

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

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# A Natureza Química dos Anticorpos

Pesquisadores injetavam coelhos com diferentes compostos

proteínas solúveis, bactérias, hemácias heterólogas....

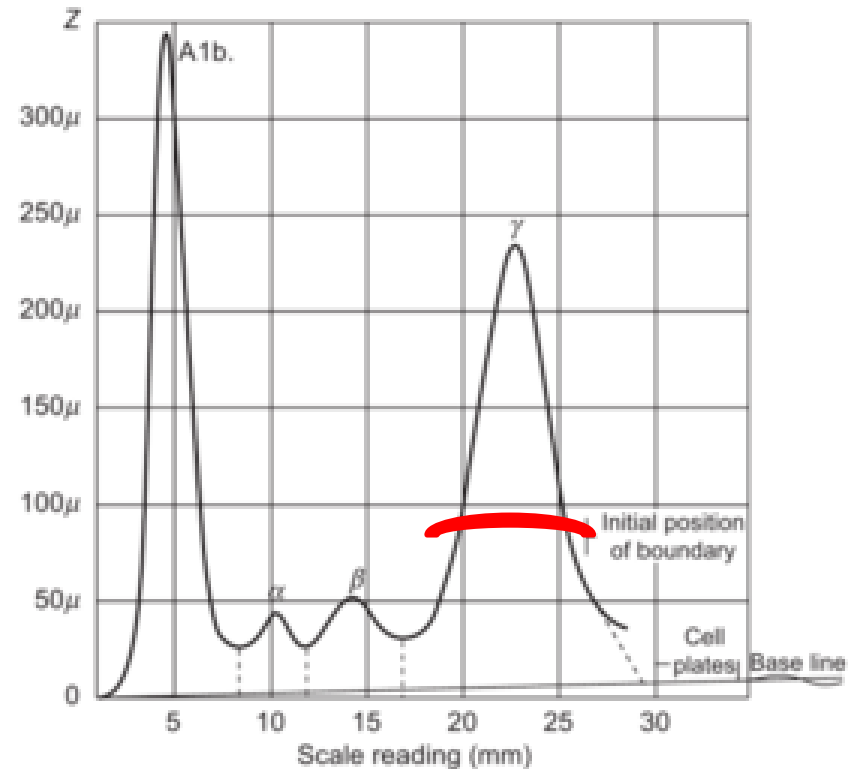
No soro obtinham funções de:

- Precipitação de toxinas - Precipitinas
- Lise - Lisinas
- Aglutinação de partículas - Aglutininas
- Fagocitose potenciada - Oponinas

## Perguntas, teorias:

Cada função do soro imune era mediada por:

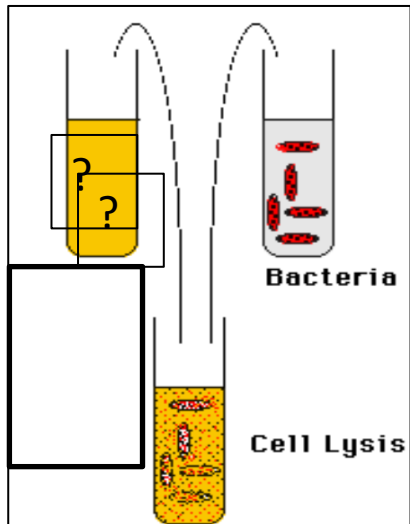
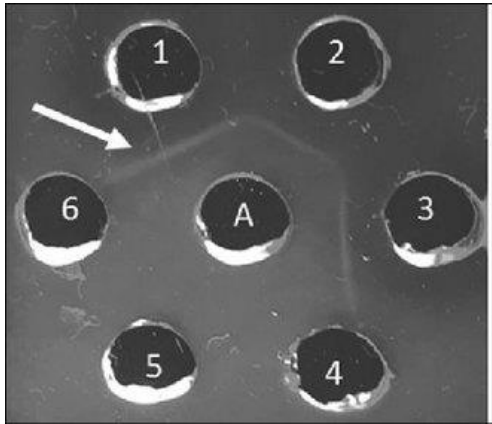
- Estruturas ≠?
- Estruturas = ("teoria unitária")?



**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).

# No soro obtinham funções de:

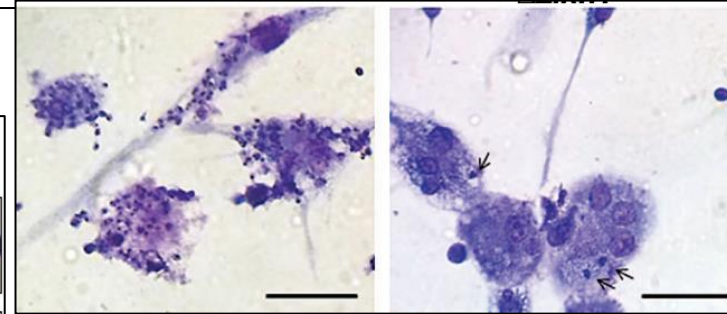
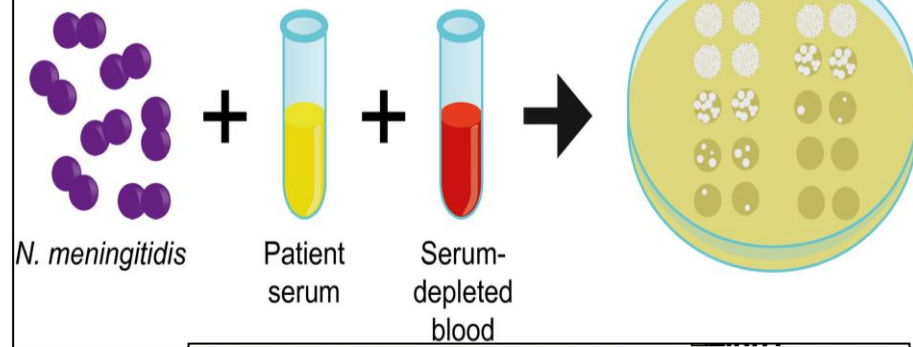
- Precipitação de toxinas - Precipitinas
- Lise - Lisinas
- Aglutinação de partículas - Aglutininas
- Fagocitose potenciada - Opsoninas



**Horizontal Card Evaluation**

Score	Positive	Negative
4		
3.5		
3		
2.5		
2		

## Whole blood killing assay

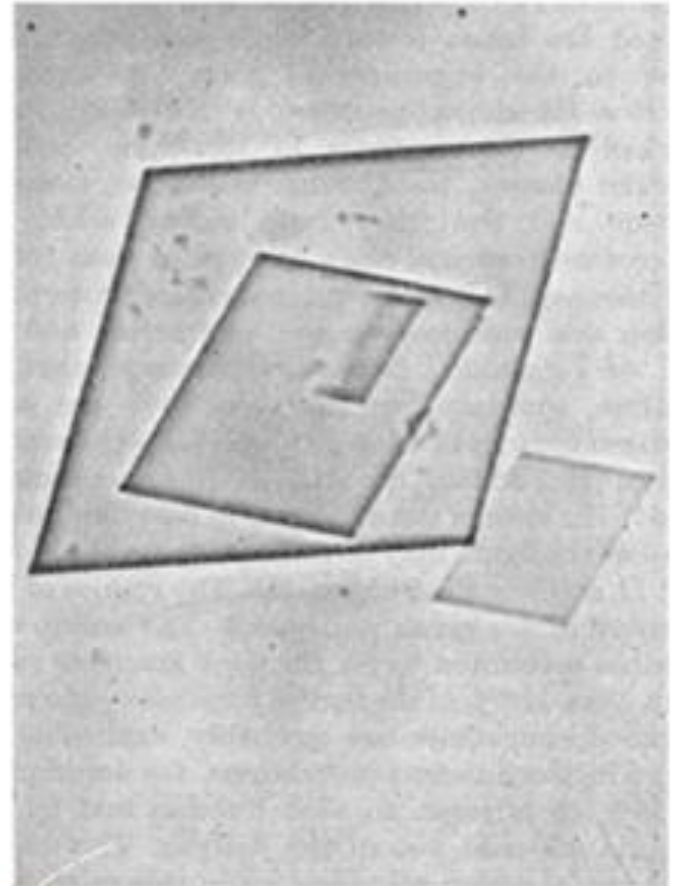


# Desenvolvimento do Modelo da Estrutura da Imunoglobulina de Quatro Cadeias

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

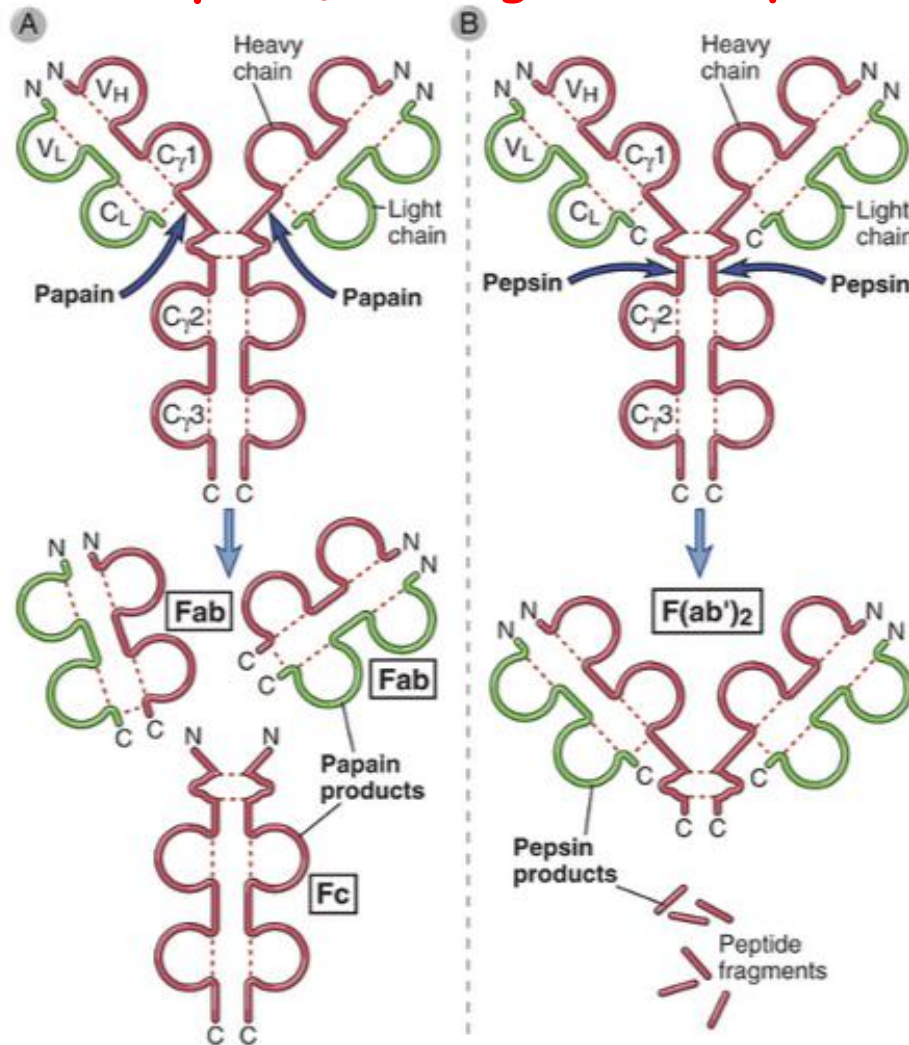


**FIGURE 11.3** Crystals of fraction III of gamma globulin treated with papain. From Porter (1959).

# Proteolytic fragments of an IgG molecule

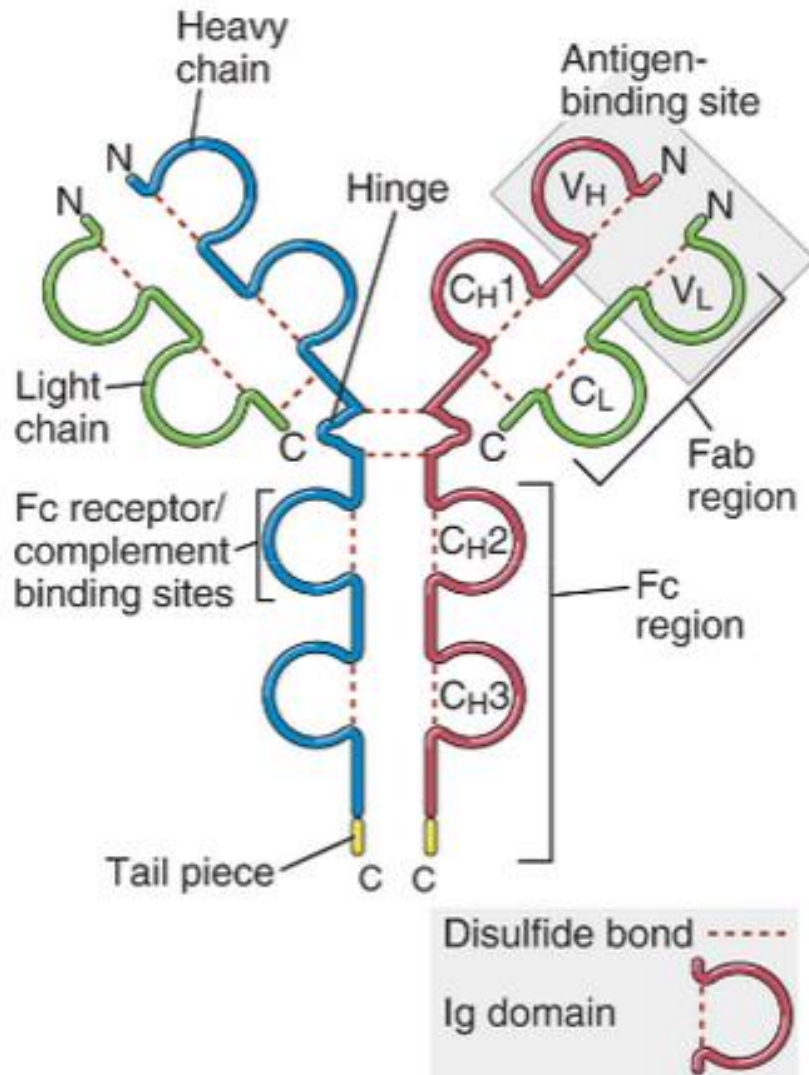
## Unraveling structure and function

**Tirando um pedaço da IgG sobra qual função?**

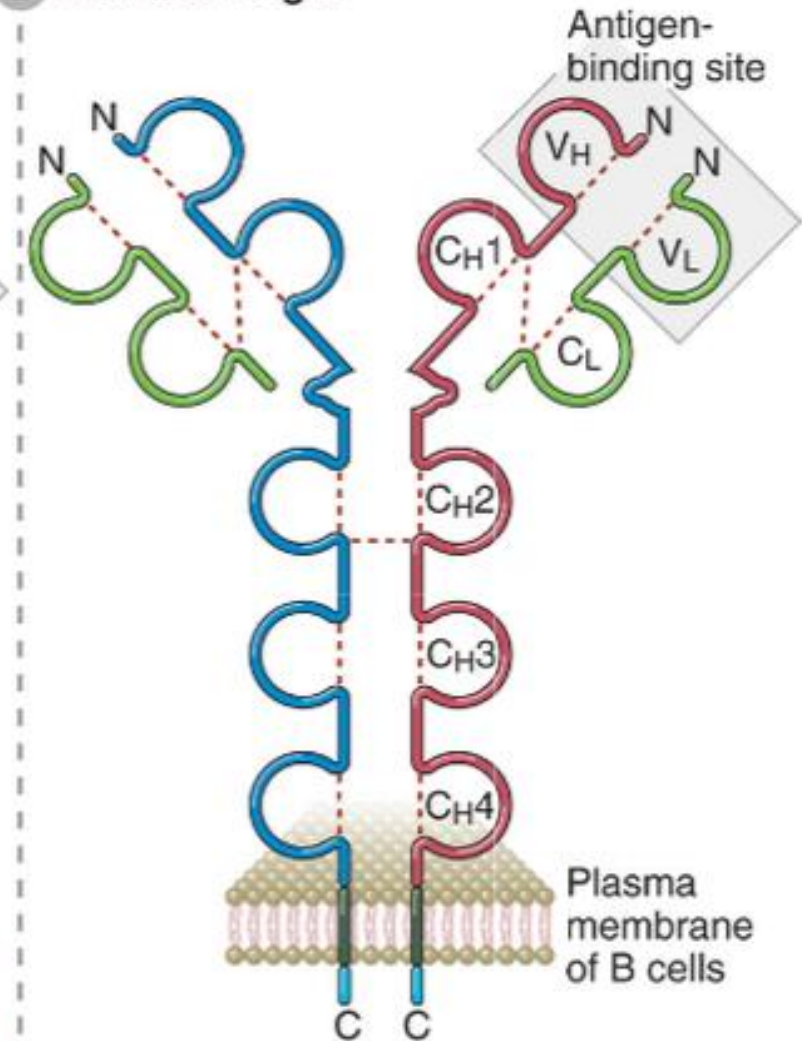


# Structure of an antibody molecule.

**A** Secreted IgG



**B** Membrane IgM



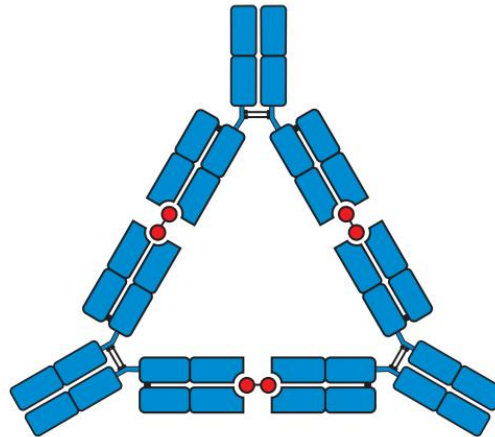
The hinge region of the immunoglobulin molecule allows flexibility in binding to multiple antigens.

**Adiante: Classes e subclasses de Igs**

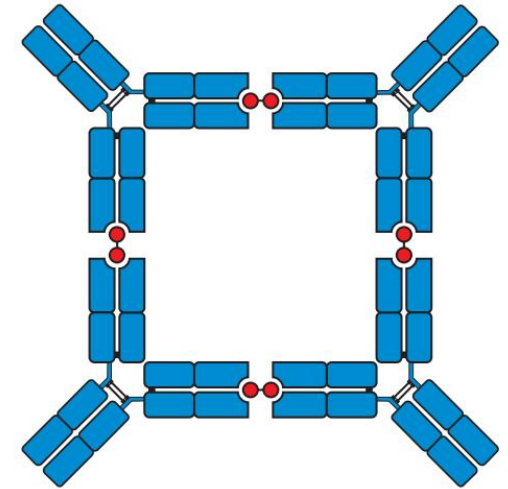
(Micrograph  $\times 300,000$ )



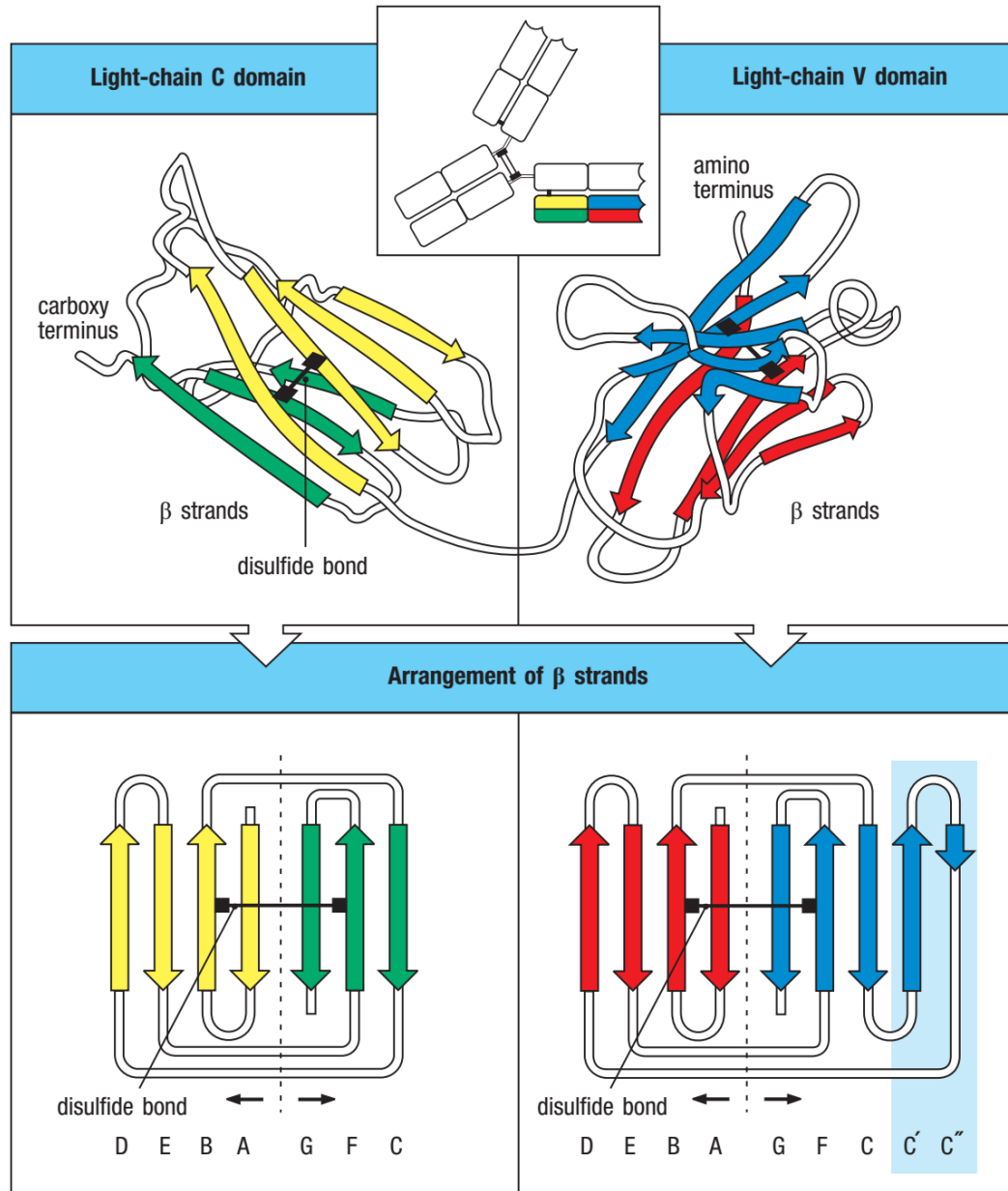
Angle between arms is  $60^\circ$



Angle between arms is  $90^\circ$



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 ( $\beta$  strands) running in opposite directions (antiparallel) pack together to form two  $\beta$  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  $\beta$  strands are lettered sequentially with respect to the order of their occurrence in the amino acid sequence of the domains; the order in each  $\beta$  sheet is characteristic of immunoglobulin domains. The  $\beta$  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.



**A região variável dos anticorpos**

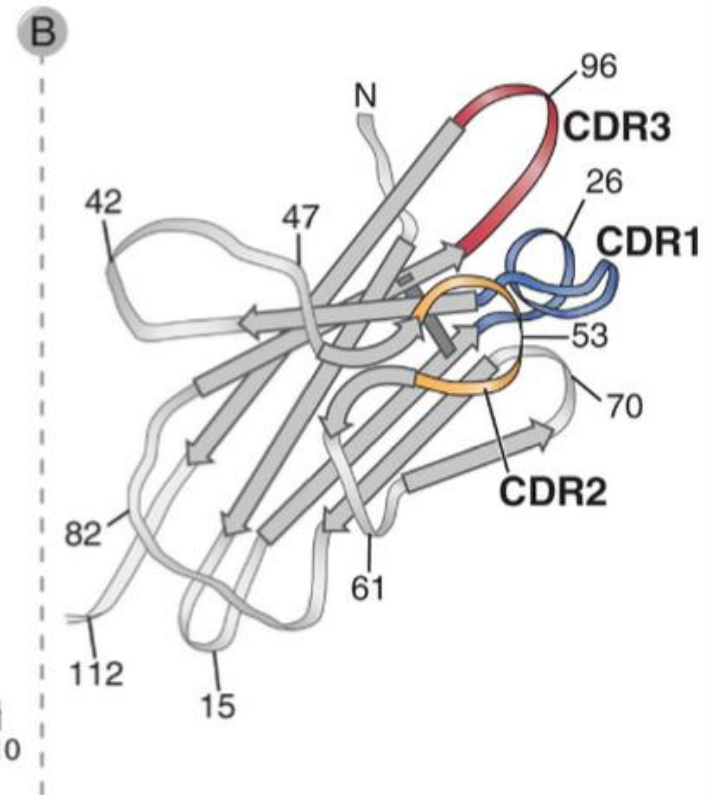
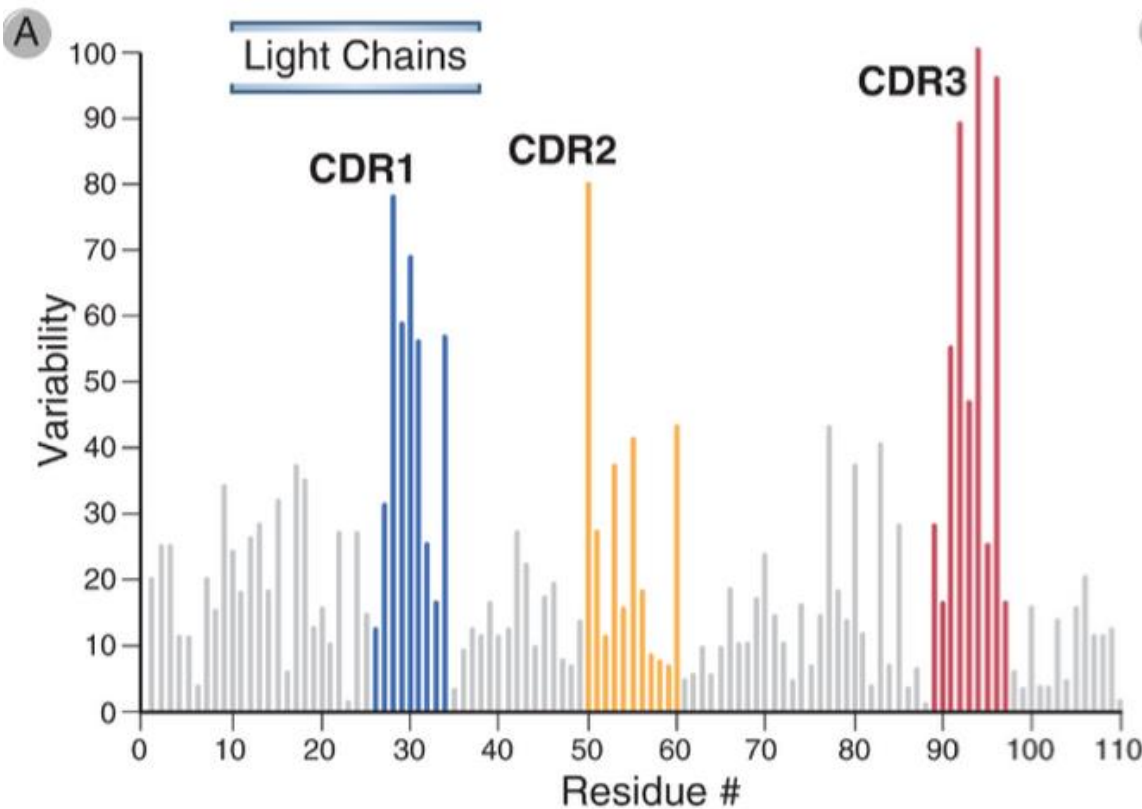
**O sítio de ligação com antígenos**

# The interaction of the antibody molecule with specific antigen.

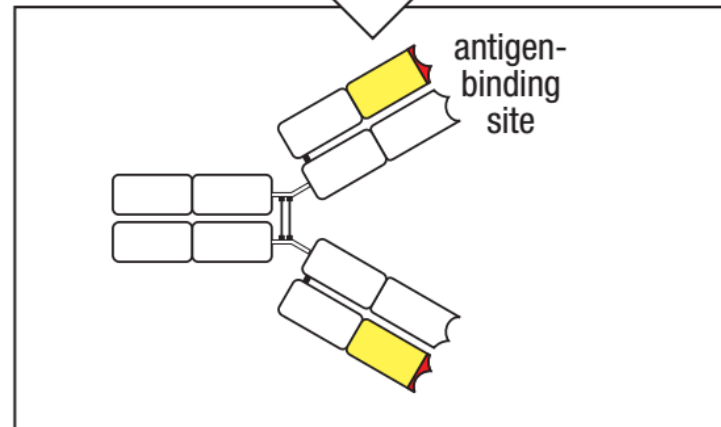
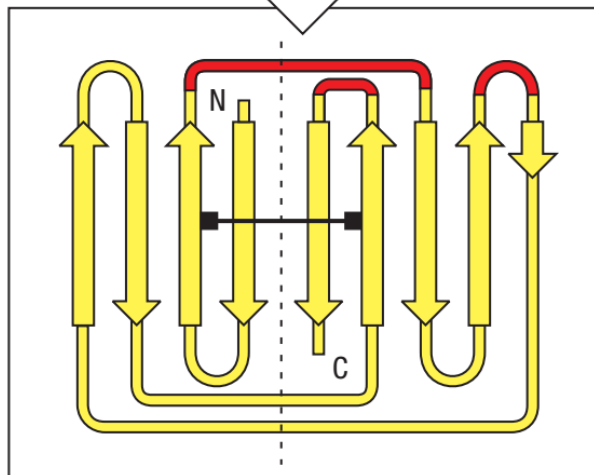
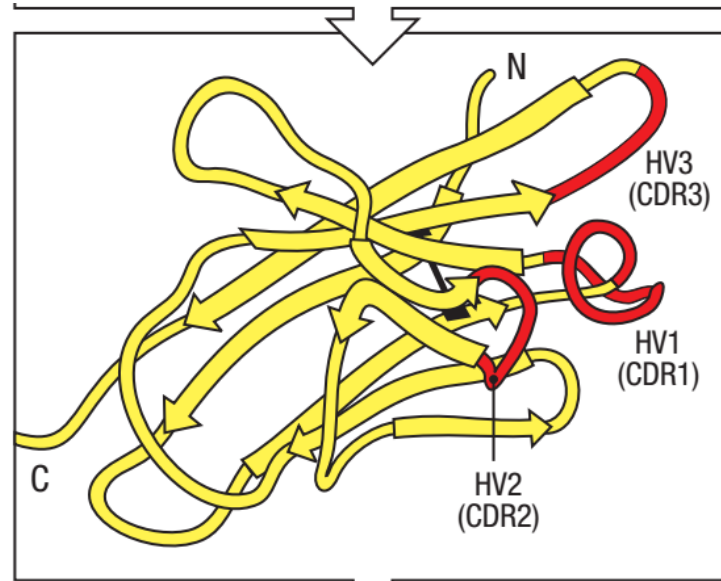
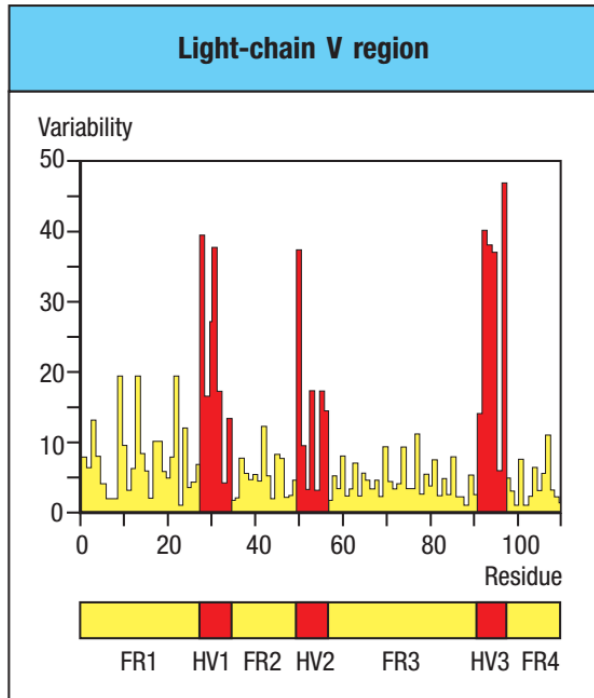
Hypervariable regions in Ig molecules  
Wu-Kabat Plots



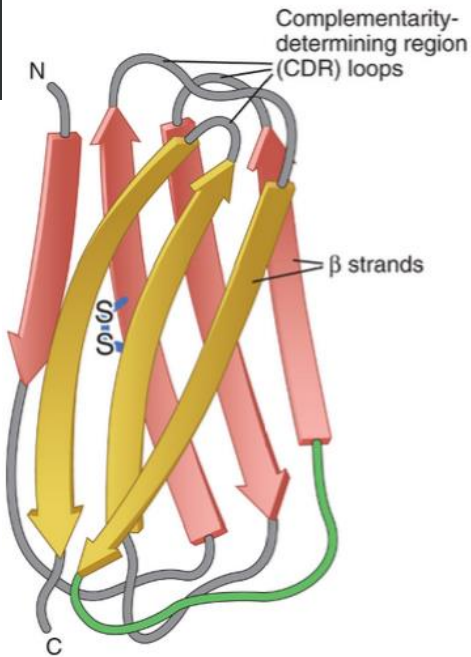
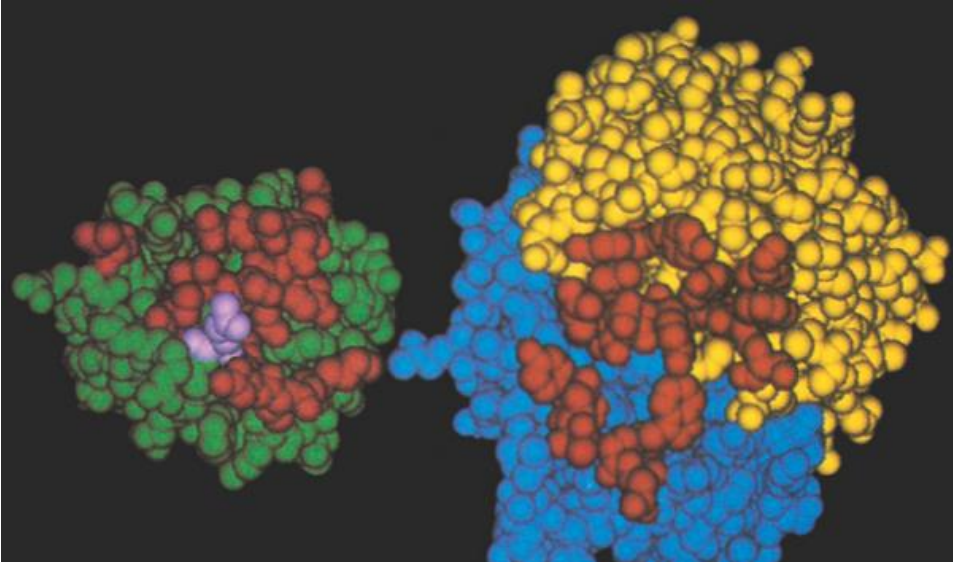
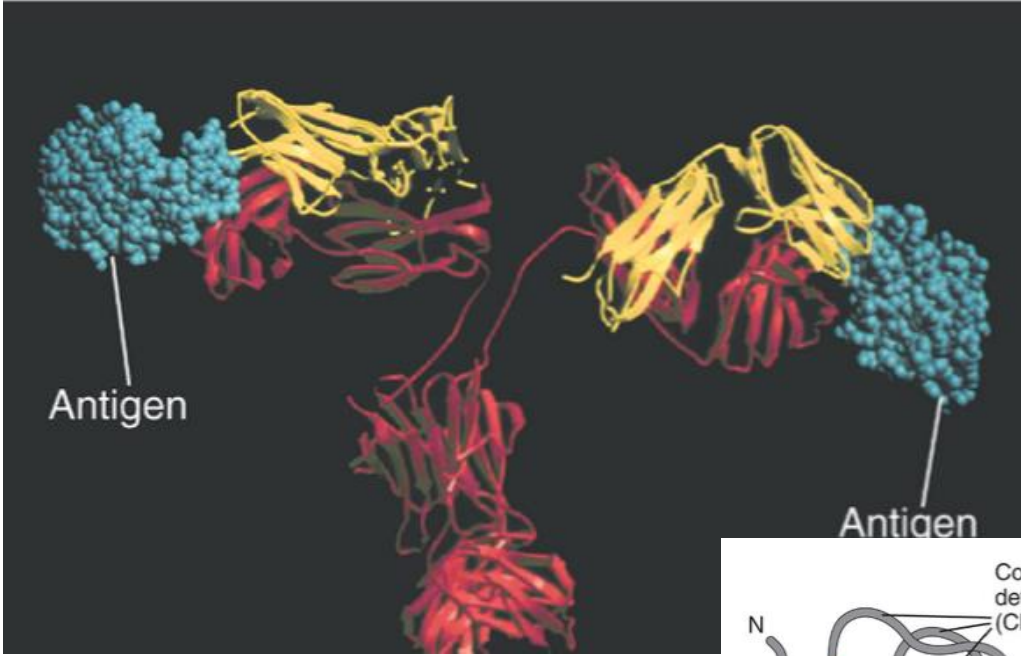
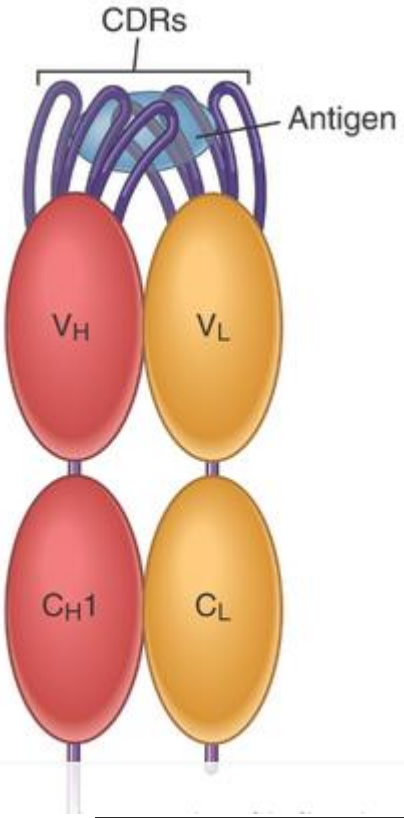
1914 – June 16, 2000



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



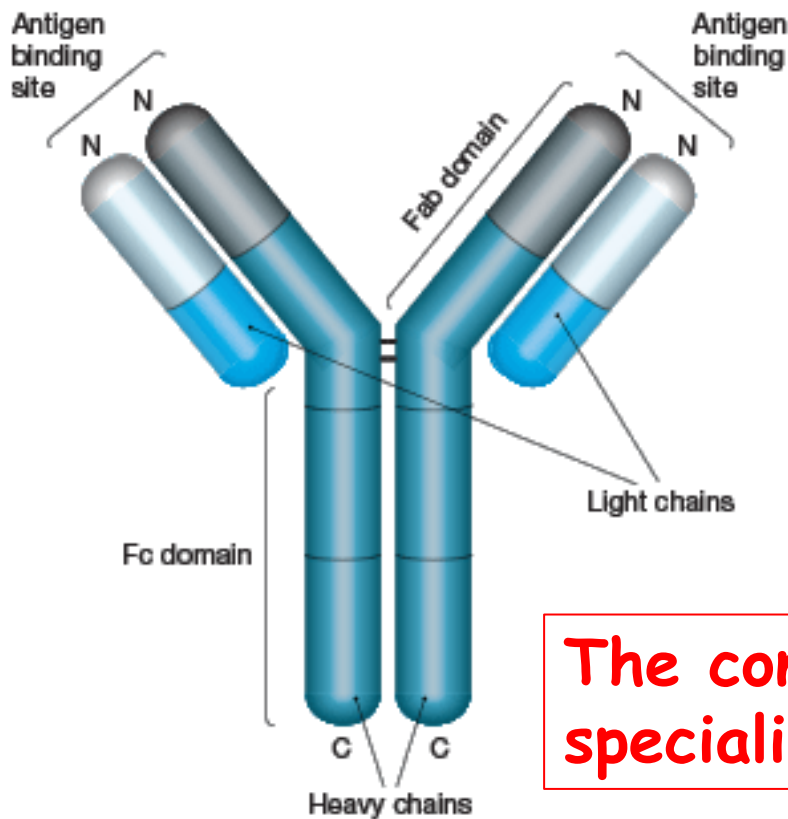
# Binding of an antigen by an antibody



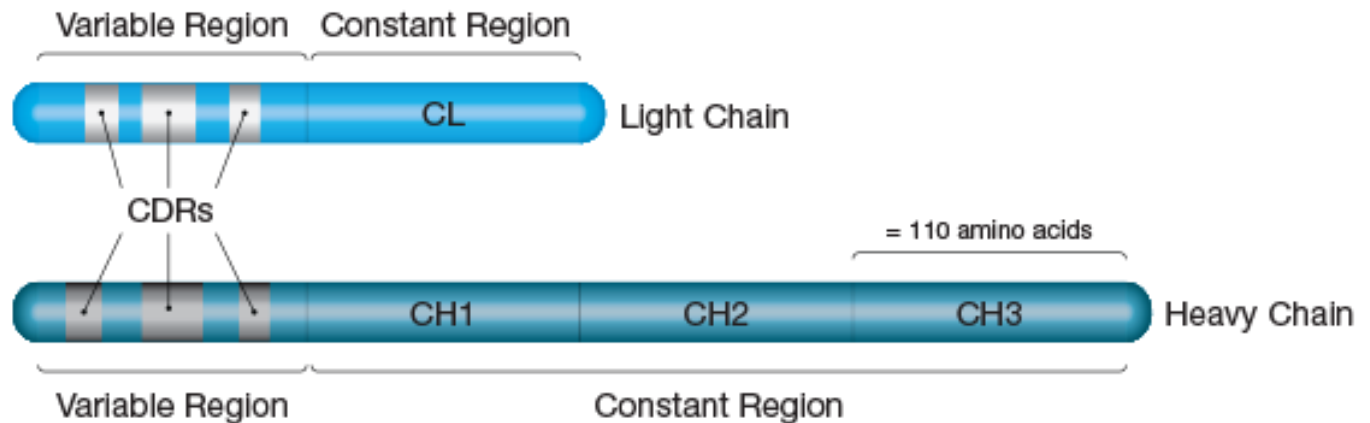
Structure of an Ig domain

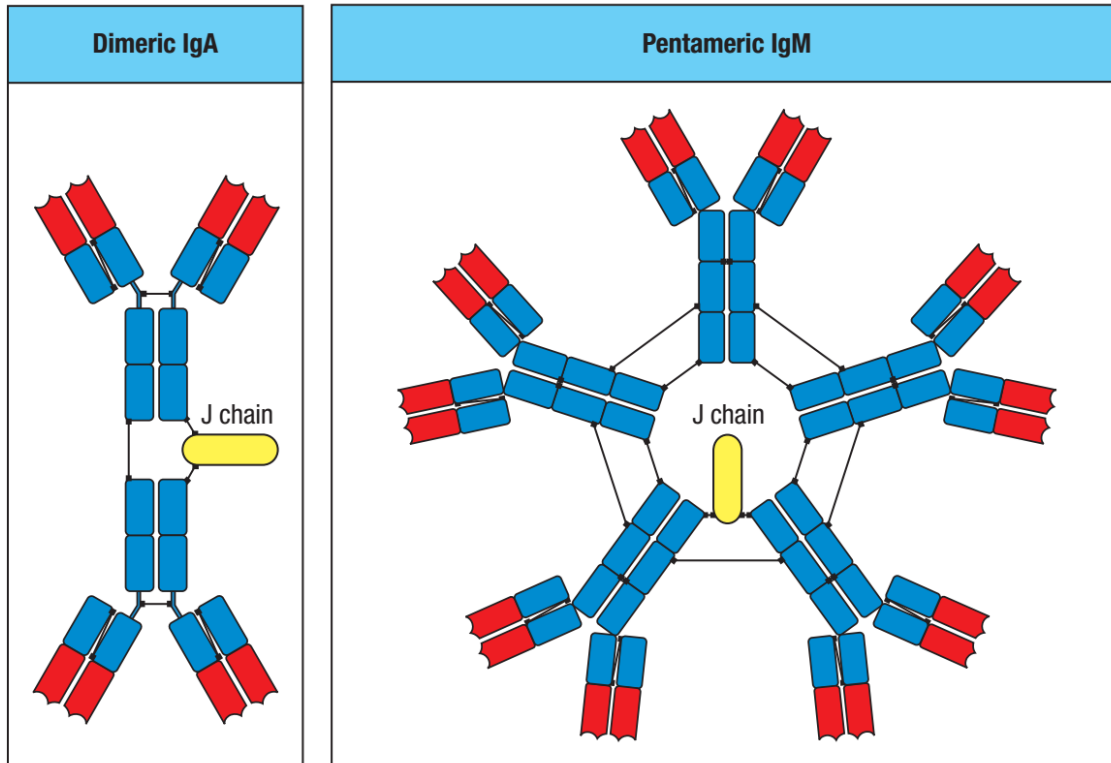
**A região constante dos anticorpos**

**Funções efetoras**



**The constant region confers functional specialization on the antibody.**



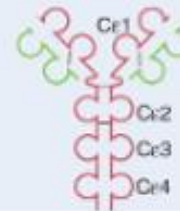


**Fig. 5.23 The IgM and IgA molecules can form multimers.** IgM and IgA are usually synthesized as multimers in association with an additional polypeptide chain, the J chain. In dimeric IgA (left panel), the monomers have disulfide bonds to the J chain as well as to each other. In pentameric IgM (right 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

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 ( $\alpha$ 1 or $\alpha$ 2)	3.5	6	IgA (dimer) Monomer, dimer, trimer	Mucosal immunity
IgD	None ( $\delta$ )	Trace	3	None	Naive B cell antigen receptor
IgE	None ( $\epsilon$ )	0.05	2	IgE Monomer	Defense against helminthic parasites, immediate hypersensitivity
IgG	IgG1-4 ( $\gamma$ 1, $\gamma$ 2, $\gamma$ 3, or $\gamma$ 4)	13.5	23	IgG1 Monomer	Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None ( $\mu$ )	1.5	5	IgM Pentamer	Naive B cell antigen receptor, complement activation





**TABLE 12-1 Functions of Antibody Isotypes**

<b>Antibody Isotype</b>	<b>Isotype-Specific Effector Functions</b>
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.	

# Human Antibody Isotypes

	Immunoglobulin								
	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Heavy chain	$\gamma_1$	$\gamma_2$	$\gamma_3$	$\gamma_4$	$\mu$	$\alpha_1$	$\alpha_2$	$\delta$	$\epsilon$
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	$5 \times 10^{-5}$
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	
<b>General</b>								
Molecular mass (kD)	146		146		170		146	
Amino acids in hinge region	15		12		62 <sup>a</sup>		12	
Inter-heavy chain disulfide bonds	2		4 <sup>b</sup>		11 <sup>a</sup>		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 <sup>a</sup>		21	
Placental transfer	++++		++		++/++++ <sup>a</sup>		+++	
<b>Antibody response to:</b>								
Proteins	++		+/-		++		++ <sup>e</sup> *	
Polysaccharides	+		+++		+/-		+/-	
Allergens	+		(-)		(-)		++	
<b>Complement activation</b>								
C1q binding	++		+		+++		-	
<b>Fc receptors</b>								
FcγRI	+++ <sup>c</sup>	65 <sup>d</sup>	-	-	++++	61	++	34
FcγRIIa <sub>H131</sub>	+++	5.2	++	0.45	++++	0.89	++	0.17
FcγRIIa <sub>R131</sub>	+++	3.5	+	0.10	++++	0.91	++	0.21
FcγRIIb/c	+	0.12	-	0.02	++	0.17	+	0.20
FcγRIIIa <sub>F158</sub>	++	1.2	-	0.03	++++	7.7	-	0.20
FcγRIIIa <sub>V158</sub>	+++	2.0	+	0.07	++++	9.8	++	0.25
FcγRIIIb	+++	0.2	-	-	++++	1.1	-	-
FcRn (at pH < 6.5)	+++		+++		++/++++ <sup>a</sup>		+++	

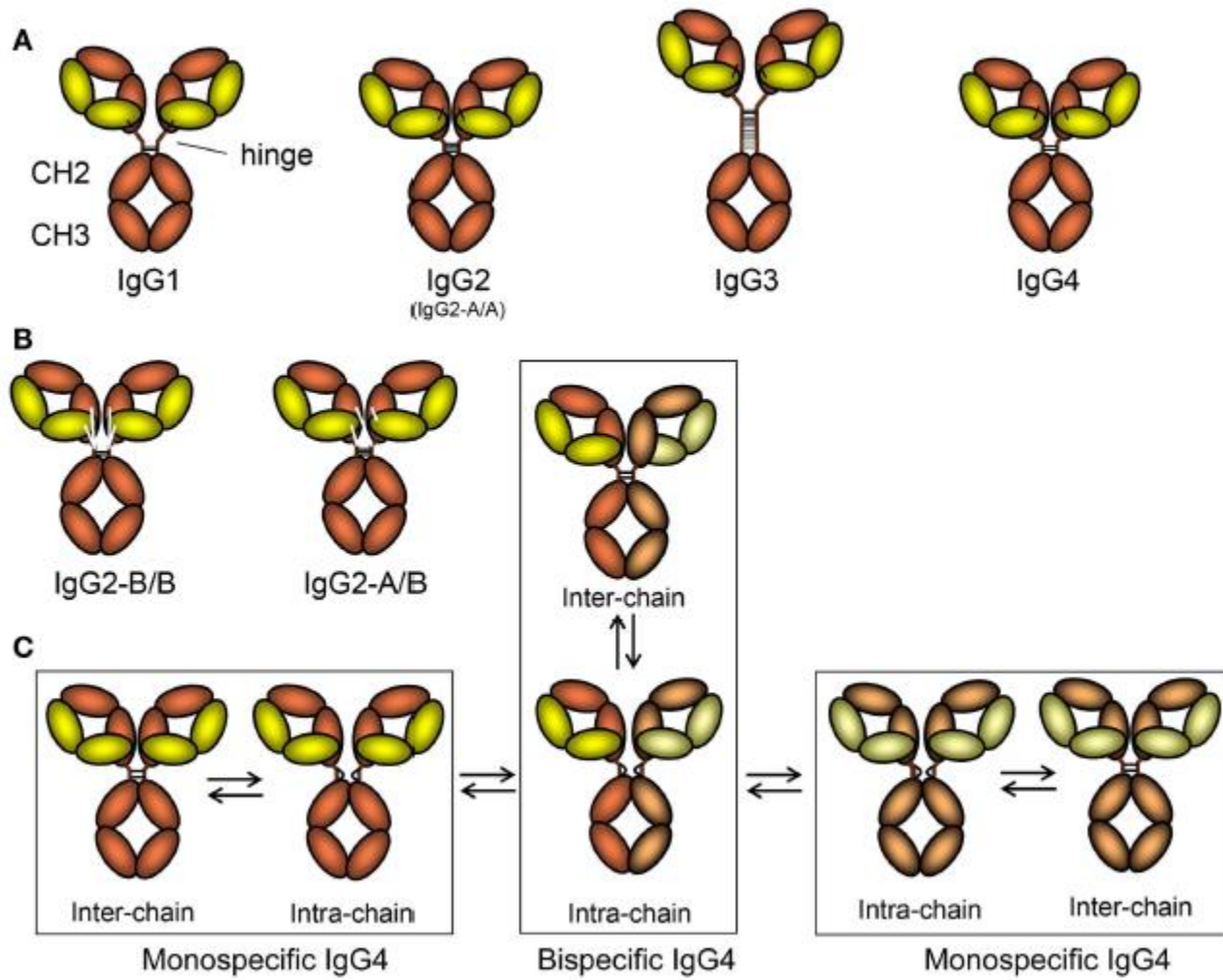
<sup>a</sup>Depends on allotype.

<sup>b</sup>For A/A isomer.

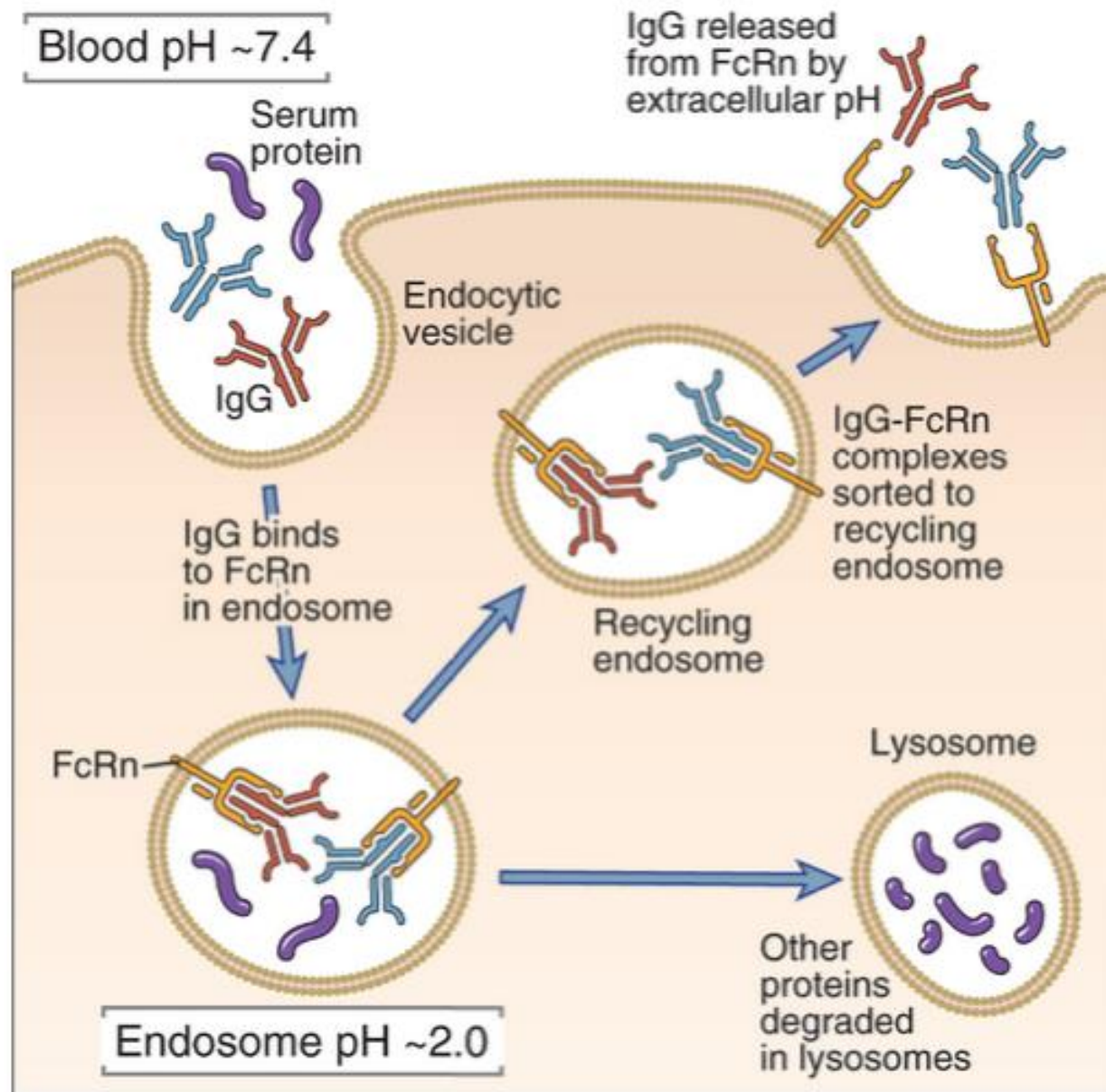
<sup>c</sup>Multivalent binding to transfected cells. Adapted from Bruhnsetal.(2).

<sup>d</sup>Association constant ( $\times 10^6 M^{-1}$ ) for monovalent binding (2).

<sup>e</sup>After repeated encounters with protein antigens, often allergens.

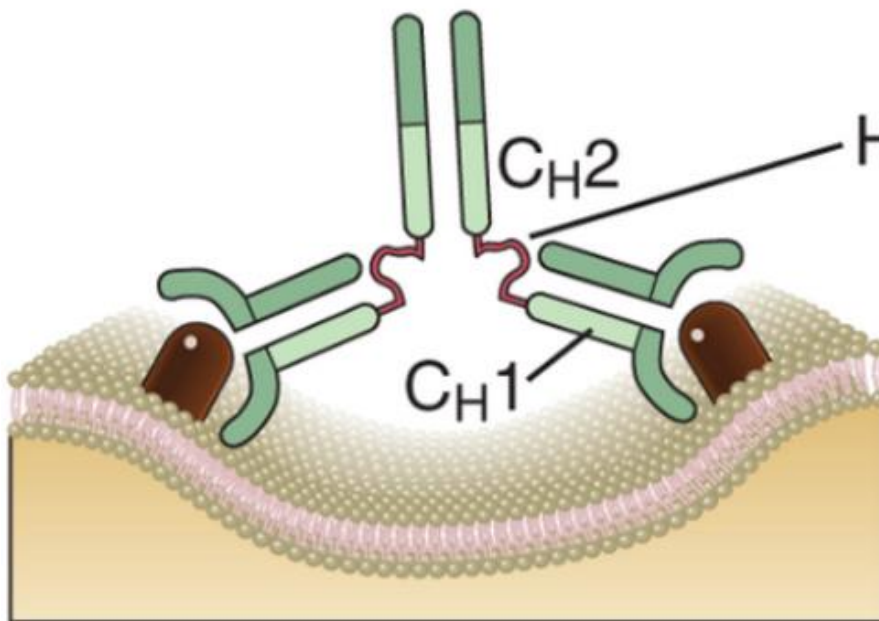


# FcRn contributes to the long half-life of IgG molecules

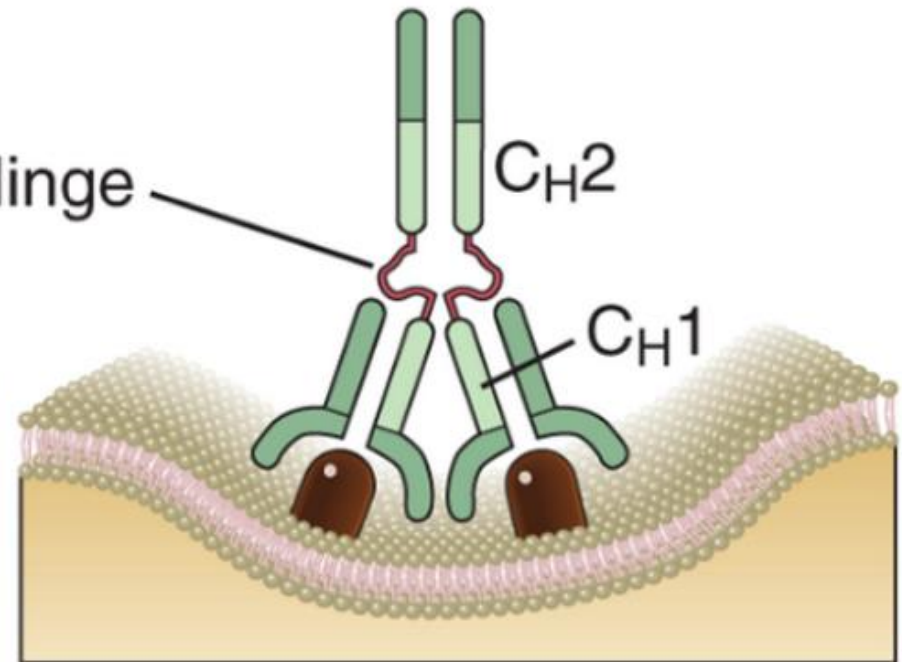


# Flexibility of antibody molecules

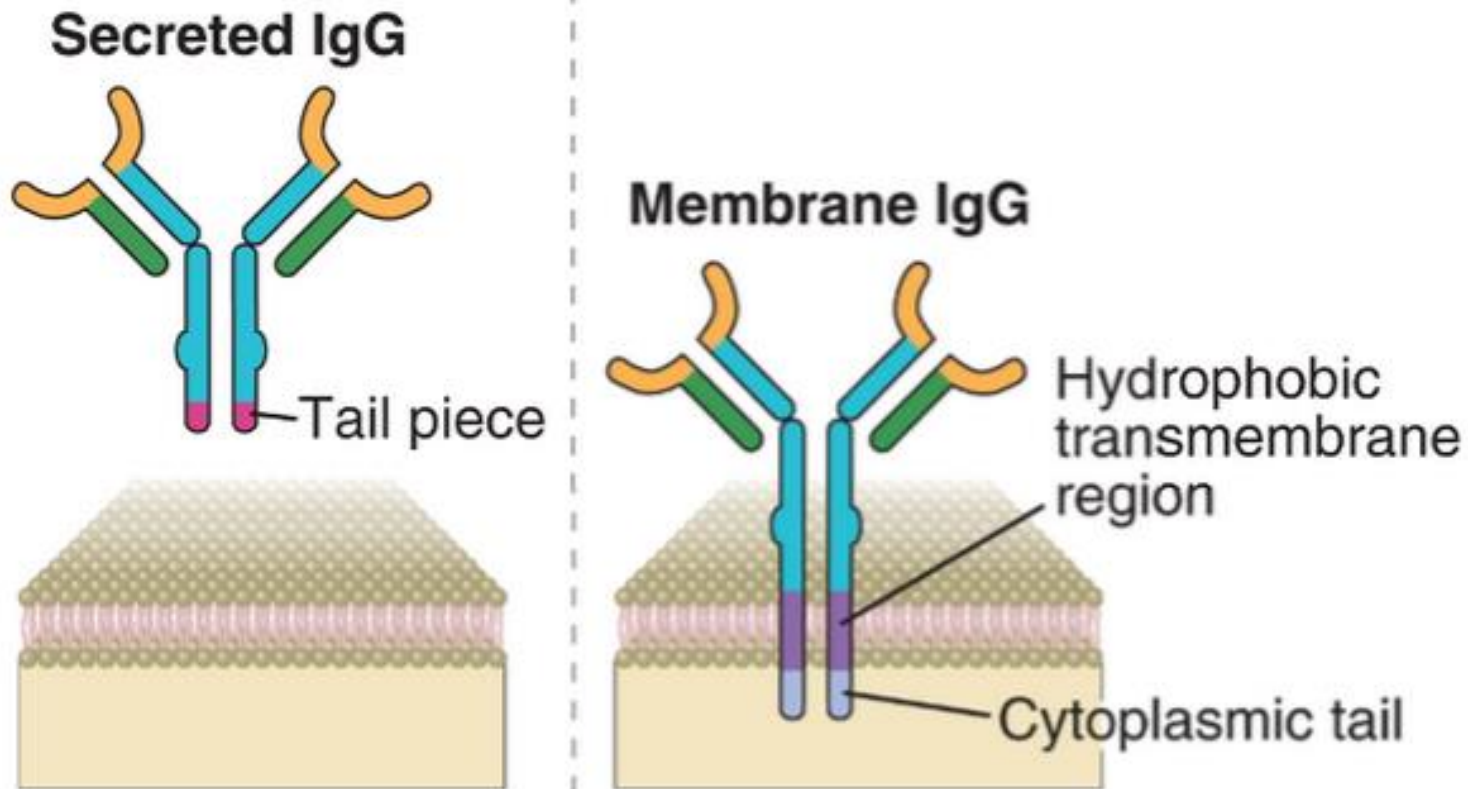
**A** Widely spaced cell surface determinants









**B** Closely spaced cell surface determinants

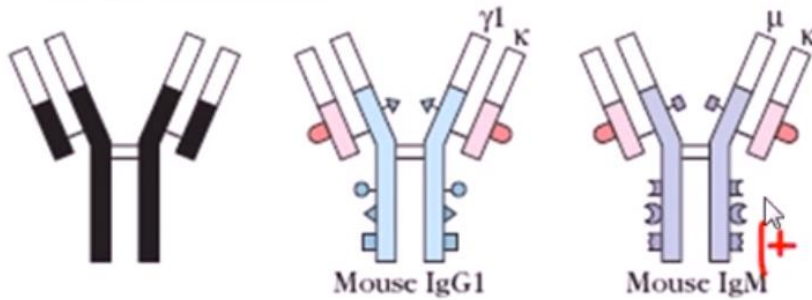


# Membrane and secreted forms of Ig heavy chains

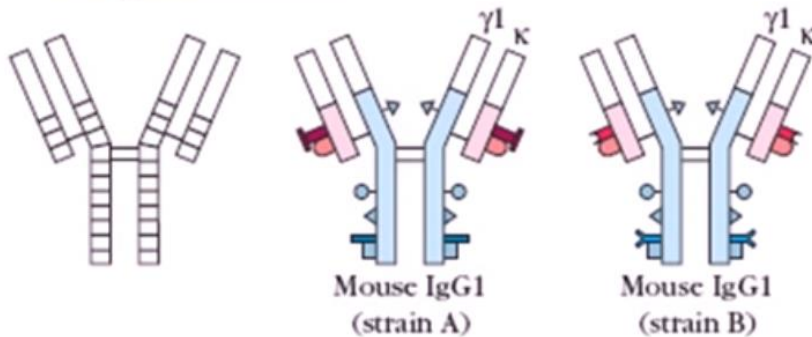


- |   |                               |   |                      |
|---|-------------------------------|---|----------------------|
|  | V region                      |  | Light chain C region |
|  | Tail piece                    |  | Cytoplasmic tail     |
|  | Transmembrane region          |   |                      |
|  | $\gamma$ heavy chain C region |   |                      |

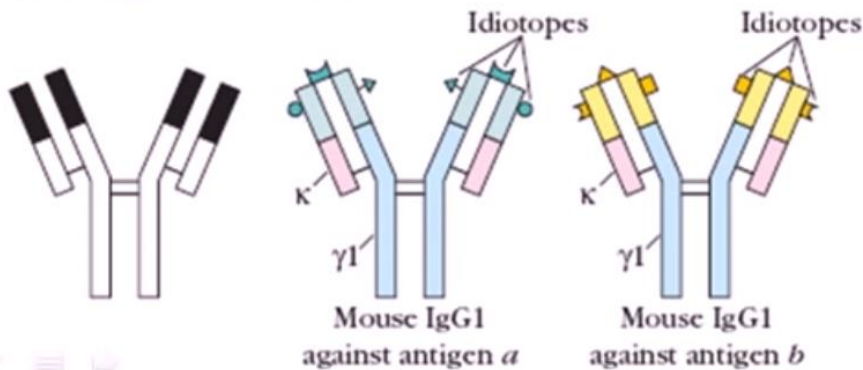
(a) Isotypic determinants



(b) Allotypic determinants



(c) Idiotypic determinants

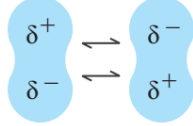
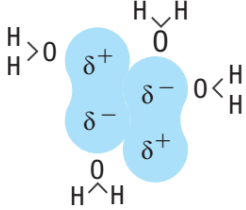
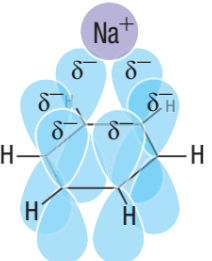


**Isotypic,  
Allotypic and  
Idiotypic  
determinants**

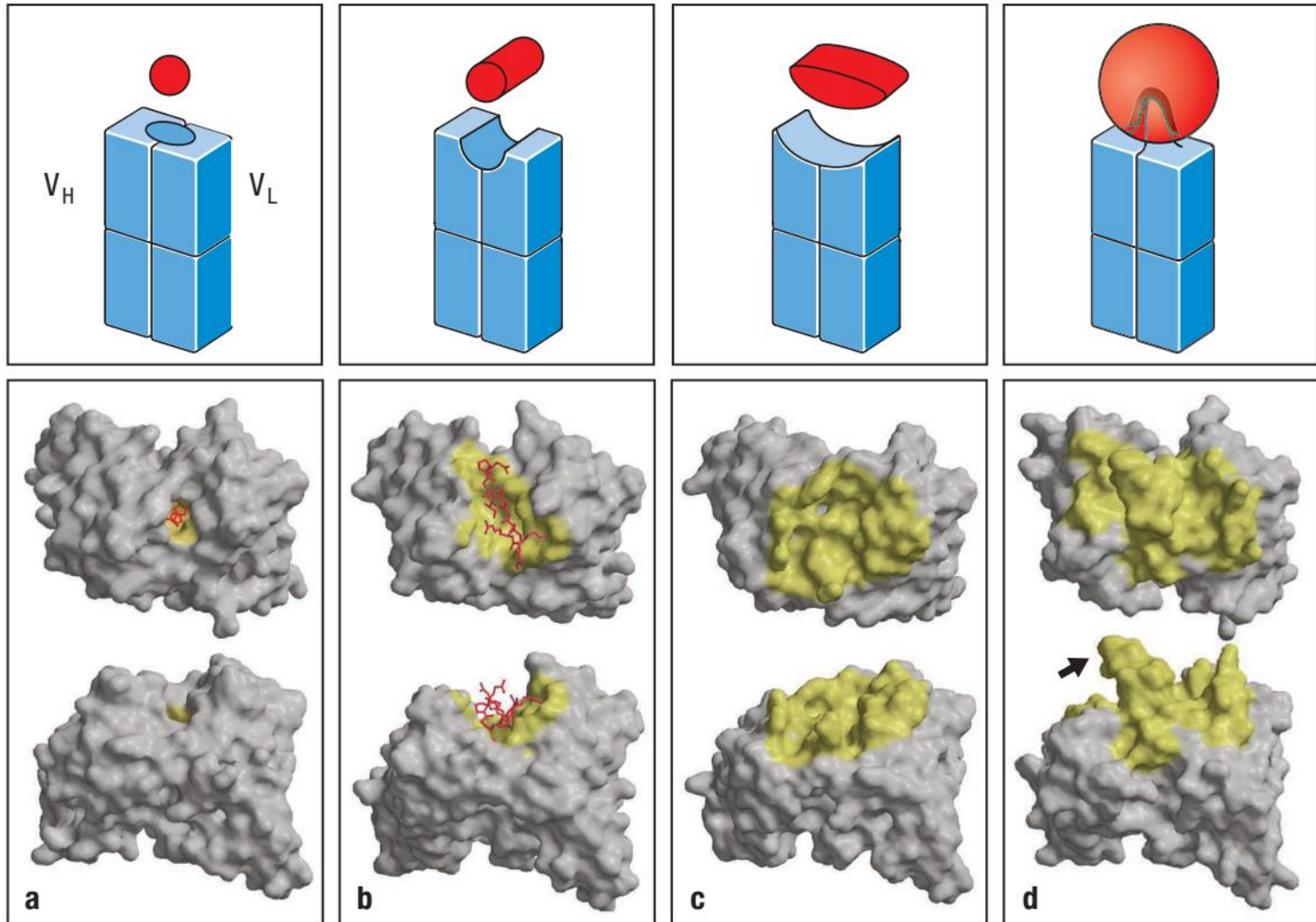
**Idiotype network: a form  
of immunoregulation?**



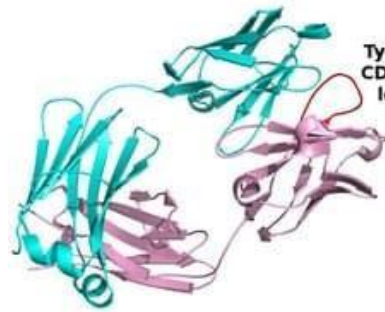
# Antibodies bind to conformational shapes on the surfaces of antigens using a variety of noncovalent forces.

Noncovalent forces	Origin	
Electrostatic forces	Attraction between opposite charges	$-\text{NH}_3^+ \quad \ominus \text{OOC}-$
Hydrogen bonds	Hydrogen shared between electronegative atoms (N, O)	$\begin{array}{c} \diagup \text{N} - \text{H} - - \text{O} = \text{C} \diagdown \\ \delta^- \quad \delta^+ \quad \delta^- \end{array}$
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	

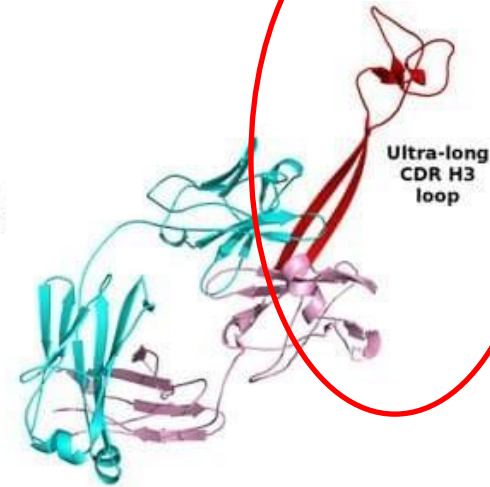
Antigens can bind in pockets, or grooves, or on extended surfaces in the binding sites of antibodies.



# Variantes de estrutura de Igs na natureza e aplicações tecnológicas



Human Antibody

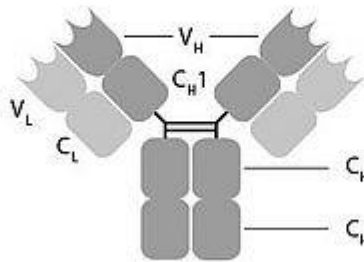


Bovine Antibody

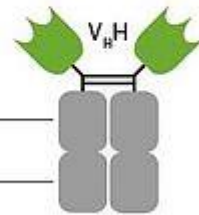
Alpaca



Conventional antibody



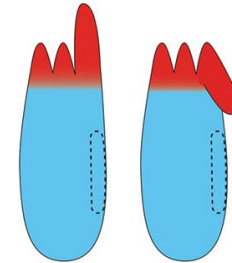
Alpaca heavy-chain antibody



Nanobody/  
V<sub>H</sub>H

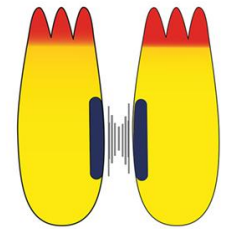


CDR 1  
CDR 2  
CDR 3



camelid VHH

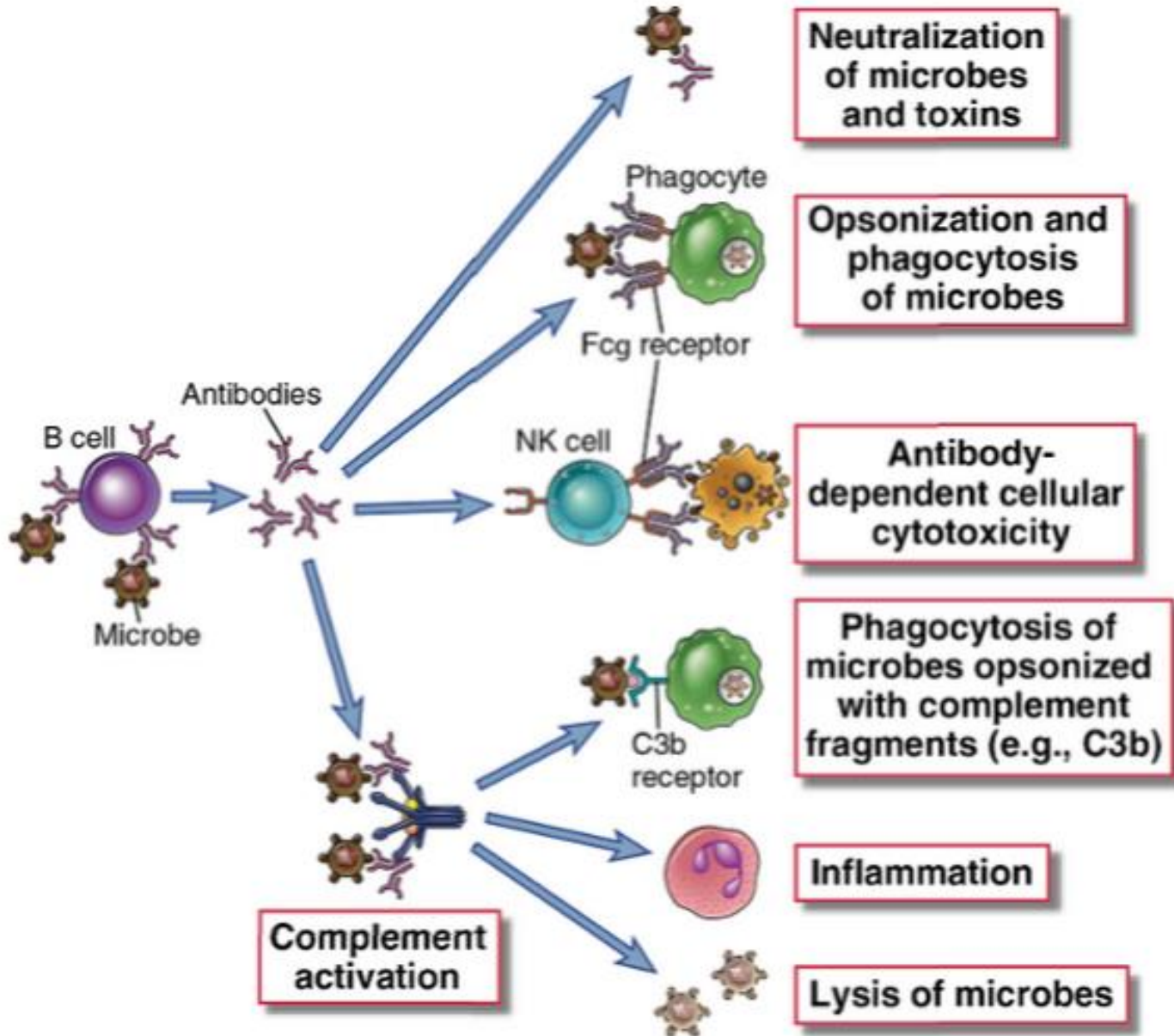
CDR 1  
CDR 2  
CDR 3



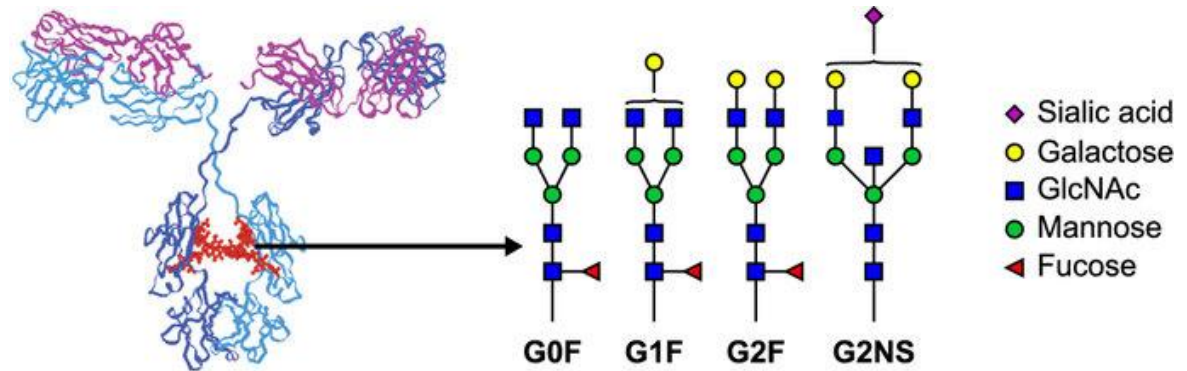
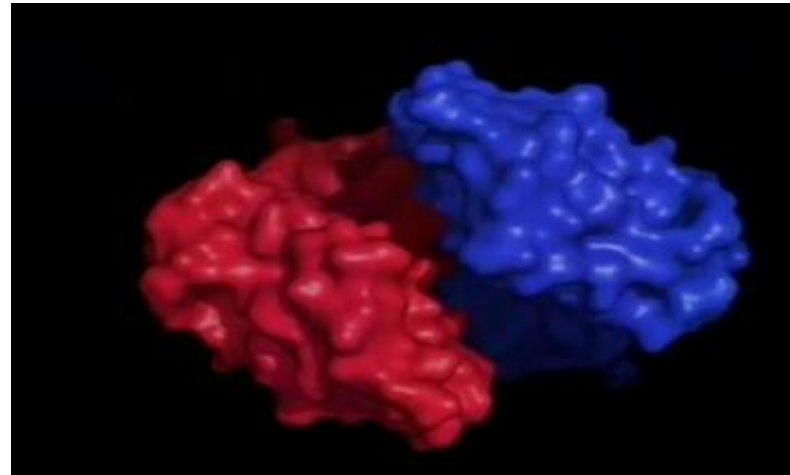
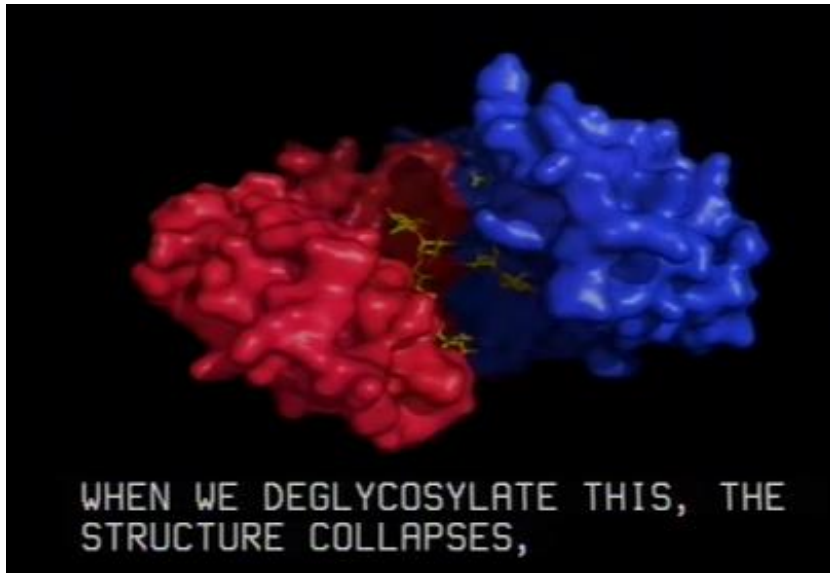
human VH

human VL

# Effector functions of antibodies



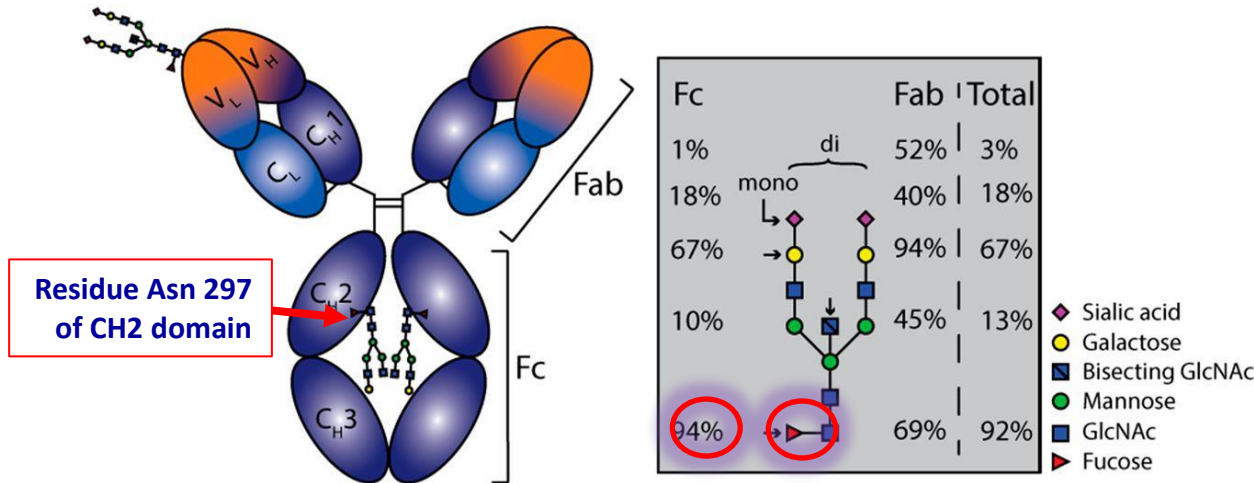
# Propriedades de Igs dependem do tipo e quantidade de glicosilação



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 in red)

# IgG antibodies present distinct profiles of N-glycans

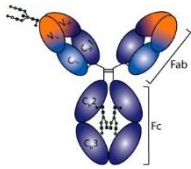
Fleur S. van de Bovenkamp et al. J Immunol 2016;196:1435-1441



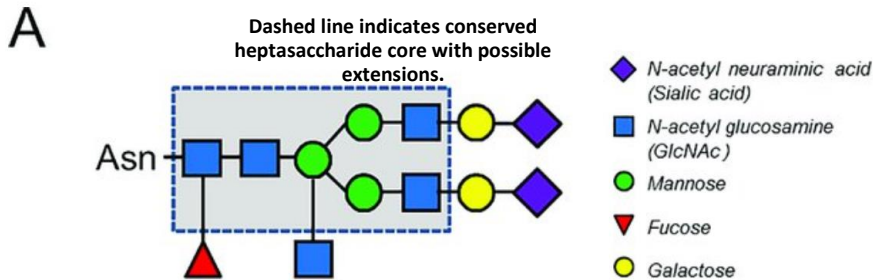
**The majority of IgGs is fucosylated, but.....!**

Marked and clinically significant **reductions** (down to ~10%) in antigen-specific IgG-Fc fucosylation are seen after **alloimmunization** against erythrocyte and platelet alloantigens

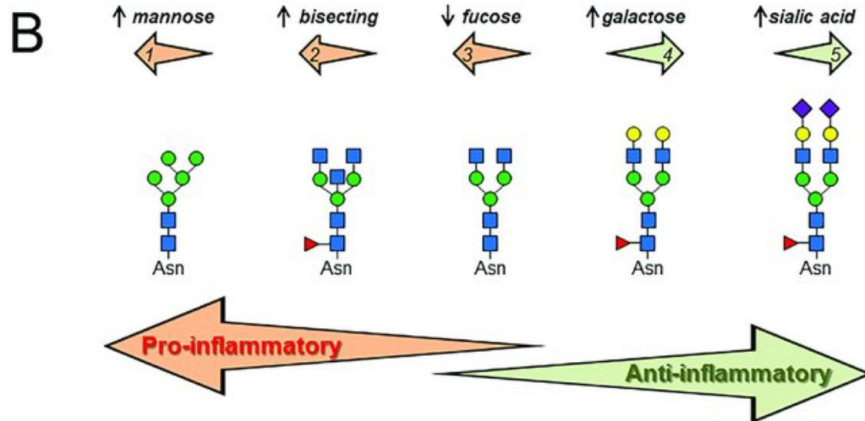
**Alloimmunity:** immune response to nonself antigens from members of the same species



# Fc N-linked glycan profiles affect the downstream effects of IgG



A: N-glycans attached at position Asn297 of IgG Fc fragment are predominantly core-fucosylated bi-antennary structures of the complex type with the arms terminating either with *N*-acetylglucosamine (GlcNAc) or *N*-acetylglucosamine-galactose (GlcNAc-Gal).



B: Five representative structures of IgG Fc glycosylation significantly impact effector functions of the IgGs.

- High-mannose N-glycans, bisecting *N*-acetyl glucosamine (GlcNAc), or **absence of core fucose**, act mainly **pro-inflammatory**
- Terminal galactose or sialic residues (*N*-acetylneuraminic acid) act mainly **anti-inflammatory**

Symbols and colors are drawn according to the Consortium for Functional Glycomics.

**Sexo, idade, ciclo hormonal, estilo de vida ... demografia...**

# Hyposialylated IgG activates endothelial IgG receptor Fc $\gamma$ RIIB to promote obesity-induced insulin resistance

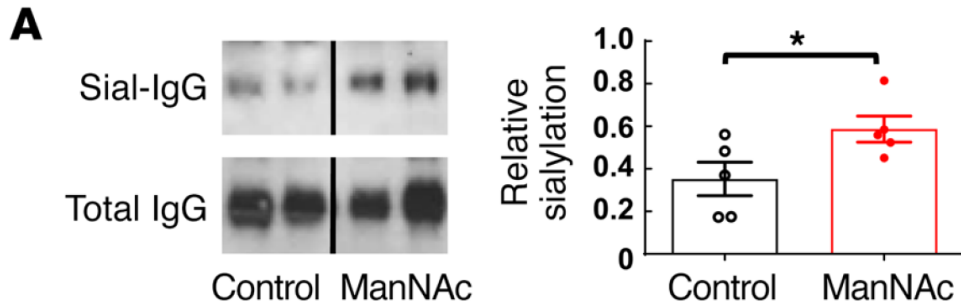
Keiji Tanigaki, ... , Philip W. Shaul, Chieko Mineo

University of Texas Southwestern Medical Center, Dallas, Texas, USA

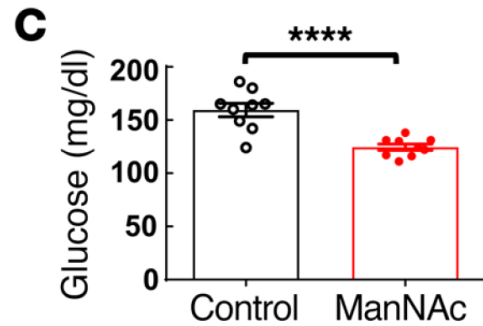
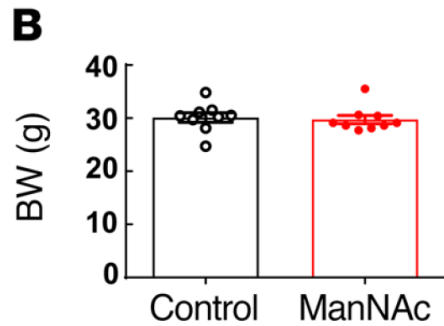
*J Clin Invest.* 2018;128(1):309-322. <https://doi.org/10.1172/JCI89333>.

- Despite becoming obese on a high-fat diet, mice lacking Fc $\gamma$ RIIB globally or selectively in endothelium were protected from insulin resistance (insulin delivery to skeletal muscle and resulting maintenance of muscle glucose disposal)
- 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.

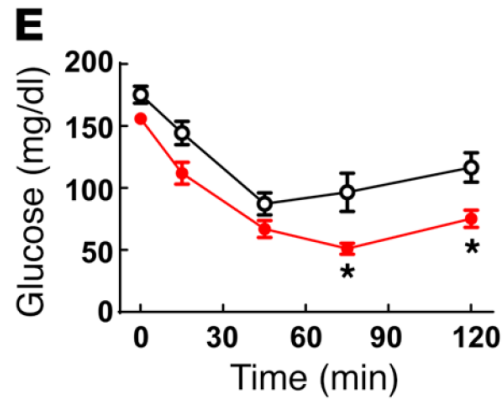
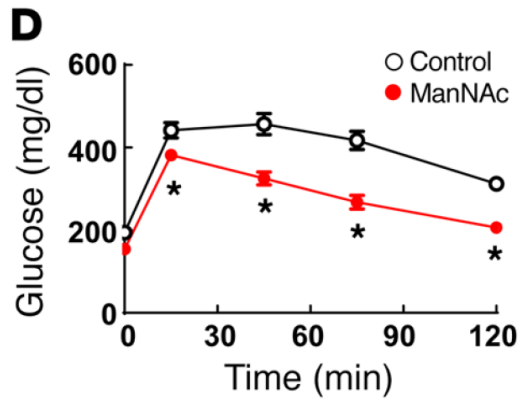




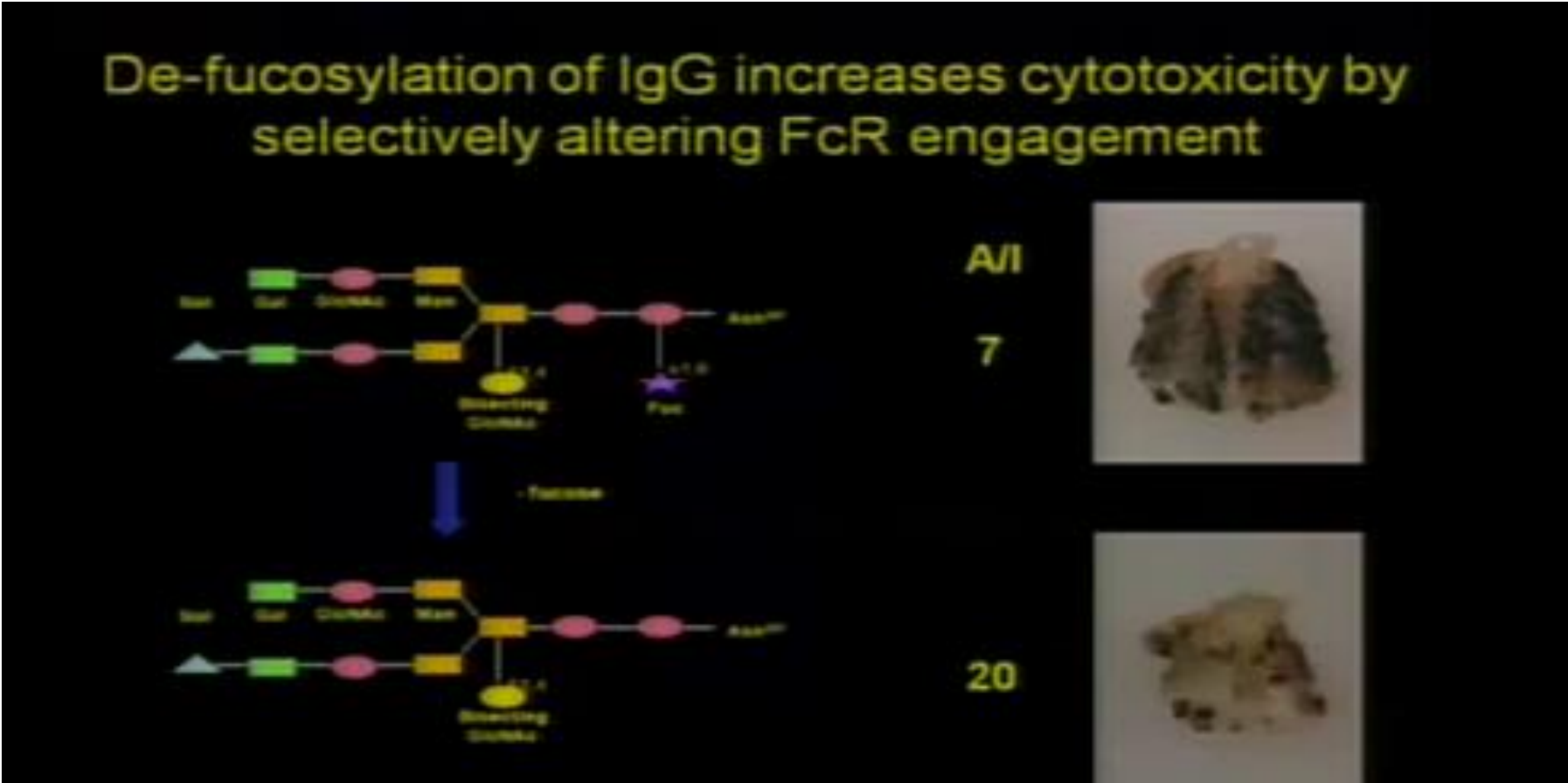
**Figure 7. ManNAc treatment protects mice from obesity-induced glucose intolerance and insulin resistance.** (A) Male WT mice were fed a HFD and either regular drinking water (control) or ManNAc-supplemented drinking water for 6 weeks. Plasma IgG was isolated, and its sialylation was evaluated by SNA-lectin 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  $\pm$  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



# Propriedades das Igs dependem do tipo e nível de glicosilação





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# Effects of low-calorie and different weight-maintenance diets on IgG glycome composition

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**Obesity-induced inflammation activates the adaptive immune system by altering immunoglobulin G (IgG) glycosylation in a way to produce more proinflammatory antibodies.**

# Antígenos

Interações com anticorpos

## **ANTIGEN (Ag)**

A molecule or molecular structure or any foreign particulate matter or a pollen grain that can bind to a **specific antibody or T-cell receptor**

## **IMMUNOGEN**

Antigens that are capable of **inducing** a humoral (antibody) or cellular immune response

# Glossary

**Epitopes** - The distinct surface features of an antigen; **antigenic determinants**

**Allergen** - A substance capable of causing an **allergic reaction**

**Superantigen** - Antigens that cause non-specific activation of T-cells, resulting in **polyclonal T-cell activation** and massive cytokine release

**Tolerogen** - A substance that invokes a specific immune non-responsiveness due to its molecular form

**Immunoglobulin-binding protein** - Proteins that are capable of binding to antibodies at positions **outside of the antigen-binding site**

**T-dependent antigen** - Antigens that require **the assistance of T cells** to induce the formation of specific antibodies

**T-independent antigen** - Antigens that stimulate B cells **directly**.

**Immunodominant antigens** - Antigens that dominate (over all others from a pathogen) in their ability to produce an immune response.

**Haptens** are small molecules that elicit an immune response only when attached to a large carrier such as a protein

**Exogenous antigens**

**Autoantigens**

**Exogenous antigens**

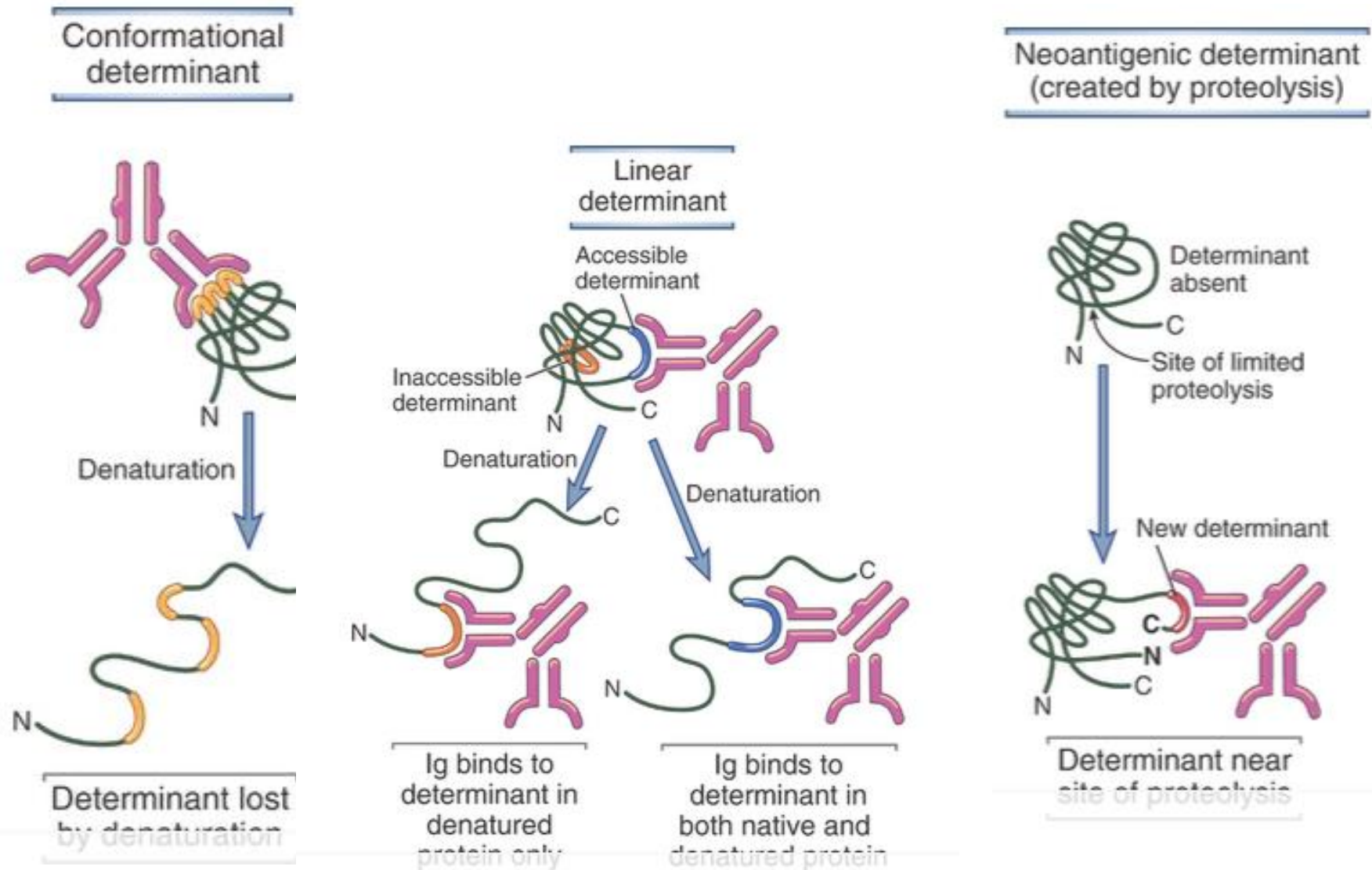
**Viral antigens, microbial antigens, etc**

**Tumor antigens**

**Neoantigens**

**Endogenous antigens**

# The nature of antigenic determinants



B: Other determinants are exposed only on protein unfolding.

## 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



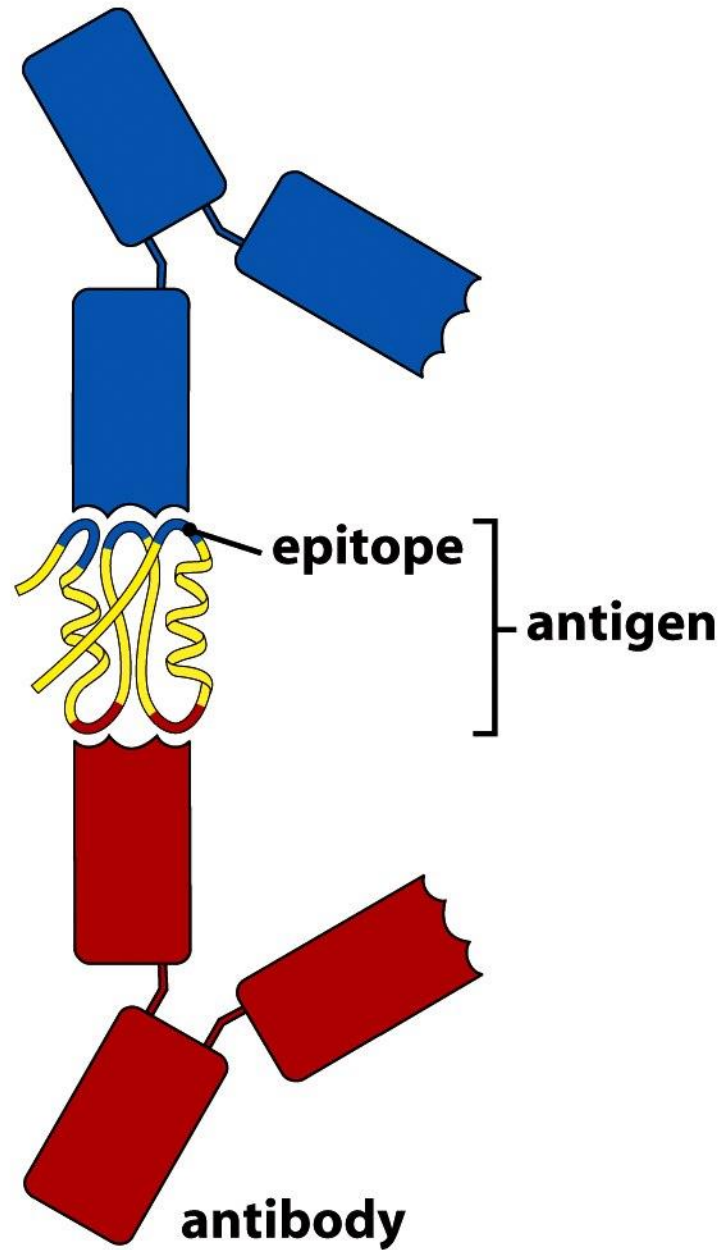
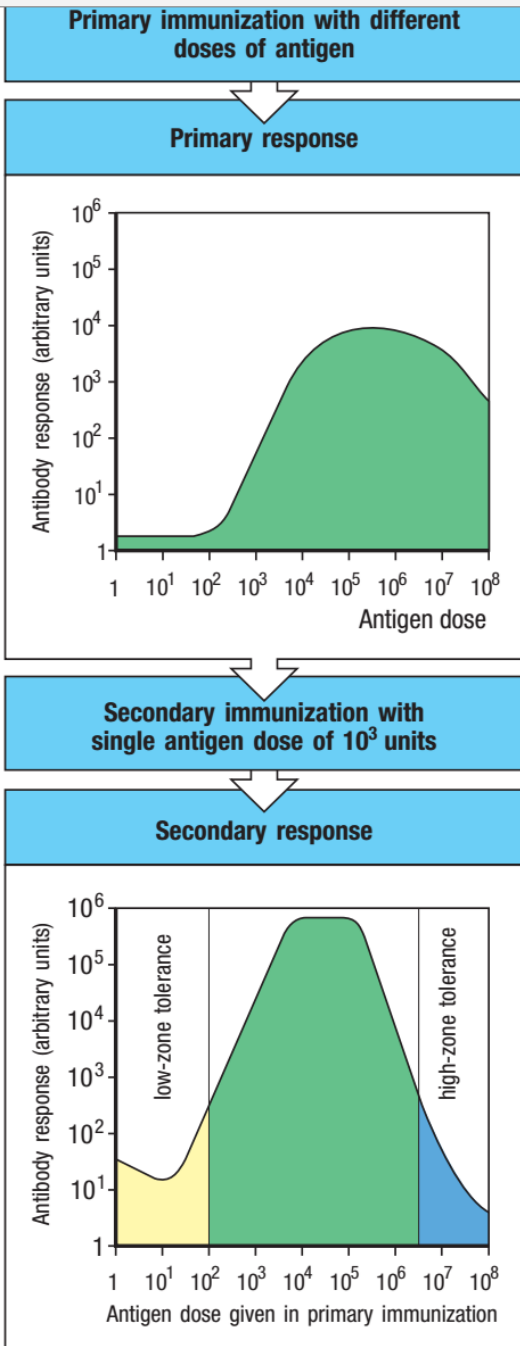
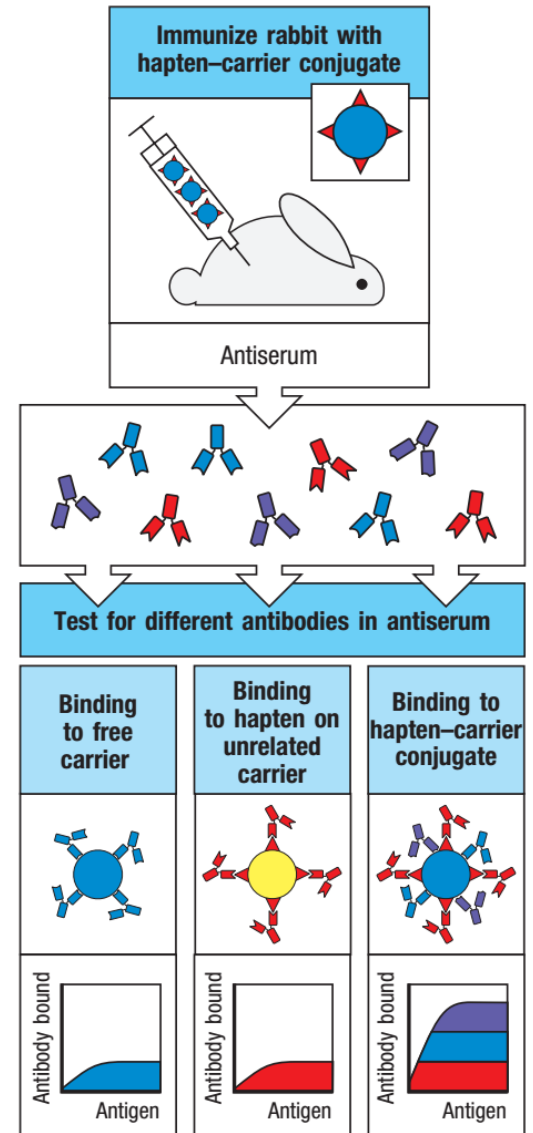


Figure 1-15 Immunobiology, 7ed. (© Garland Science 2008)

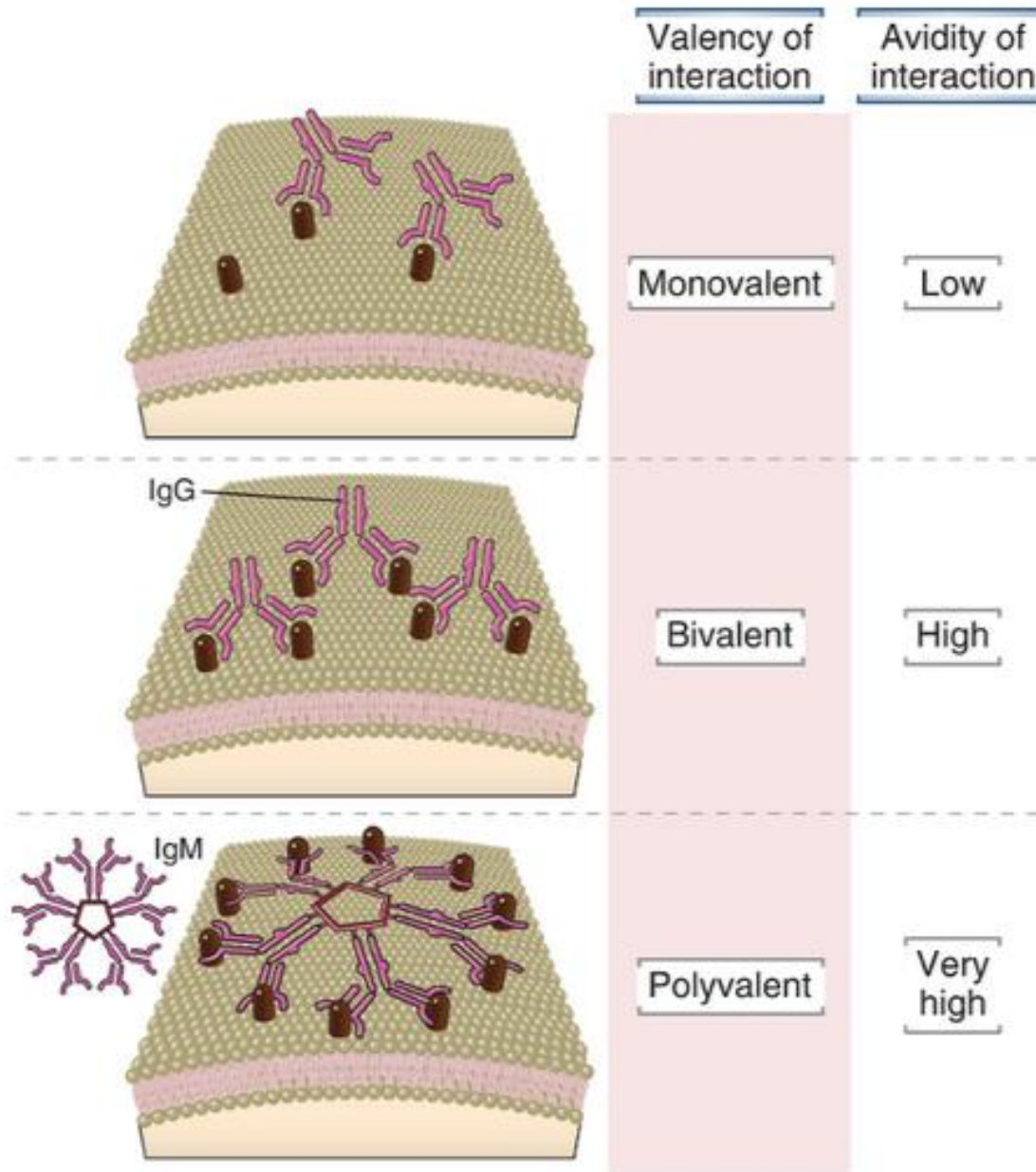


The dose of antigen used in an initial immunization affects the primary and the secondary antibody response.

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

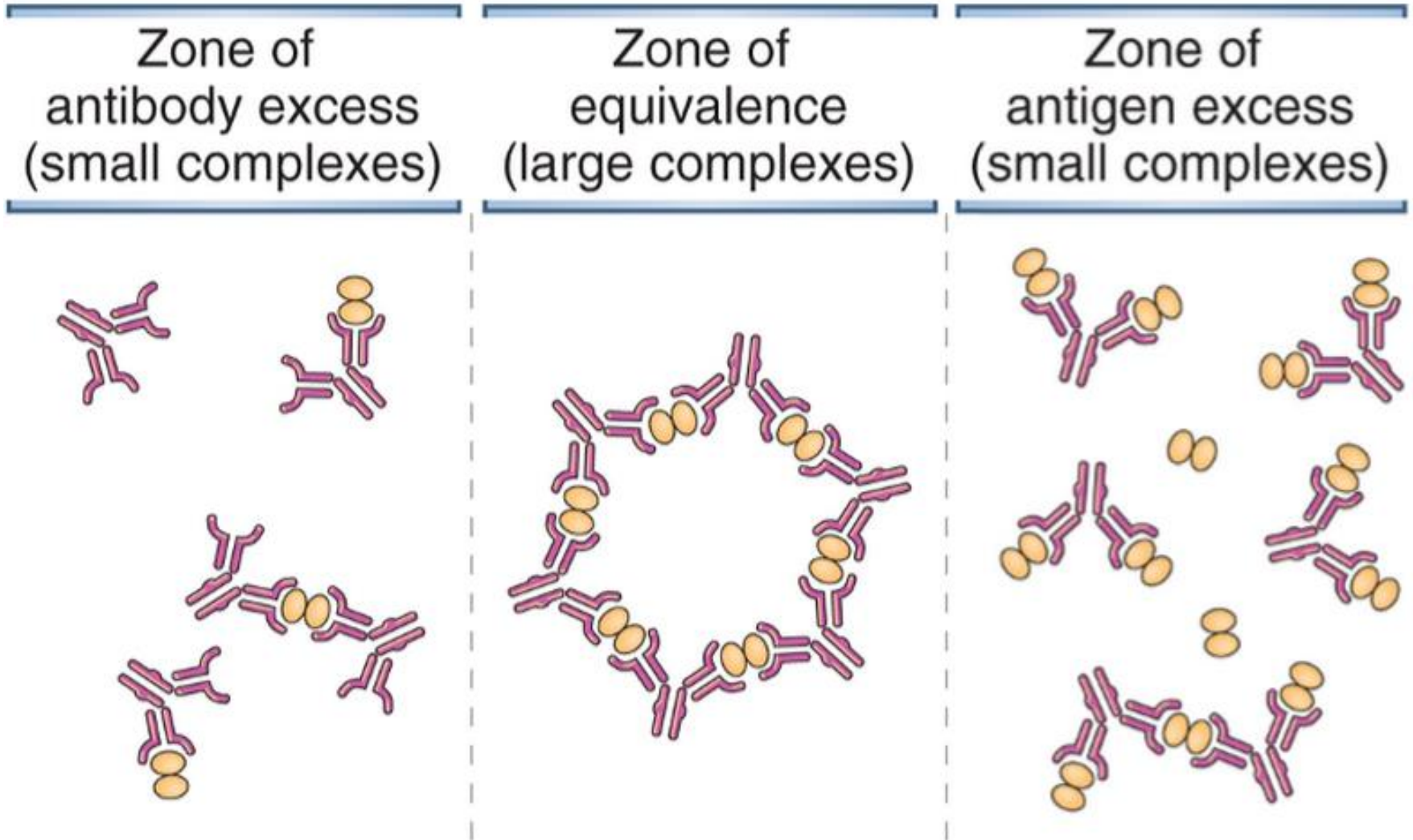


# Valency and avidity of antibody-antigen interactions

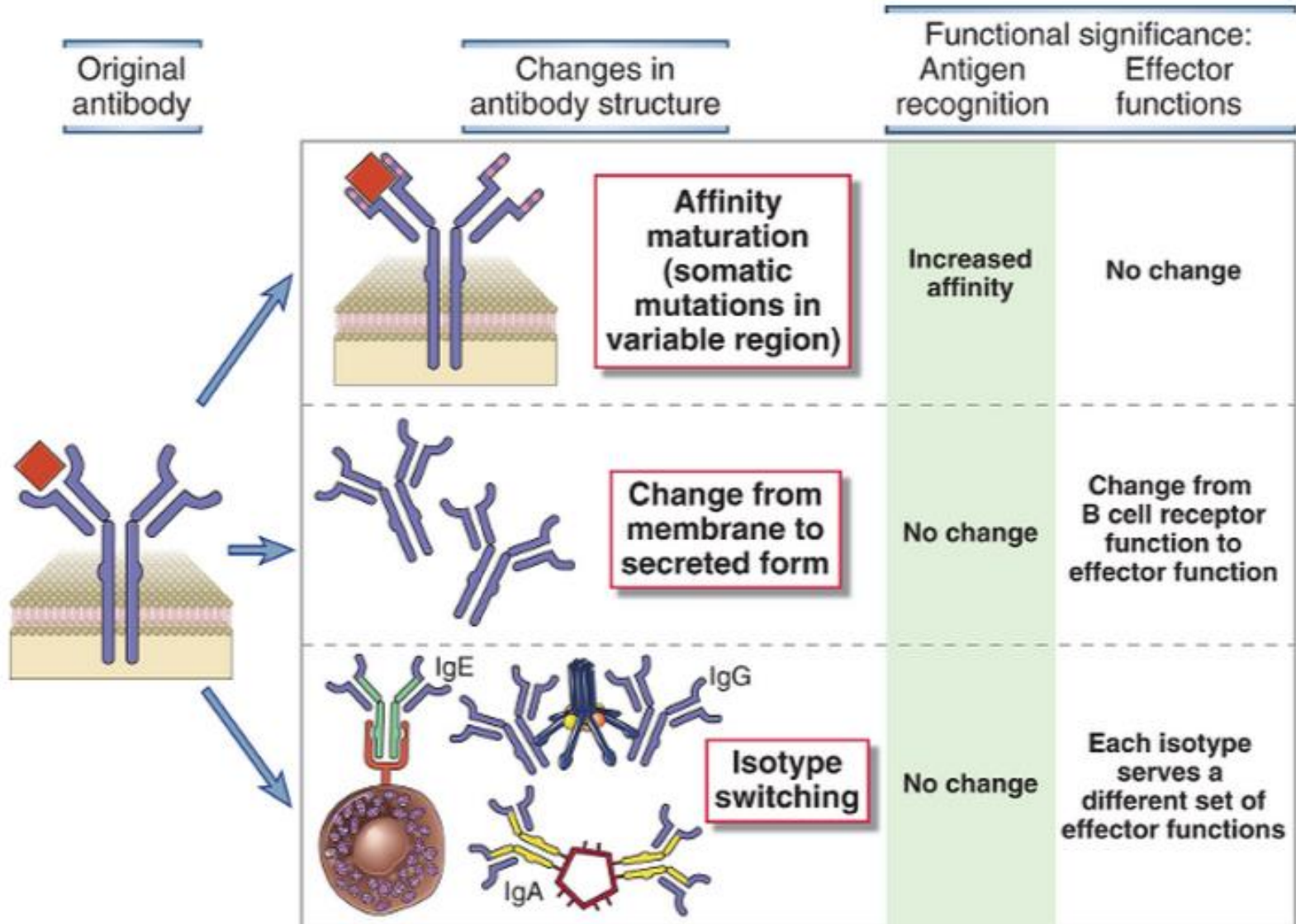


# Antigen-antibody complexes

The basis of immunopathology of immune-mediated diseases

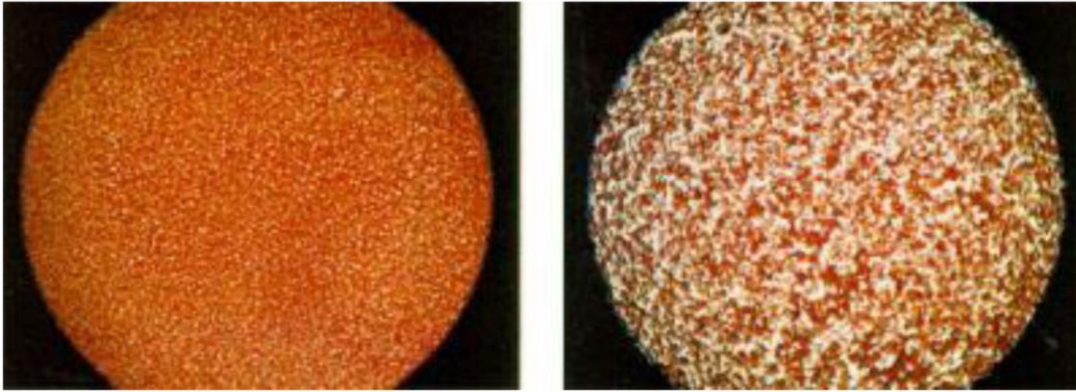


# Changes in antibody structure during humoral immune responses



**Como detectar e medir Anticorpos e suas interações com Antígenos?**

## Antígenos particulados - Aglutinação



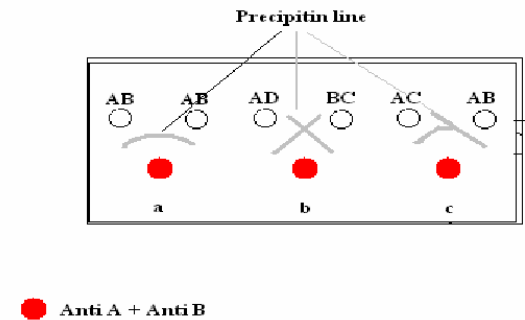
Exemplo: Reação de Widal para o diagnóstico da febre tifóide

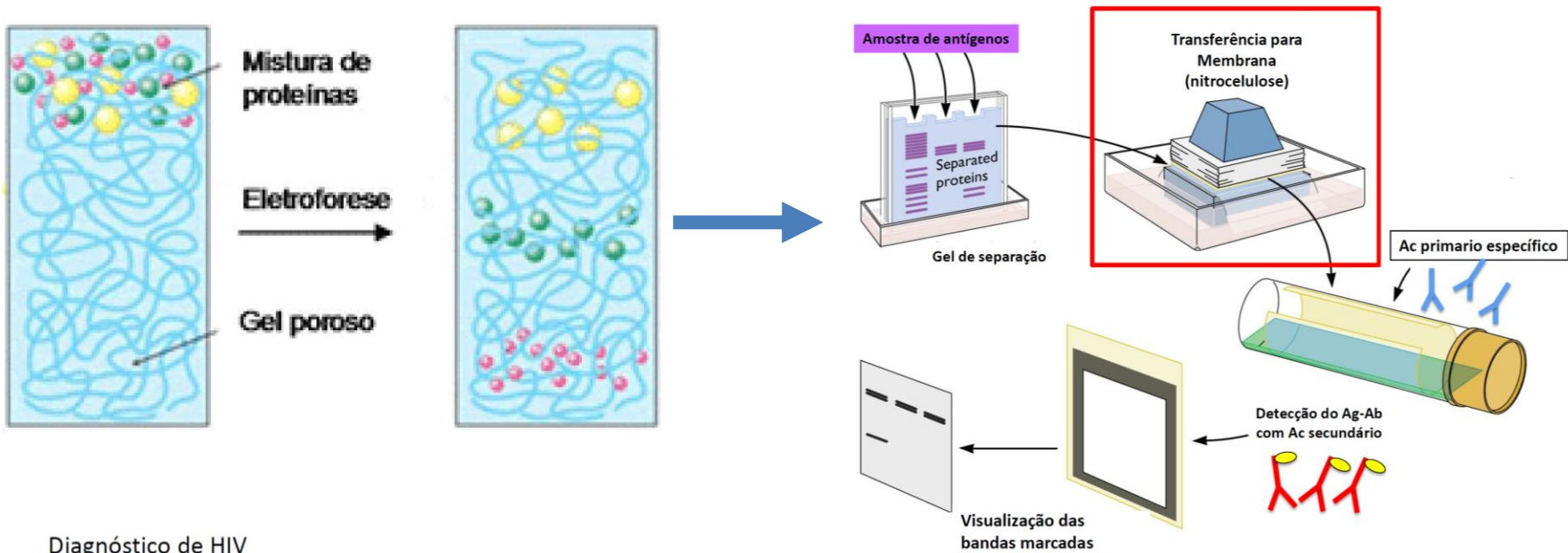
## Antígenos solúveis - Precipitação



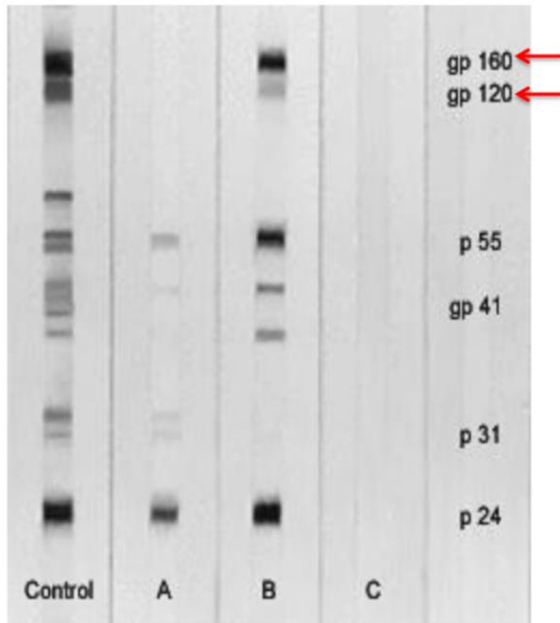
The Ouchterlony reaction:

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

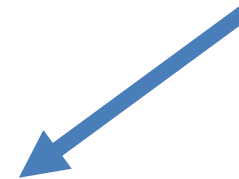
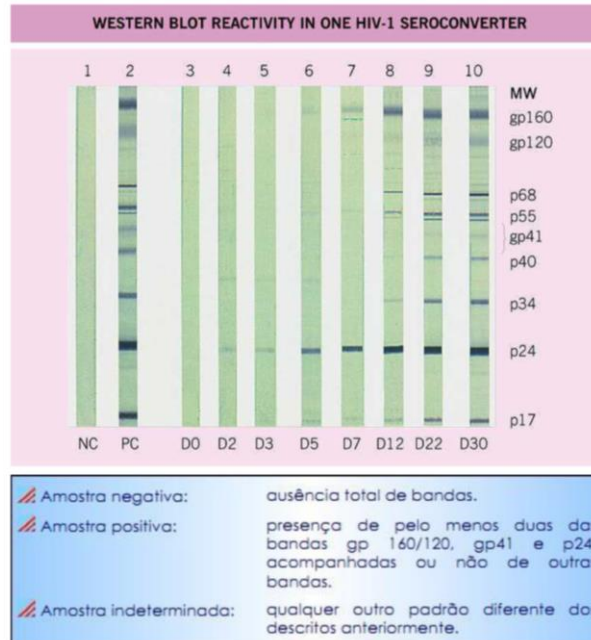




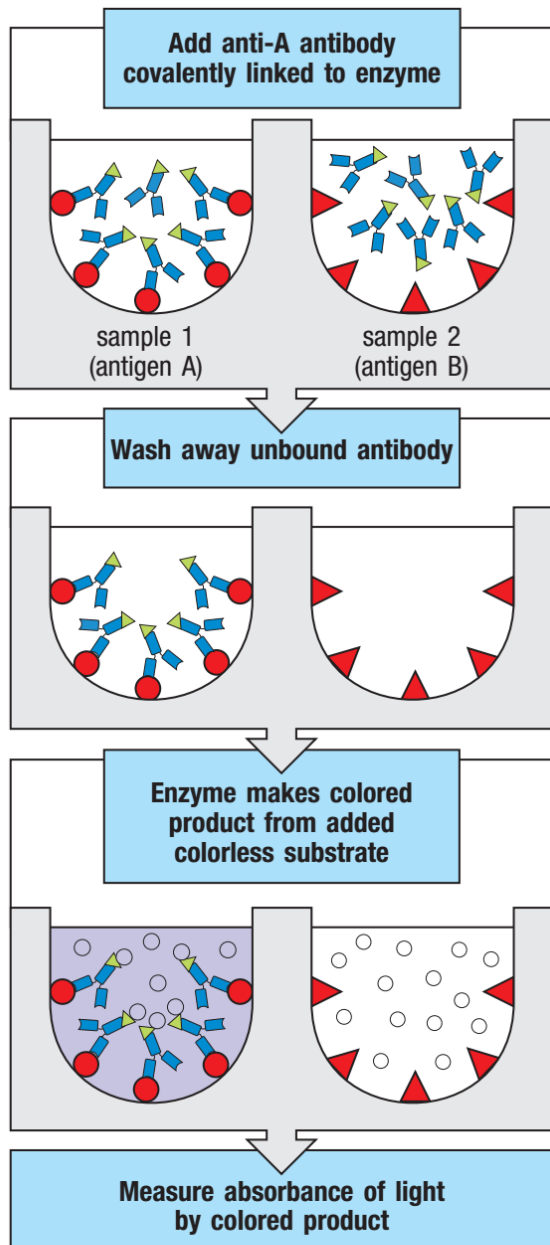
### Diagnóstico de HIV



Pacientes







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