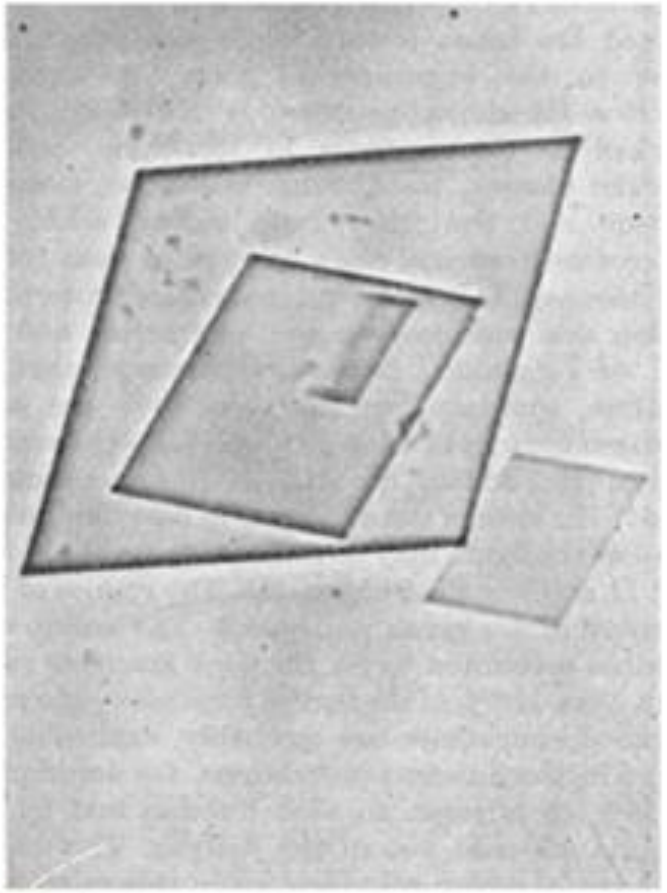
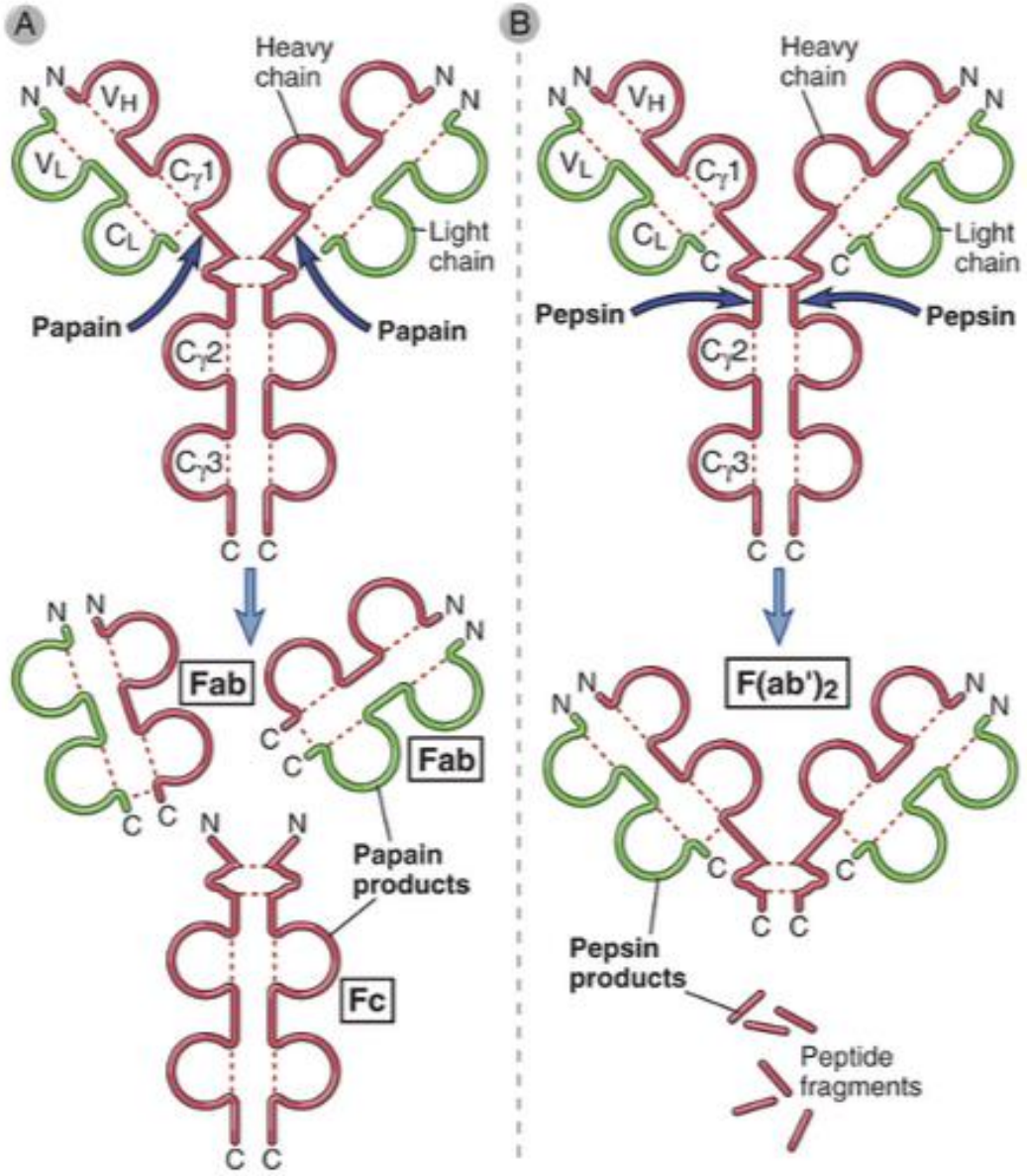
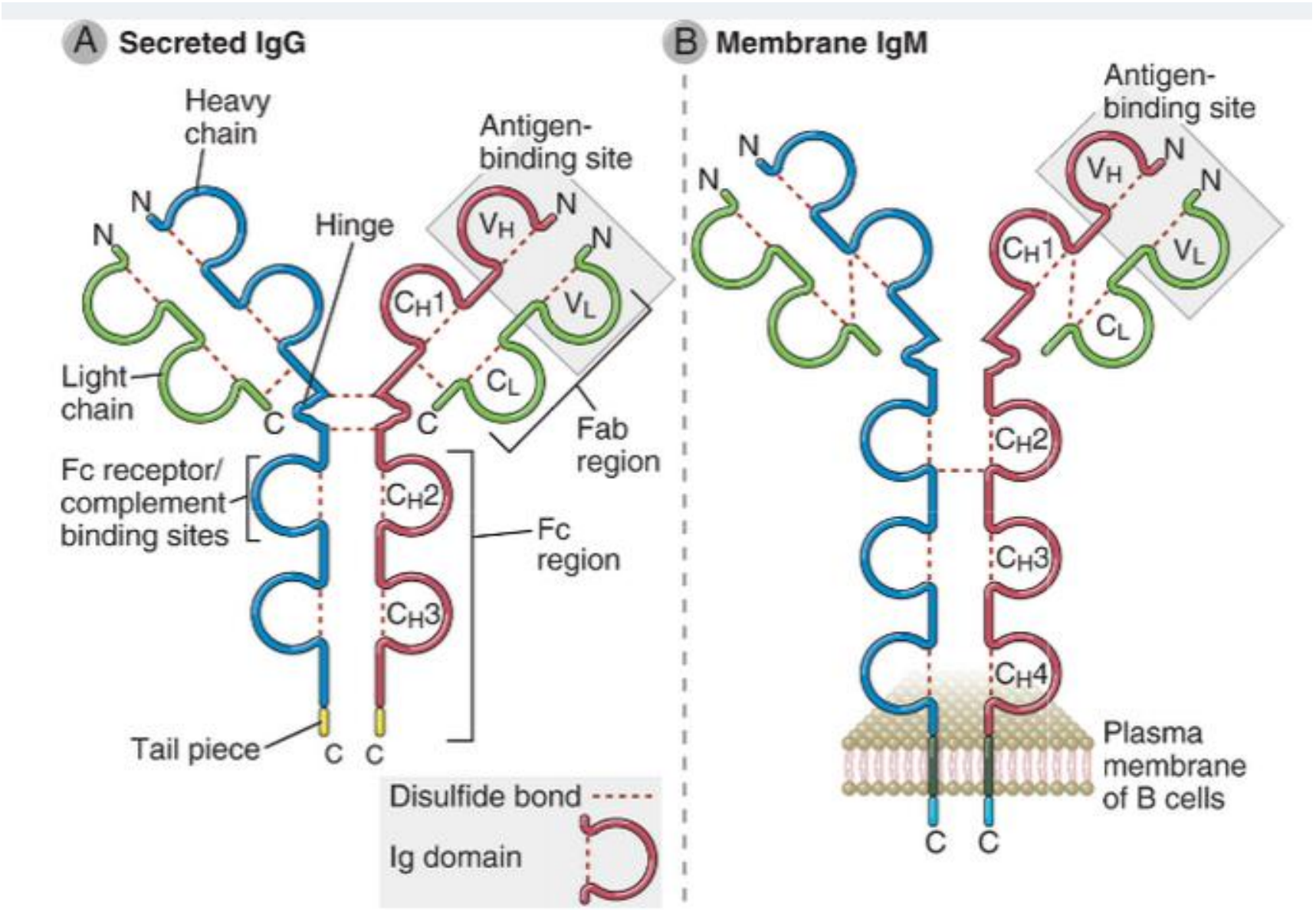


FIGURE 11.3 Crystals of fraction III of gamma globulin treated with papain. *From Porter (1959).*

Proteolytic fragments of an IgG molecule
Unraveling structure and function

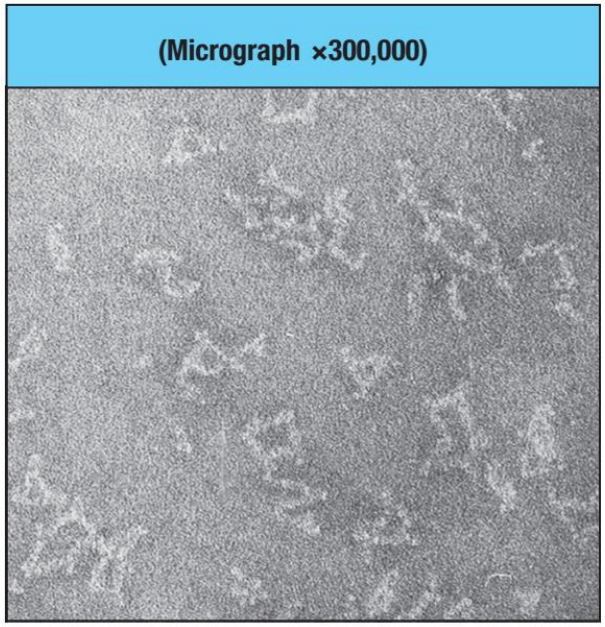


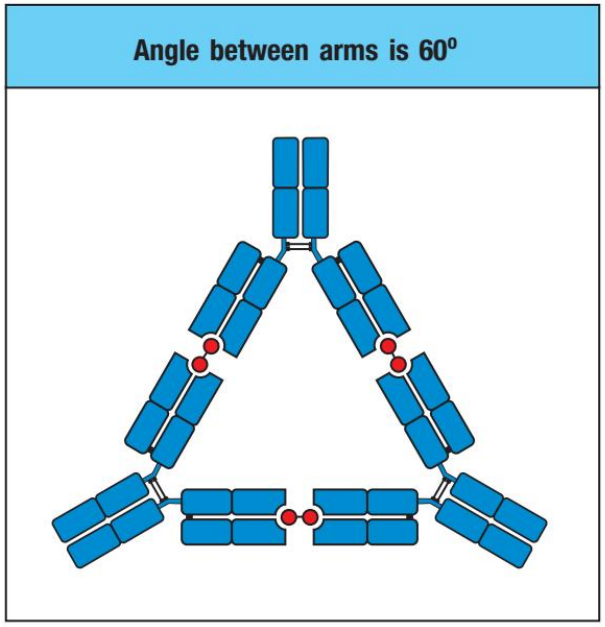
Structure of an antibody molecule.

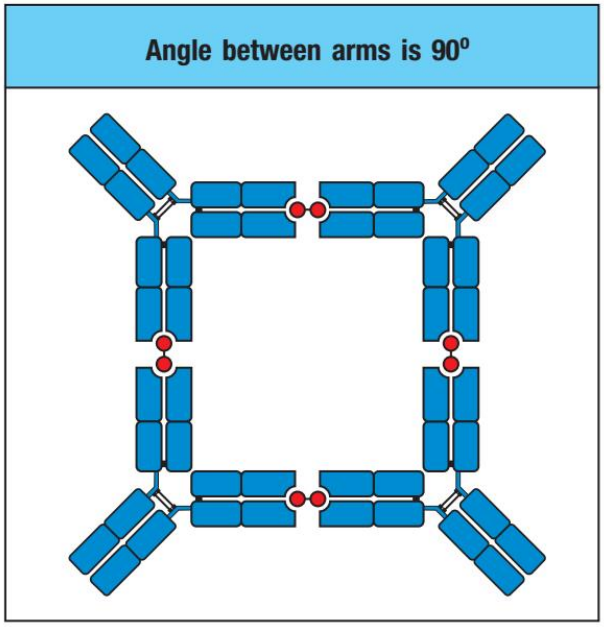


The hinge region of the immunoglobulin molecule allows flexibility in binding to multiple antigens.

Adiante: Classes e subclasses de Igs







immunoglobulin-like domains: **present in many proteins of the immune system** (KIRs of NK cells; involved in cell–cell recognition and adhesion. Together with the immunoglobulins and the T-cell receptors, these proteins make up the extensive immunoglobulin superfamily

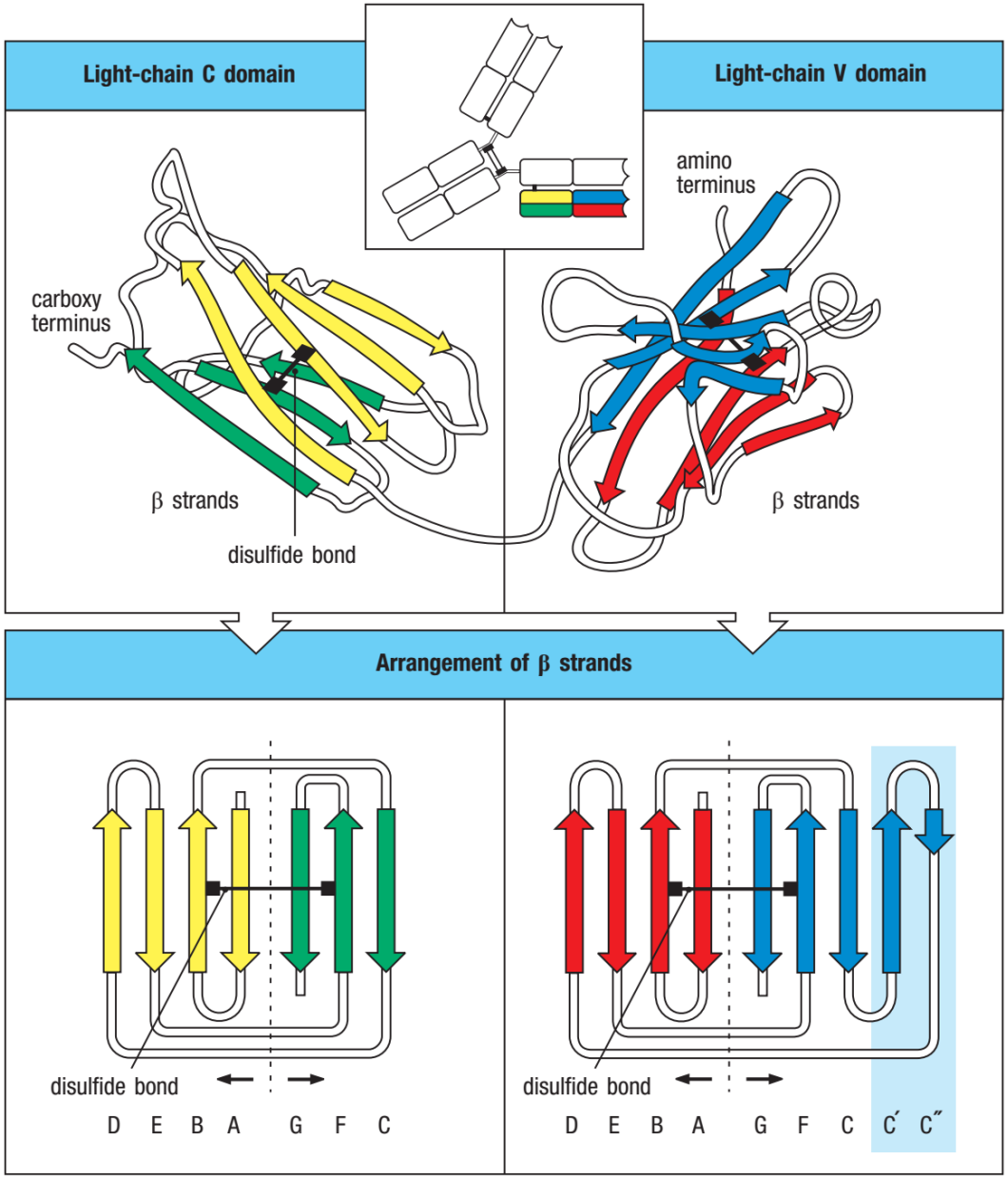
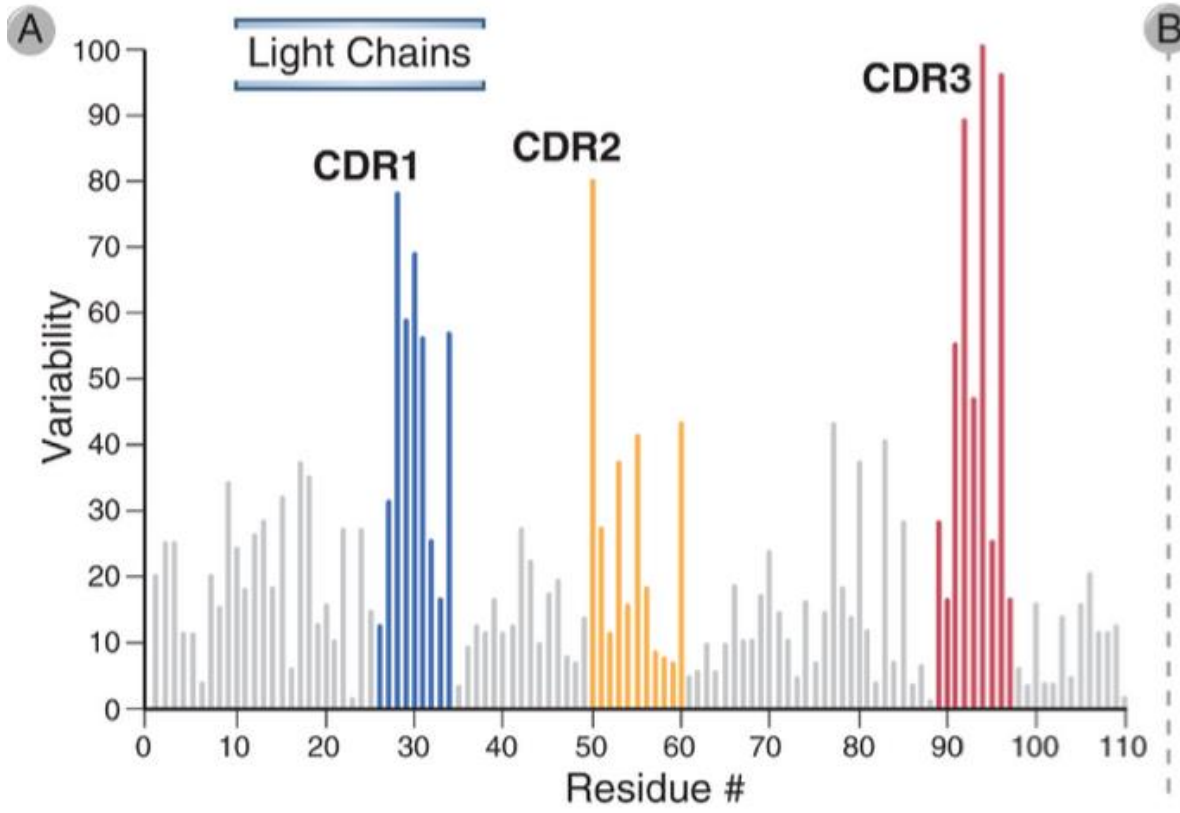


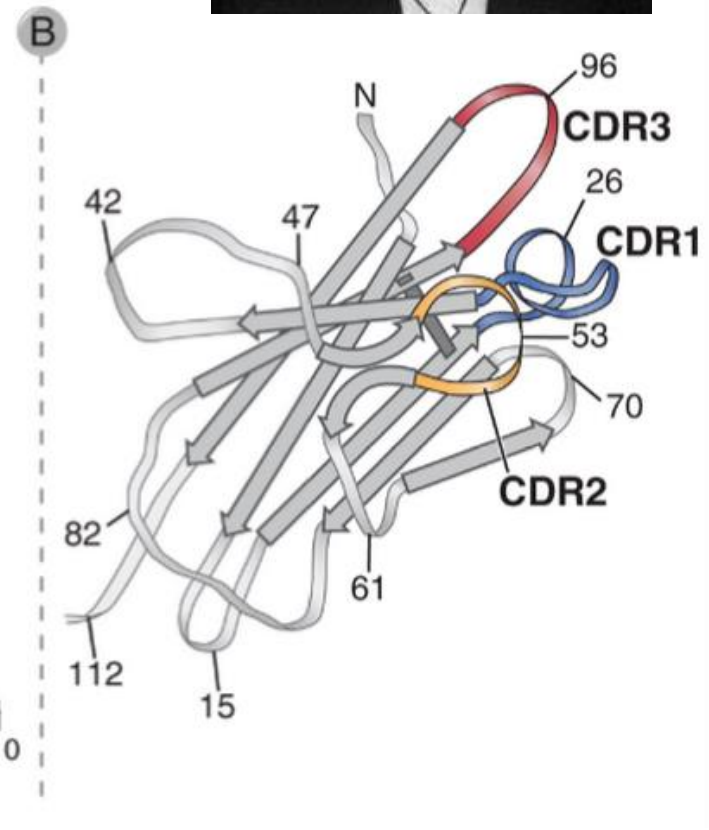
Fig. 4.3 The structure of immunoglobulin constant and variable domains. The upper panels show schematically the folding pattern of the constant (C) and variable (V) domains of an immunoglobulin light chain. Each domain is a barrel-shaped structure in which strands of polypeptide chain (β strands) running in opposite directions (antiparallel) pack together to form two β sheets (shown in yellow and green for the C domain and red and blue for the V domain), which are held together by a disulfide bond. The way in which the polypeptide chain folds to give the final structure can be seen more clearly when the sheets are opened out, as shown in the lower panels. The β strands are lettered sequentially with respect to the order of their occurrence in the amino acid sequence of the domains; the order in each β sheet is characteristic of immunoglobulin domains. The β strands C′ and C″ that are found in the V domains but not in the C domains are indicated by a blue-shaded background. The characteristic four-strand plus three-strand (C-region type domain) or four-strand plus five-strand (V-region type domain) arrangements are typical immunoglobulin superfamily domain building blocks, found in a whole range of other proteins as well as antibodies and T-cell receptors.

O sítio de ligação com antígenos

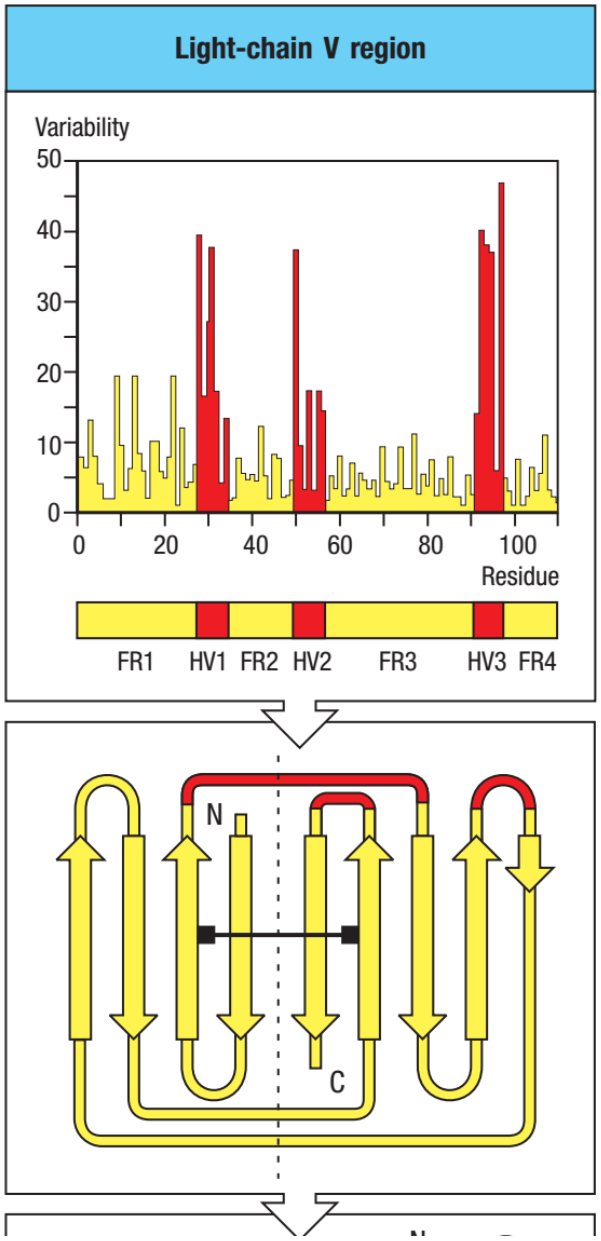
The interaction of the antibody molecule with specific antigen.

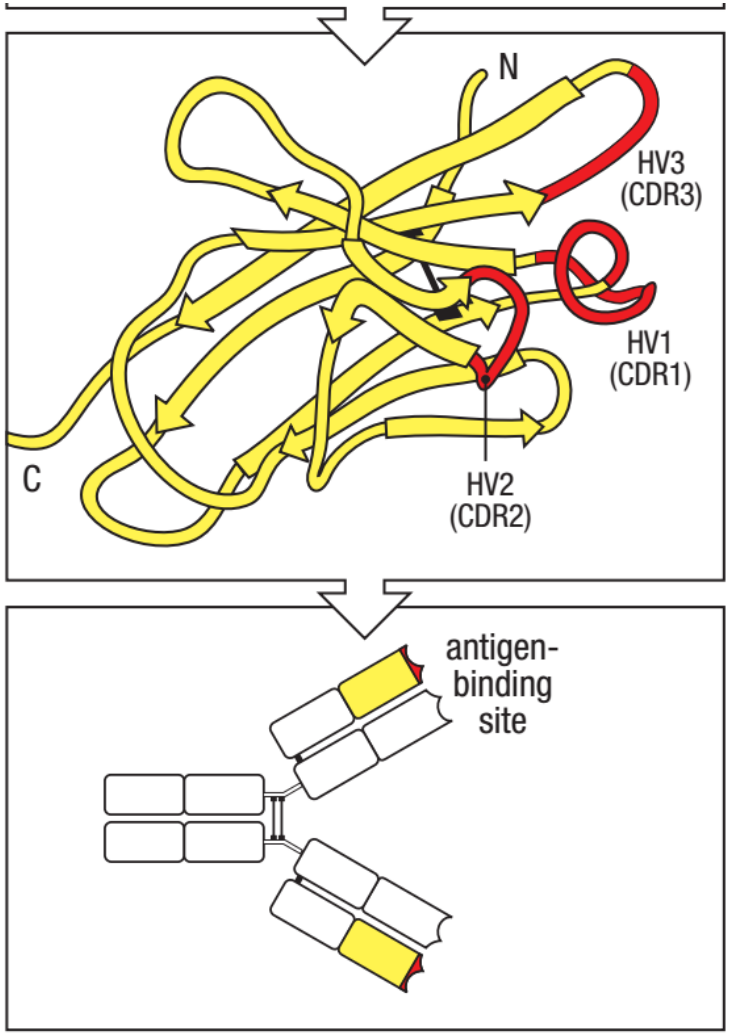
Hypervariable regions in Ig molecules
Wu-Kabat Plots



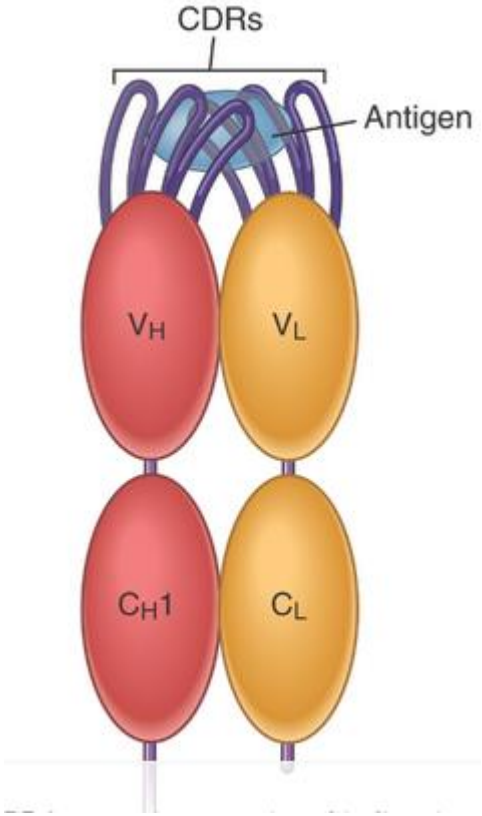


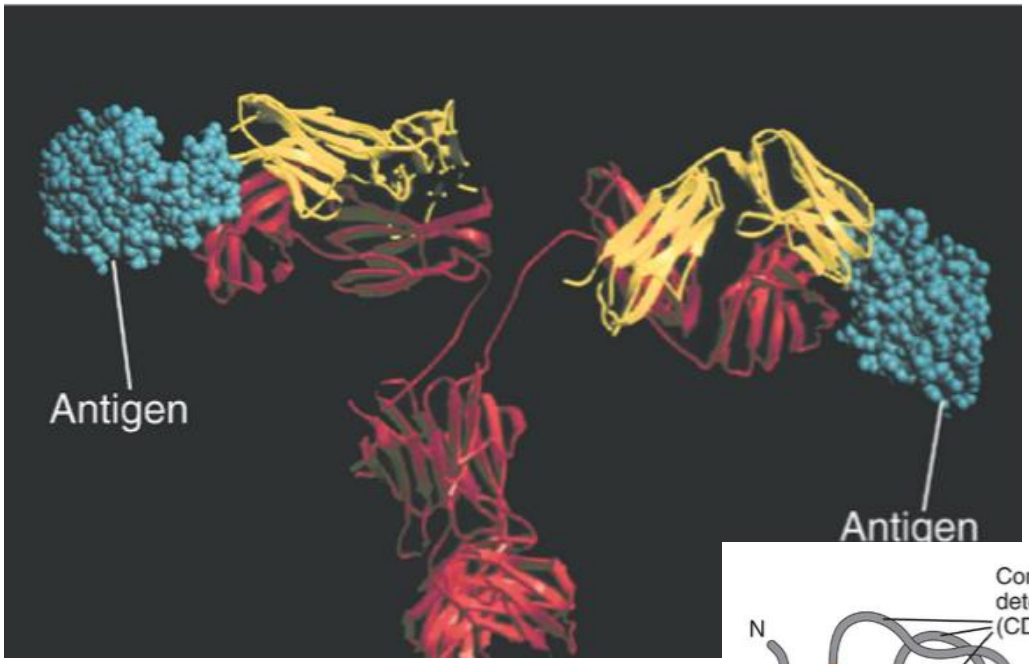
The hypervariable regions lie in discrete loops of the folded structure.

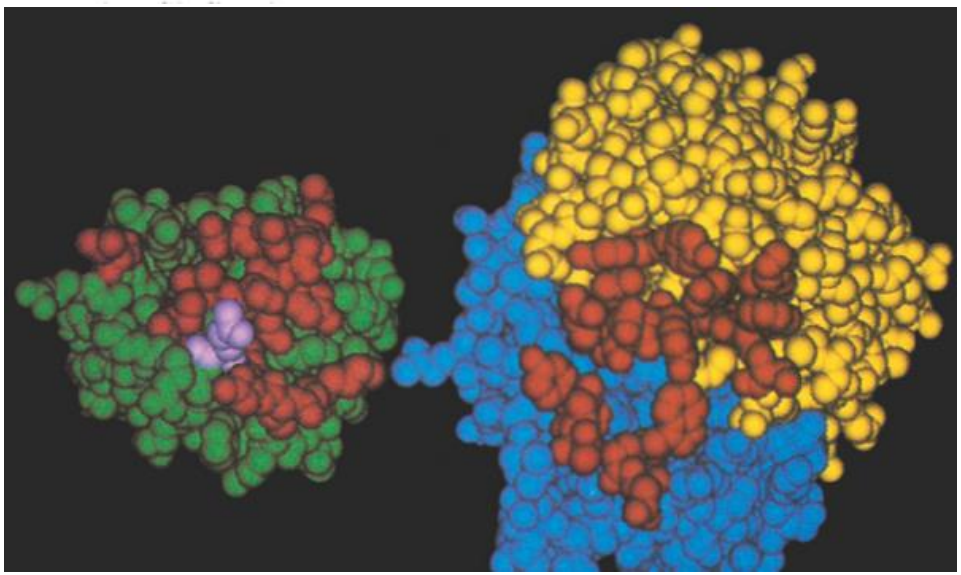


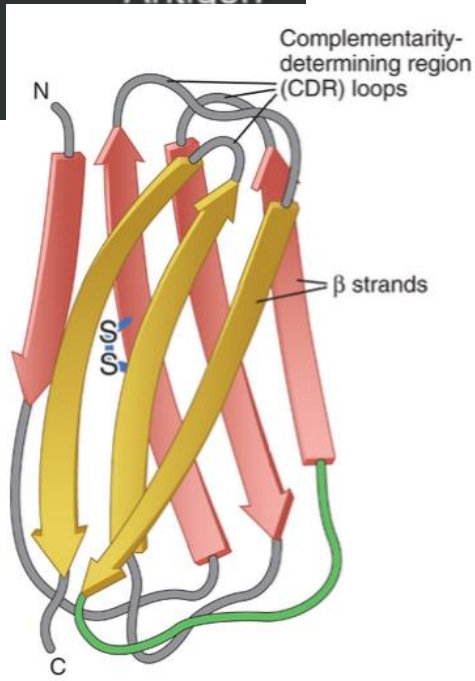


Binding of an antigen by an antibody

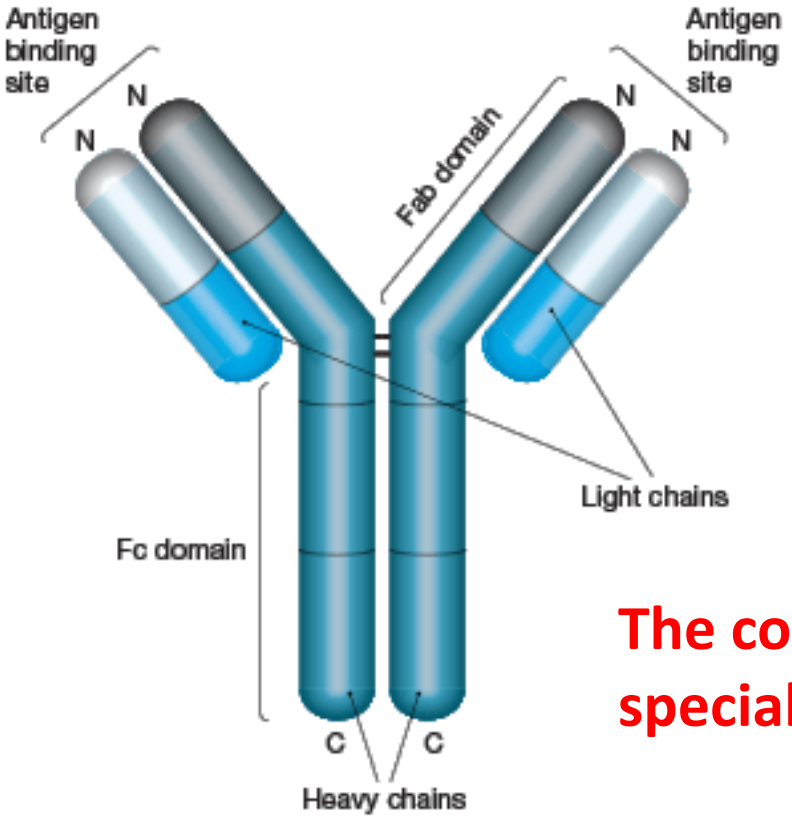




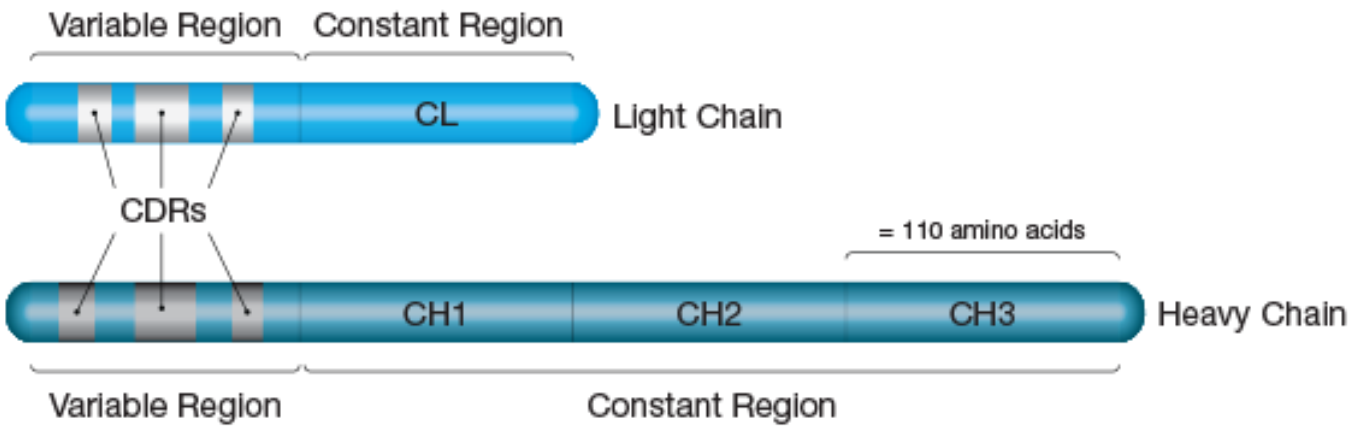




Structure of an Ig domain

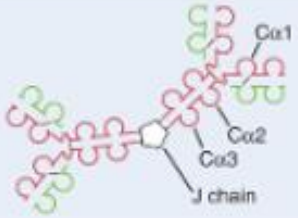
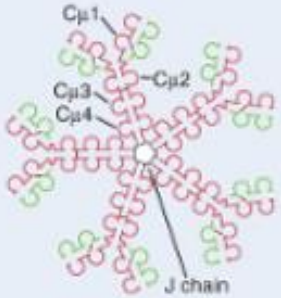


The constant region confers functional specialization on the antibody.



Human Antibody Isotypes

TABLE 5–2 Human Antibody Isotypes

Isotope of Antibody	Subtypes (H Chain)	Serum Concentration (mg/mL)	Serum Half-life (days)	Secreted Form		Functions
IgA	IgA1,2 (α1 or α2)	3.5	6	IgA (dimer) Monomer, dimer, trimer		Mucosal immunity
IgD	None (δ)	Trace	3	None		Naive B cell antigen receptor
IgE	None (ε)	0.05	2	IgE Monomer		Defense against helminthic parasites, immediate hypersensitivity
IgG	IgG1-4 (γ1, γ2, γ3, or γ4)	13.5	23	IgG1 Monomer		Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None (μ)	1.5	5	IgM Pentamer		Naive B cell antigen receptor, complement activation

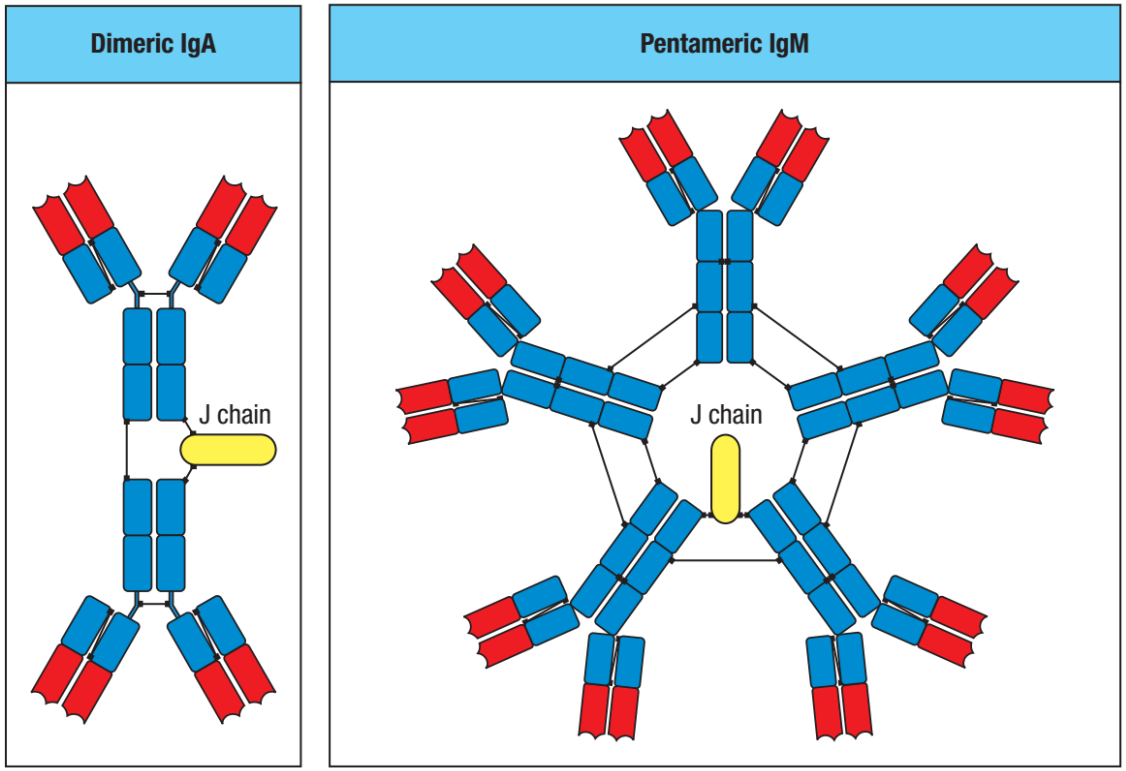


Fig. 5.23 The IgM and IgA molecules can form multimers. IgM and IgA are usually synthesized as multimers in association with an additional polypeptide chain, the J chain. In dimeric IgA (left panel), the monomers have disulfide bonds to the J chain as well as to each other. In pentameric IgM (right panel), the monomers are cross-linked by disulfide bonds to each other and to the J chain. IgM can also form hexamers that lack a J chain (not shown).

Human Antibody Isotypes

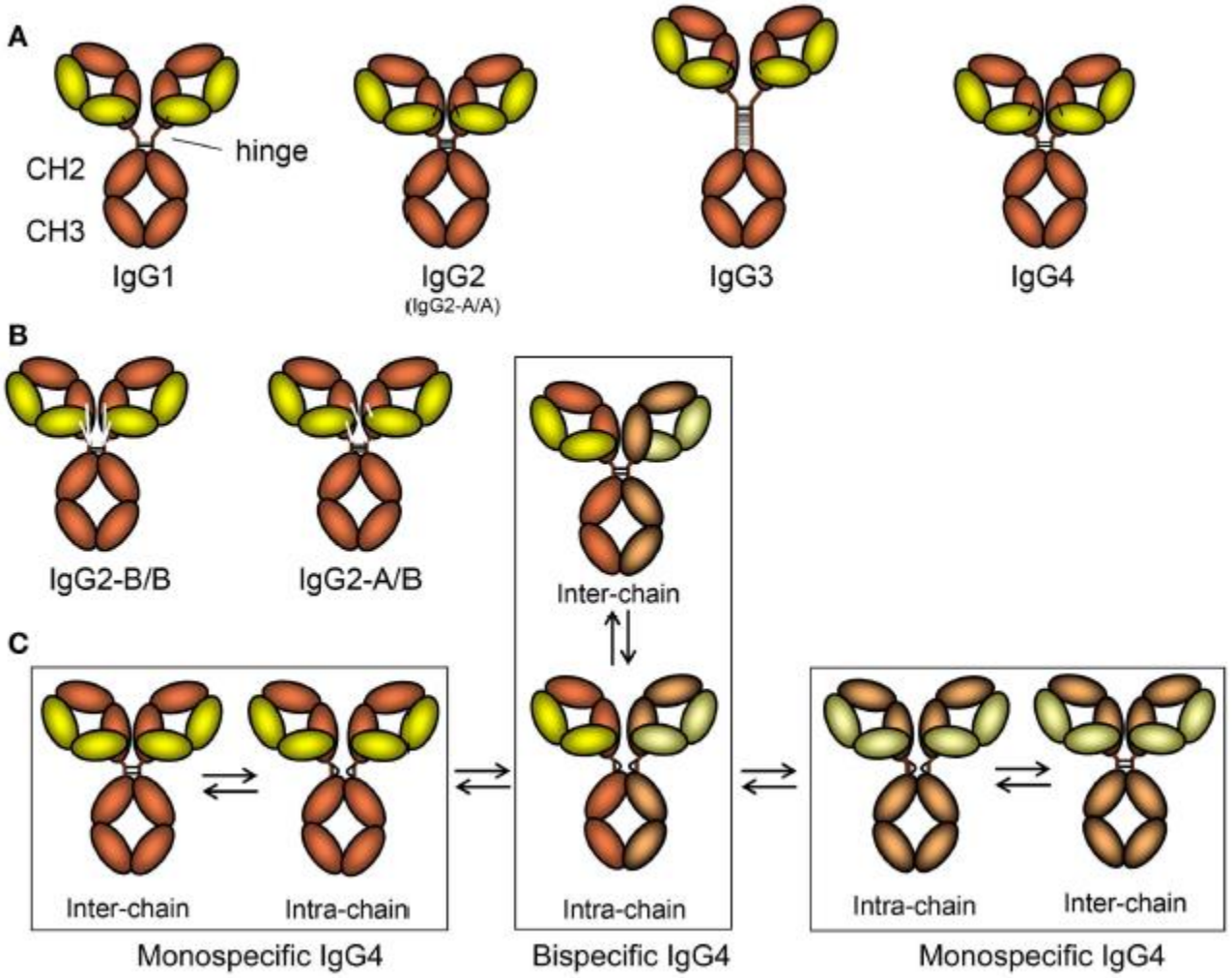
	Immunoglobulin								
	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Heavy chain	γ_1	γ_2	γ_3	γ_4	μ	α_1	α_2	δ	ε
Molecular weight (kDa)	146	146	165	146	970	160	160	184	188
Serum level (mean adult mg/ml)	9	3	1	0.5	1.5	3.0	0.5	0.03	5×10^{-5}
Half-life in serum (days)	21	20	7	21	10	6	6	3	2
Classical pathway of complement activation	++	+	+++	–	++++	–	–	–	–
Alternative pathway of complement activation	–	–	–	–	–	+	–	–	–
Placental transfer	+++	+	++	–/+	–	–	–	–	–
Binding to macrophage and phagocyte Fc receptors	+	–	+	–/+	–	+	+	–	+
High-affinity binding to mast cells and basophils	–	–	–	–	–	–	–	–	+++
Reactivity with staphylococcal Protein A	+	+	–/+	+	–	–	–	–	–

Fig. 5.20 The physical and functional properties of the human immunoglobulin isotypes. IgM is so called because of its size: although monomeric IgM is only 190 kDa, it normally forms pentamers, known as macroglobulin (hence the M), of very large molecular weight (see Fig. 5.23). IgA dimerizes to give an approximate molecular weight of around 390 kDa in secretions. IgE antibody is associated with immediate-type hypersensitivity. When fixed to tissue mast cells, IgE has a much longer half-life than its half-life in plasma shown here. The relative activities of the various isotypes are compared for several functions, ranging from inactive (–) to most active (++++).

Table 1 | Properties of human IgG subclasses.

	IgG1		IgG2		IgG3		IgG4	
General								
Molecular mass (kD)	146		146		170		146	
Amino acids in hinge region	15		12		62[a]		12	
Inter-heavy chain disulfide bonds	2		4[b]		11[a]		2	
Mean adult serum level (g/l)	6.98		3.8		0.51		0.56	
Relative abundance (%)	60		32		4		4	
Half-life (days)	21		21		7/~21[a]		21	
Placental transfer	++++		++		++/++++[a]		+++	
Antibody response to:								
Proteins	++		+/–		++		++[e] *	
Polysaccharides	+		+++		+/–		+/–	
Allergens	+		(–)		(–)		++	
Complement activation								
C1q binding	++		+		+++		–	
Fc receptors								
FcγRI	+++[c]	65[d]	–	–	++++	61	++	34
$Fc\gamma RIIa_{H131}$	+++	5.2	++	0.45	++++	0.89	++	0.17
$Fc\gamma RIIa_{R131}$	+++	3.5	+	0.10	++++	0.91	++	0.21
FcγRIIb/c	+	0.12	–	0.02	++	0.17	+	0.20
$Fc\gamma RIIIa_{F158}$	++	1.2	–	0.03	++++	7.7	–	0.20
$Fc\gamma RIIIa_{V158}$	+++	2.0	+	0.07	++++	9.8	++	0.25
FcγRIIIb	+++	0.2	–	–	++++	1.1	–	–
FcRn (at pH < 6.5)	+++		+++		++/+++[a]		+++	

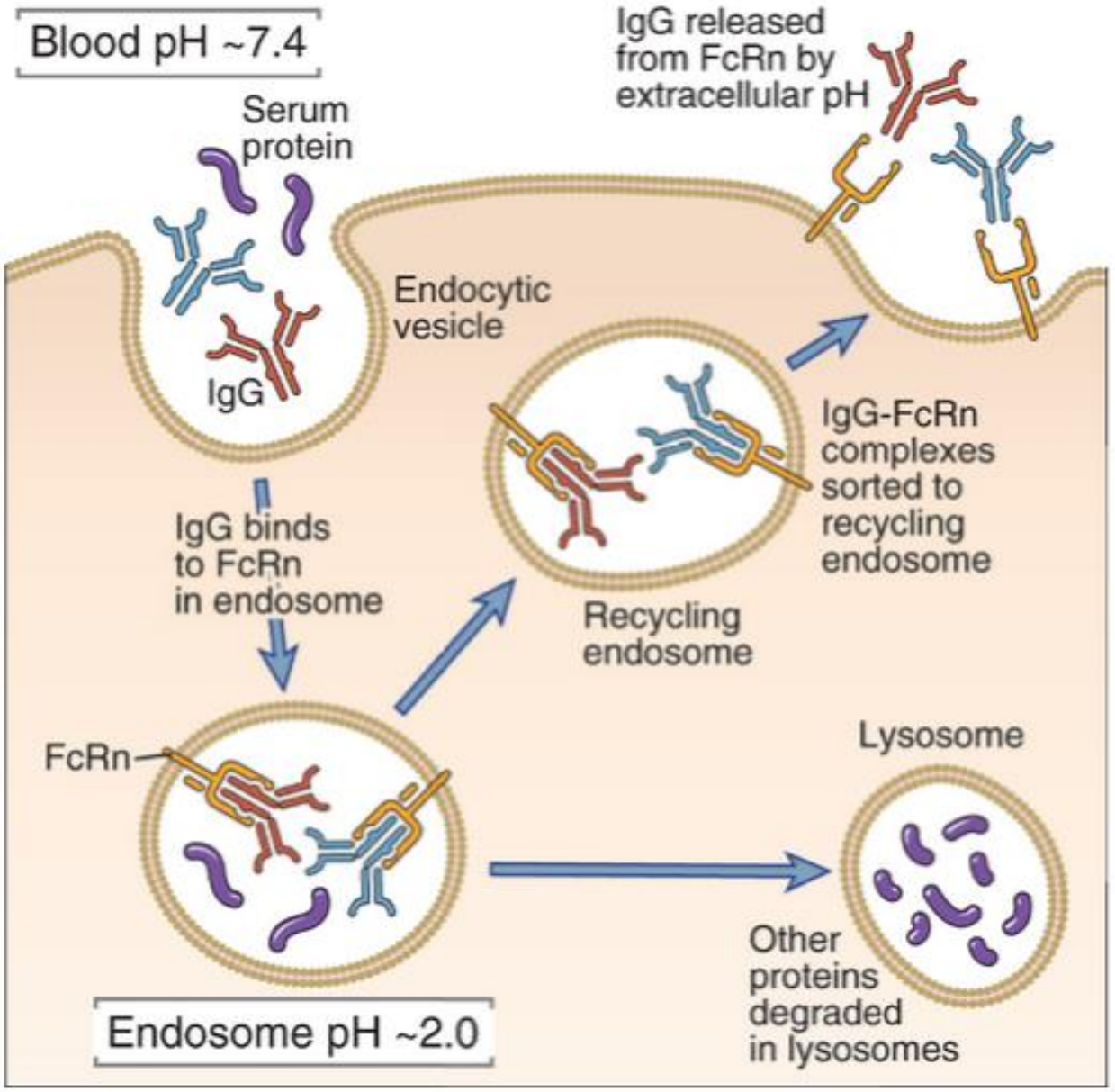
[a]Depends onallotype.
[b]ForA/Aisomer.
[c]Multivalent binding to transfected cells. Adapted from Bruhnsetal.(2).
[d]Association constant (X 10^6 M^{-1}) for monovalent binding (2).
[e]After repeated encounters with protein antigens, often allergens.


A
hinge
CH2
CH3
IgG1
IgG2
(IgG2-A/A)
IgG3
IgG4
B
IgG2-B/B
IgG2-A/B
Inter-chain
C
Inter-chain
Intra-chain
Monospecific IgG4
Intra-chain
Bispecific IgG4
Intra-chain
Inter-chain
Monospecific IgG4

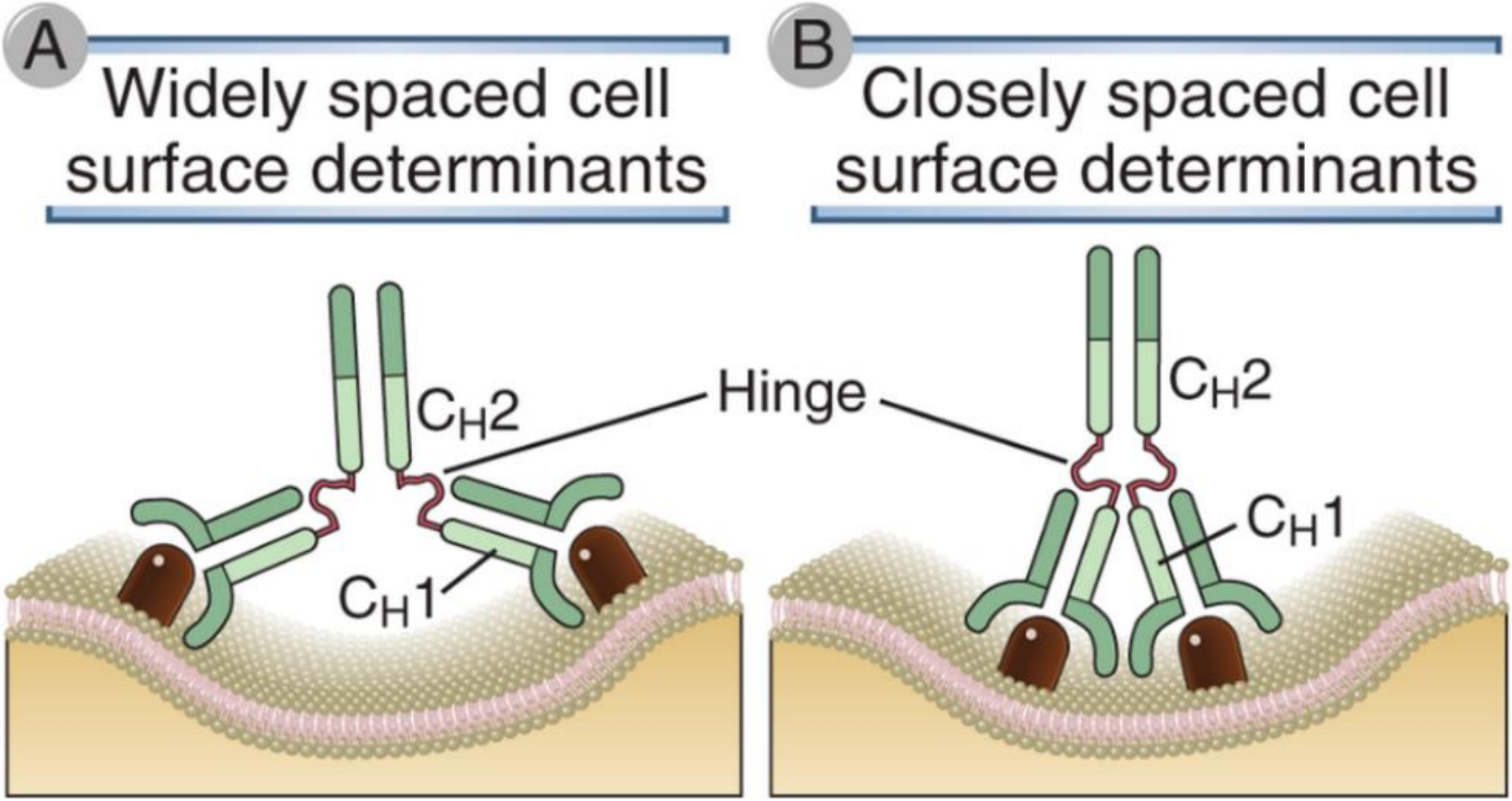
IgG facts and figures

Heavy chains:	*γ1 γ2 γ3 γ4 - Gamma 1 - 4*			
Half-life:	*IgG1* *IgG3*	*21 - 24 days* *7 - 8 days*	*IgG2* *IgG4*	*21 - 24 days* *21 - 24 days*
Serum level (mgml^{-1}):	*IgG1* *IgG3*	*5 - 12* *0.5 - 1*	*IgG2* *IgG4*	*2 - 6* *0.2 - 1*
% of Ig in serum:	*IgG1* *IgG3*	*45 - 53* *3 - 6*	*IgG2* *IgG4*	*11 - 15* *1 - 4*
Complement activation:	*IgG1* *IgG3*	*+++* *++++*	*IgG2* *IgG4*	*+* *No*
Interactions with cells:	*All subclasses via IgG receptors on macrophages and phagocytes*			
Transplacental transfer:	*IgG1* *IgG3*	*++* *++*	*IgG2* *IgG4*	*+* *++*

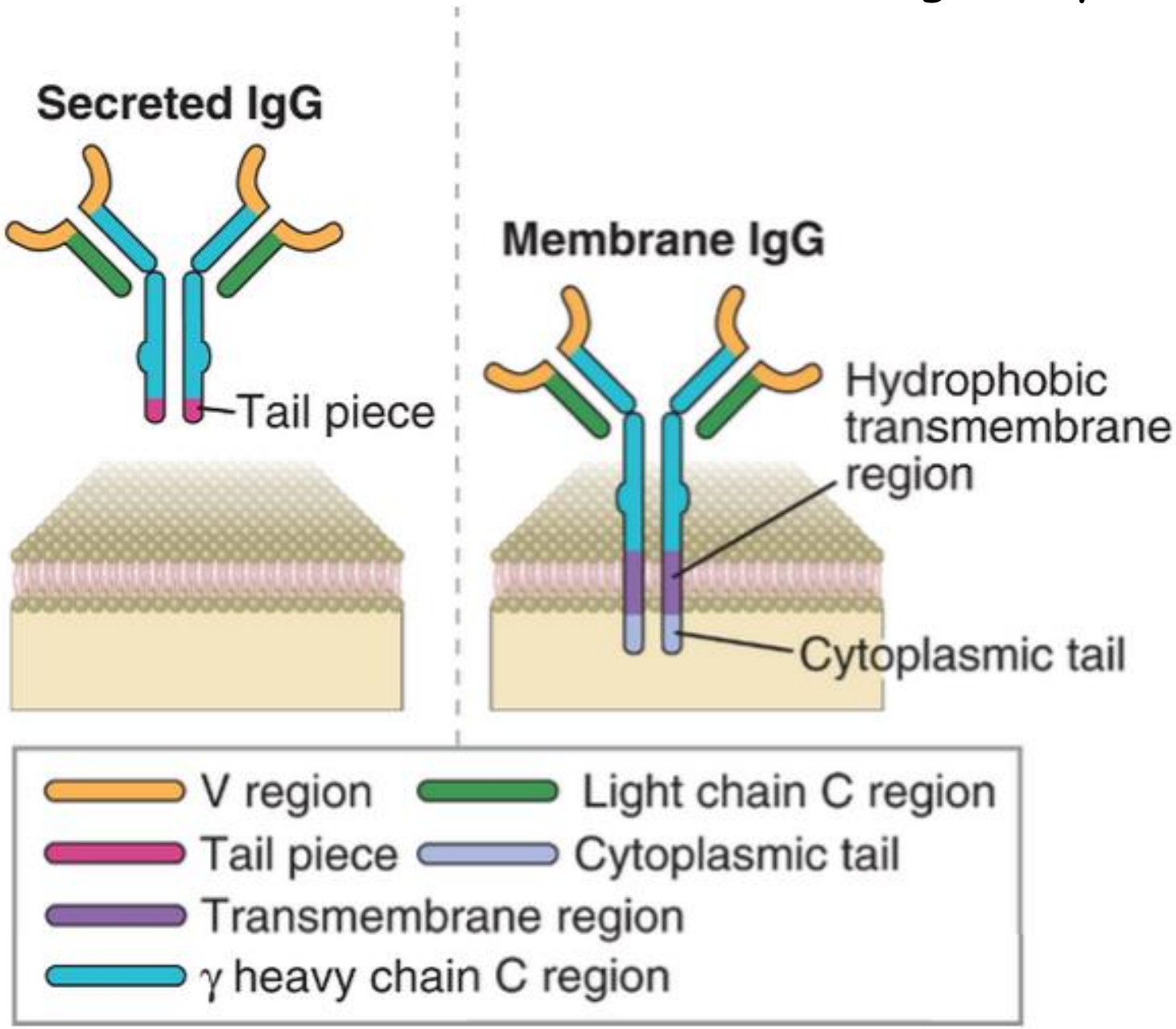
FcRn contributes to the long half-life of IgG molecules

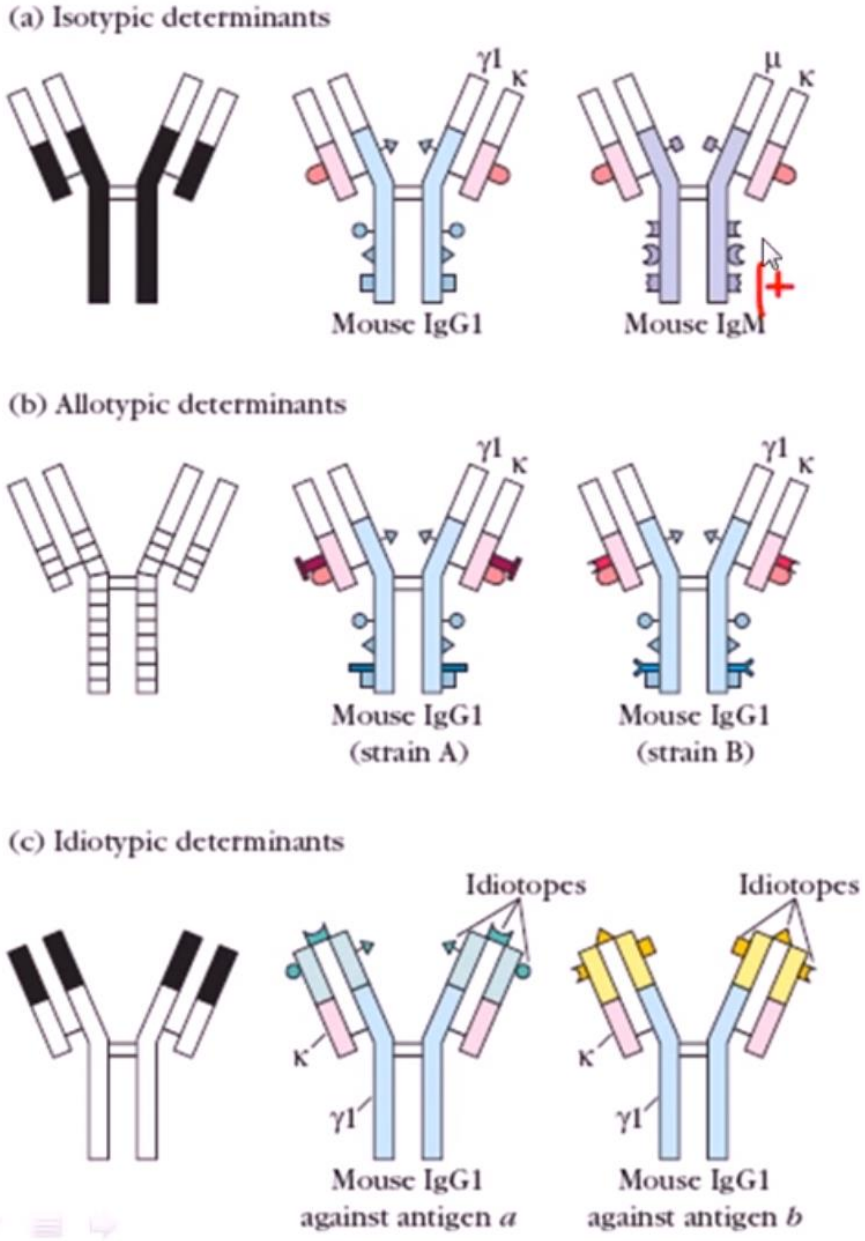


Flexibility of antibody molecules



Membrane and secreted forms of Ig heavy chains

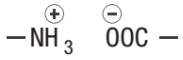
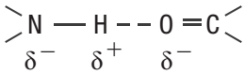
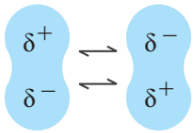
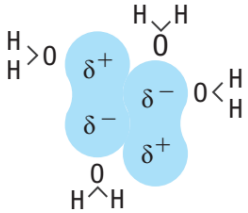
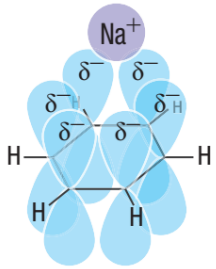




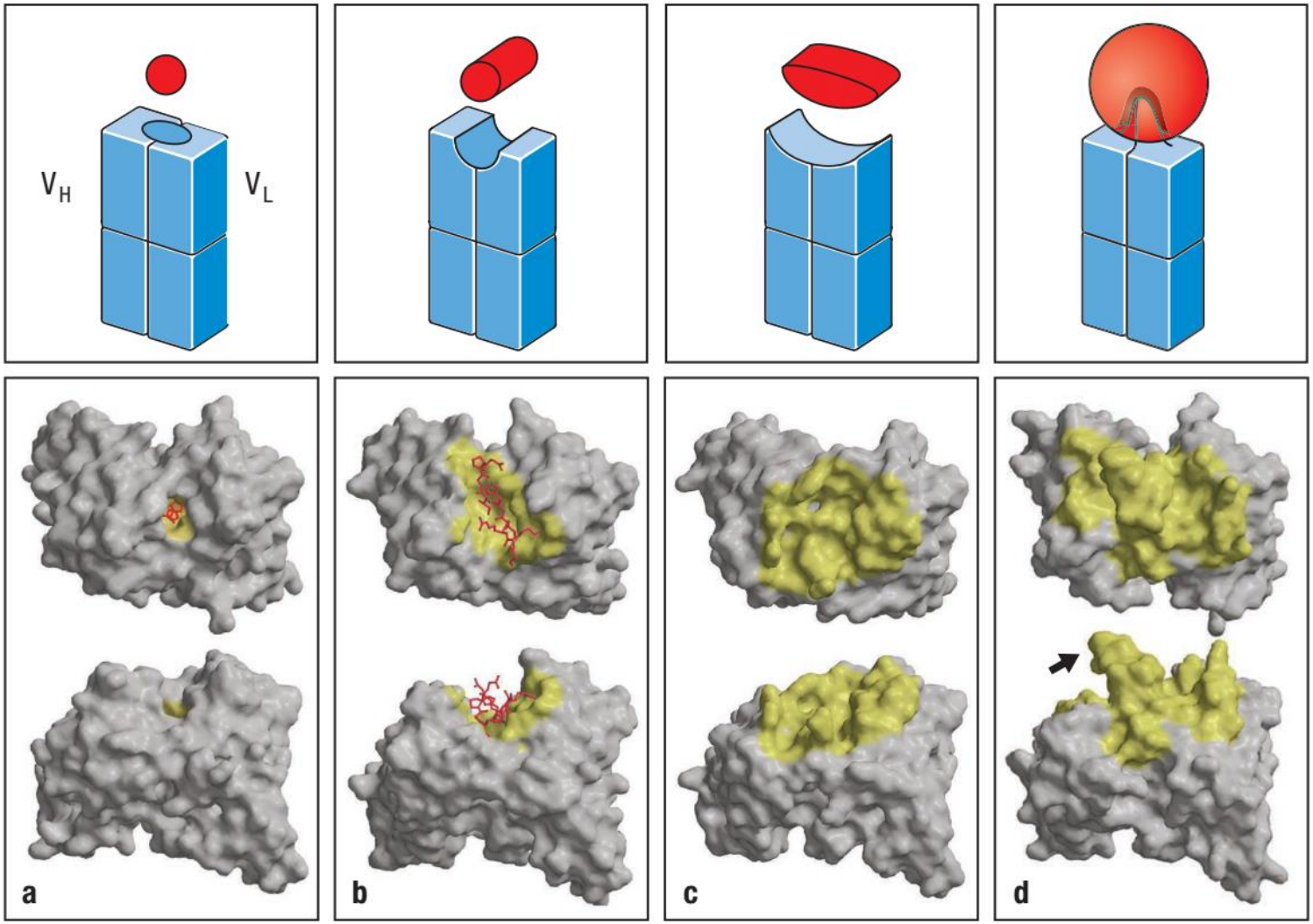
Isotypic, Allotypic and Idiotypic determinants

Idiotype network: a form of immunoregulation?

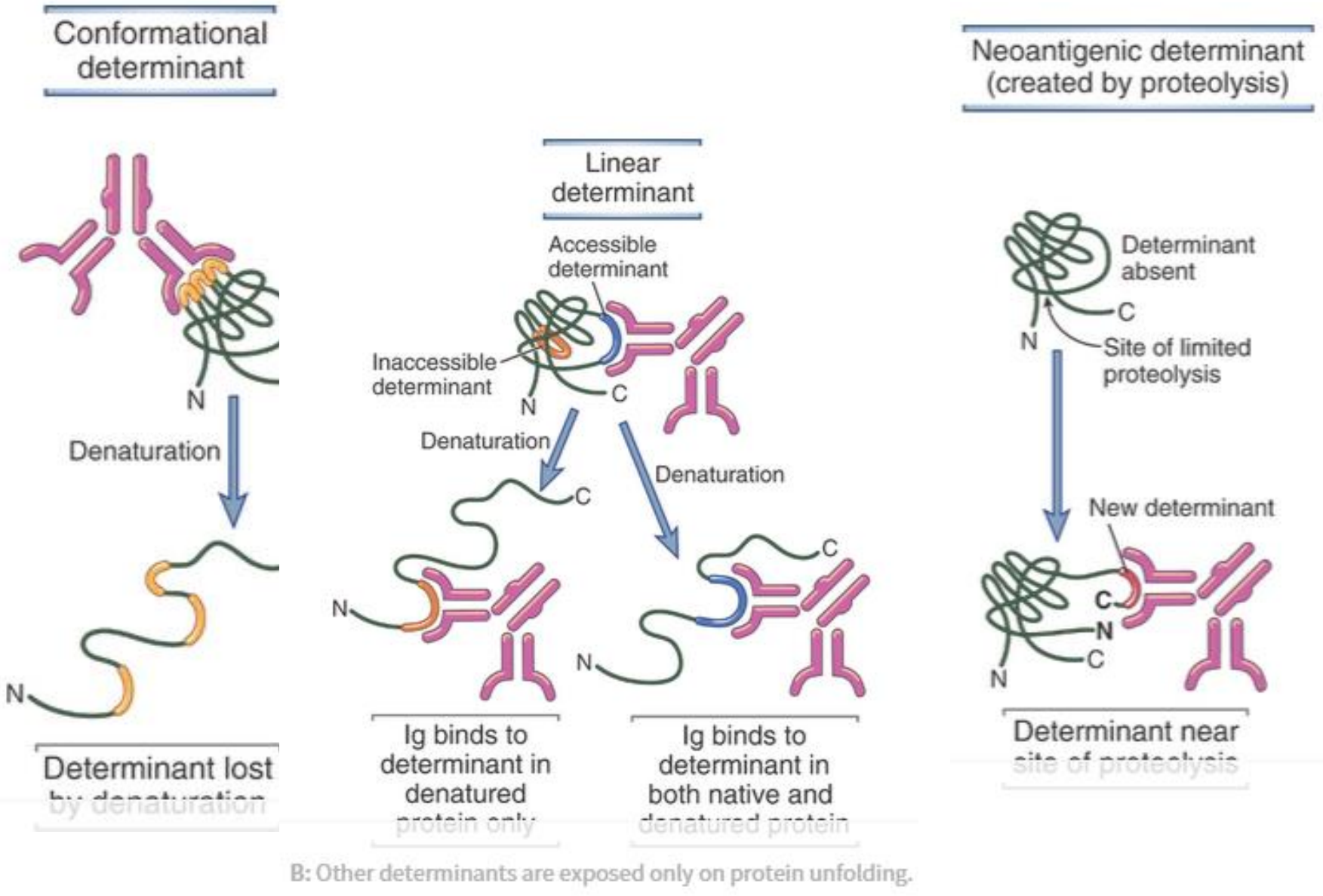
Antibodies bind to conformational shapes on the surfaces of antigens using a variety of noncovalent forces.

Noncovalent forces	Origin	
Electrostatic forces	Attraction between opposite charges	
Hydrogen bonds	Hydrogen shared between electronegative atoms (N, O)	
Van der Waals forces	Fluctuations in electron clouds around molecules polarize neighboring atoms oppositely	
Hydrophobic forces	Hydrophobic groups interact unfavorably with water and tend to pack together to exclude water molecules. The attraction also involves van der Waals forces	
Cation-pi interaction	Non-covalent interaction between a cation and an electron cloud of a nearby aromatic group	

Antigens can bind in pockets, or grooves, or on extended surfaces in the binding sites of antibodies.



The nature of antigenic determinants



B: Other determinants are exposed only on protein unfolding.

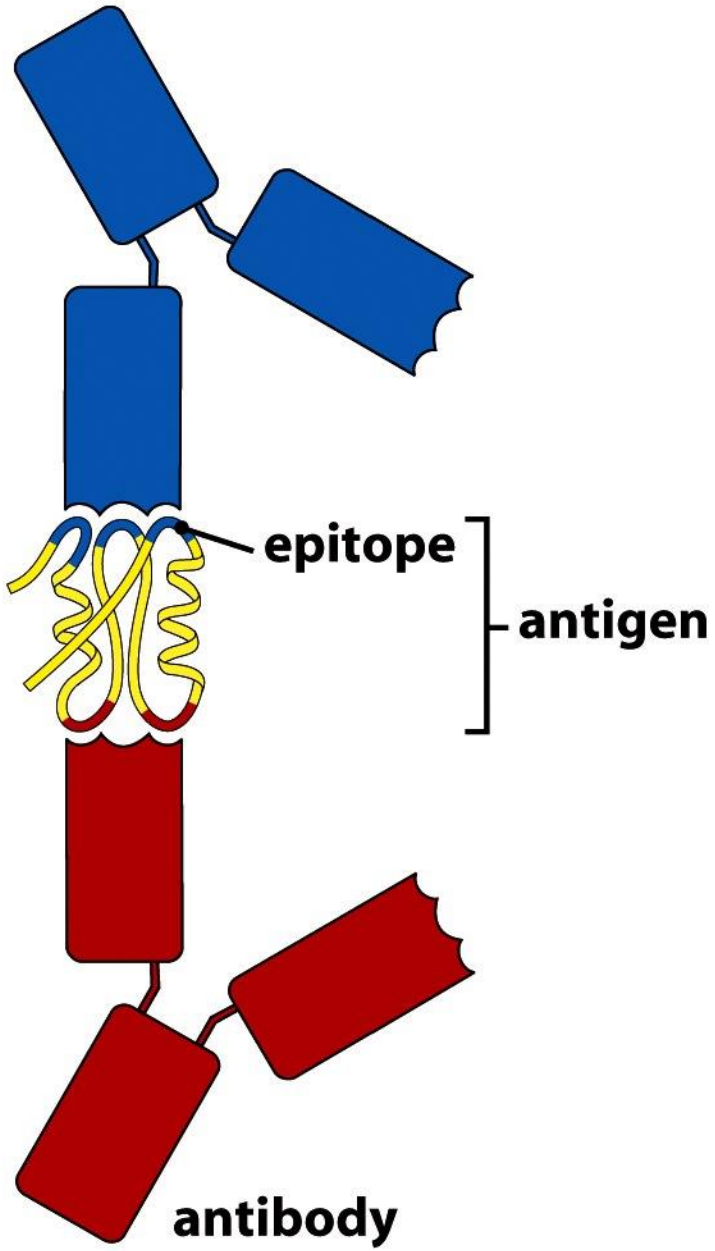
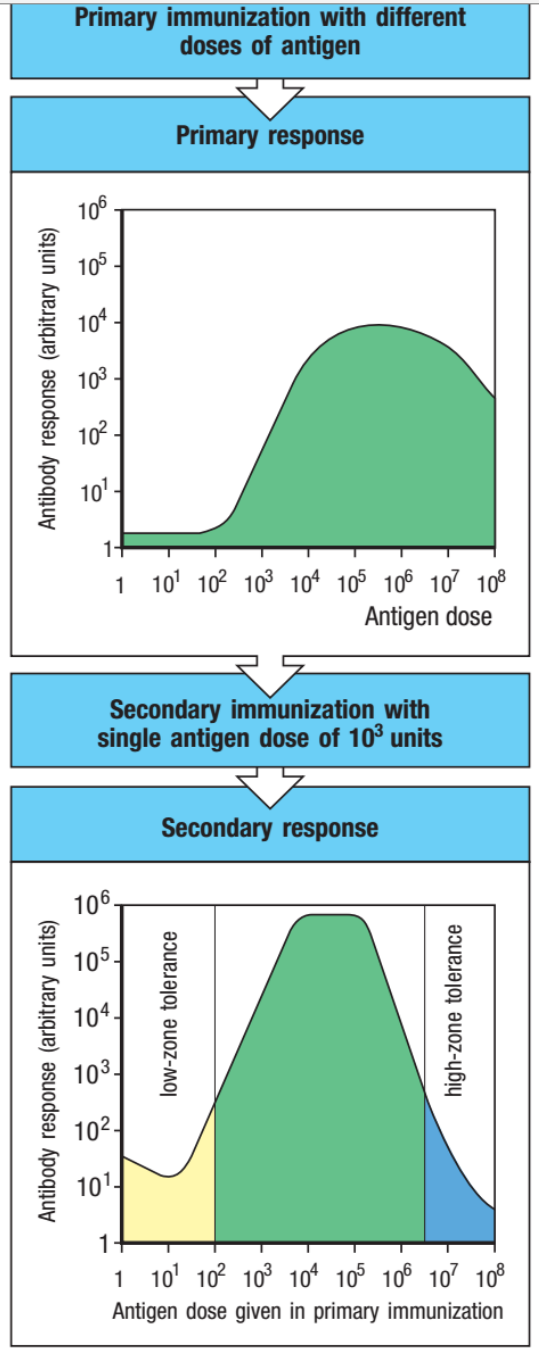
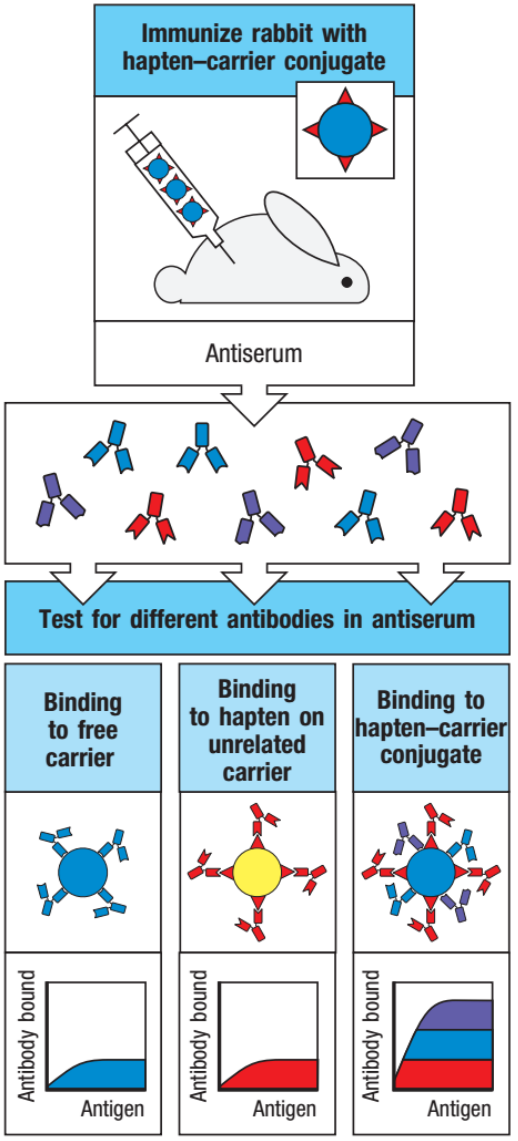


Figure 1-15 Immunobiology, 7ed. (© Garland Science 2008)



The dose of antigen used in an initial immunization affects the primary and the secondary antibody response.

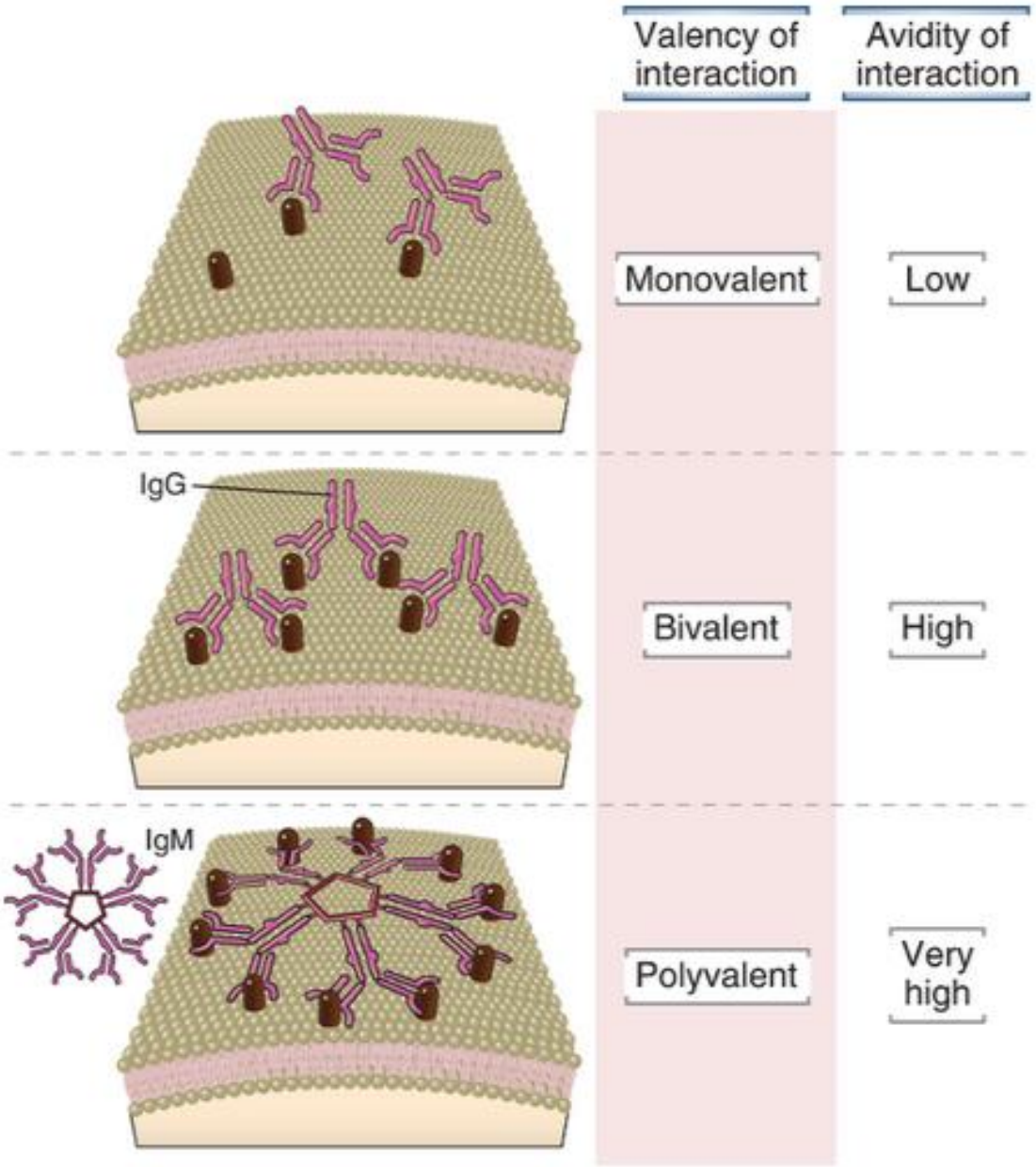


Antibodies can be elicited by small chemical groups called **haptens** only when the hapten is linked to an immunogenic protein carrier.

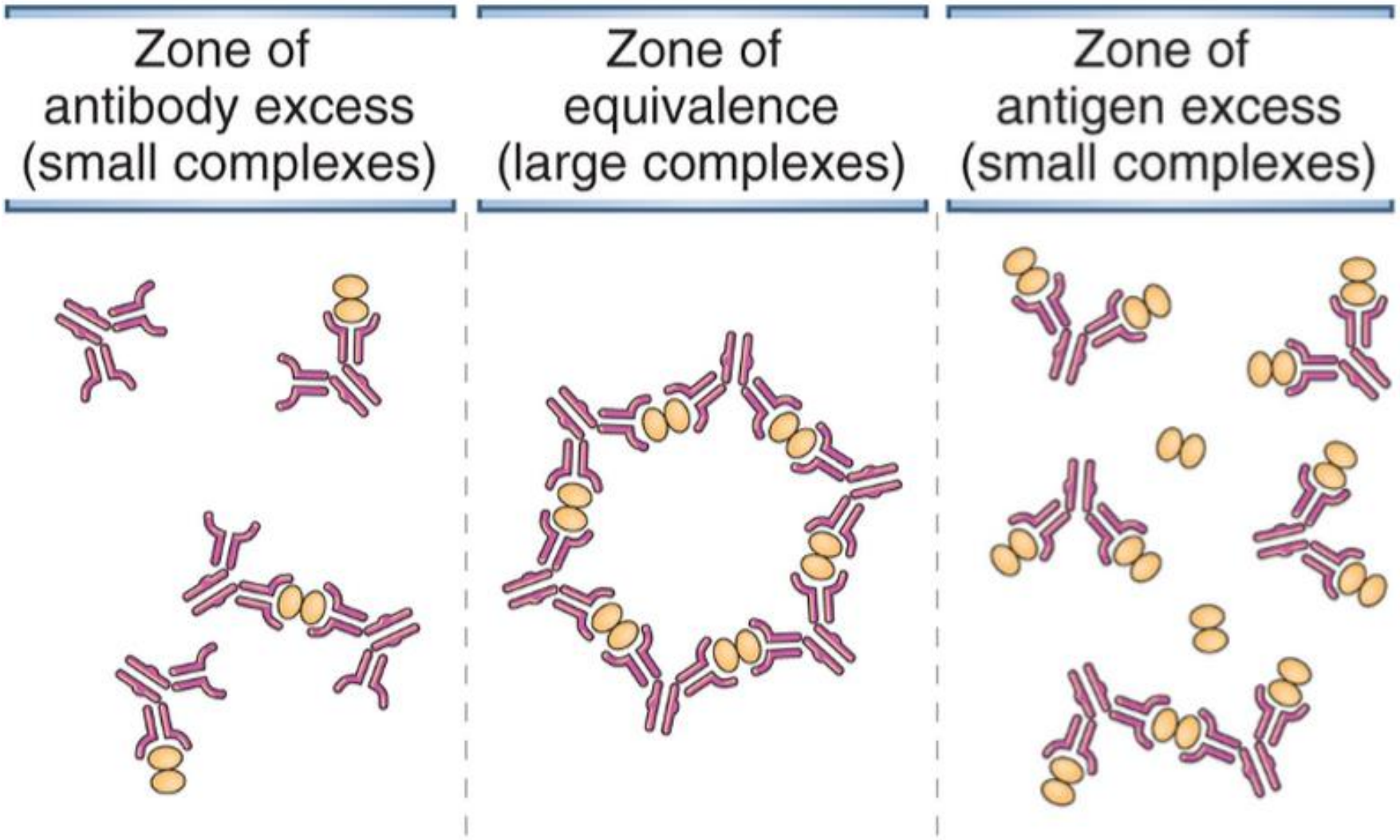
Factors that influence the immunogenicity of proteins

Parameter	Increased immunogenicity	Decreased immunogenicity
Size	Large	Small (MW<2500)
Dose	Intermediate	High or low
Route	Subcutaneous > intraperitoneal > intravenous or intragastric	
Composition	Complex	Simple
Form	Particulate	Soluble
	Denatured	Native
Similarity to self protein	Multiple differences	Few differences
Adjuvants	Slow release	Rapid release
	Bacteria	No bacteria
Interaction with host MHC	Effective	Ineffective

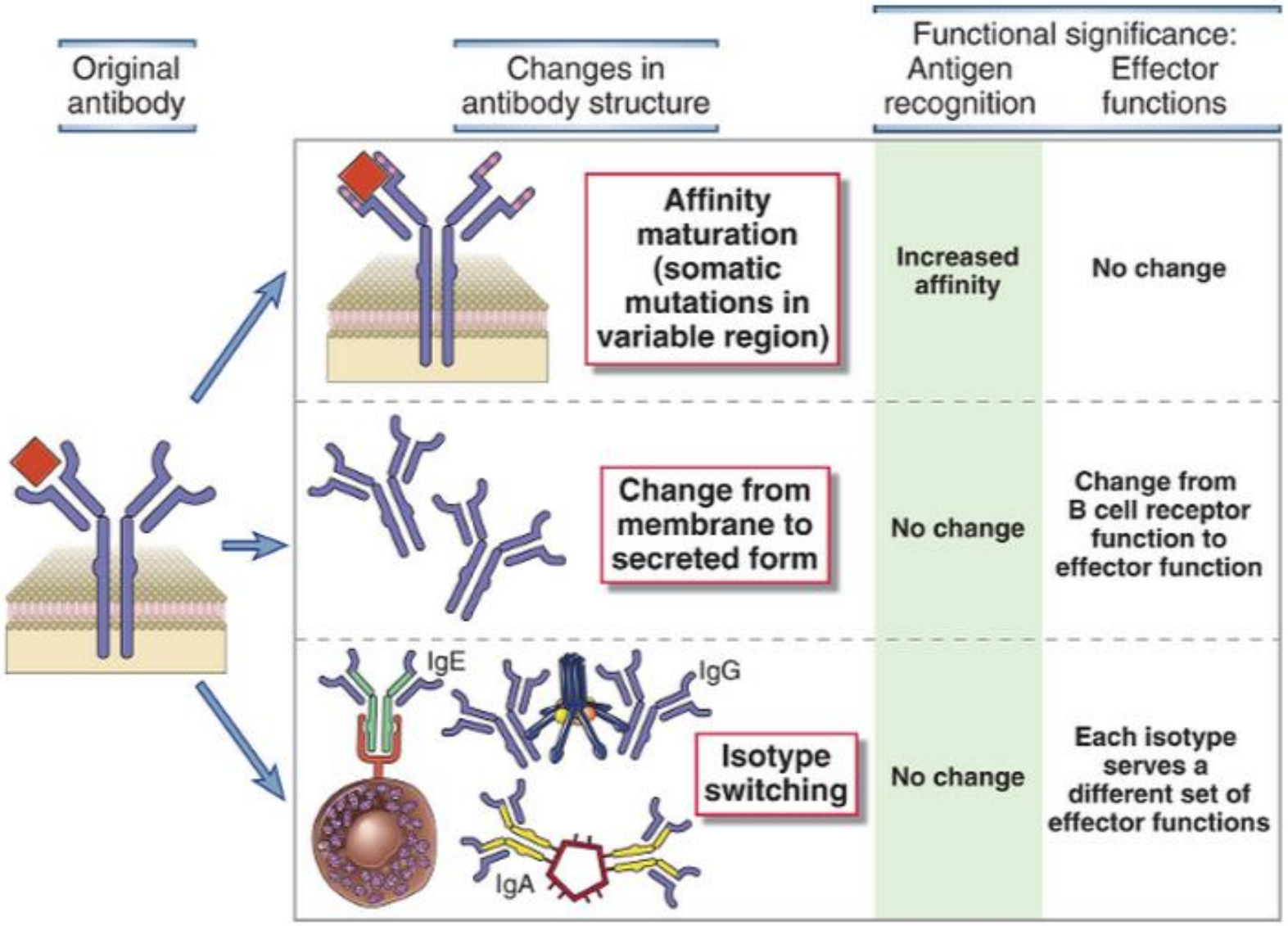
Valency and avidity of antibody-antigen interactions



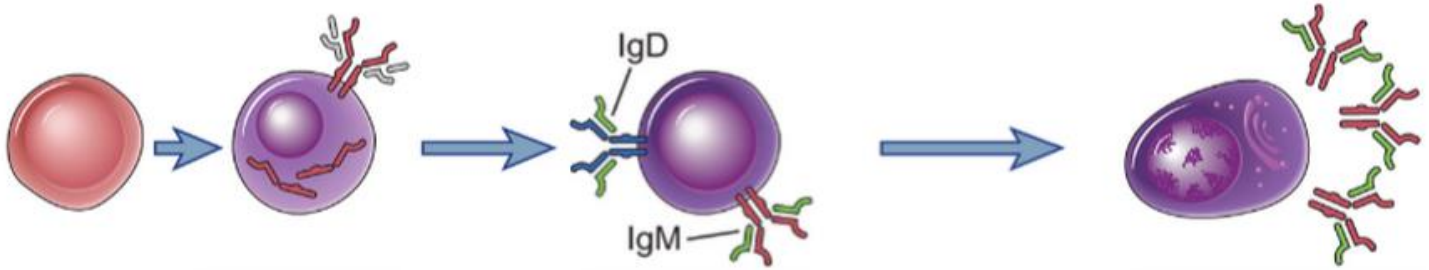
Antigen-antibody complexes
The basis of immunopathology of immune-mediated diseases



Changes in antibody structure during humoral immune responses

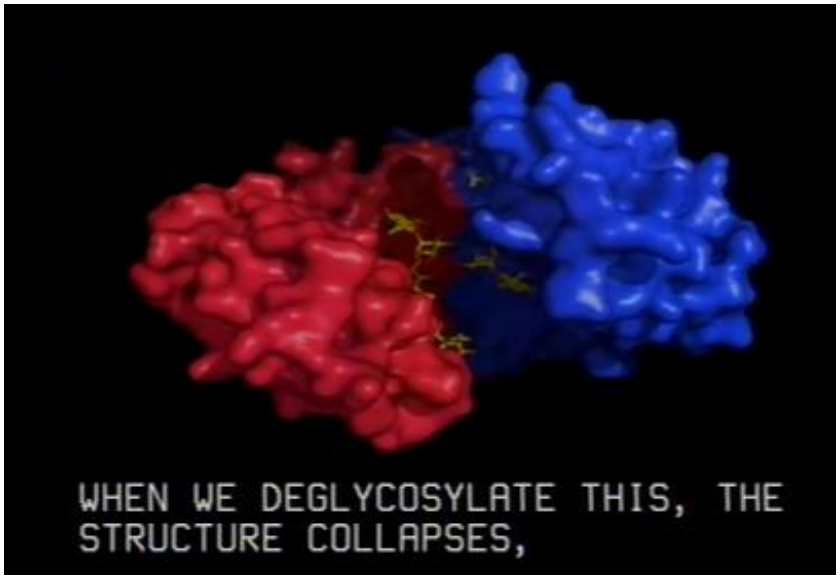


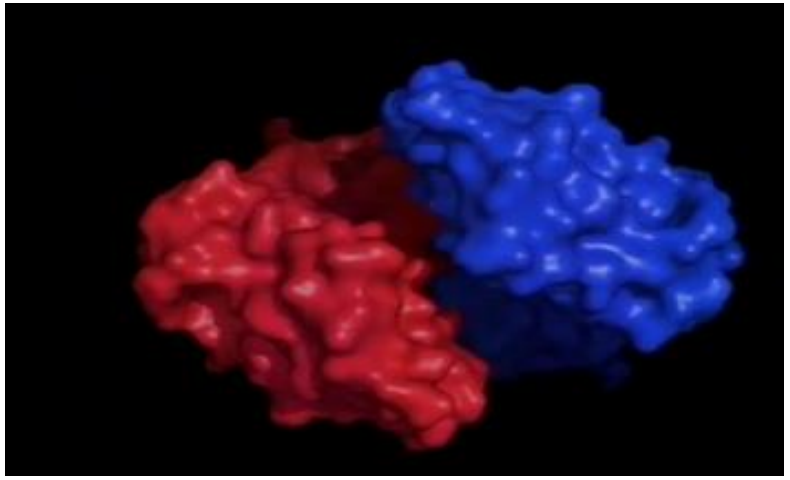
Ig expression during B lymphocyte maturation

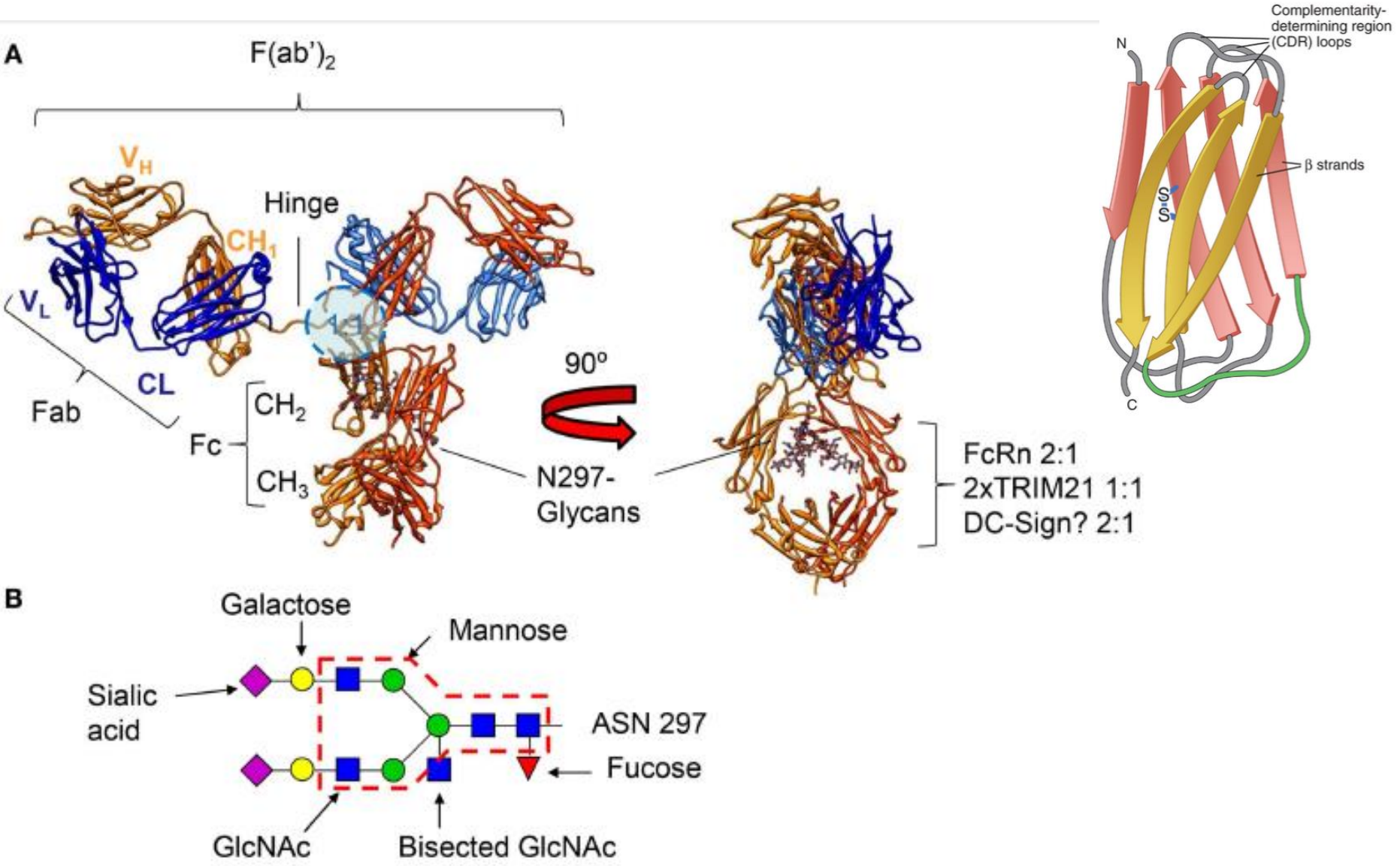


Stage of maturation	Stem cell	Pre-B cell	Immature B cell	Mature B cell	Activated B cell	Antibody-secreting cell
Pattern of immunoglobulin production	None	Cytoplasmic μ heavy chain and pre-B receptor	Membrane IgM	Membrane IgM, IgD	Low rate Ig secretion; heavy chain isotype switching; affinity maturation	High rate Ig secretion; reduced membrane Ig

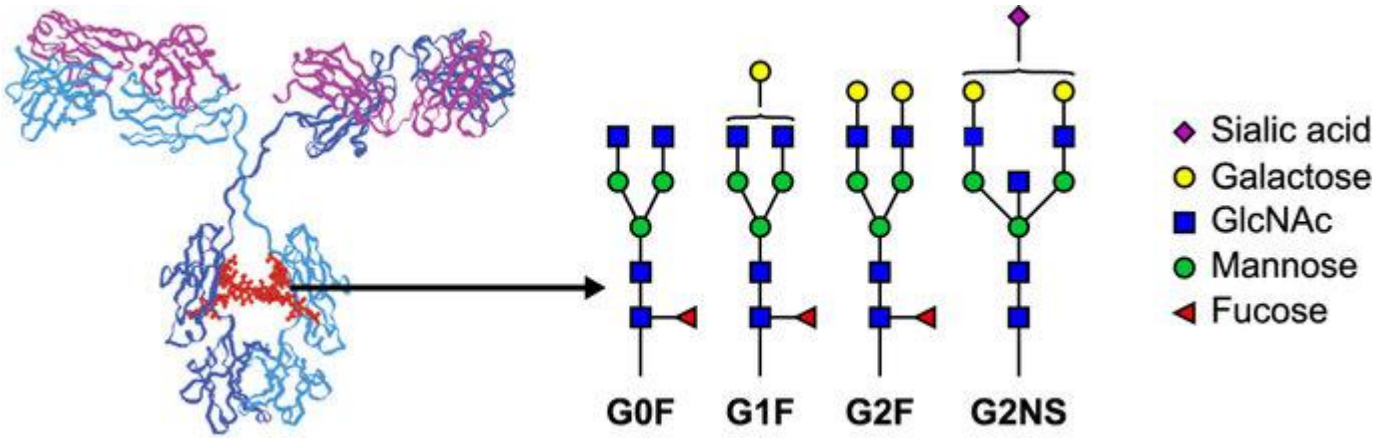
Propriedades das Igs dependem do tipo e nível de glicosilação



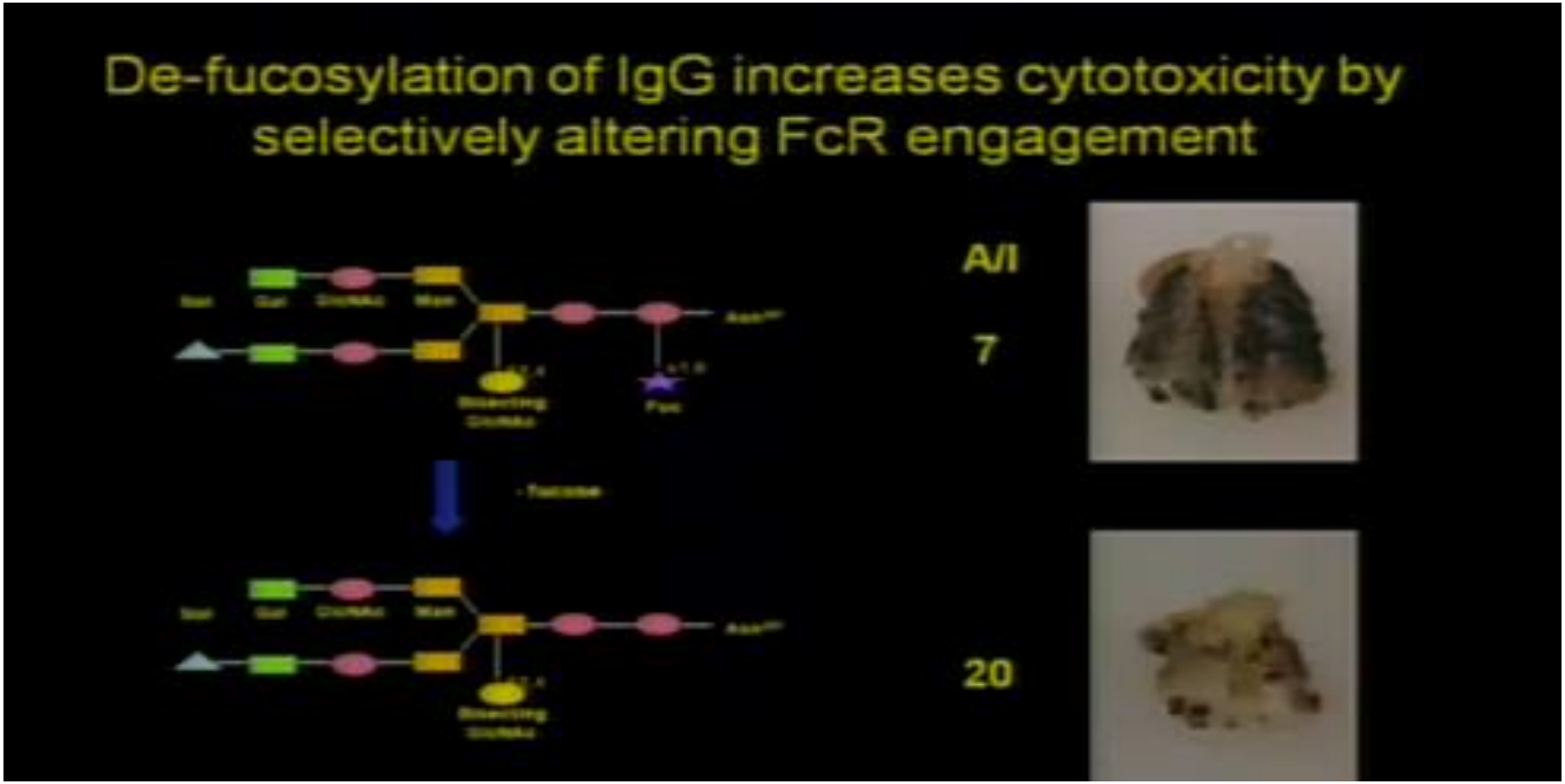




Crystal structure of na human IgG1 molecule viewed from two different angles, demonstrating theflexibility of the two Fab fragments with respect to each other and the Fc tail.
The N-linked glycan found at position 297 can be found as a core structure, common to all IgG found in human beings and rodents (core structure indicated with a red dashed line)


G0F
G1F
G2F
G2NS
Sialic acid
Galactose
GlcNAc
Mannose
Fucose

Propriedades das Igs dependem do tipo e nível de glicosilação



JCI The Journal of Clinical Investigation

Hyposialylated IgG activates endothelial IgG receptor FcγRIIB to promote obesity-induced insulin resistance

Keiji Tanigaki, … , Philip W. Shaul, Chieko Mineo
University of Texas Southwestern Medical Center, Dallas, Texas, USA
J Clin Invest. 2018;128(1):309-322. https://doi.org/10.1172/JCI89333.

- Type 2 diabetes mellitus is a common complication of obesity
- Activation of the IgG receptor FcgRIIB in endothelium by hyposialylated IgG has a role in obesity-induced insulin resistance
- Despite becoming obese on a high-fat diet, mice lacking FcgRIIB globally or selectively in endothelium were protected from insulin resistance (insulin delivery to skeletal muscle and resulting maintenance of muscle glucose disposal)
- IgG transferred from patients with T2DM but not from metabolically healthy subjects caused insulin resistance in IgG-deficient mice via FcgRIIB
- IgG from T2DM patients was hyposialylated.
- In HFD-fed mice, supplementation with a sialic acid precursor restored IgG sialylation and preserved insulin sensitivity without affecting weight gain.

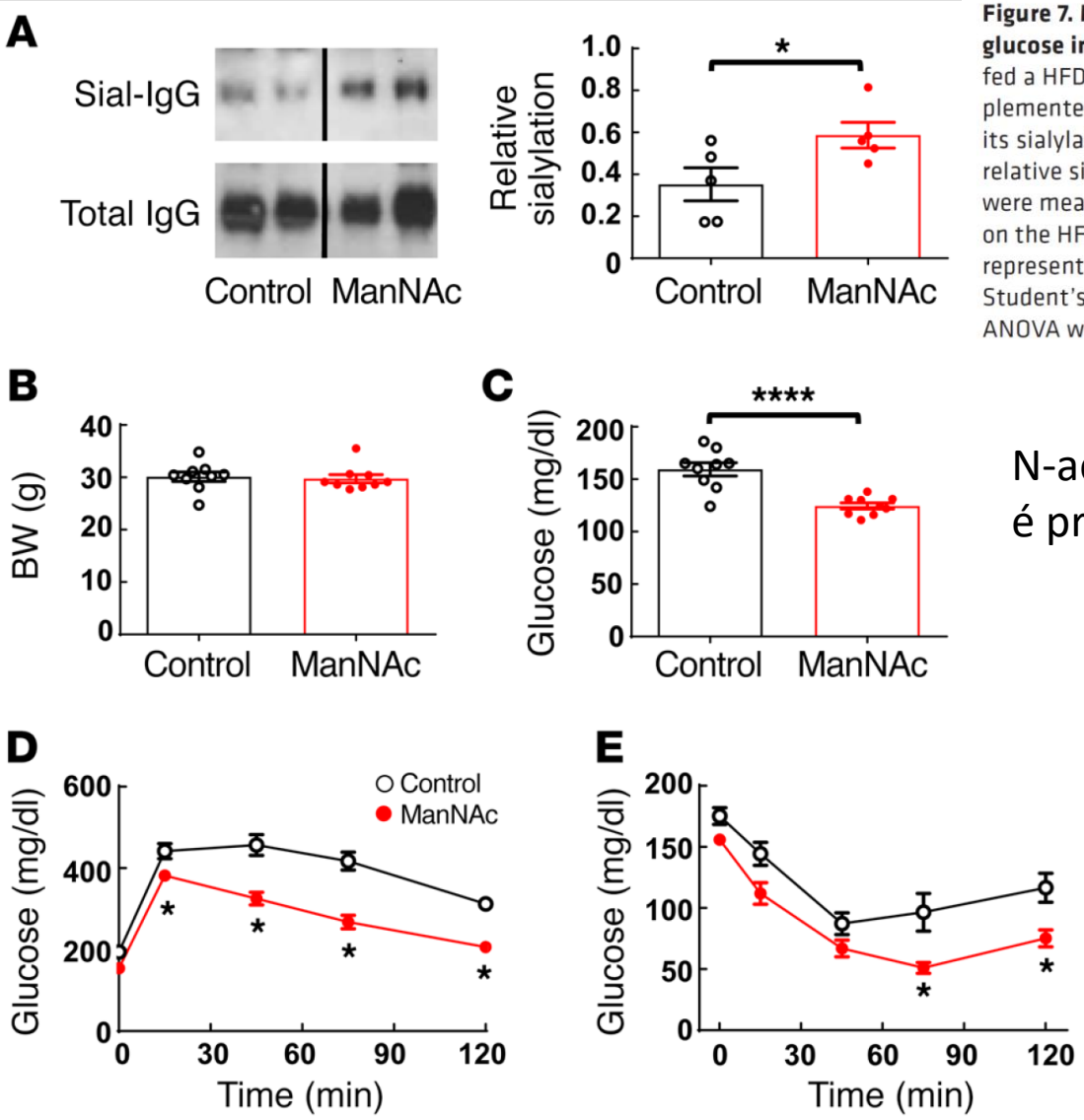
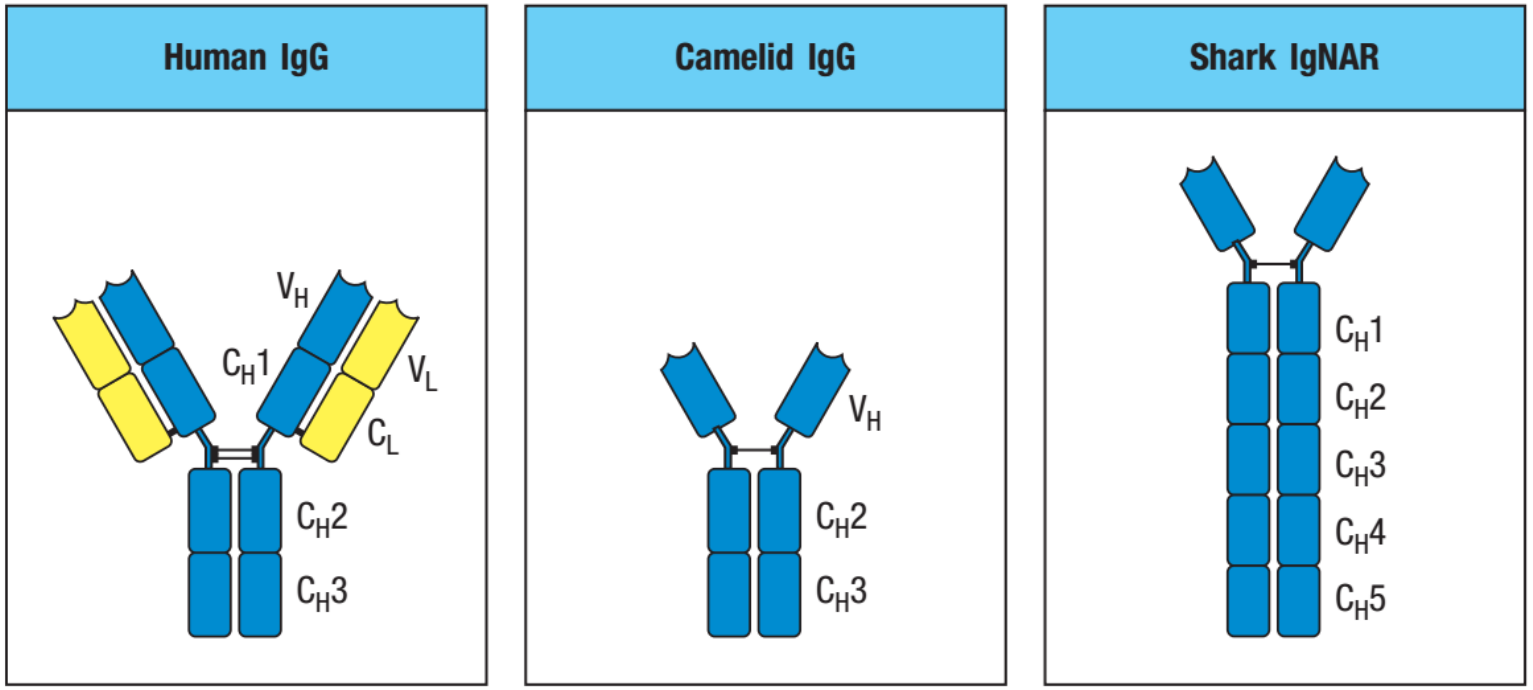
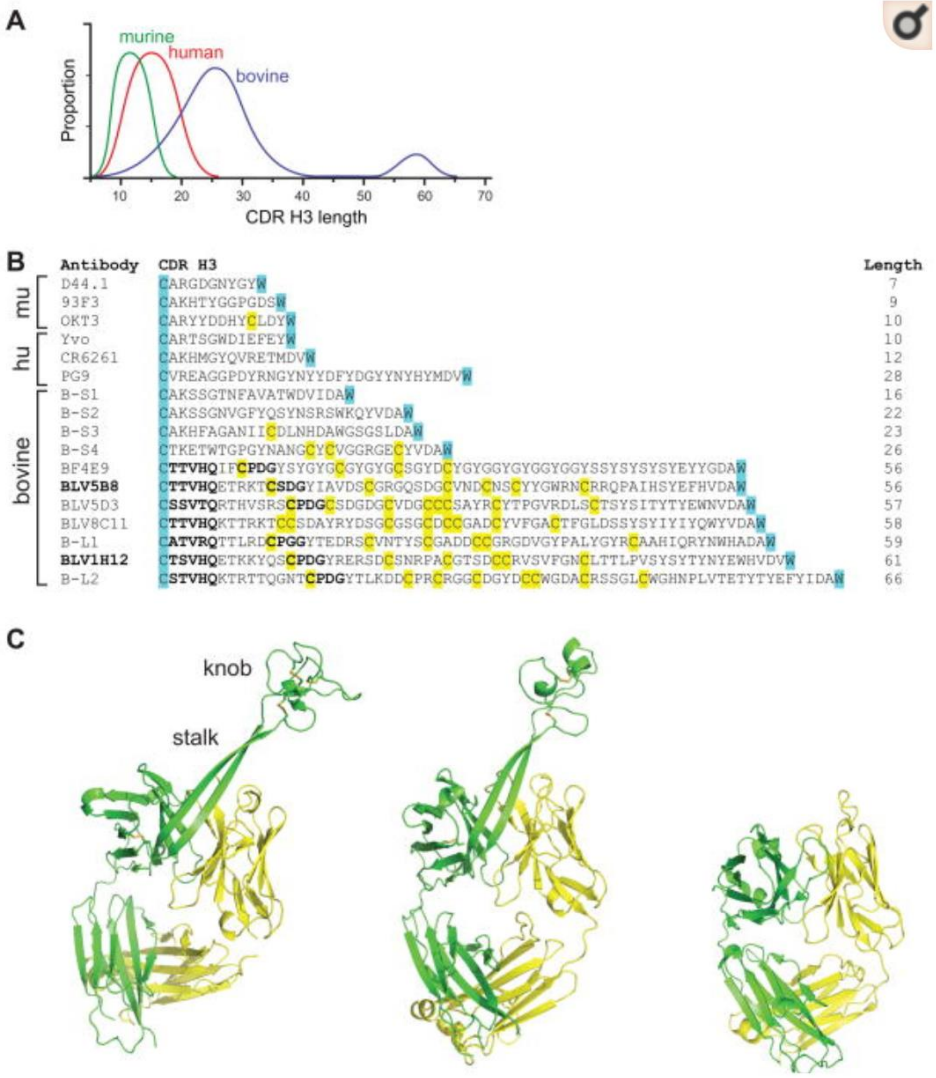


Figure 7. ManNAc treatment protects mice from obesity-induced glucose intolerance and insulin resistance. (**A**) Male WT mice were fed a HFD and either regular drinking water (control) or ManNAc-supplemented drinking water for 6 weeks. Plasma IgG was isolated, and its sialylation was evaluated by SNA-lectin blotting. Graph depicts the relative sialylation. $n = 5$. (**B**) BW and (**C**) fasting plasma glucose levels were measured, and (**D**) a GTT was performed. (**E**) Mice were continued on the HFD, and an ITT was performed 1 week later. $n = 9$. (**A**–**E**) Values represent the mean ± SEM. (**A**–**C**) $^*P < 0.05$ and $^{****}P < 0.001$, by Student's t test; (**D** and **E**) $^*P < 0.05$, ManNAc versus control, by 2-way ANOVA with Tukey's post-hoc test.

N-acetil-D-manosamina (ManNAc) é precursor de ácido siálico

Some species generate antibodies with alternative structures.





nature
International journal of science

Letter | Published: 20 July 2017

Rapid elicitation of broadly neutralizing antibodies to HIV by immunization in cows

Devin Sok, Khoa M. Le, Melissa Vadnais, Karen L. Saye-Francisco, Joseph G. Jardine, Jonathan L. Torres, Zachary T. Berndsen, Leopold Kong, Robyn Stanfield, Jennifer Ruiz, Alejandra Ramos, Chi-Hui Liang, Patricia L. Chen, Michael F. Criscitiello, Waithaka Mwangi, Ian A. Wilson, Andrew B. Ward, Vaughn V. Smider & Dennis R. Burton

Nature **548**, 108–111 (03 August 2017) | Download Citation

Abstract

No immunogen to date has reliably elicited broadly neutralizing antibodies to HIV in humans or animal models. Advances in the design of immunogens that antigenically mimic the HIV envelope glycoprotein (Env), such as the soluble cleaved trimer BG505 SOSIP[1], have improved the elicitation of potent isolate-specific antibody responses in rabbits[2] and macaques[3], but so far failed to induce broadly neutralizing antibodies. One possible reason for this failure is that the relevant antibody repertoires are poorly suited to target the conserved epitope regions on Env, which are somewhat occluded relative to the exposed variable epitopes. Here, to test this hypothesis, we immunized four

Editorial Summary - **Milking cows for their antibodies**

HIV infection gives rise to the generation of broadly neutralizing antibodies in a subset of infected subjects. However, it has been difficult to induce such antibodies through vaccination in humans and a variety of animal models. Four cows were immunized with a recombinant HIV envelope protein and broadly neutralizing antibodies developed rapidly after repeated immunization. For example, one cow developed cross-neutralizing activity just 42 days after a vaccine boost.

Effector functions of antibodies

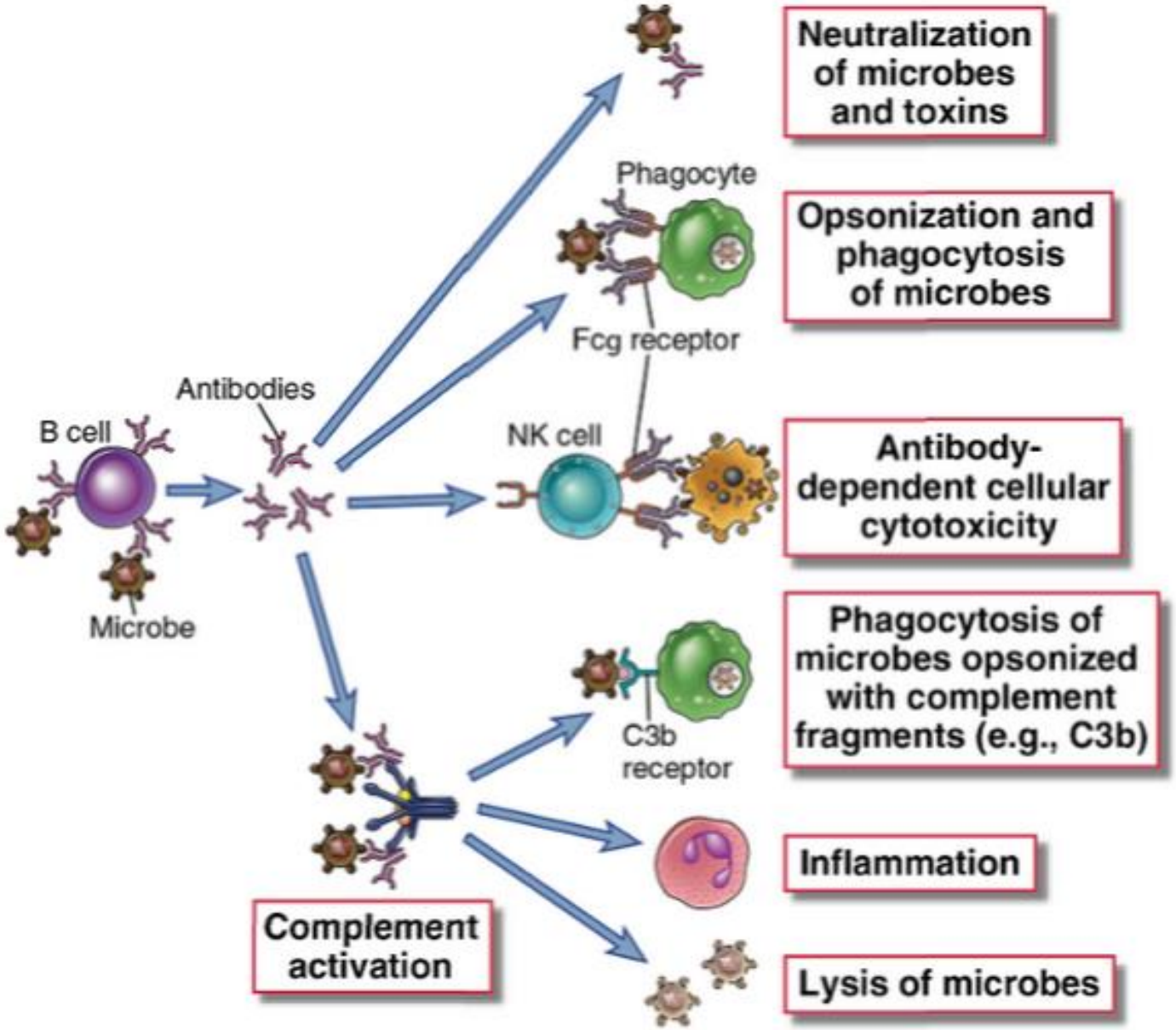


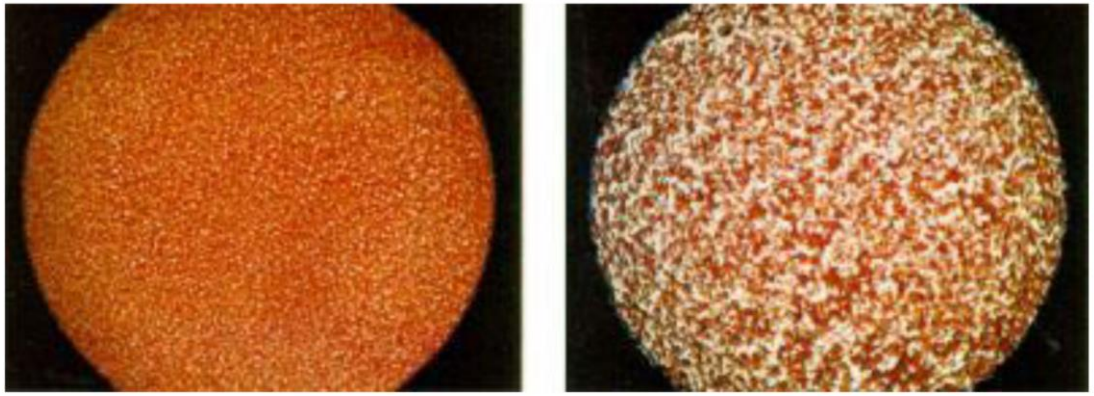
TABLE 12–1 Functions of Antibody Isotypes

Antibody Isotype	Isotype-Specific Effector Functions
IgG	Opsonization of antigens for phagocytosis by macrophages and neutrophils Activation of the classical pathway of complement Antibody-dependent cell-mediated cytotoxicity mediated by natural killer cells Neonatal immunity: transfer of maternal antibody across the placenta and gut Feedback inhibition of B cell activation
IgM	Activation of the classical pathway of complement Antigen receptor of naive B lymphocytes*
IgA	Mucosal immunity: secretion of IgA into the lumens of the gastrointestinal and respiratory tracts Activation of complement by the lectin pathway or by the alternative pathway
IgE	Mast cell degranulation (immediate hypersensitivity reactions)
IgD	Antigen receptor of naive B lymphocytes*

*These functions are mediated by membrane-bound and not secreted antibodies.

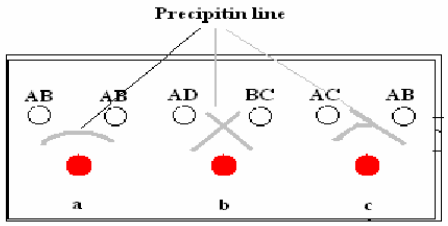
Como detectar e medir Anticorpos e suas interações com Antigenos?

Antígenos particulados - Aglutinação



Exemplo: Reação de Widal para o diagnóstico da febre tifóide

Antígenos solúveis - Precipitação



Anti A + Anti B

The Ouchterlony reaction:

- Detect, identify, and quantify antibody and antigen
- Test the similarity between antigens
  - For disease diagnosis

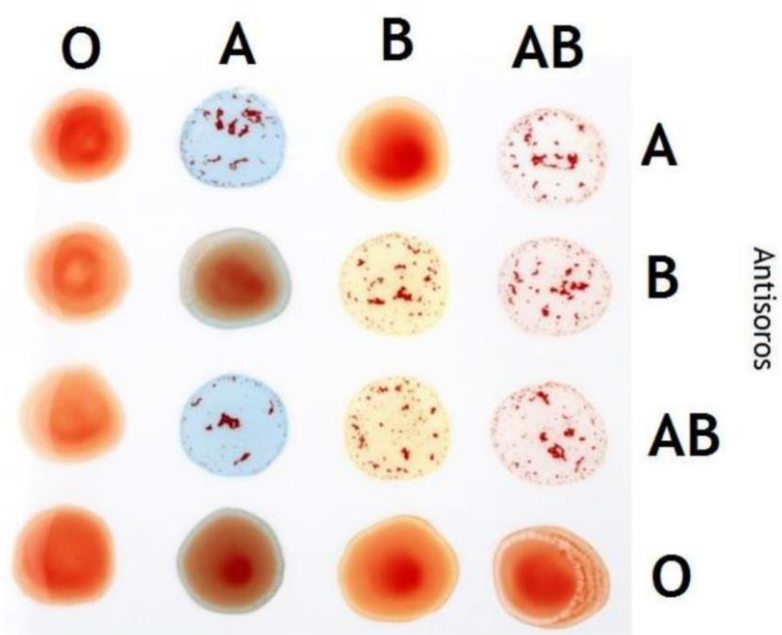
Dr. Karl Landsteiner

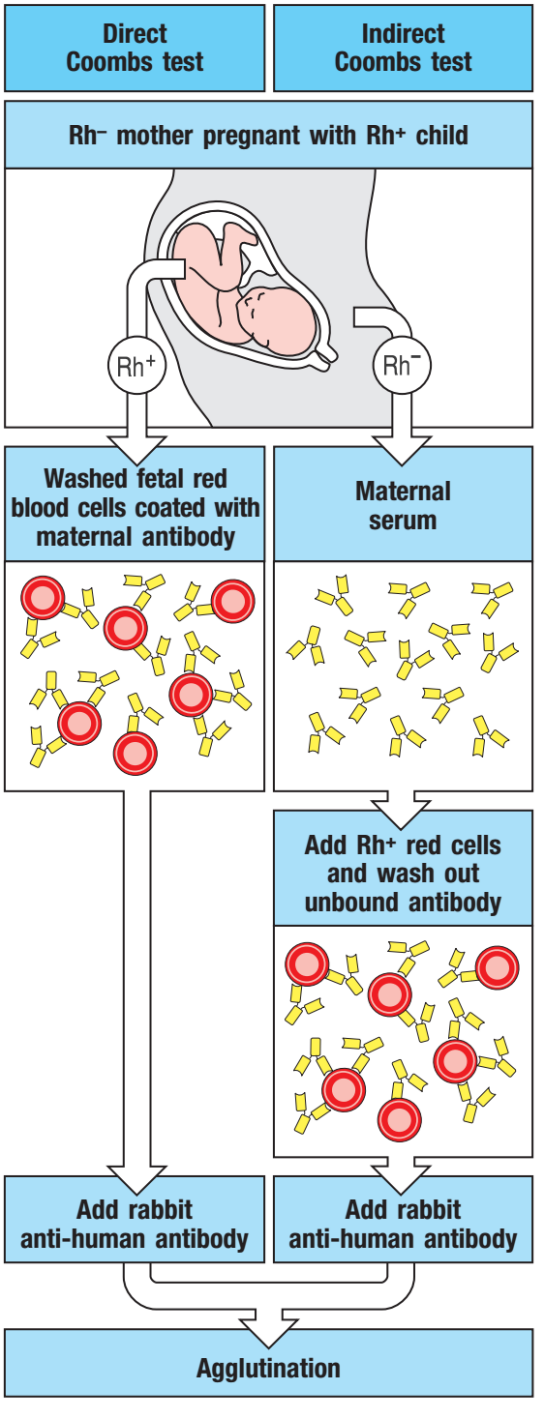
Nobel de Medicina de 1930

The ABO Blood System

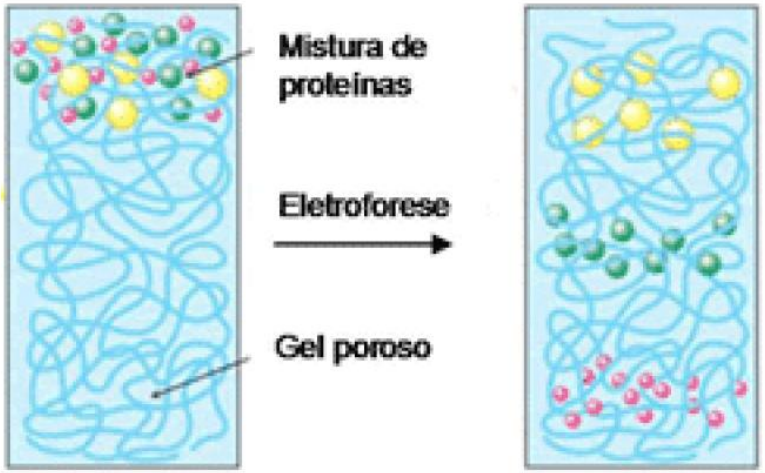
Blood Type (genotype)	Type A (AA, AO)	Type B (BB, BO)	Type AB (AB)	Type O (OO)
Red Blood Cell Surface Proteins (phenotype)	A agglutinogens only	B agglutinogens only	A and B agglutinogens	No agglutinogens
Plasma Antibodies (phenotype)	b agglutinin only	a agglutinin only	No agglutinin	a and b agglutinin

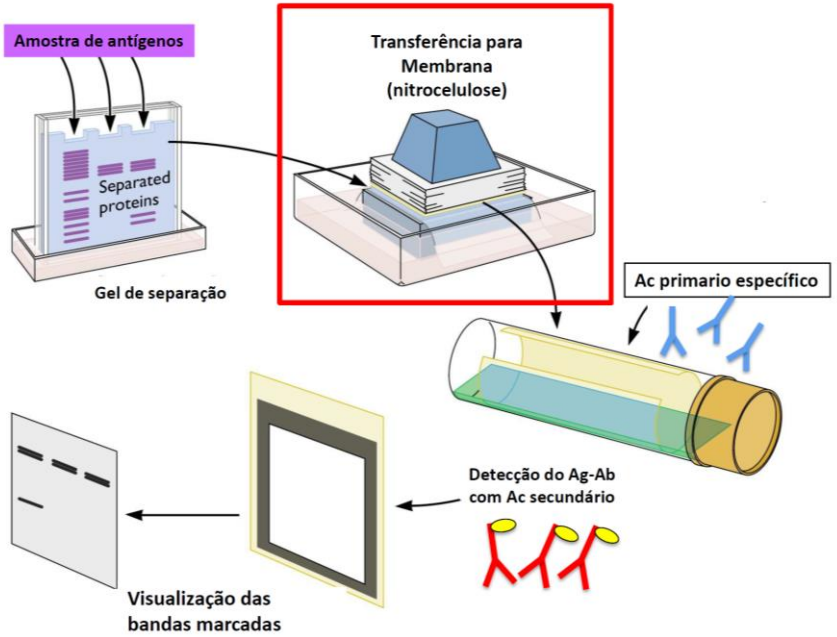
Tipo de Sangue



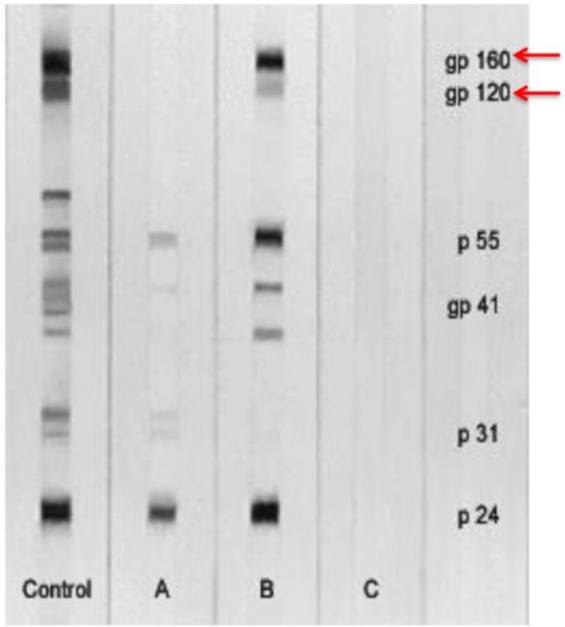


The Coombs direct and indirect anti-globulin tests for antibody against red blood cell antigens.

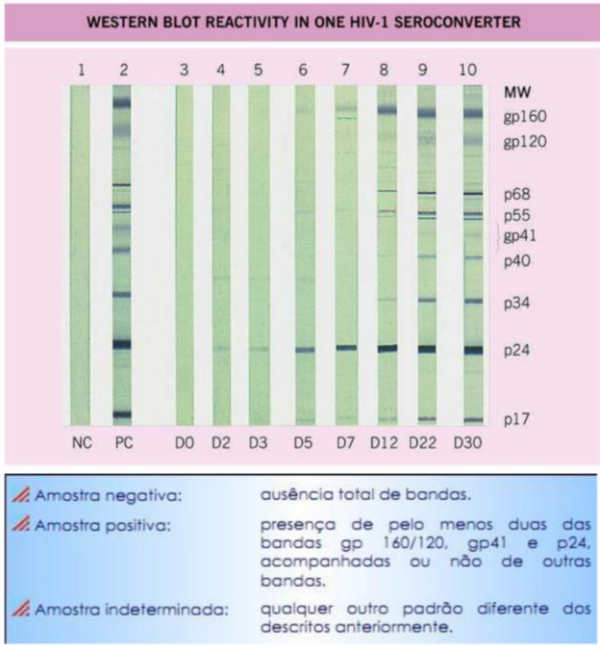




Diagnóstico de HIV



Pacientes



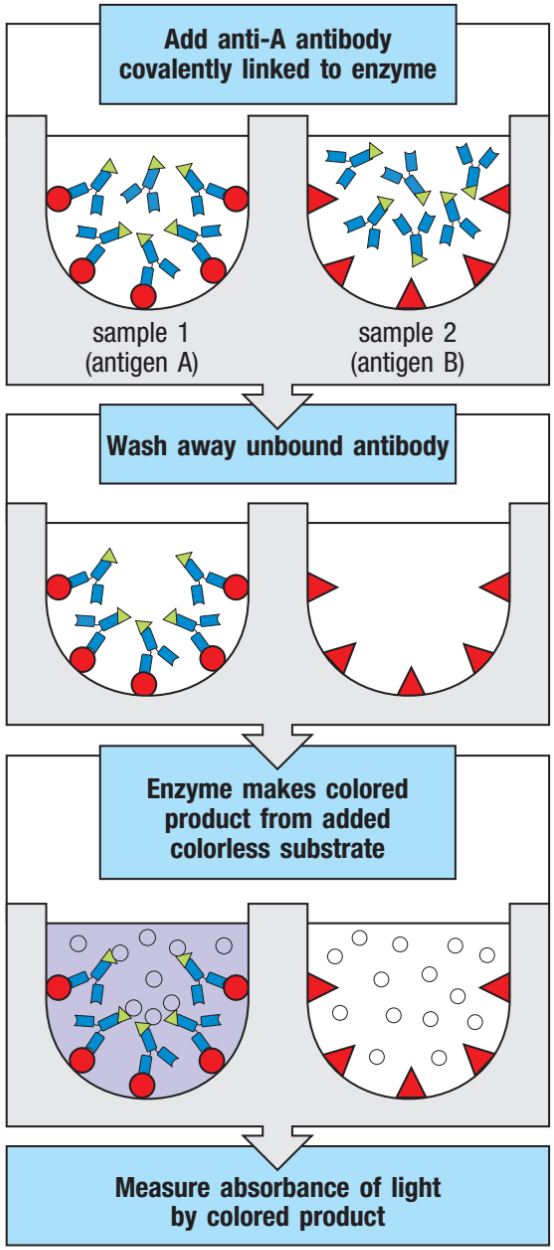


Fig. A.5 The principle of the enzyme-linked immunosorbent assay (ELISA).