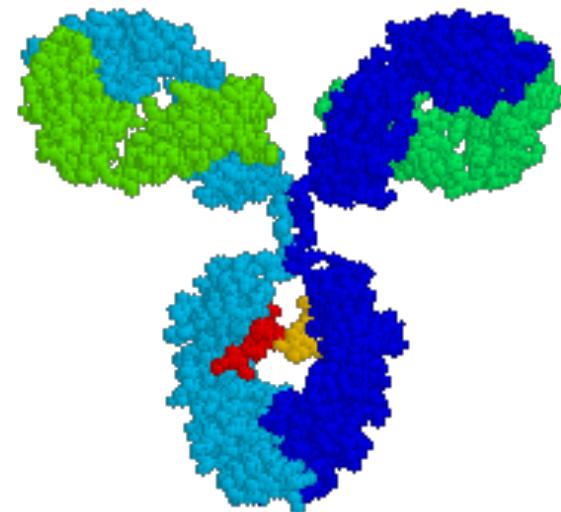


Corso di Immunologia - III anno
Prof. Paolini

Lezione 22/11/2024

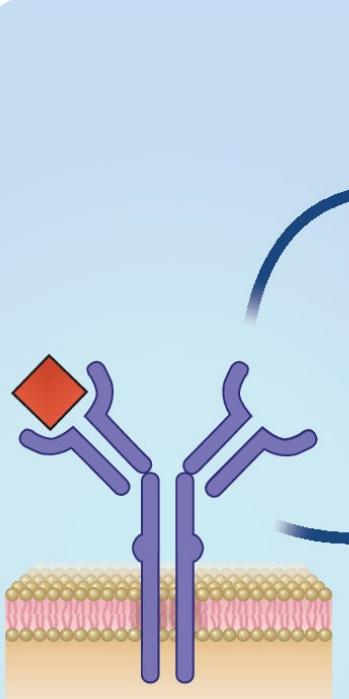
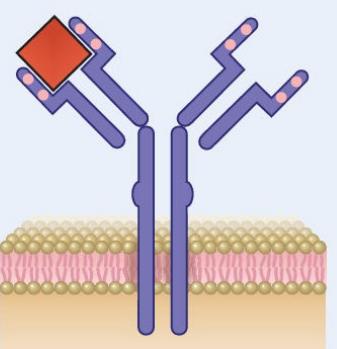
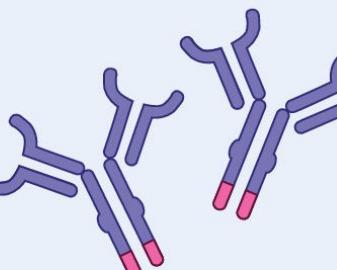
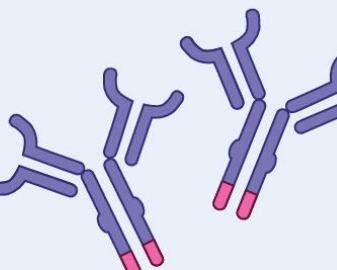
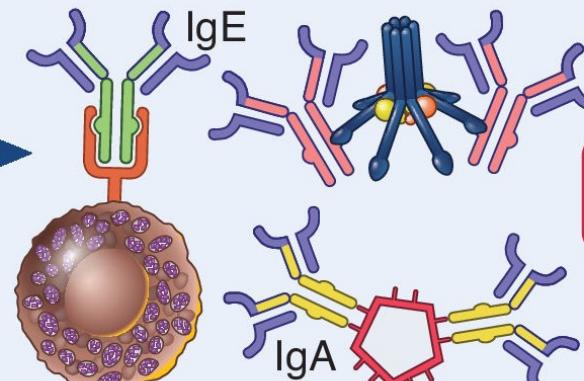
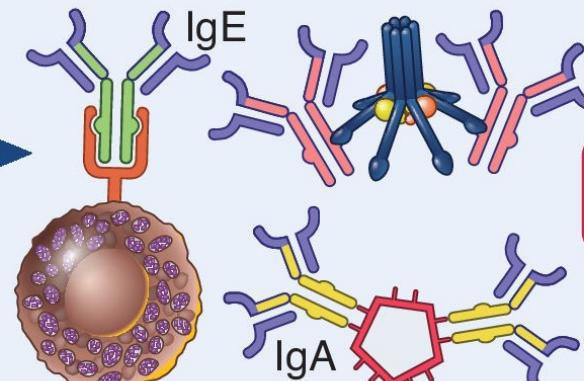
“Le funzioni effettive degli anticorpi”



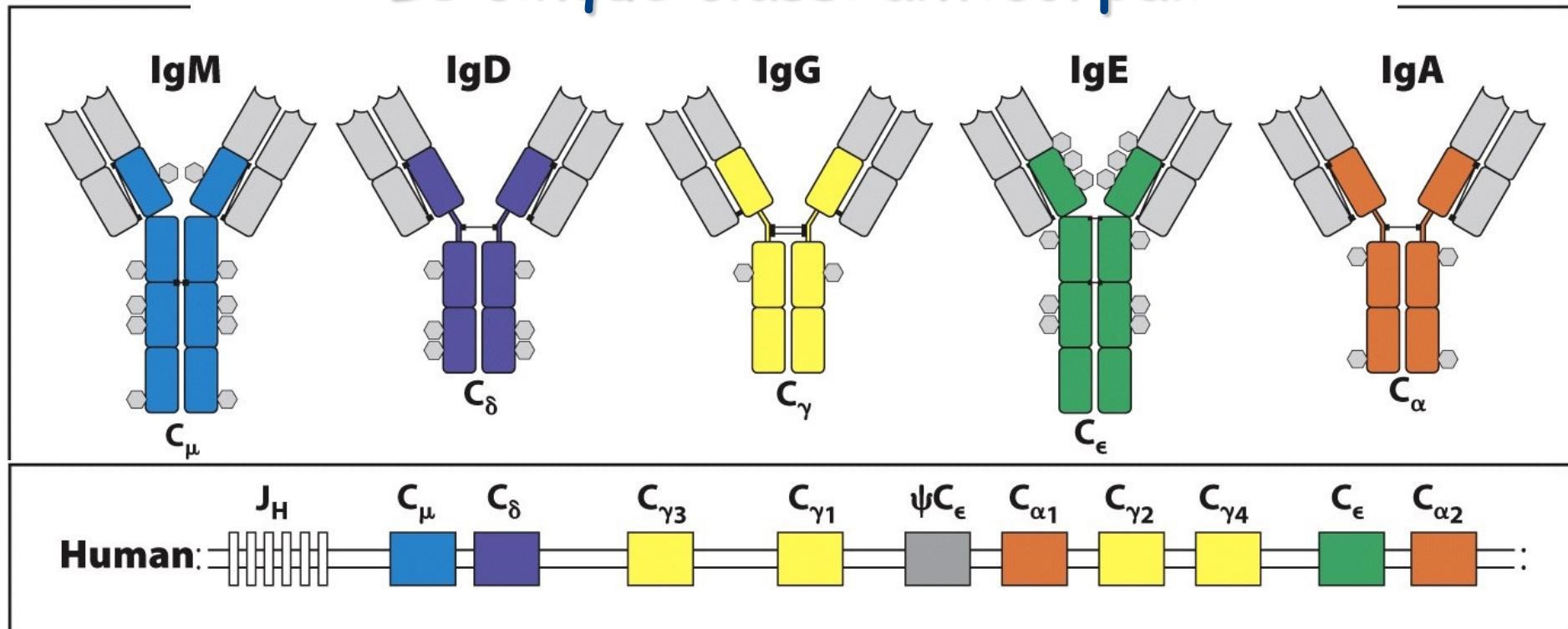
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**Il materiale presente in questo documento viene distribuito
esclusivamente ad uso interno e per scopi didattici.**

Durante la risposta umorale il recettore del linfocita B cambia

Anticorpo originale	Cambiamenti nella struttura dell'anticorpo	Significato funzionale
	 <p>Maturazione dell'affinità (mutazioni somatiche nella regione variabile)</p>	Affinità aumentata Nessun cambiamento
	 <p>Passaggio dalla forma di membrana a quella secreta</p>	Nessun cambiamento Cambiamento dalla funzione da recettore del linfocita B ad anticorpo effettore
	 <p>Scambio isotipico</p>	Nessun cambiamento Ogni isotipo svolge diverse funzioni effettive

Le cinque classi anticorpali

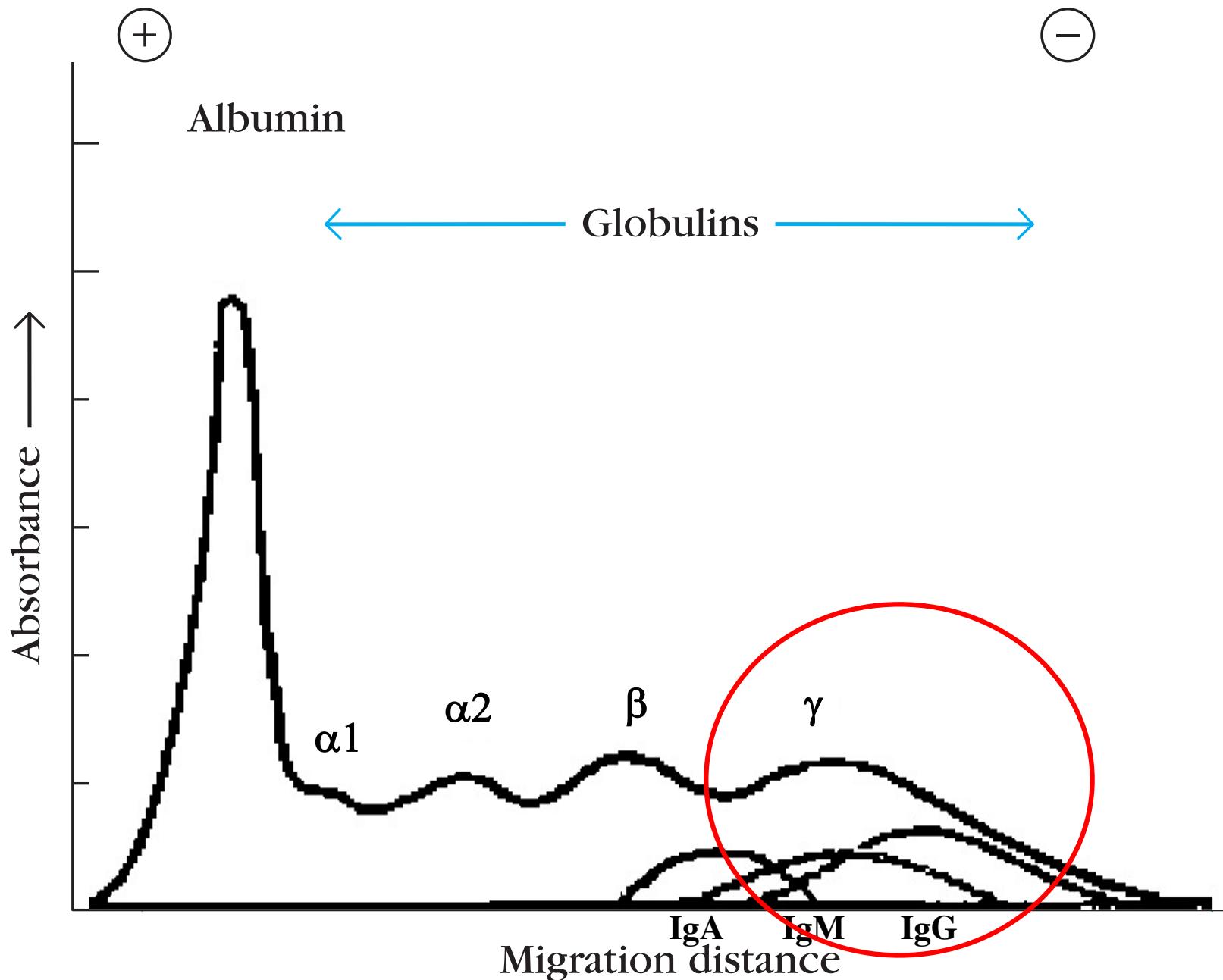


	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Heavy chain	γ_1	γ_2	γ_3	γ_4	μ	α_1	α_2	δ	ϵ
Molecular weight (kDa)	146	146	165	146	970	160	160	184	188
Serum level (mean adult mg/ml)	9	3	1	0.5	1.5	3.0	0.5	0.03	5×10^{-5}
Half-life in serum (days)	21	20	7	21	10	6	6	3	2

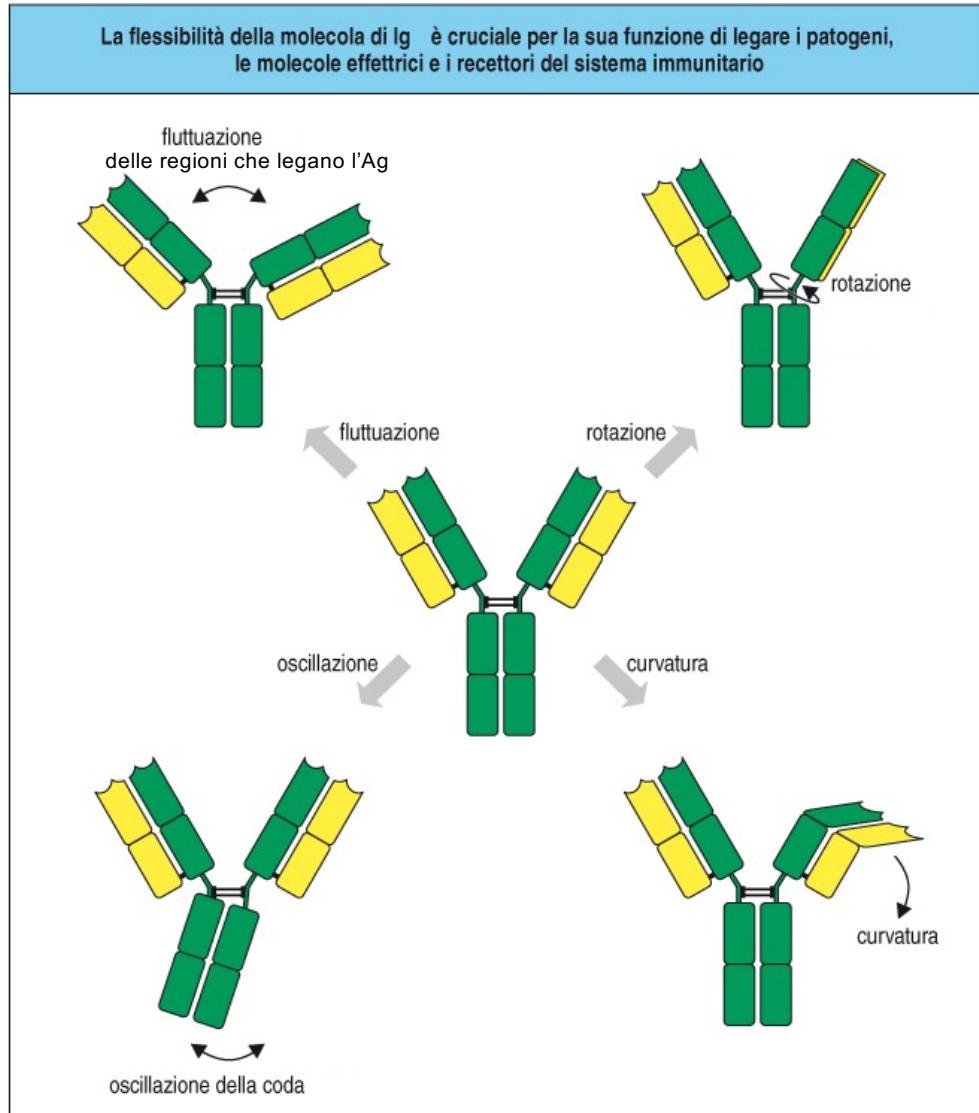
Livelli sierici

IgG>IgA>IgM>IgD>IgE

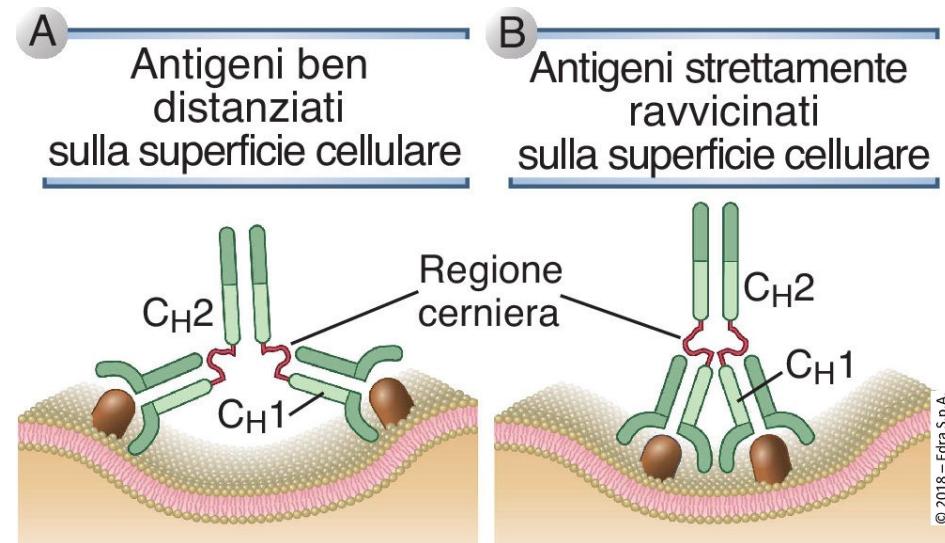
Elettroforesi delle proteine sieriche



La regione cerniera conferisce flessibilità agli anticorpi...

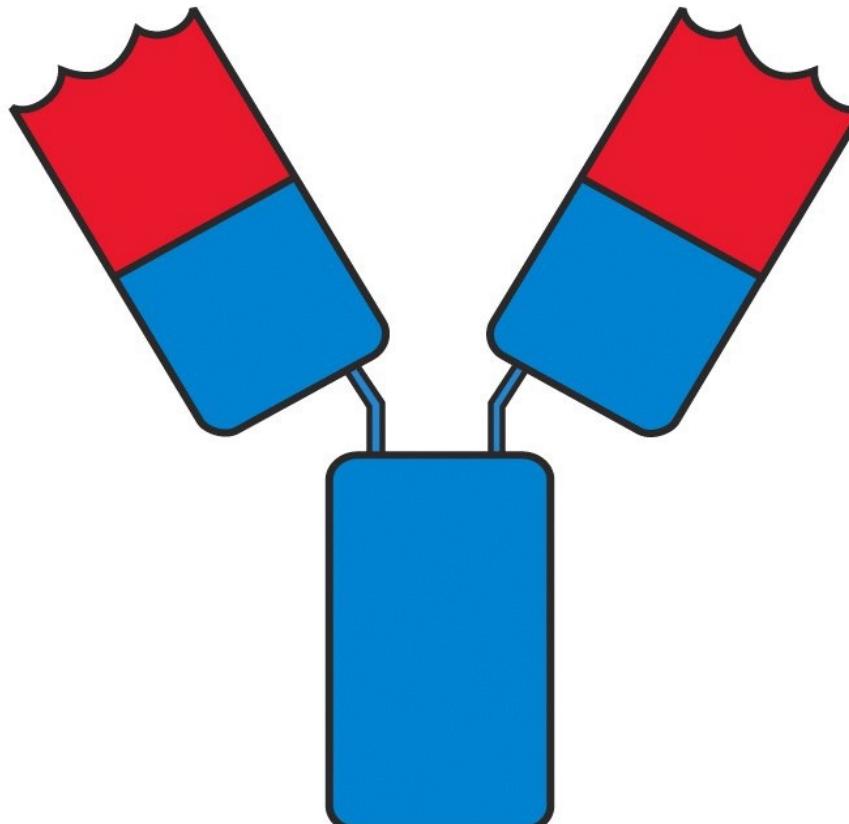


... che possono legare contemporaneamente due antigeni anche distanti tra loro



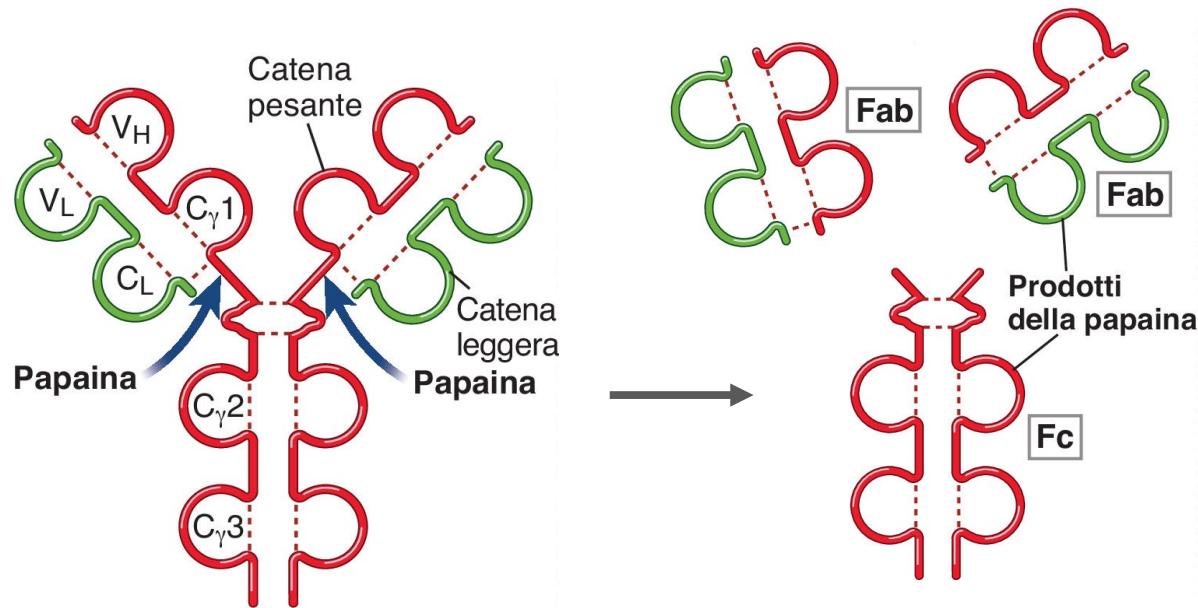
The antibody couples the **specific** recognition of antigen to the activation of **non-specific effector mechanisms**

variable regions
(antigen-binding sites)



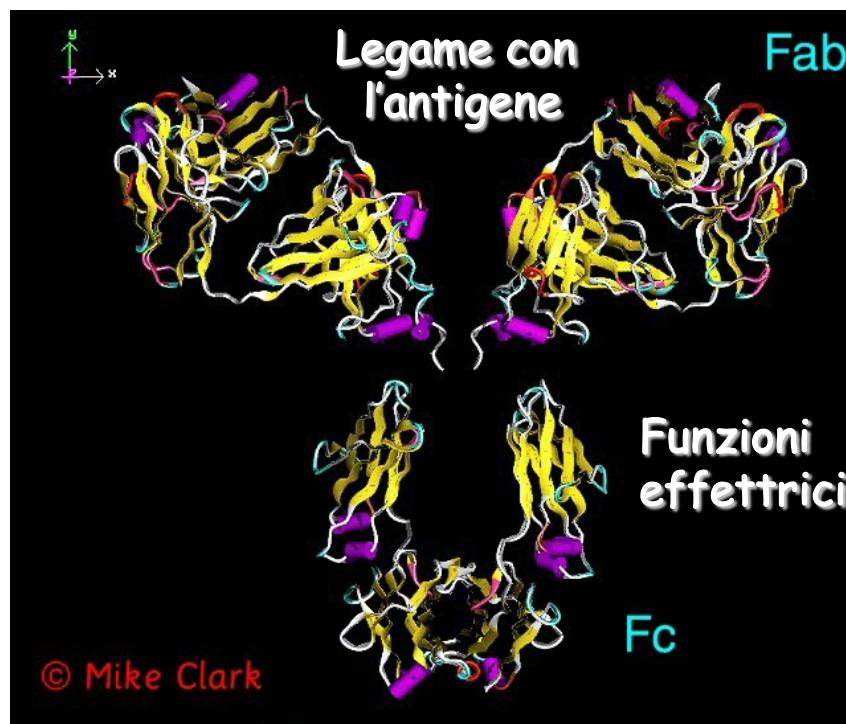
constant region
(effector function)

L'Ig può venire scissa in tre frammenti, due identici tra loro chiamati Fab ed un terzo frammento Fc

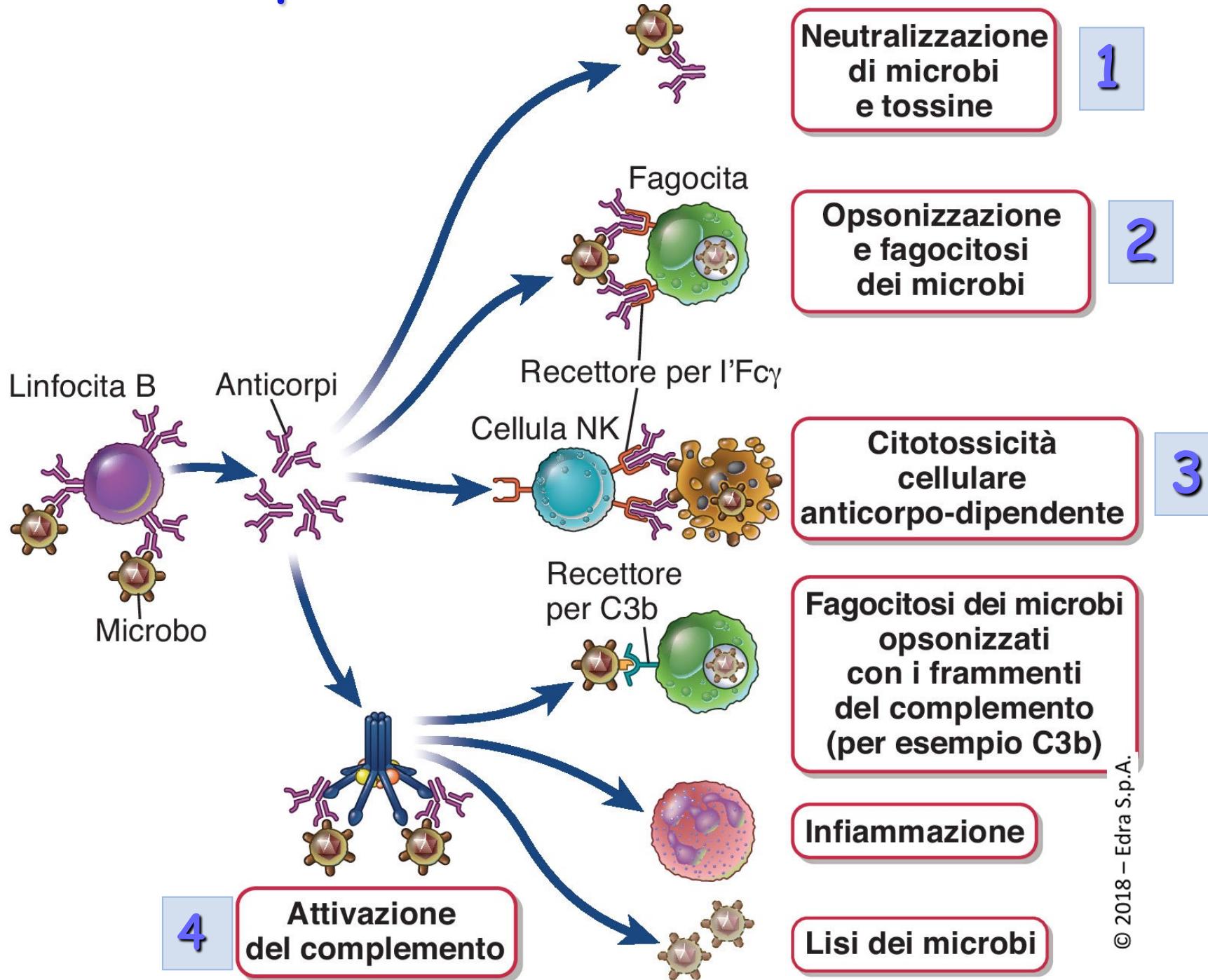


Fab = Fragment
Antigen Binding

Fc = Frammento
Cristallizzabile



Antibodies perform several effector functions



1. Antibodies can neutralize the infectious ability of pathogens

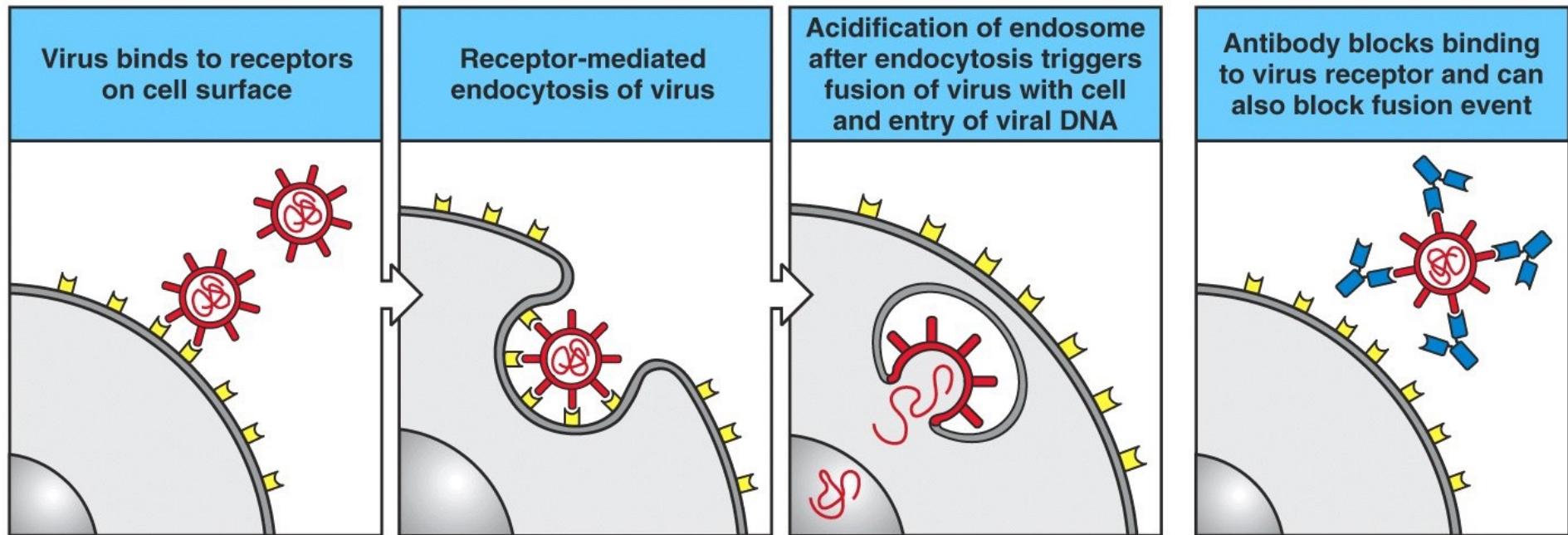
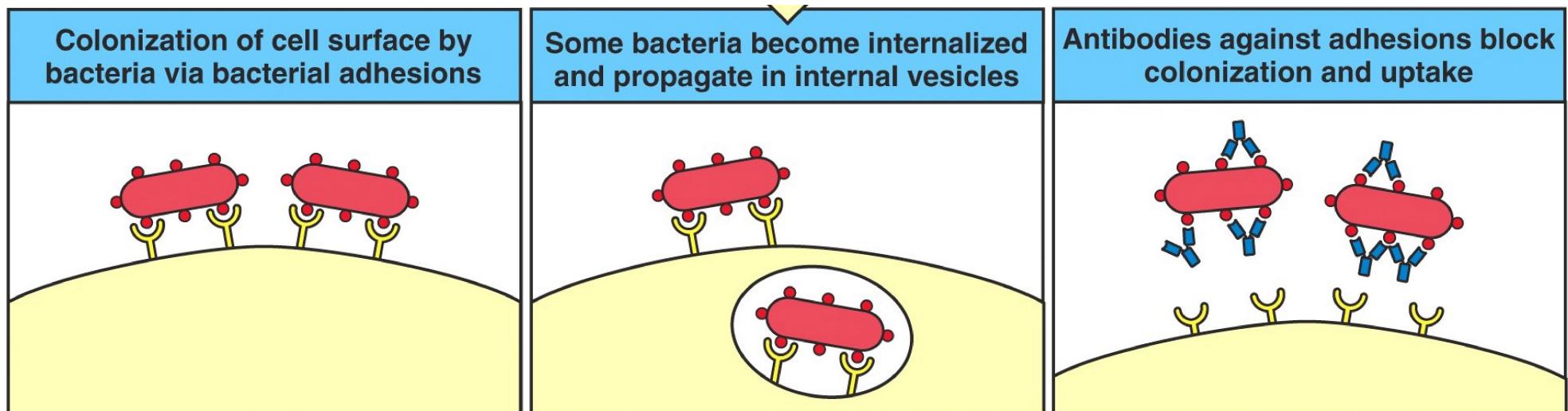


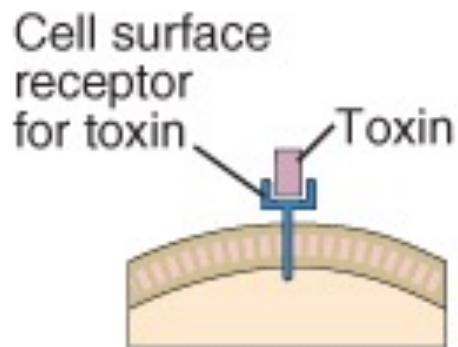
Figure 9-25 Immunobiology, 6/e. (© Garland Science 2005)



1. Antibodies can neutralize pathogen-derived toxins

C

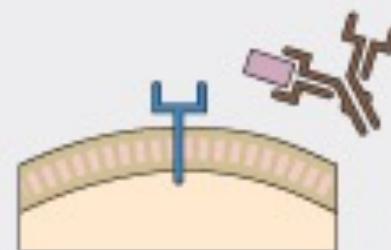
Pathologic effect of toxin



Pathologic effect
of toxin (e.g.,
cell necrosis)



**Antibody blocks
binding of toxin
to cellular receptor**

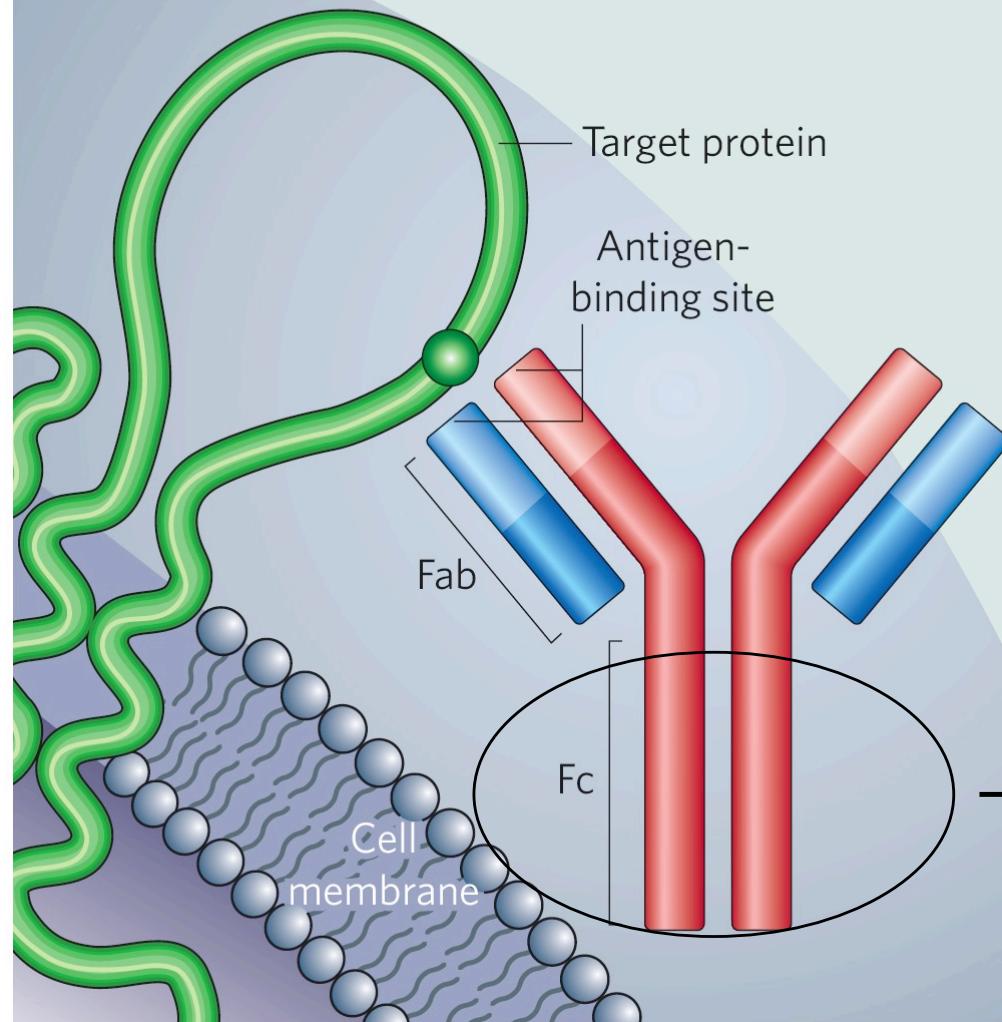


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The antibody couples the **specific** recognition of antigen to the activation of **non-specific** effector mechanisms

ANTIBODY STRUCTURE

Typical antibodies have a Y-shaped configuration.



→ Effector functions

I recettori Fc

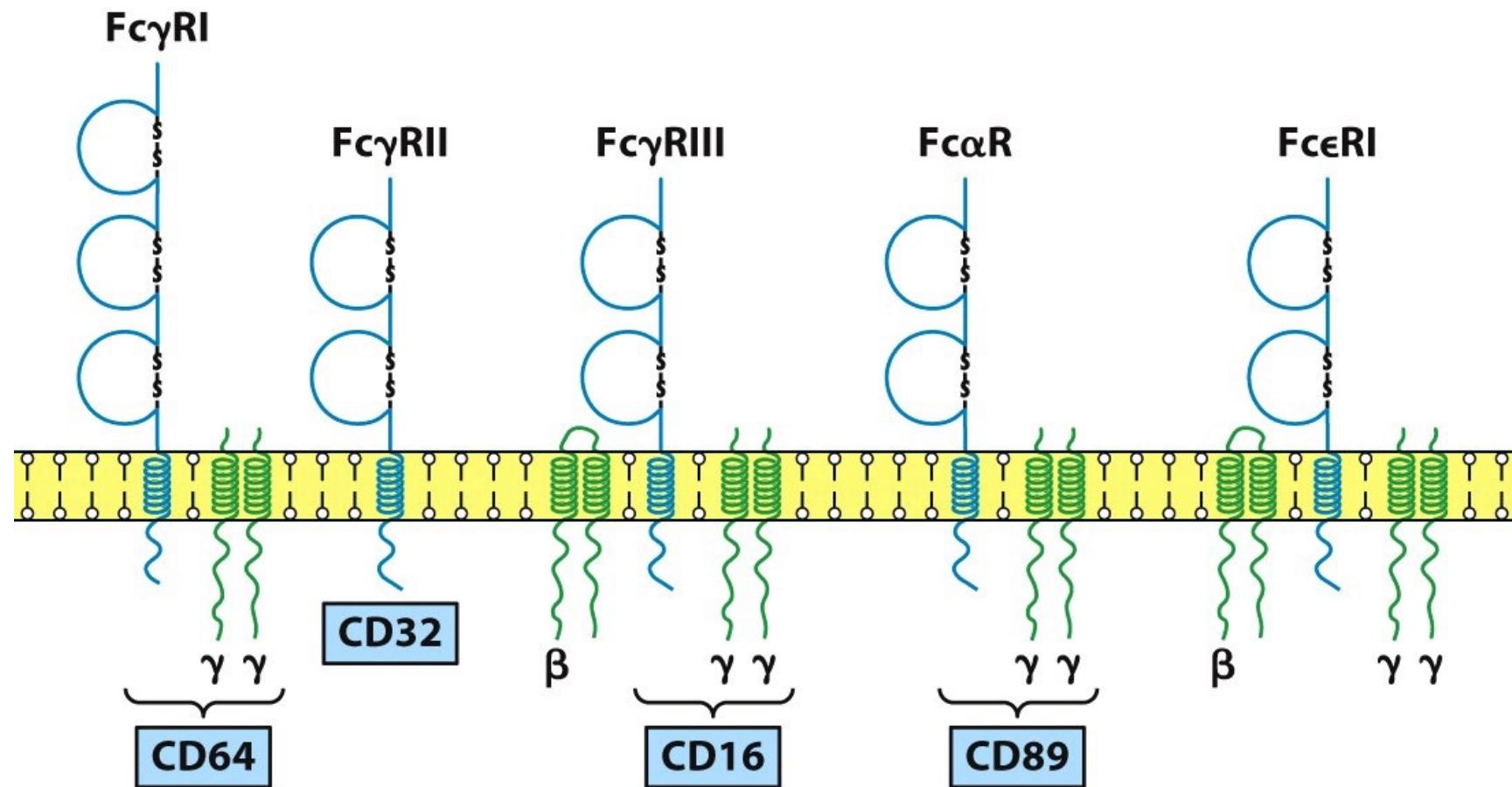
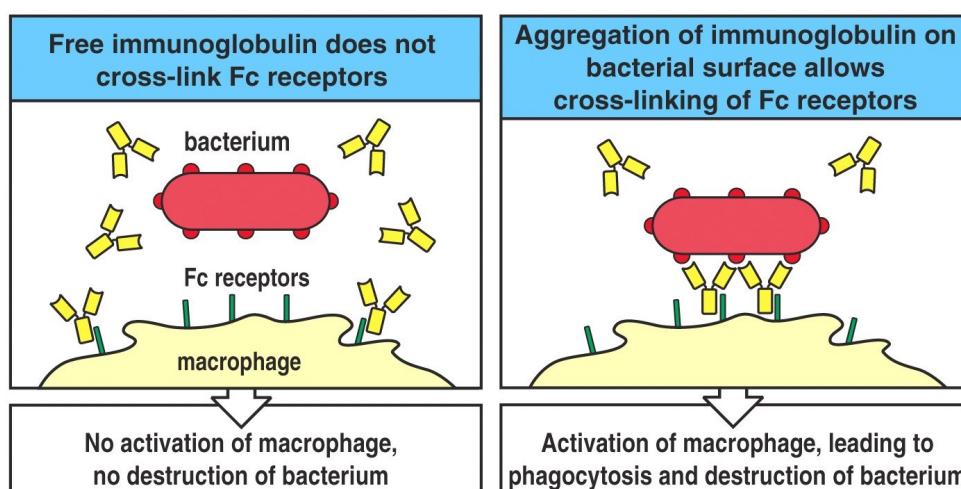
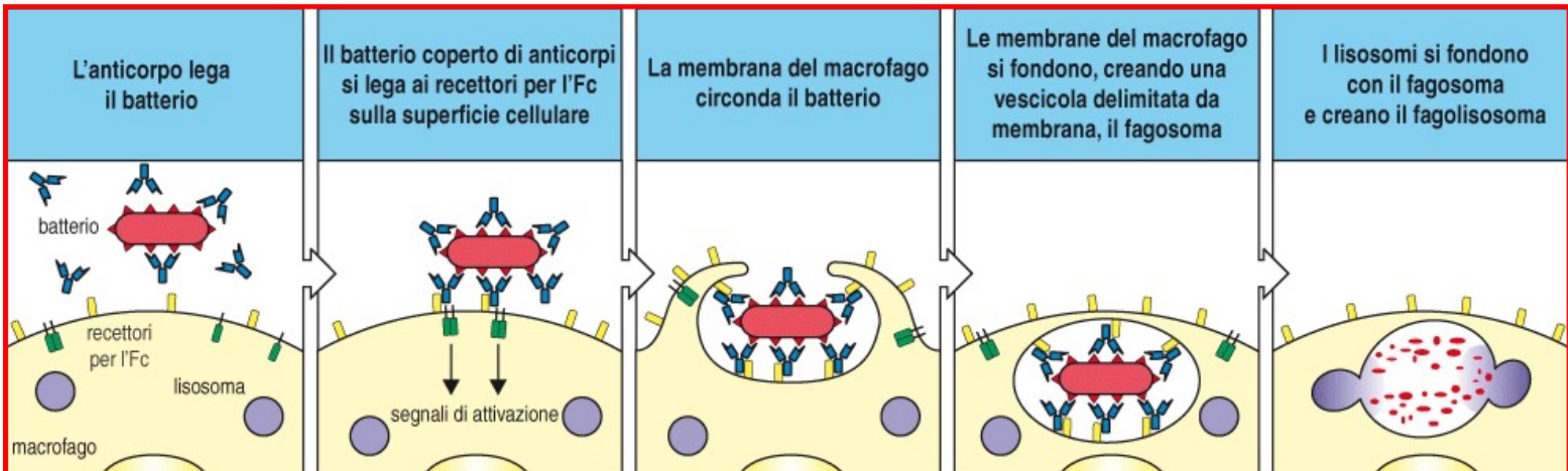


Figure 4-23
Kuby IMMUNOLOGY, Sixth Edition
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MΦ, PMN

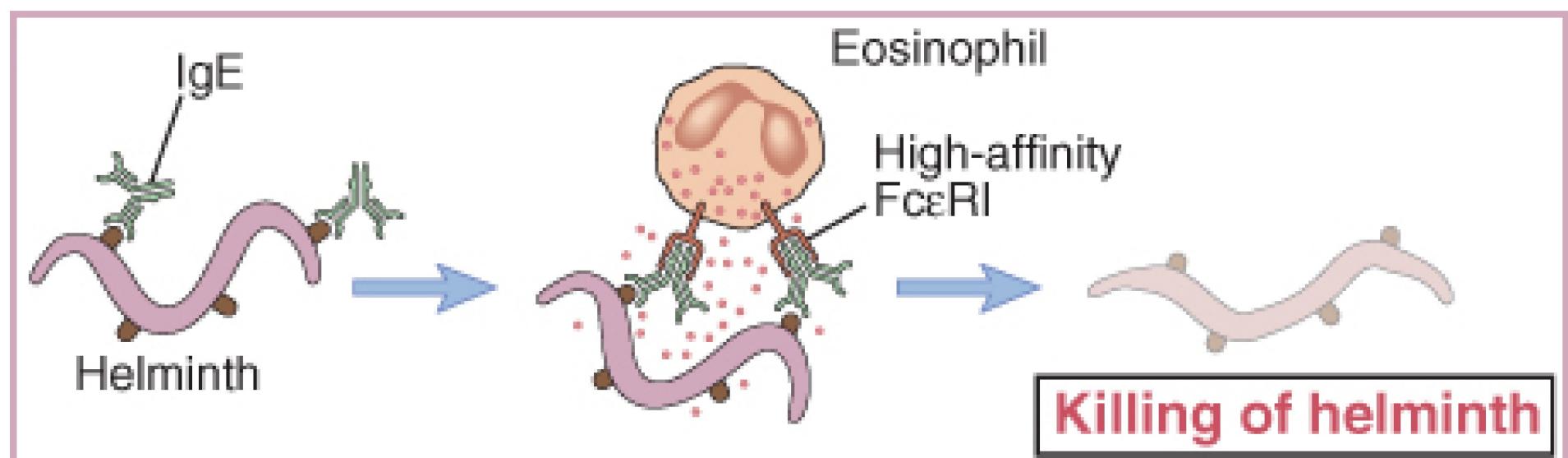
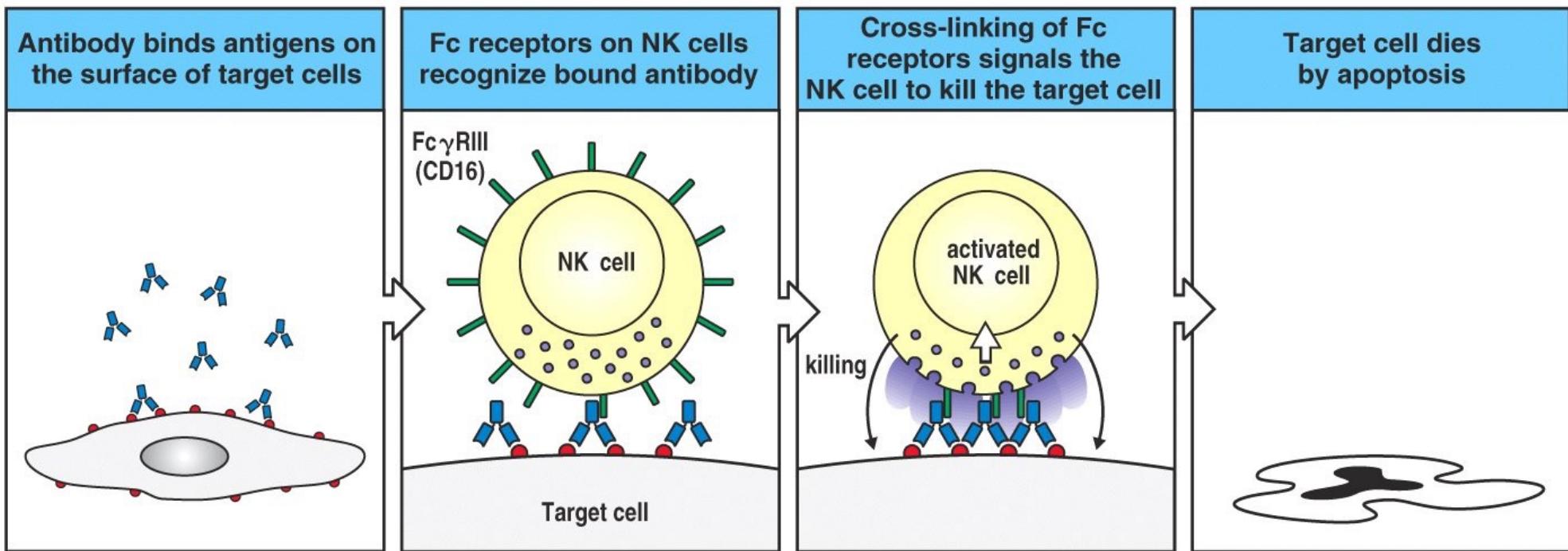
Mastociti, Basofili,
Eosinofili

2. Antibodies can enhance phagocytosis of pathogens (OPSONIZATION)



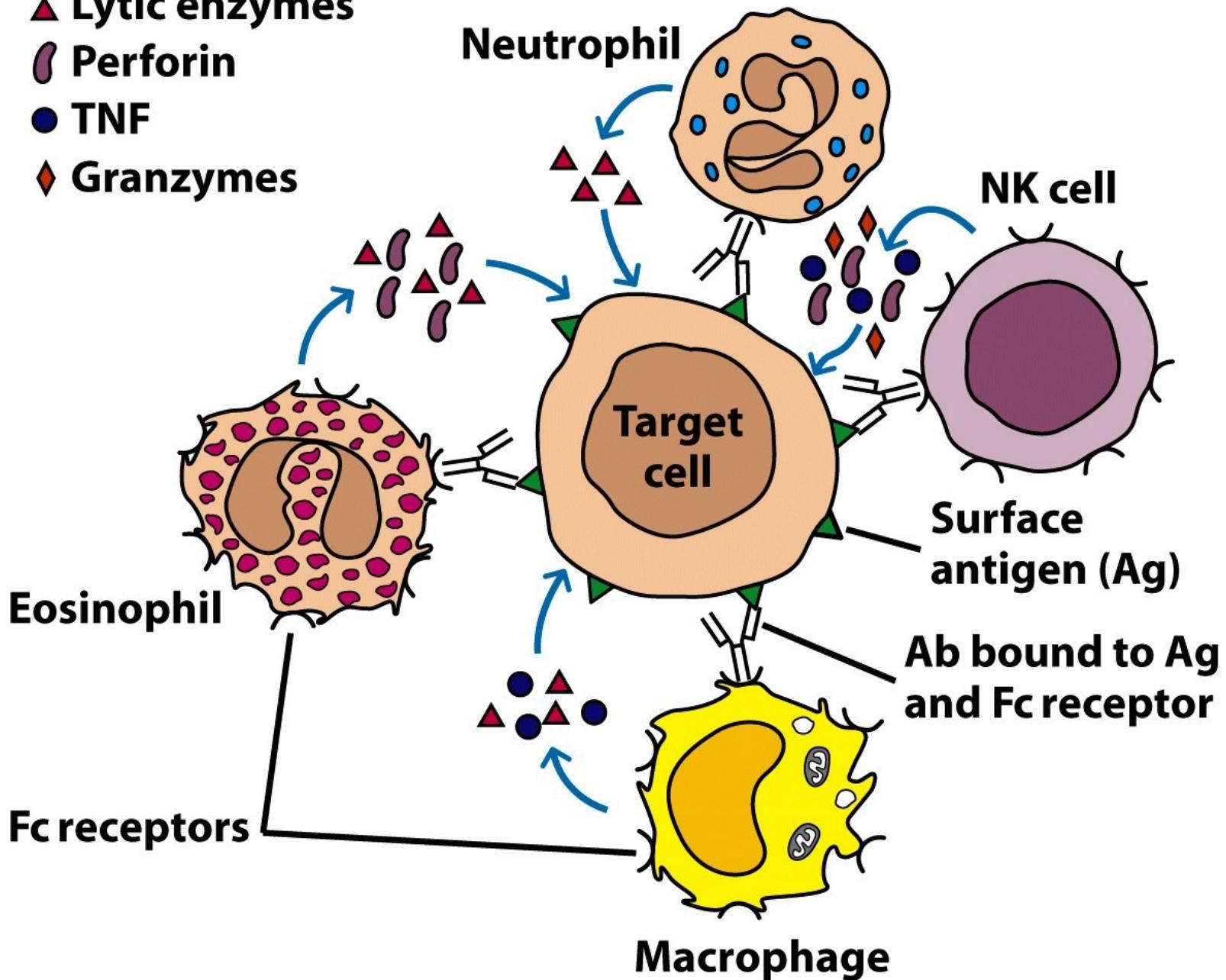
Antibody-mediated crosslinking of Fc receptors is necessary for phagocyte activation!

3. Antibodies can induce cytotoxicity by innate immunity cells (Antibody Dependent Cytotoxicity-ADCC)

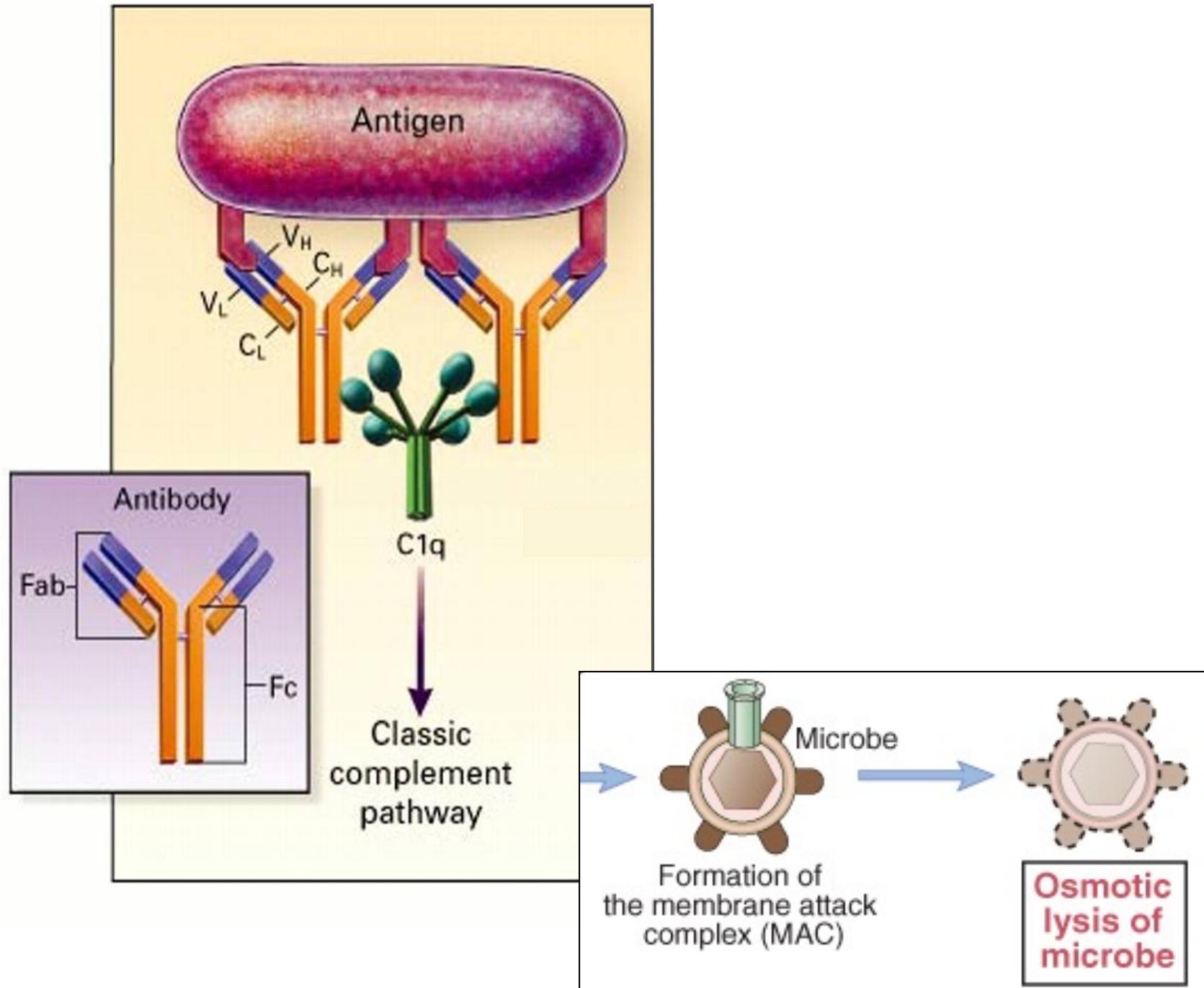


Many innate cell populations are capable of ADCC

- ▲ Lytic enzymes
- ▨ Perforin
- TNF
- ◆ Granzymes



4. Antibodies can induce the activation of COMPLEMENT



Five different Ig classes (ISOTYPES)

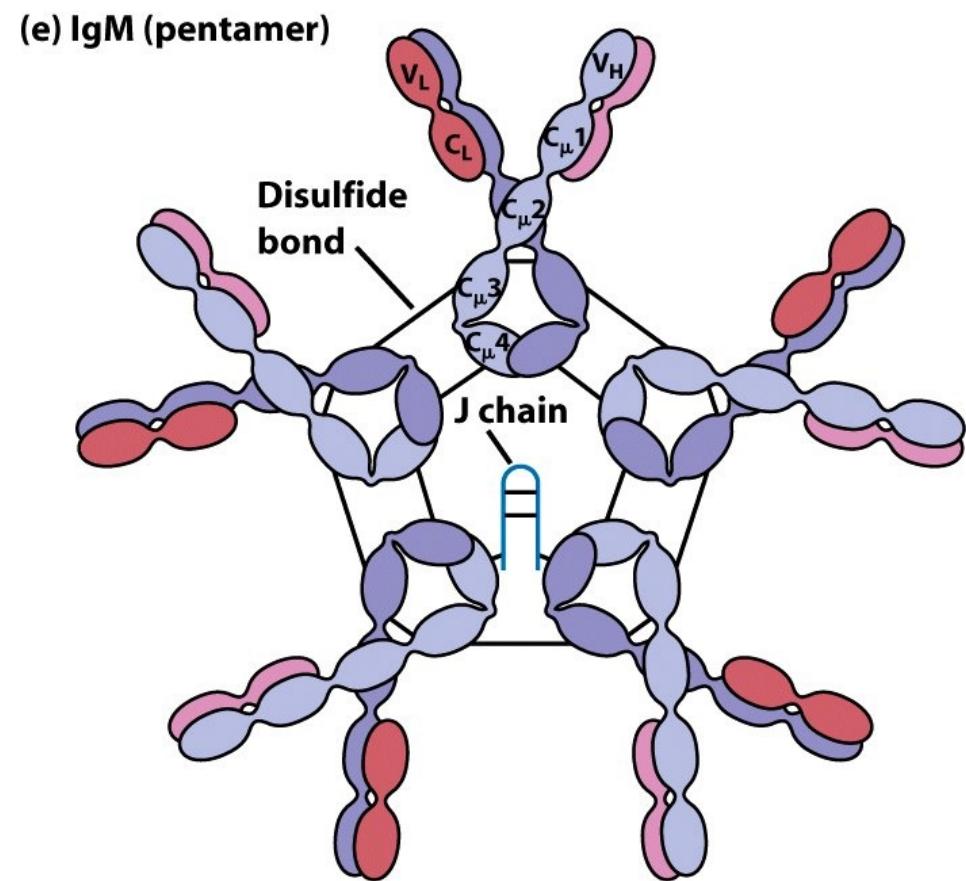
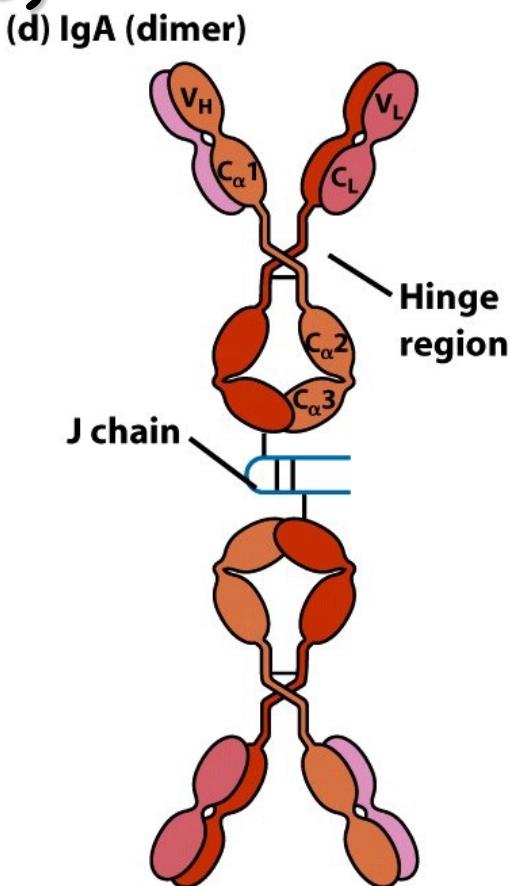
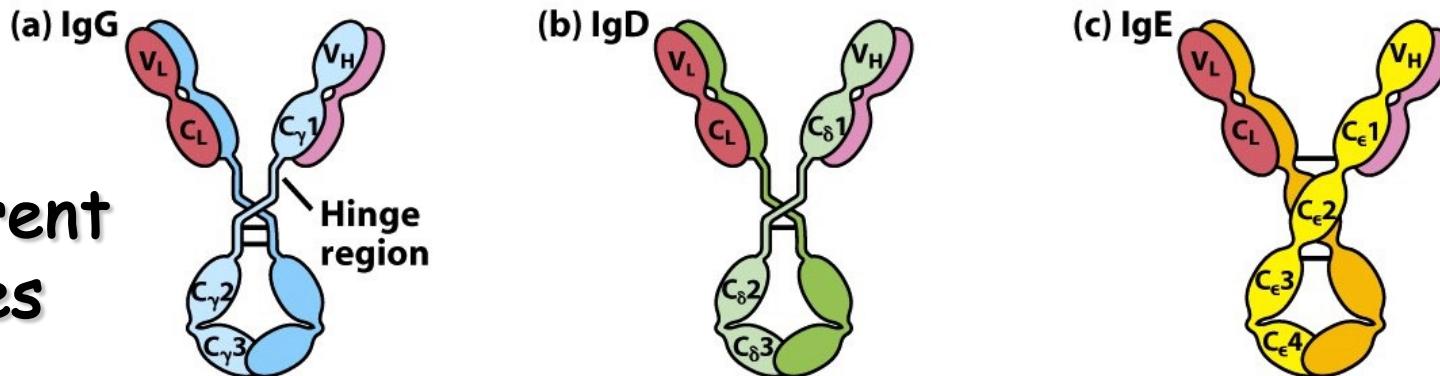
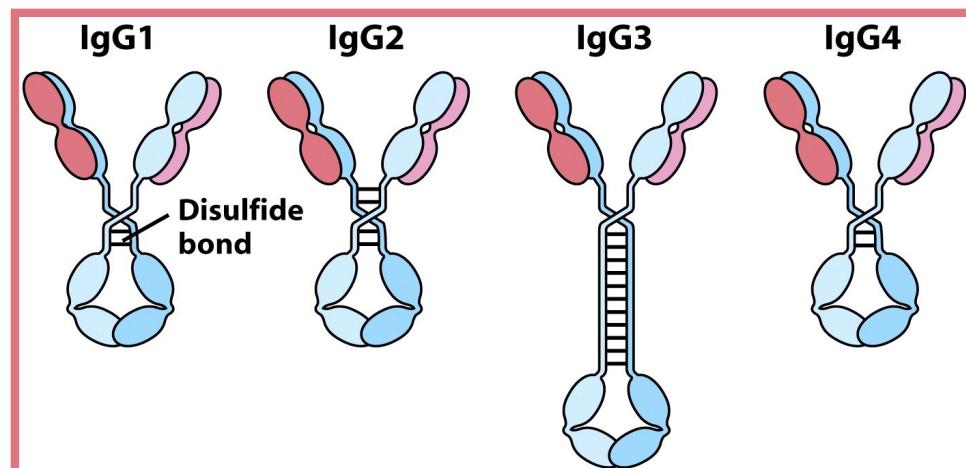


Figure 4-17
Kuby IMMUNOLOGY, Sixth Edition
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IgG

- Most abundant Ig in body
- Highest concentration in serum (75%)
- Major form produced in secondary response
- Four subclasses: IgG1-4



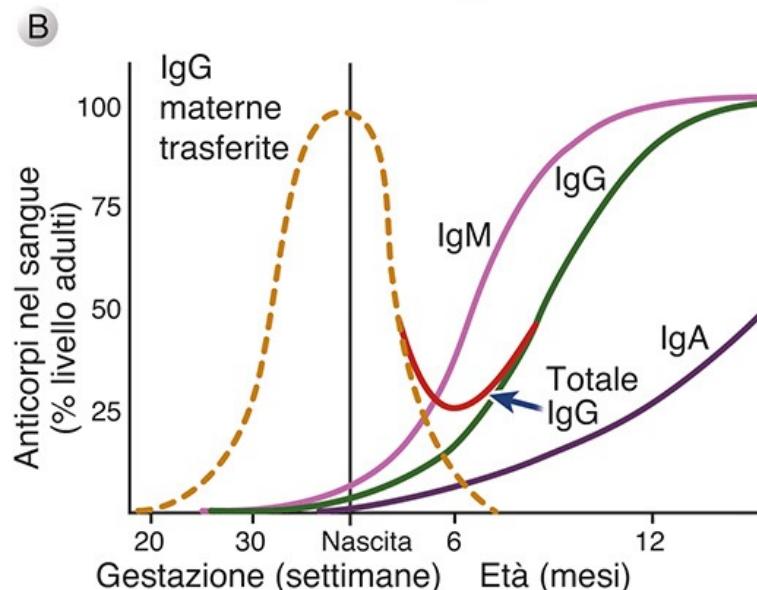
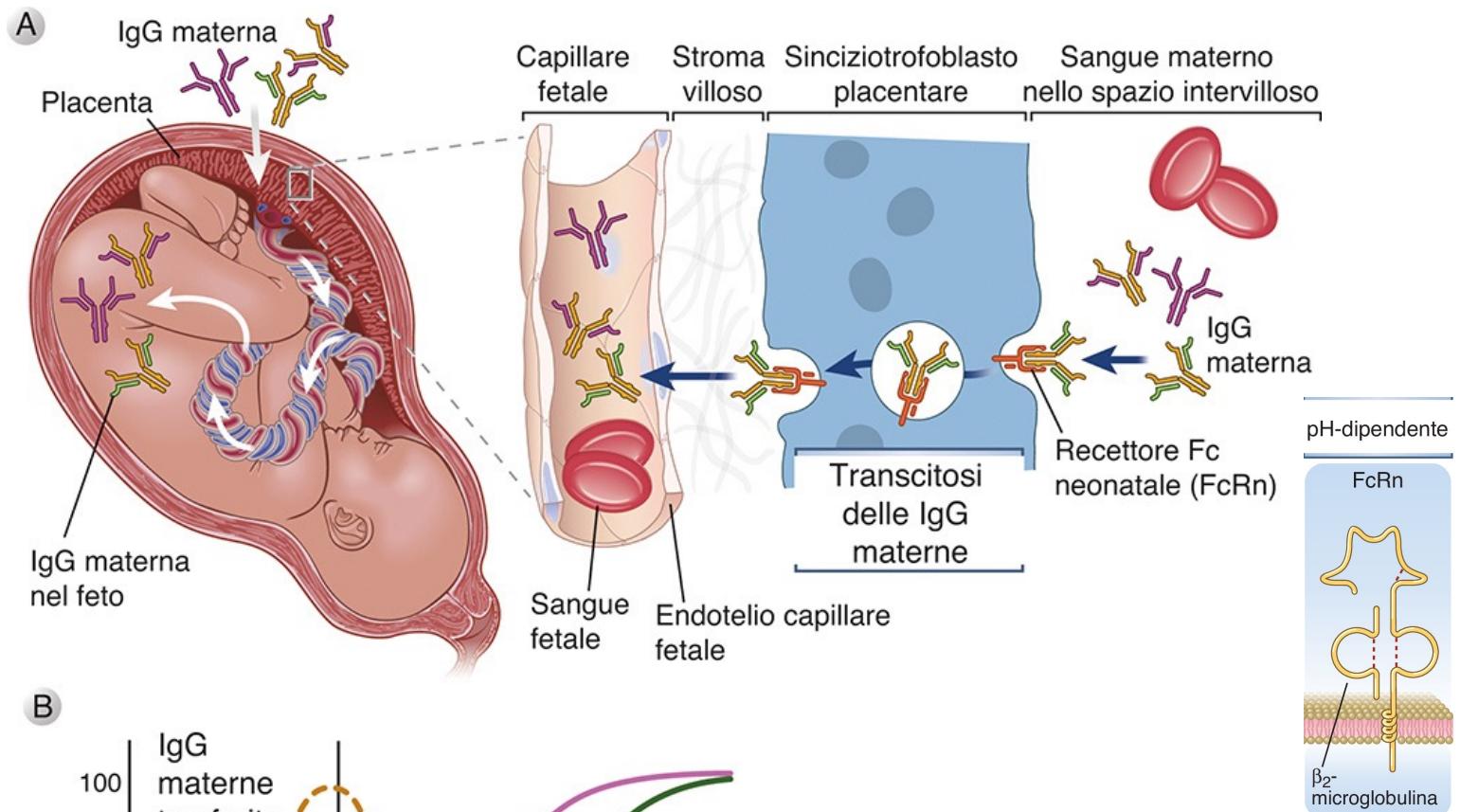
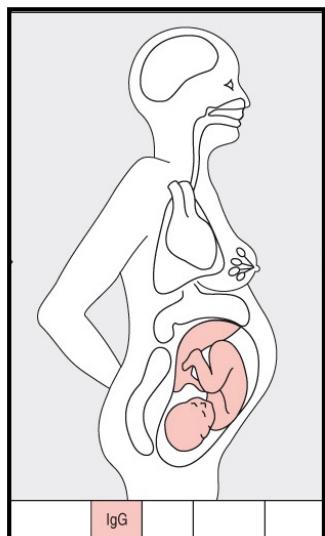
Proporzione delle IgG totali (%)	45–75	16–48	2–8	1–12
Lunghezza della cerniera della catena pesante (aminoacidi)	15	12	62	12
Numero di legami disolfuro nella cerniera	2	4	11	2
Suscettibilità della cerniera alla proteolisi	++	+	+++	+
Emivita nel siero (giorni)	21	21	7	21

IgG

Proprietà e funzioni

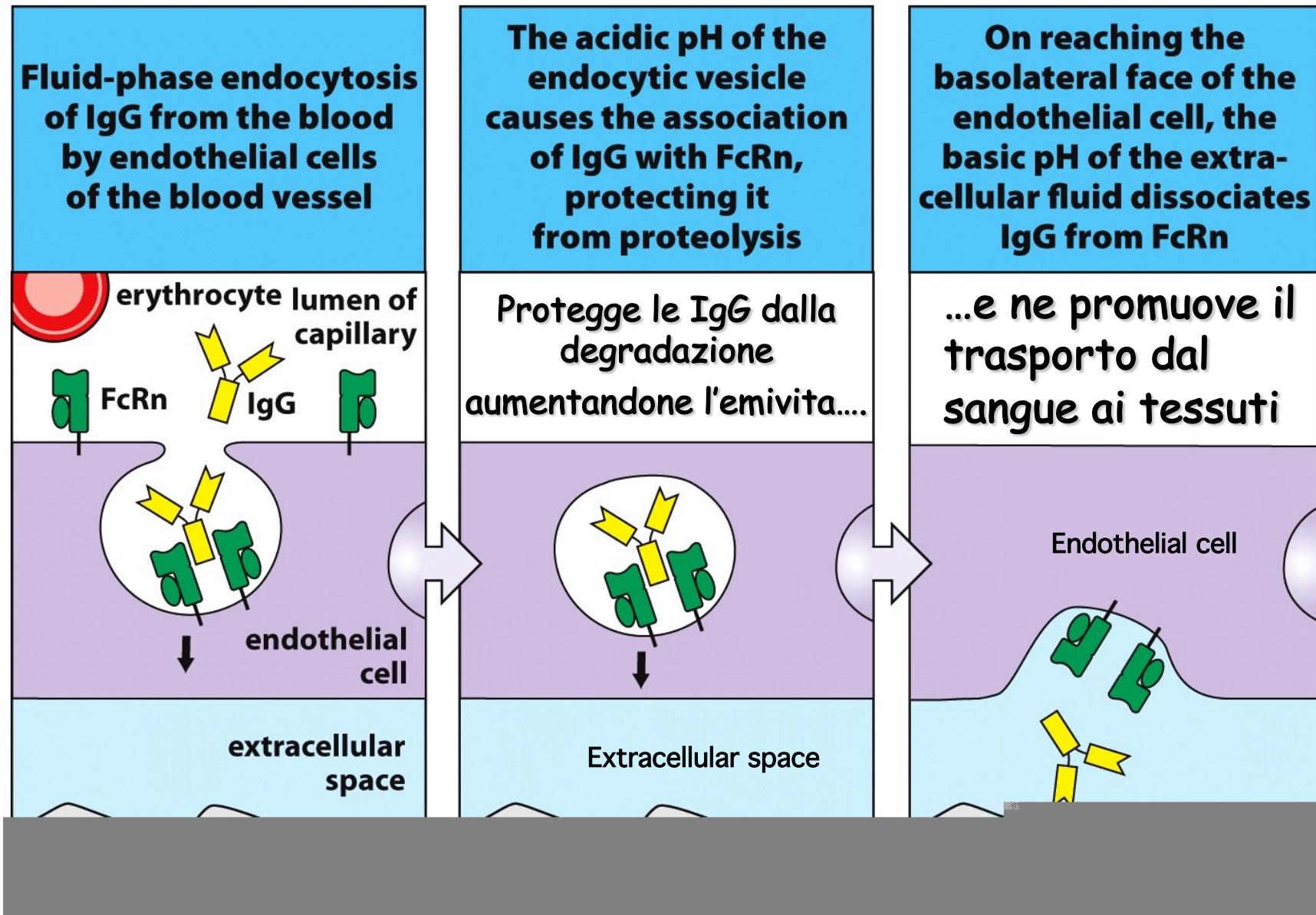
- Possono attraversare la placenta
- Neutralizzano patogeni e tossine batteriche
- Attivano il complemento
- Legano recettori Fc
 - Fagocitosi - opsonizzazione
 - ADCC

Il recettore Fc neonatale (FcRn) media il trasferimento delle IgG dalla madre al feto



Le IgG materne trasferite proteggono il neonato nei primi mesi di vita!!

Il recettore FcRn è espresso anche nell'adulto a livello delle cellule endoteliali



Le IgG sono l'isotipo più abbondante negli spazi extracellulari !

IgG

Proprietà e funzioni

- Possono attraversare la placenta
- Neutralizzano patogeni e tossine batteriche
- Attivano il complemento
- Legano recettori Fc
 - Fagocitosi - opsonizzazione
 - ADCC

IgG

Proprietà e funzioni

- Possono attraversare la placenta
- Neutralizzano patogeni e tossine batteriche
- Attivano il complemento

IgG3>IgG1>IgG2

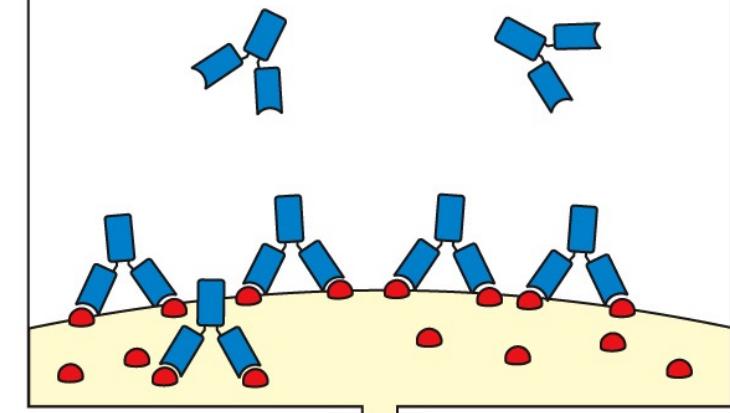
- Legano recettori Fc
 - Fagocitosi - opsonizzazione
 - ADCC

I frammenti Fab legano l'antigene e il frammento Fc rimane accessibile.....

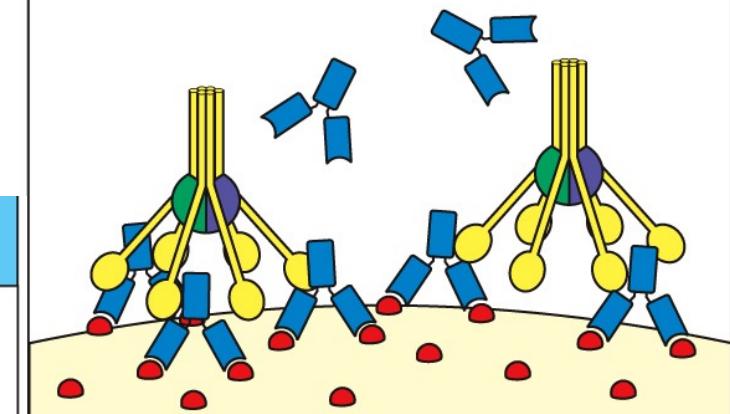
...ma per attivare la via classica sono necessarie due molecole di IgG che devono simultaneamente legare una molecola C1

Anche antigeni solubili possono attivare il complemento !

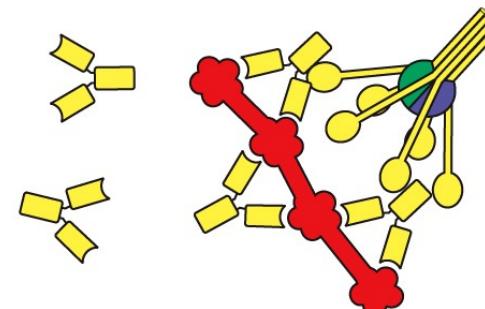
IgG molecules bind to antigens on bacterial surface

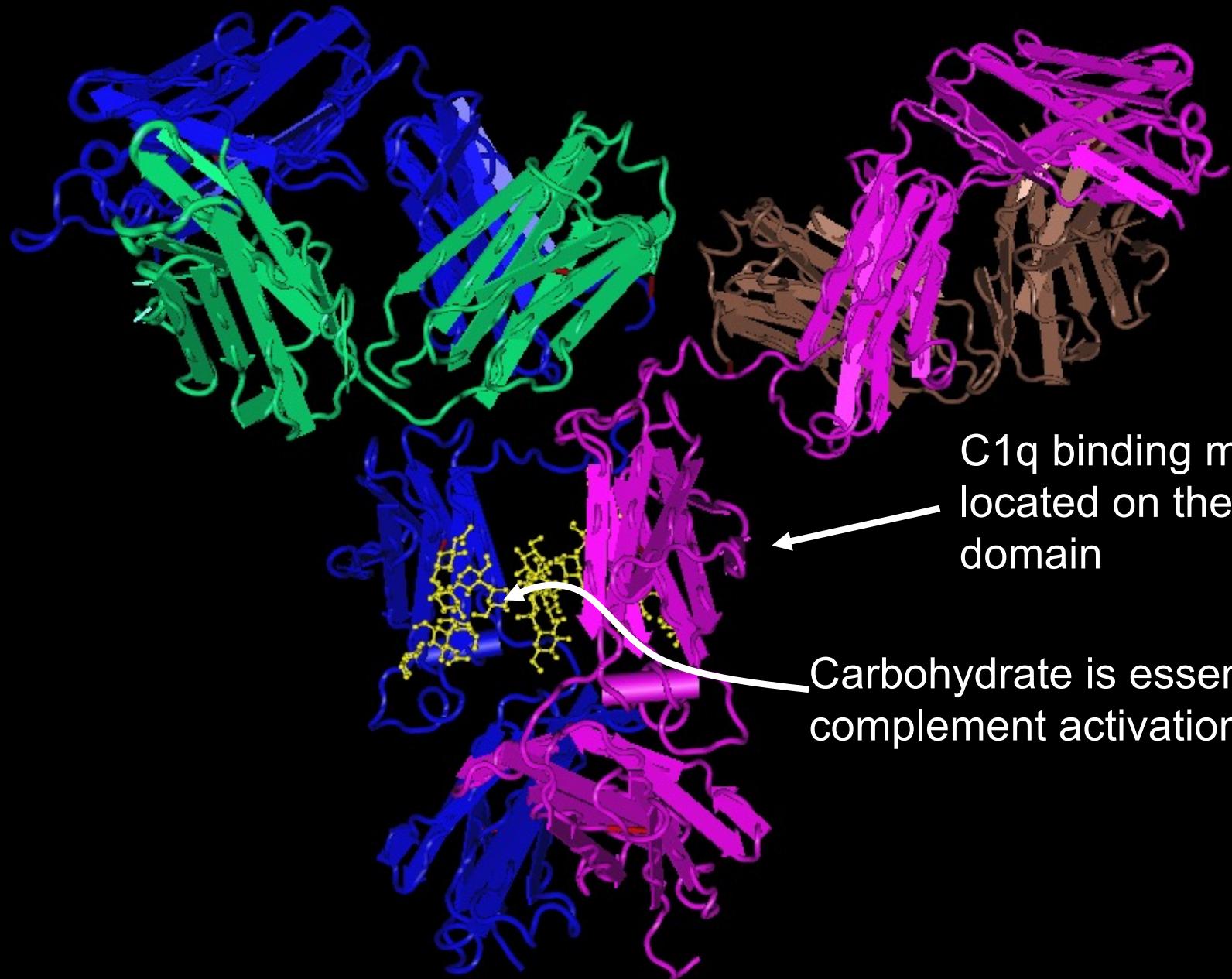


C1q binds to two or more IgG molecules and initiates complement activation



C1q binds to soluble immune complex and initiates complement activation





C1q binding motif is
located on the C γ 2
domain

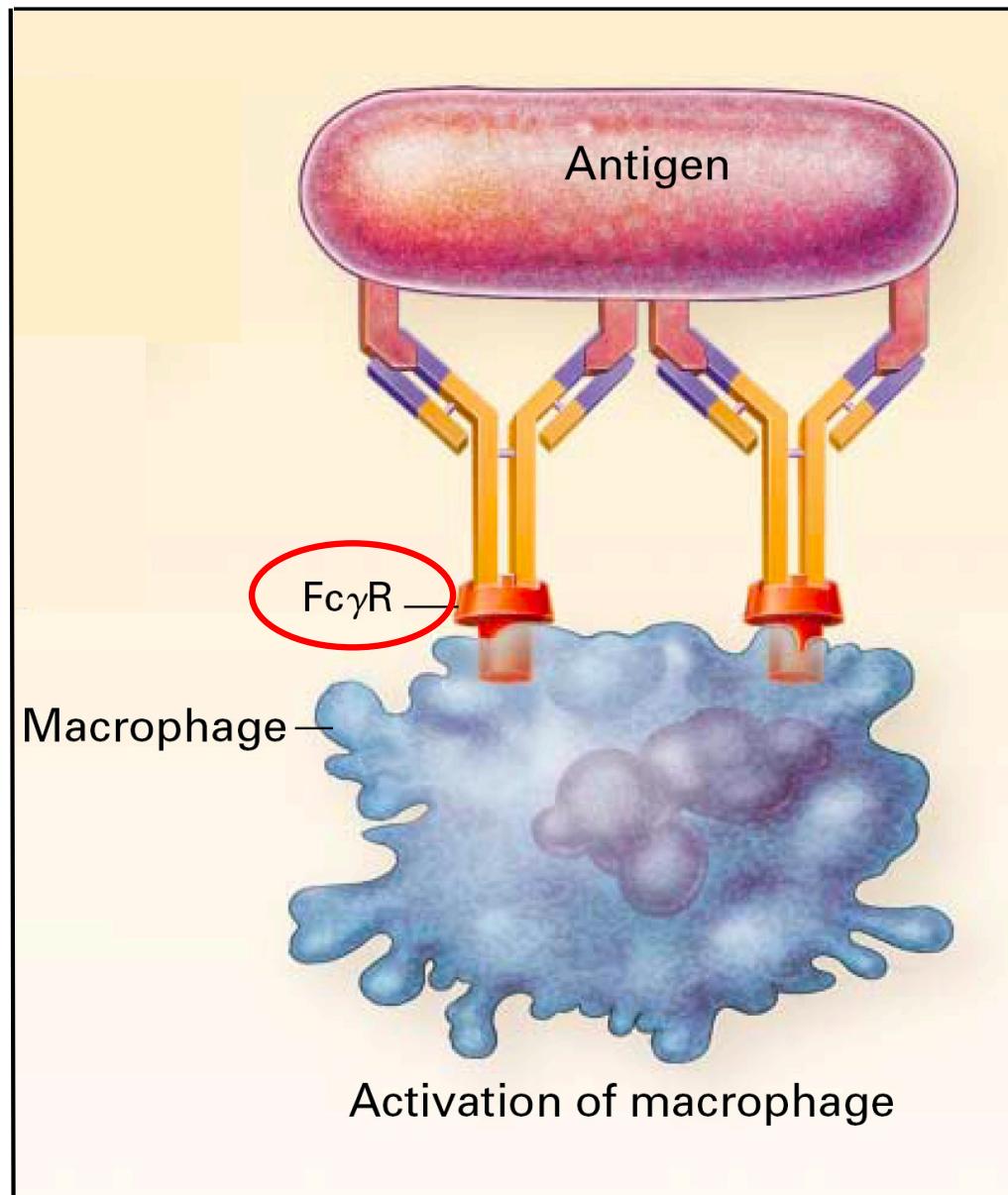
Carbohydrate is essential for
complement activation

IgG

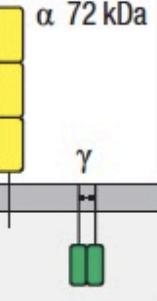
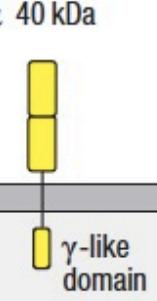
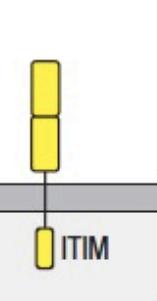
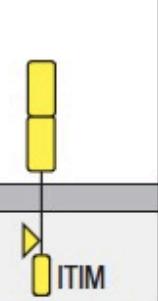
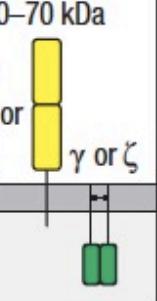
Proprietà e funzioni

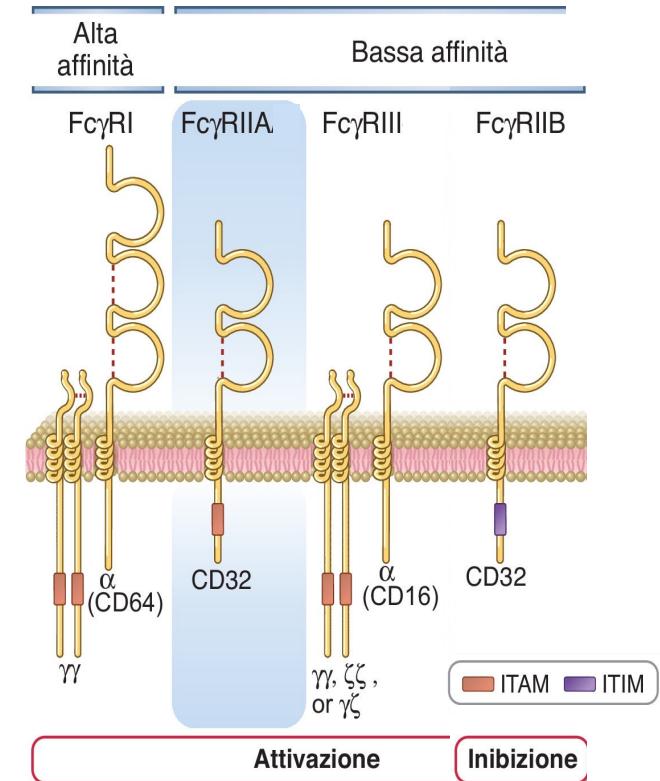
- Possono attraversare la placenta
- Neutralizzano patogeni e tossine batteriche
- Attivano il complemento
- Legano recettori Fc
 - Fagocitosi - opsonizzazione IgG1>IgG3
 - ADCC IgG1, IgG3

La regione costante delle IgG è riconosciuta da recettori specifici: Fc γ R

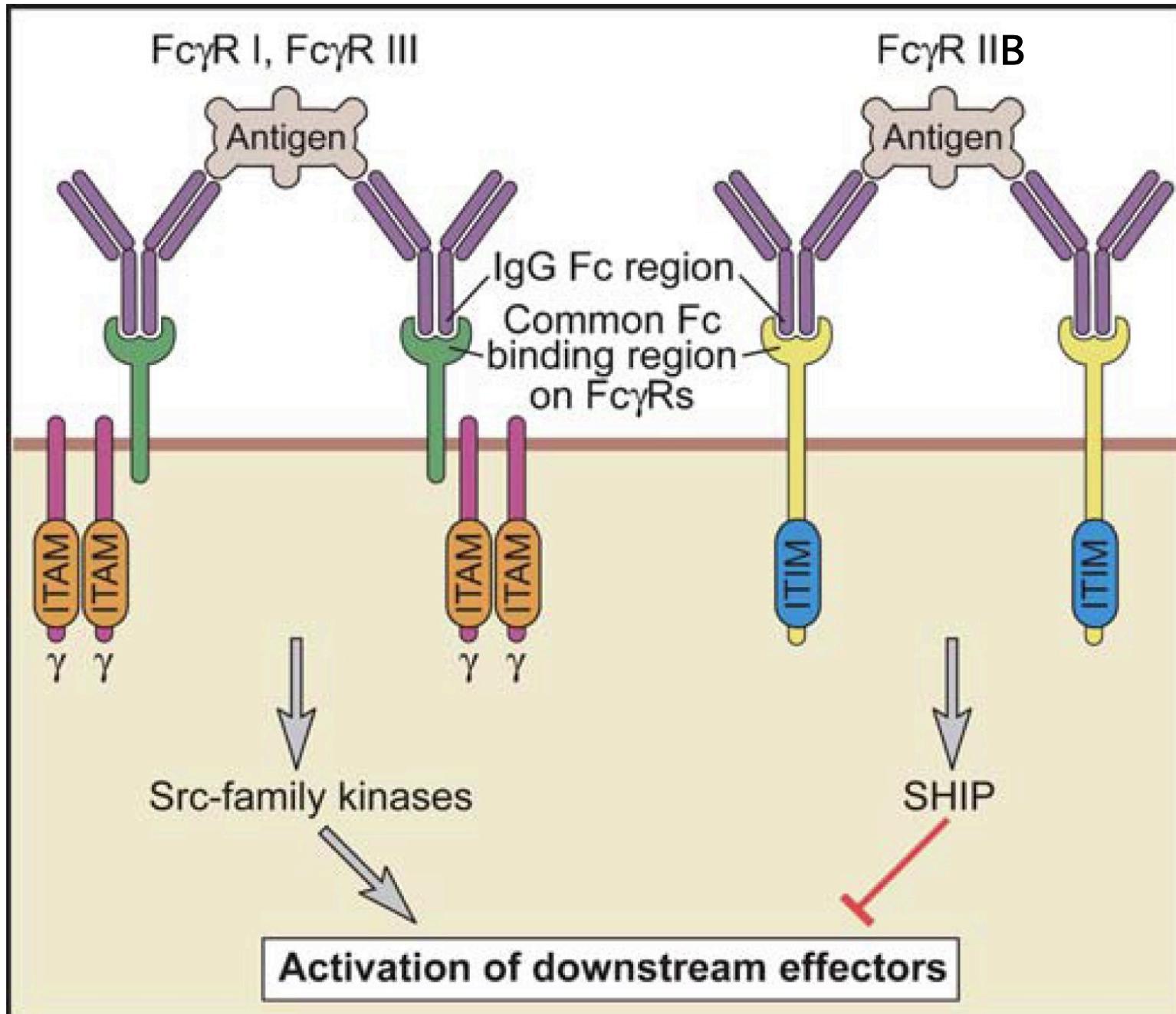


A family of Fc γ receptors

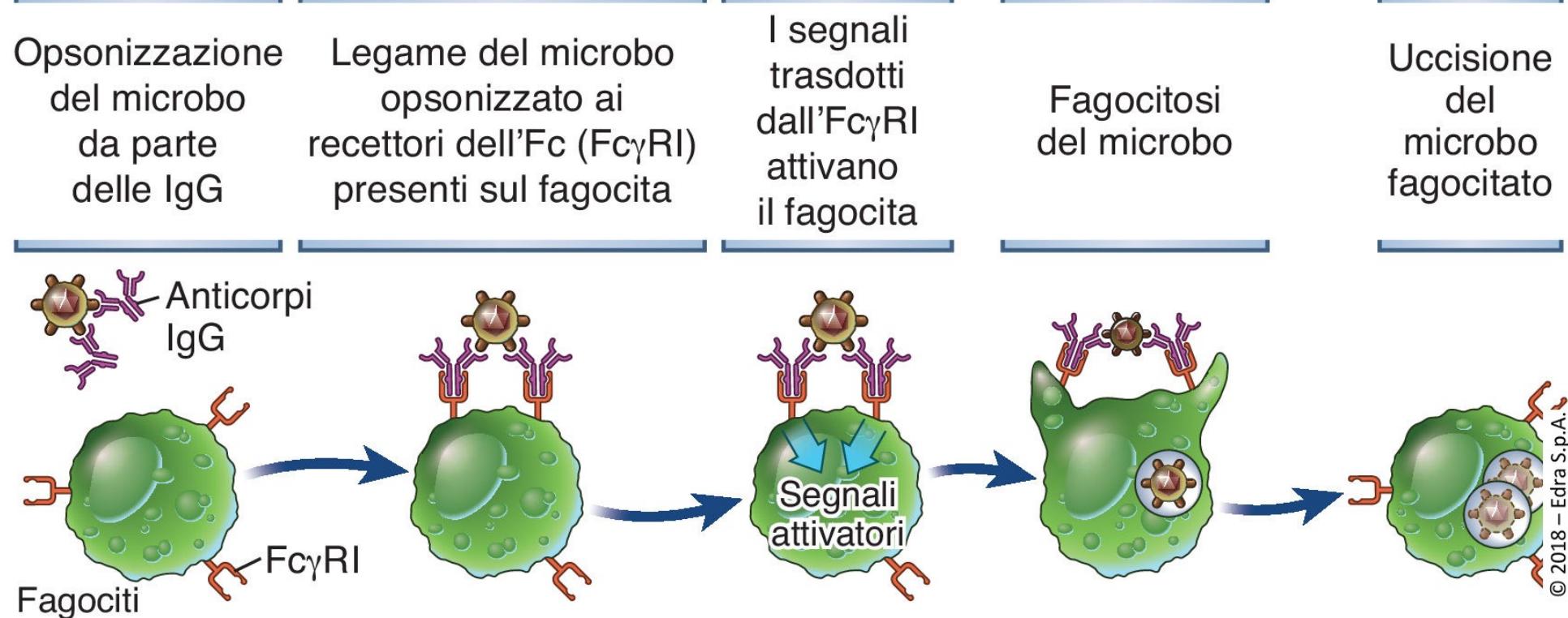
HIGH Affinity $10^{-9} M$		Medium to low affinity (10^{-6} - $10^{-5} M$)			
Receptor	Fc γ RI (CD64)	Fc γ RII-A (CD32)	Fc γ RII-B2 (CD32)	Fc γ RII-B1 (CD32)	Fc γ RIII (CD16)
Structure	α 72 kDa 	α 40 kDa 	α 40 kDa 	α 40 kDa 	α 50-70 kDa 
Binding	IgG1 $10^8 M^{-1}$ 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 $2 \times 10^6 M^{-1}$ 1) IgG1 2) IgG3=IgG2* 3) IgG4	IgG1 $2 \times 10^6 M^{-1}$ 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 $2 \times 10^6 M^{-1}$ 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 $5 \times 10^5 M^{-1}$ IgG1=IgG3
Order of affinity					
Cell type	Macrophages Neutrophils Eosinophils	Macrophages Neutrophils Eosinophils Platelets Langerhans cells	Macrophages Neutrophils Eosinophils	B cells Mast cells	NK cells Eosinophils Macrophages Neutrophils Mast cells
Effect of ligation	Uptake Stimulation Activation of respiratory burst Induction of killing	Uptake Granule release (eosinophils)	Uptake Inhibition of stimulation	No uptake Inhibition of stimulation	Induction of killing (NK cells)



Recettori Fc γ attivatori e inibitori



Gli anticorpi IgG sono potenti opsonine: potenziano la fagocitosi dei patogeni extracellulari perché sono riconosciuti dai recettori Fcγ attivatori



Gli anticorpi IgG promuovono la citotossicità cellulare anticorpo dipendente (ADCC)

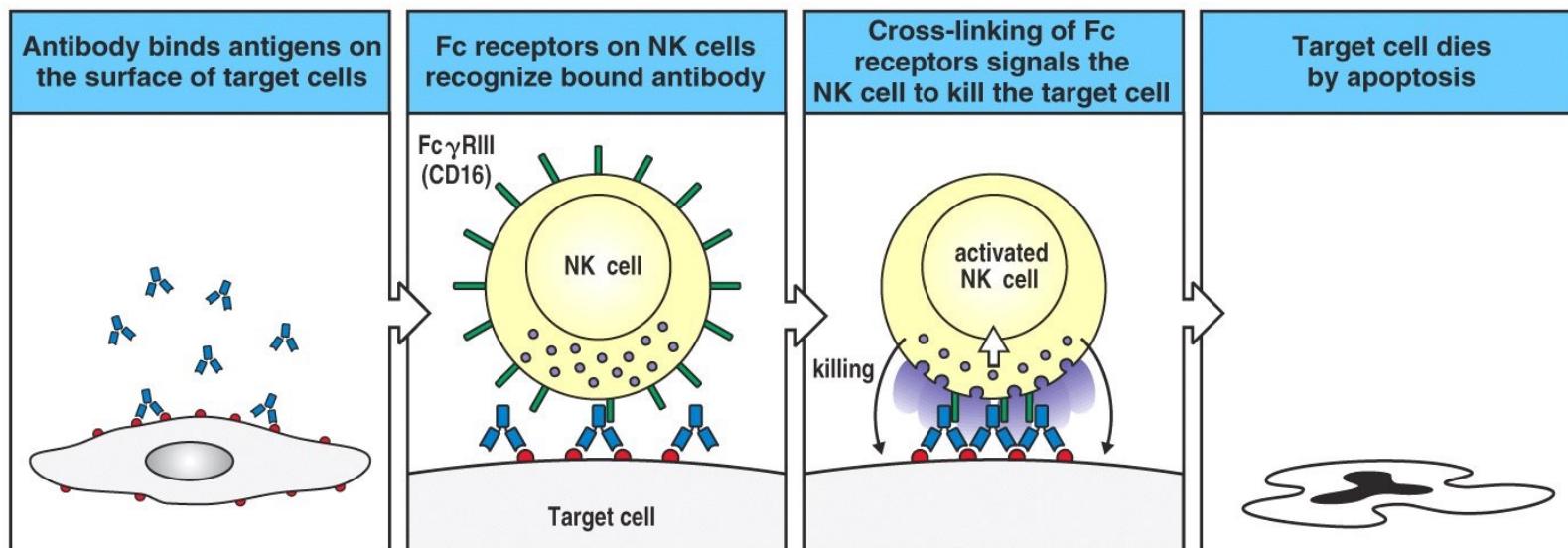
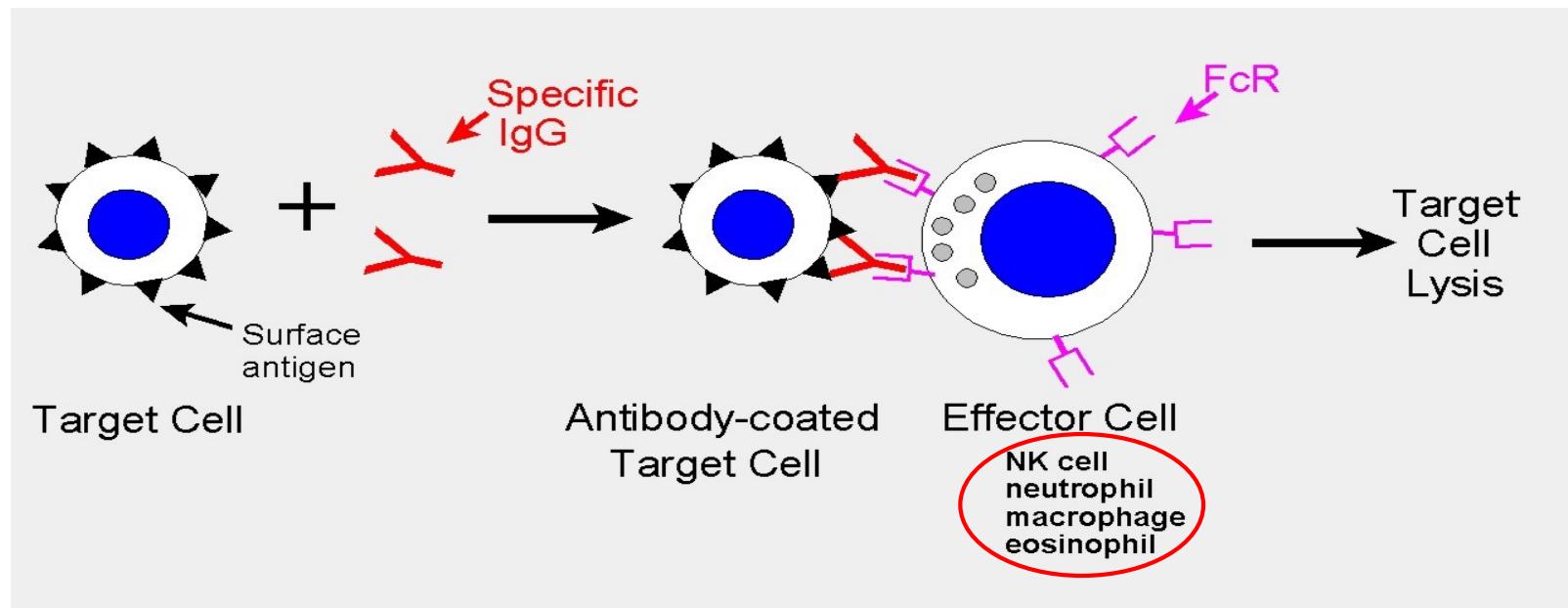
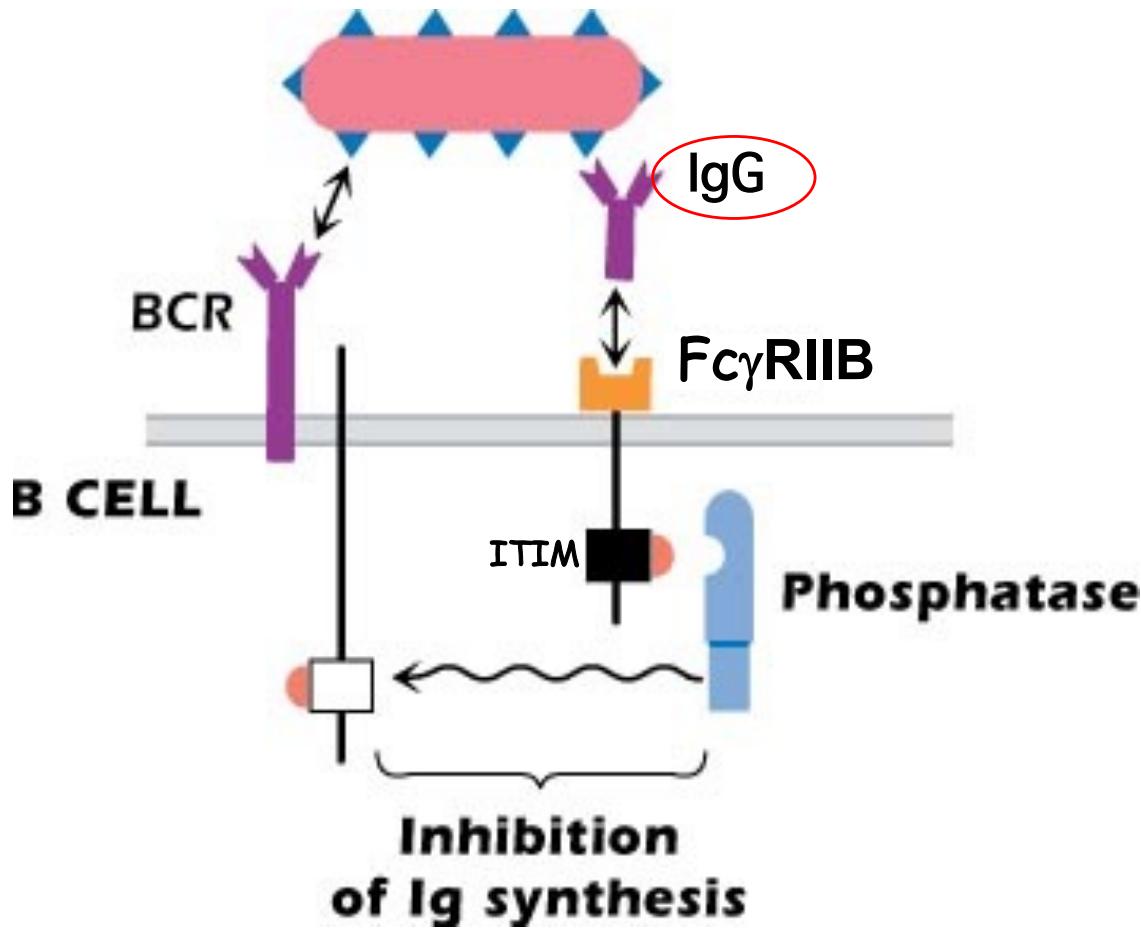
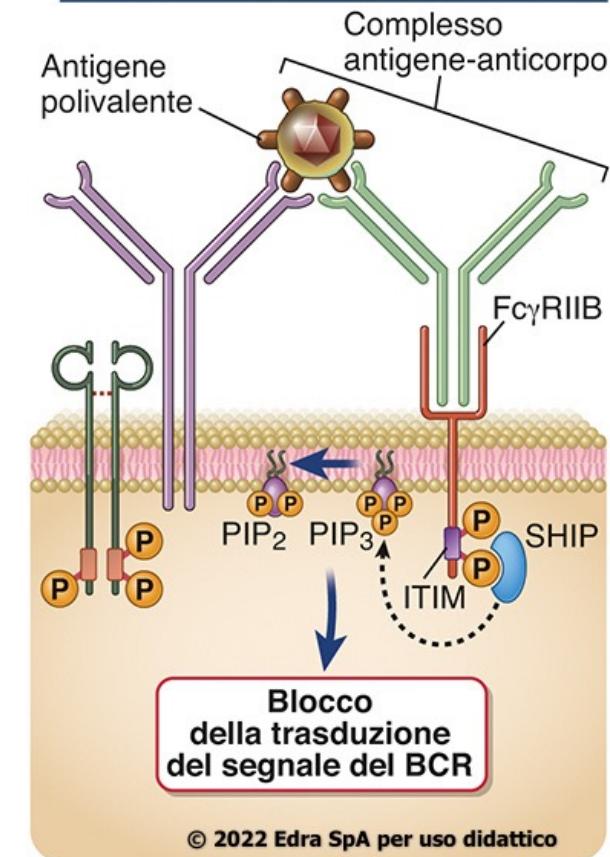


Figure 9-34 Immunobiology, 6/e. (© Garland Science 2005)

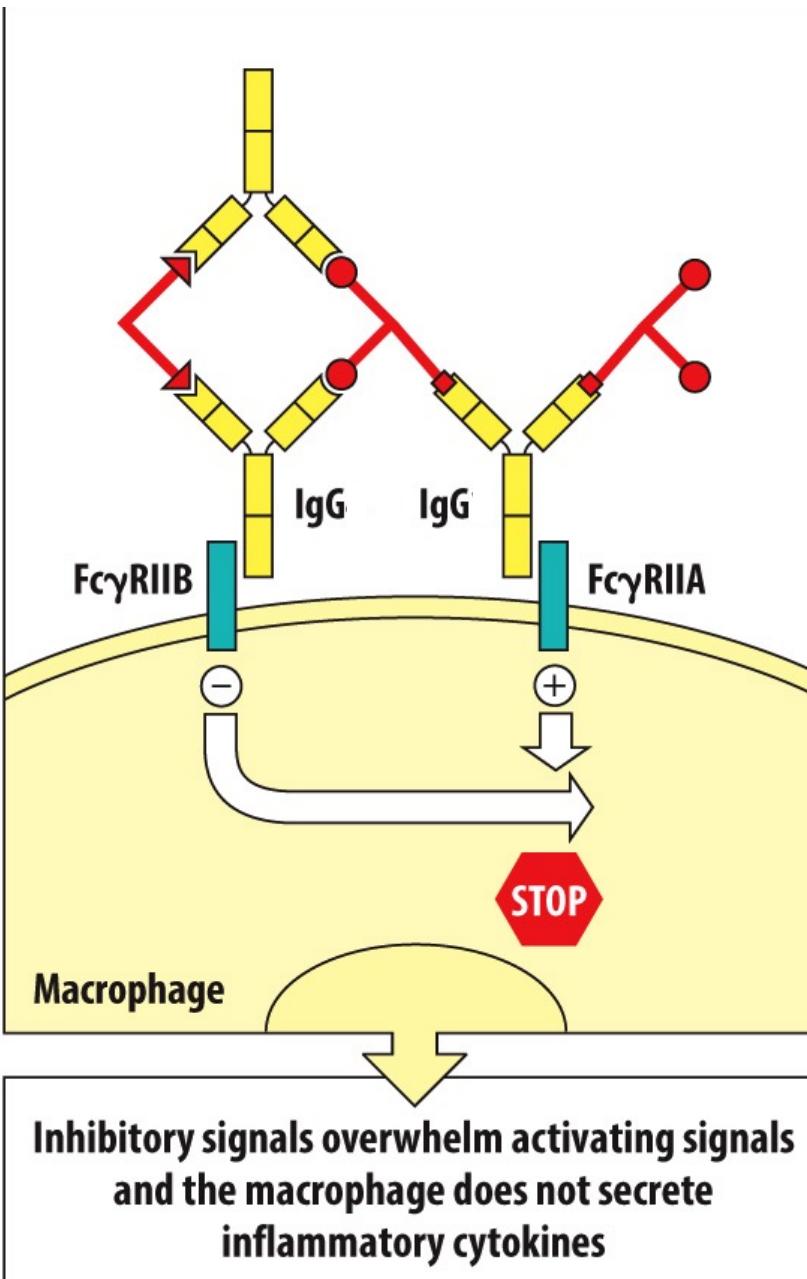
Le IgG contribuiscono allo spegnimento del segnale trasdotto dal BCR ingaggiando il recettore Fc γ inibitorio



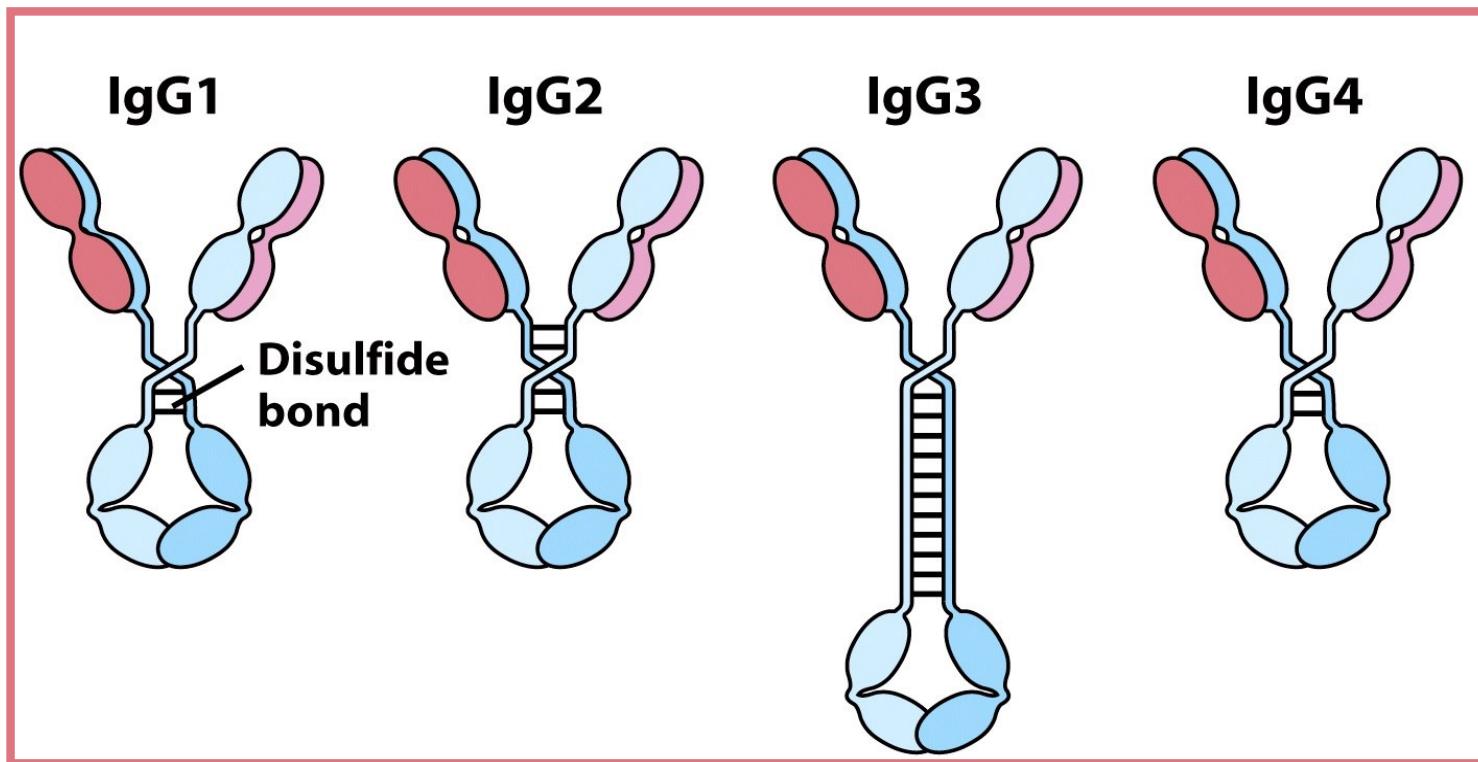
La fosfatasi associata al recettore Fc, SHIP, converte PIP₃ in PIP₂ nel complesso recettoriale dei linfociti B, bloccando la trasduzione del segnale



Nei macrofagi il recettore Fc γ RIIB blocca il segnale trasdotto dalla controparte attivatoria

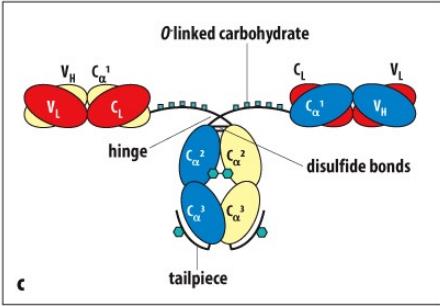


Functions of IgG subclasses



+++	+	++	+/-	Transport across placenta
++	+	+++	-	Complement activation
++	+/-	++	+	Binding to Fc receptors

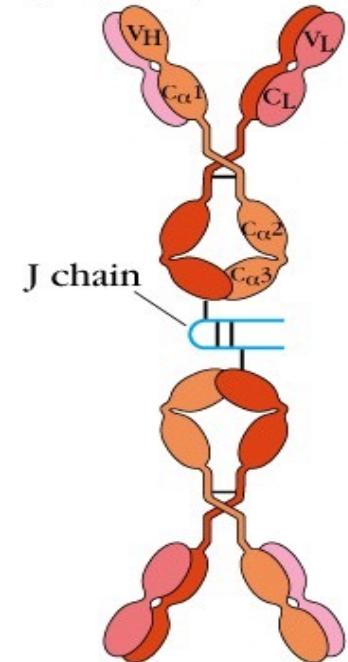
IgA



Le IgA possono essere rilasciate sia in forma monomerica che in forma dimerica.

Il dимерo è stabilizzato dalla presenza di una catena invariante aggiuntiva, la catena J.

IgA (dimer)



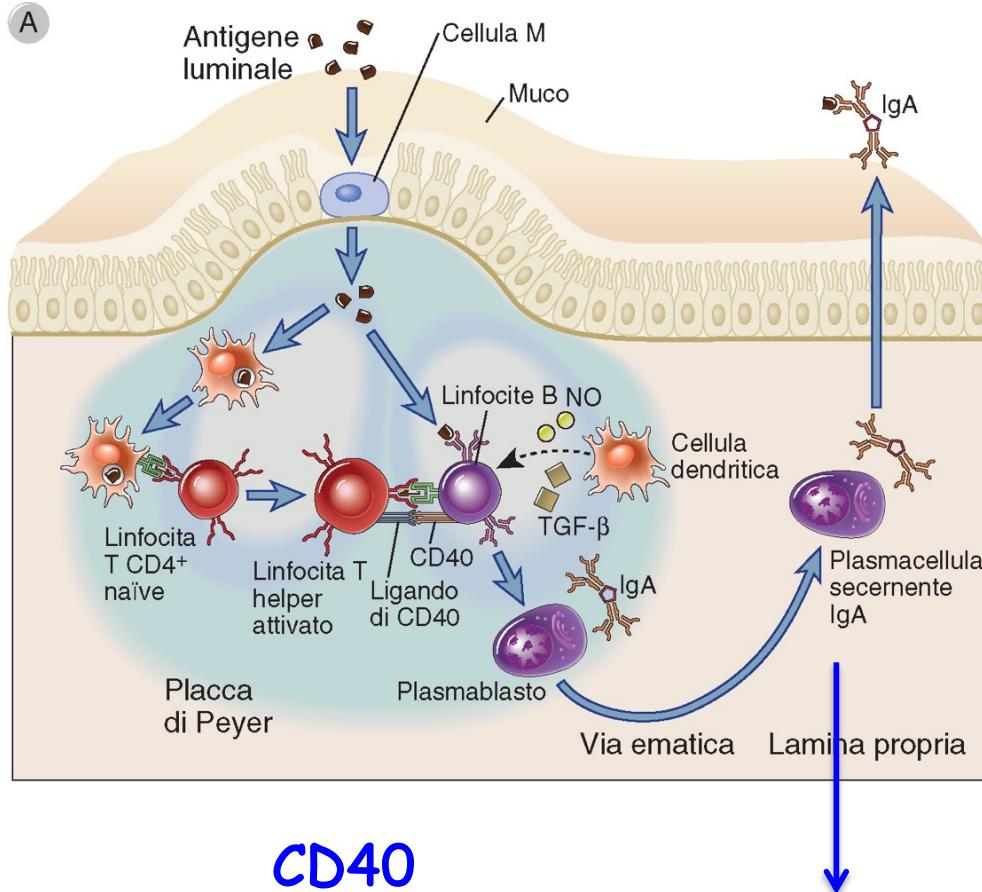
• **IgA1** è più abbondante nel sangue (90%)

IgA1:IgA2 10:1; principalmente monomero

• **IgA2** è molto abbondante nelle secrezioni in particolare nel colon (60%) dove i batteri sono presenti in gran quantità.



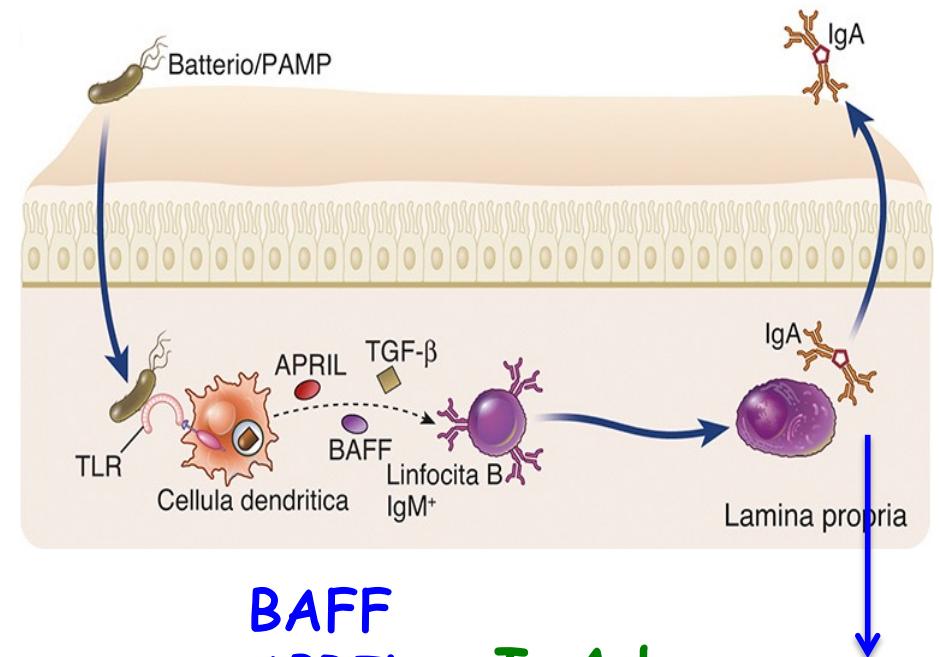
Scambio isotipico T-dipendente



**CD40
TGF-β**

**IgA alta affinità
vs patogeni e tossine**

Scambio isotipico T-indipendente

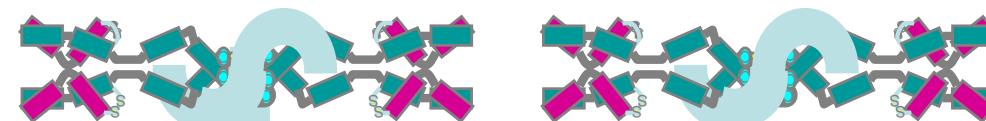
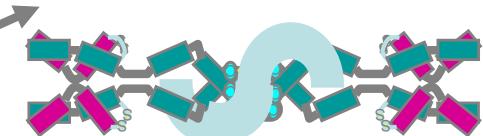


**BAFF
APRIL**

**IgA bassa
affinità**

Secretory IgA and transcytosis

'Stalk' of the pIgR is degraded to release IgA containing part of the pIgR - the secretory component



IgA and pIgR are transported to the apical surface in vesicles

Epithelial cell

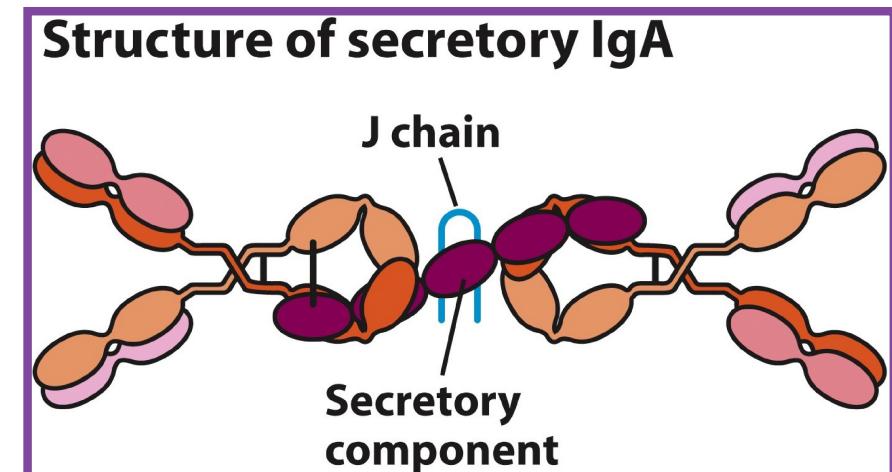
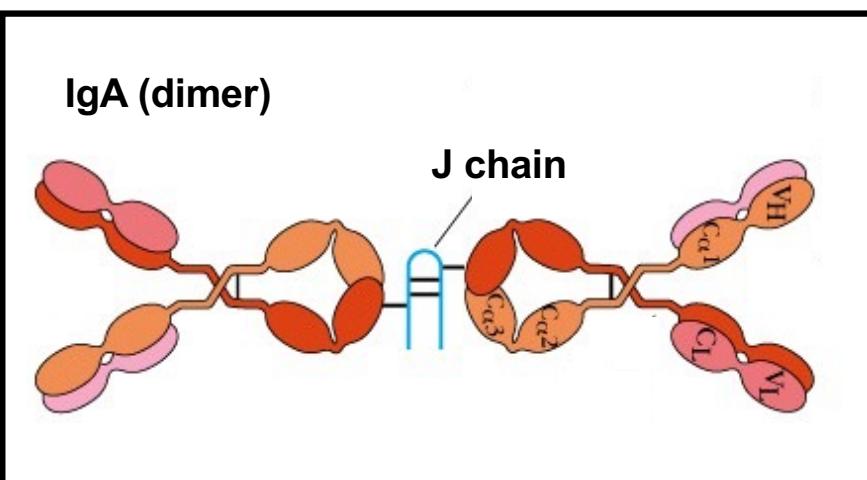
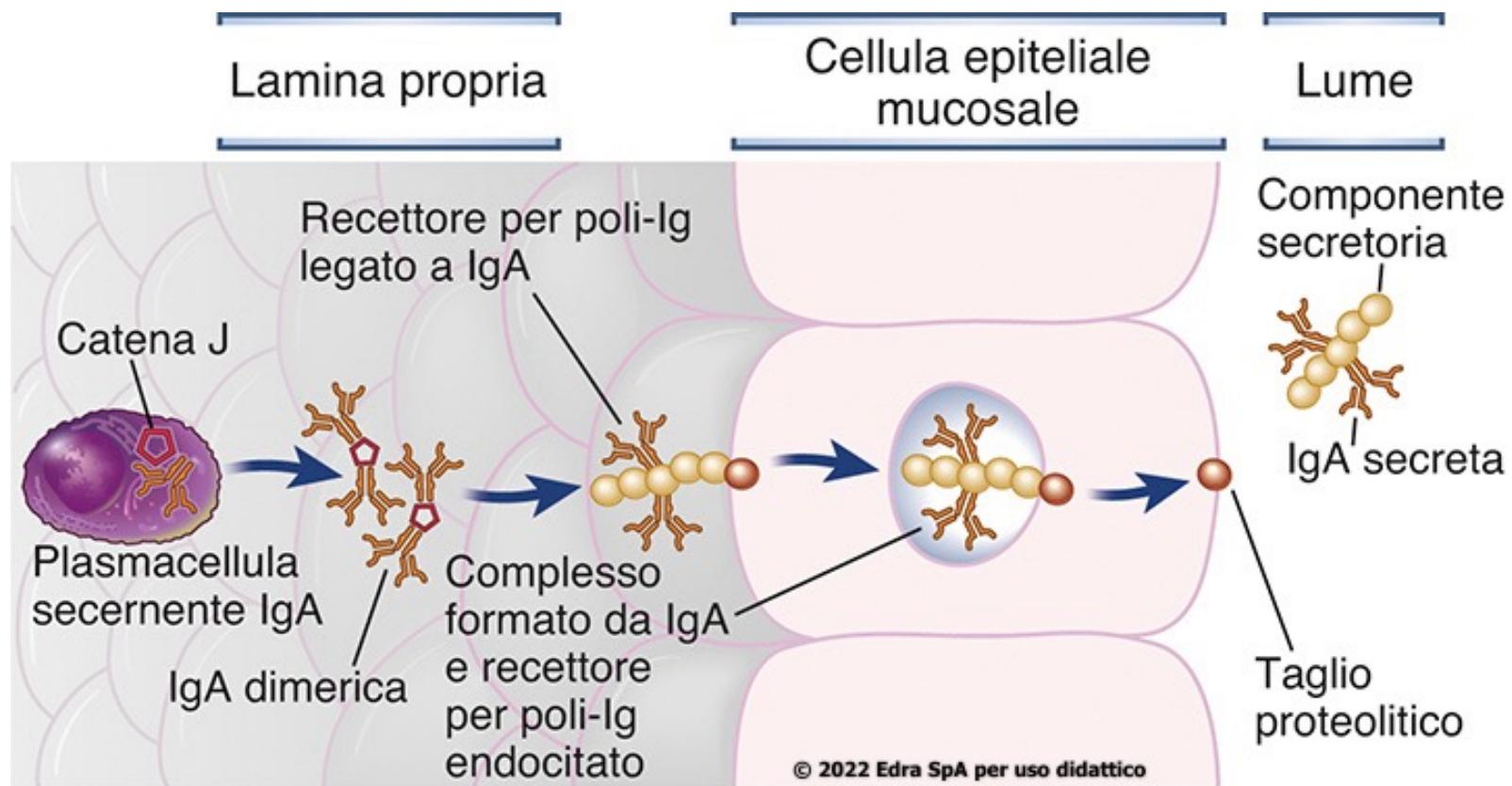
pIgR & IgA are internalised

Polymeric Ig receptors are expressed on the basolateral surface of epithelial cells to capture IgA produced in the mucosa

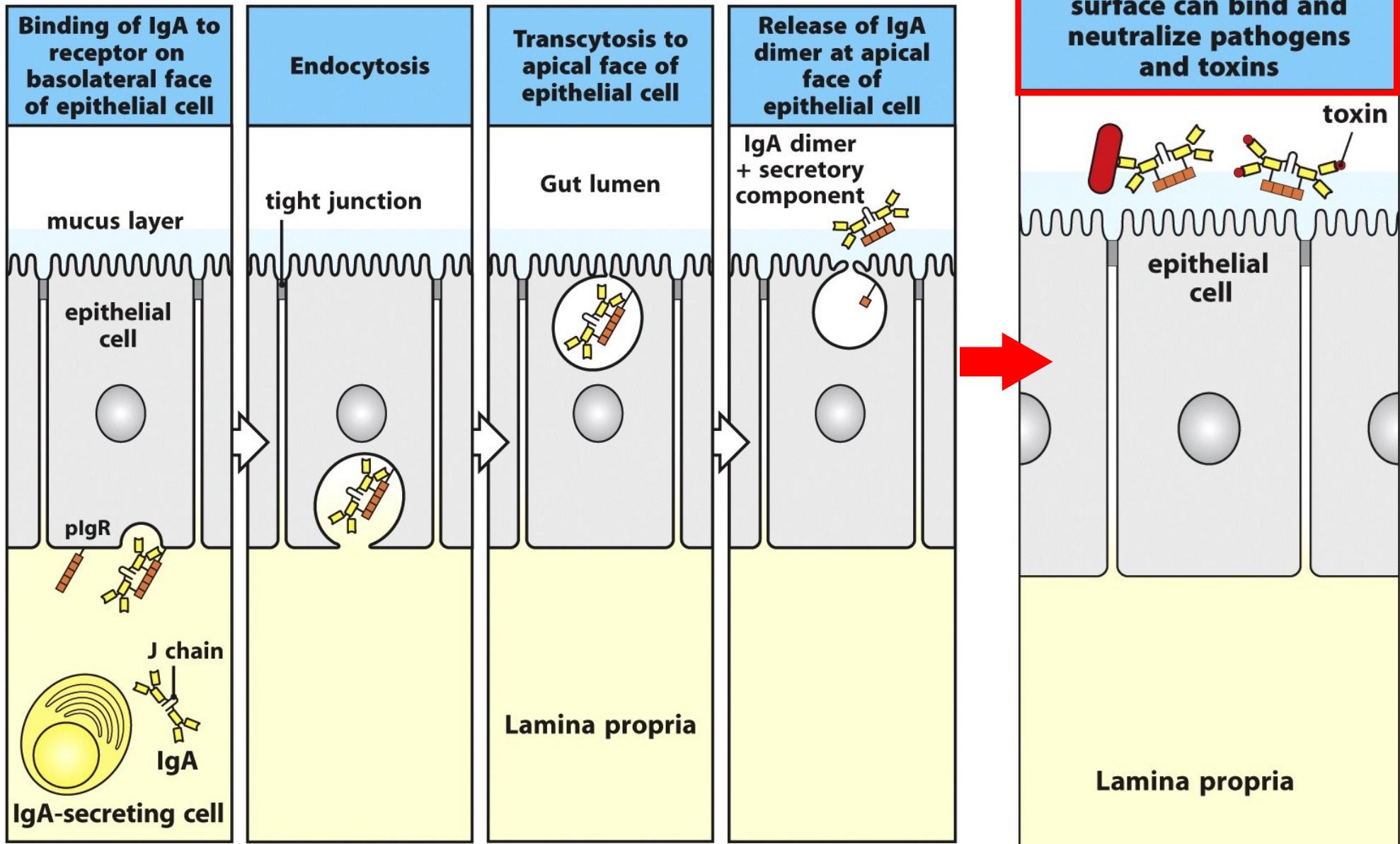
B

B cells located in the submucosa produce dimeric IgA

Poly Ig receptor-mediated transport of IgA through epithelial cells



Poly Ig receptor-mediated transport of IgA



IgA Functions

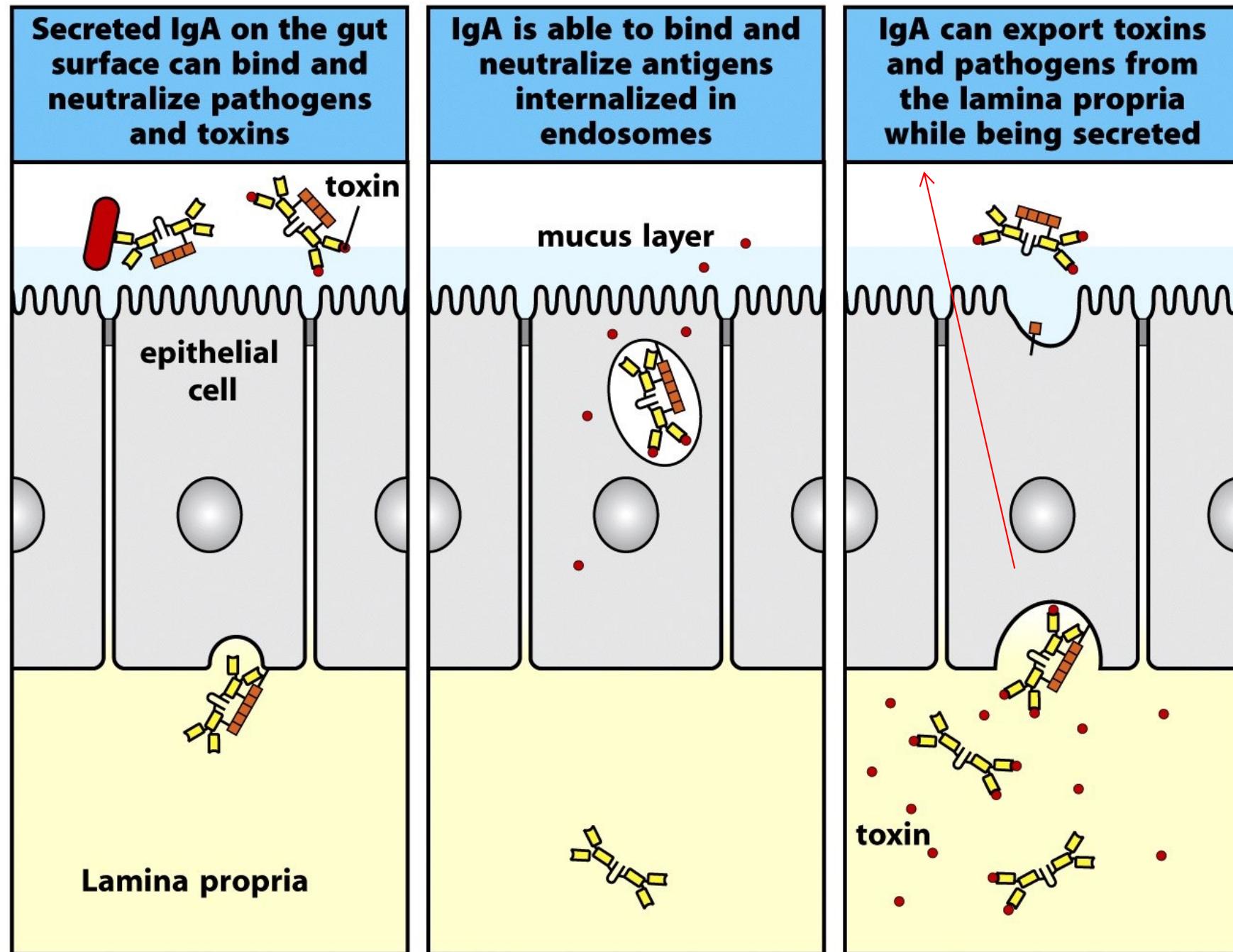


Figure 11-15 Immunobiology, 7ed. (© Garland Science 2008)

IgM

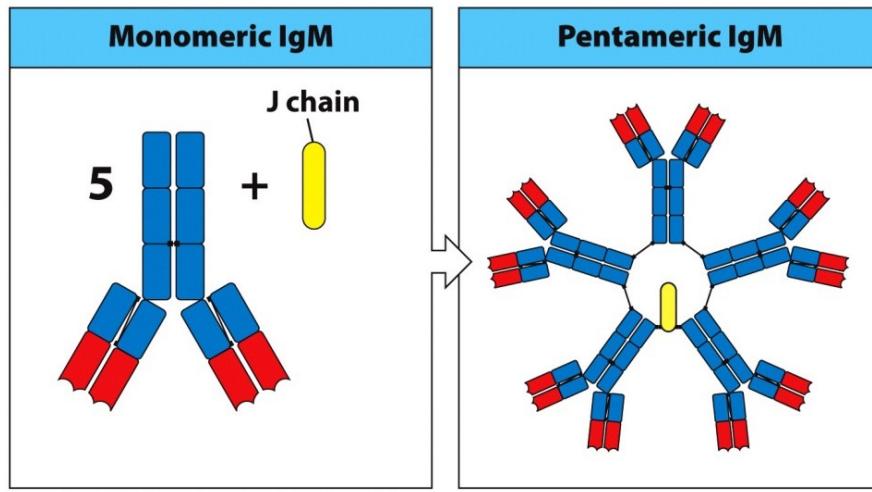
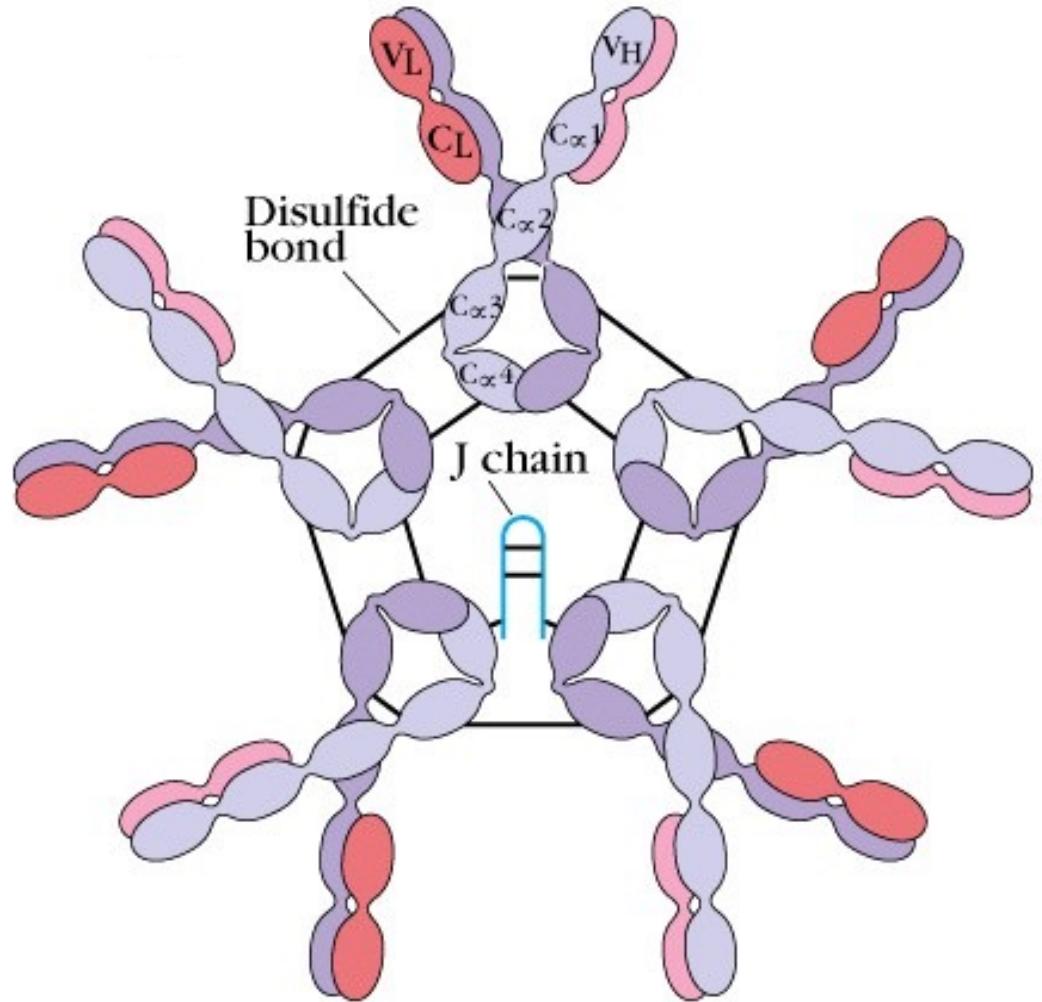
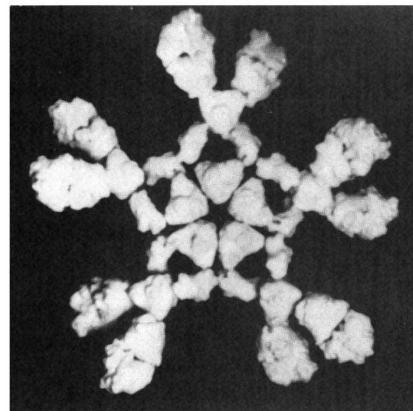
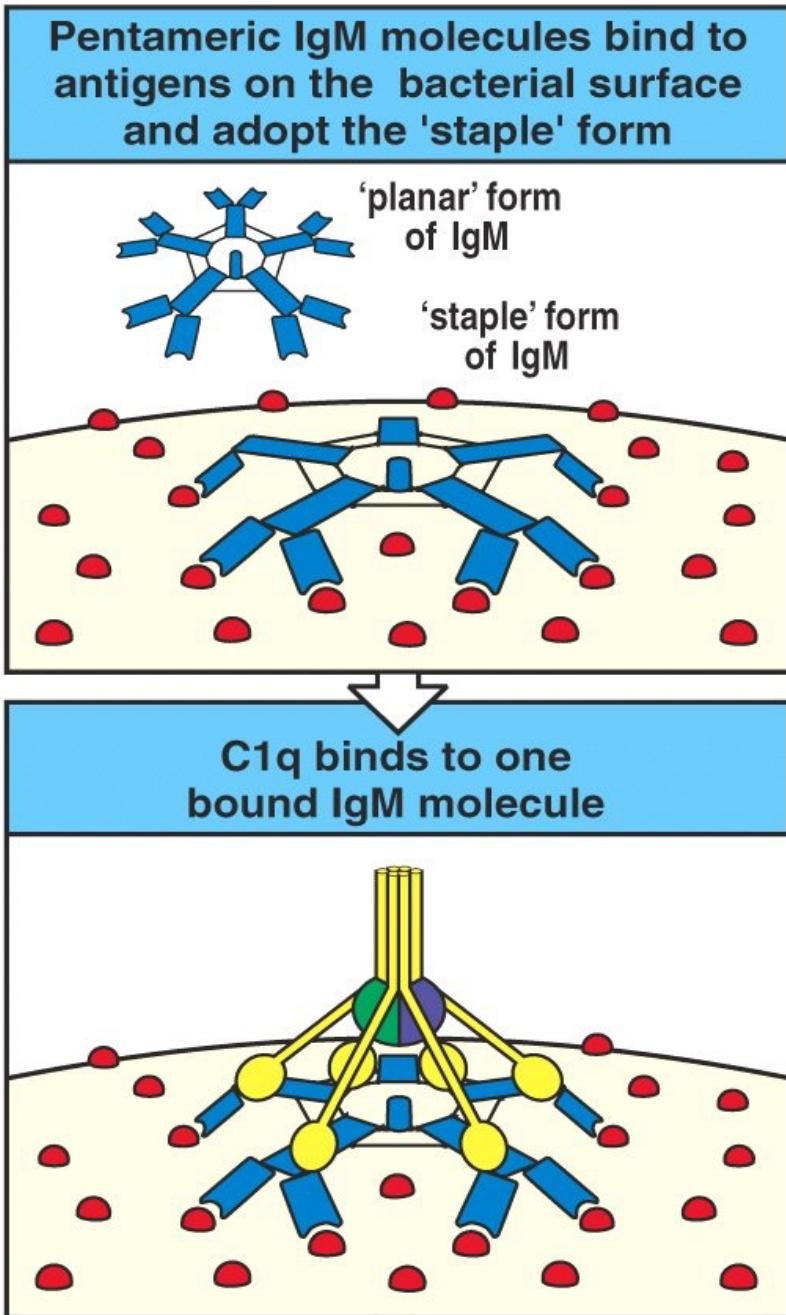


Figure 4.29 part 1 of 2 The Immune System, 3ed. (© Garland Science 2009)

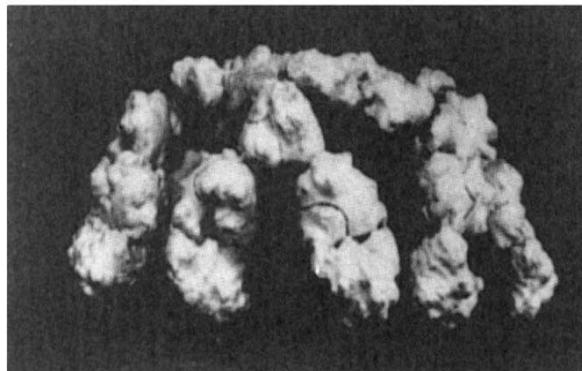


- E' sempre secreta in forma pentamerica
- E' l'isotipo più abbondante nella risposta primaria
- E' l'isotipo più efficiente nell'attivare il complemento

Il legame con l'antigene cambia la conformazione dell'IgM



Le IgM libere hanno una conformazione planare e non sono in grado di fissare il complemento.



Il legame con l'antigene induce un cambiamento conformazionale che rende accessibile la porzione costante.

L'IgM complessata lega il componente C1q del complemento attivandolo.

Le IgM complessate all'antigene attivano la via classica del complemento

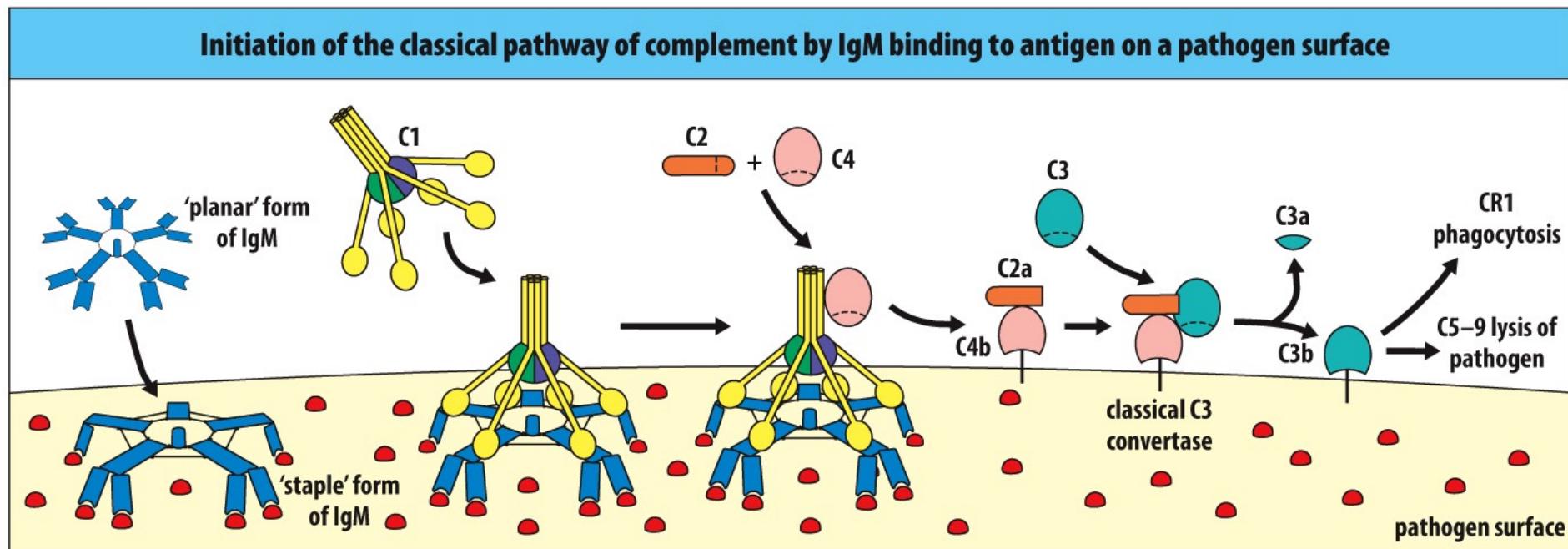
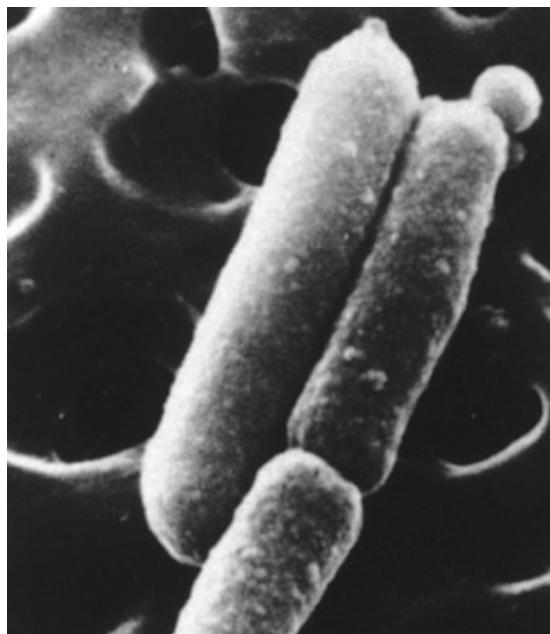


Figure 9.28 The Immune System, 4th ed. (© Garland Science 2015)

Electron micrographs of the effect of antibodies and complement upon bacteria



Healthy E. coli

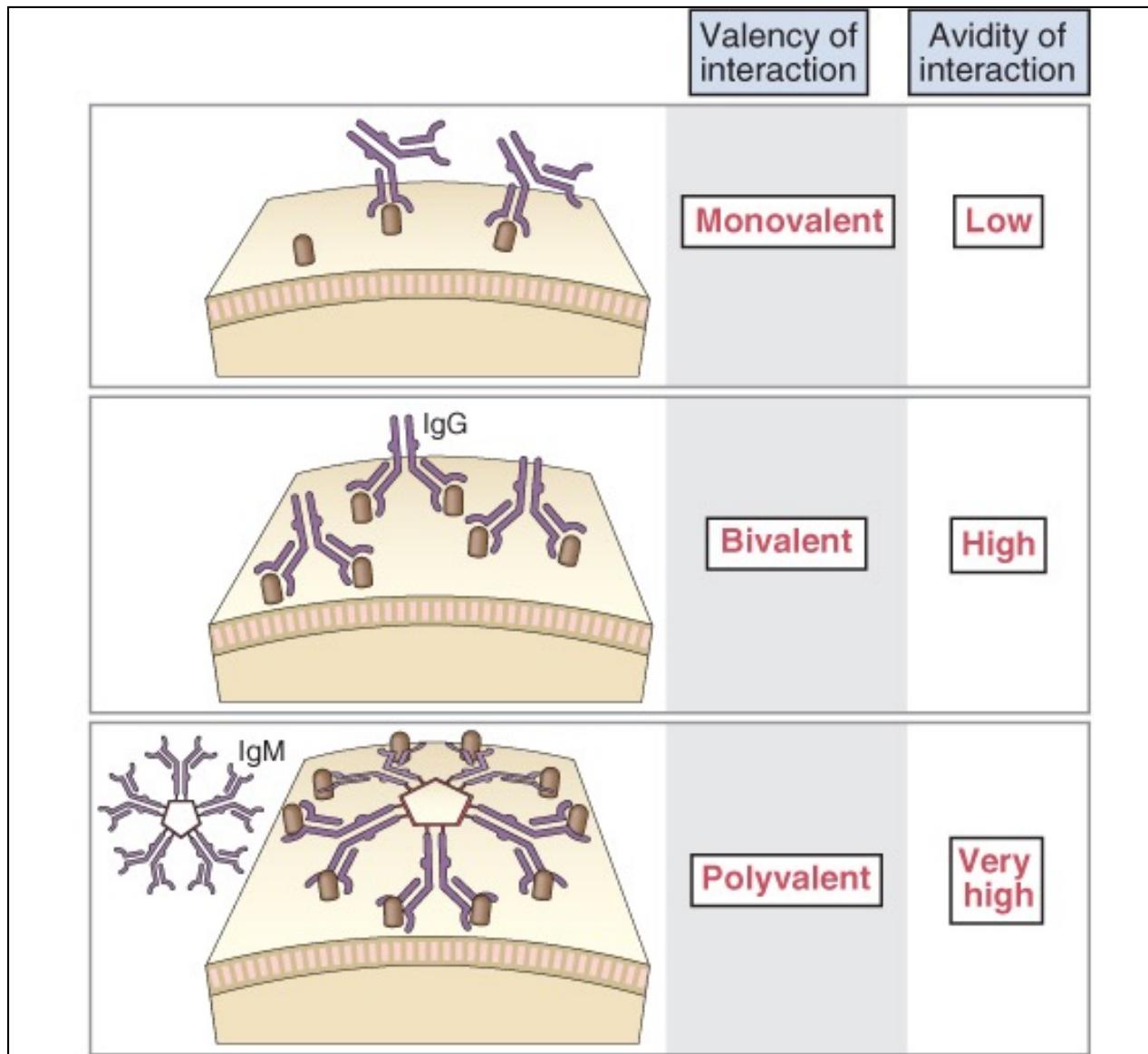


Antibody + complement- mediated
damage to E. coli

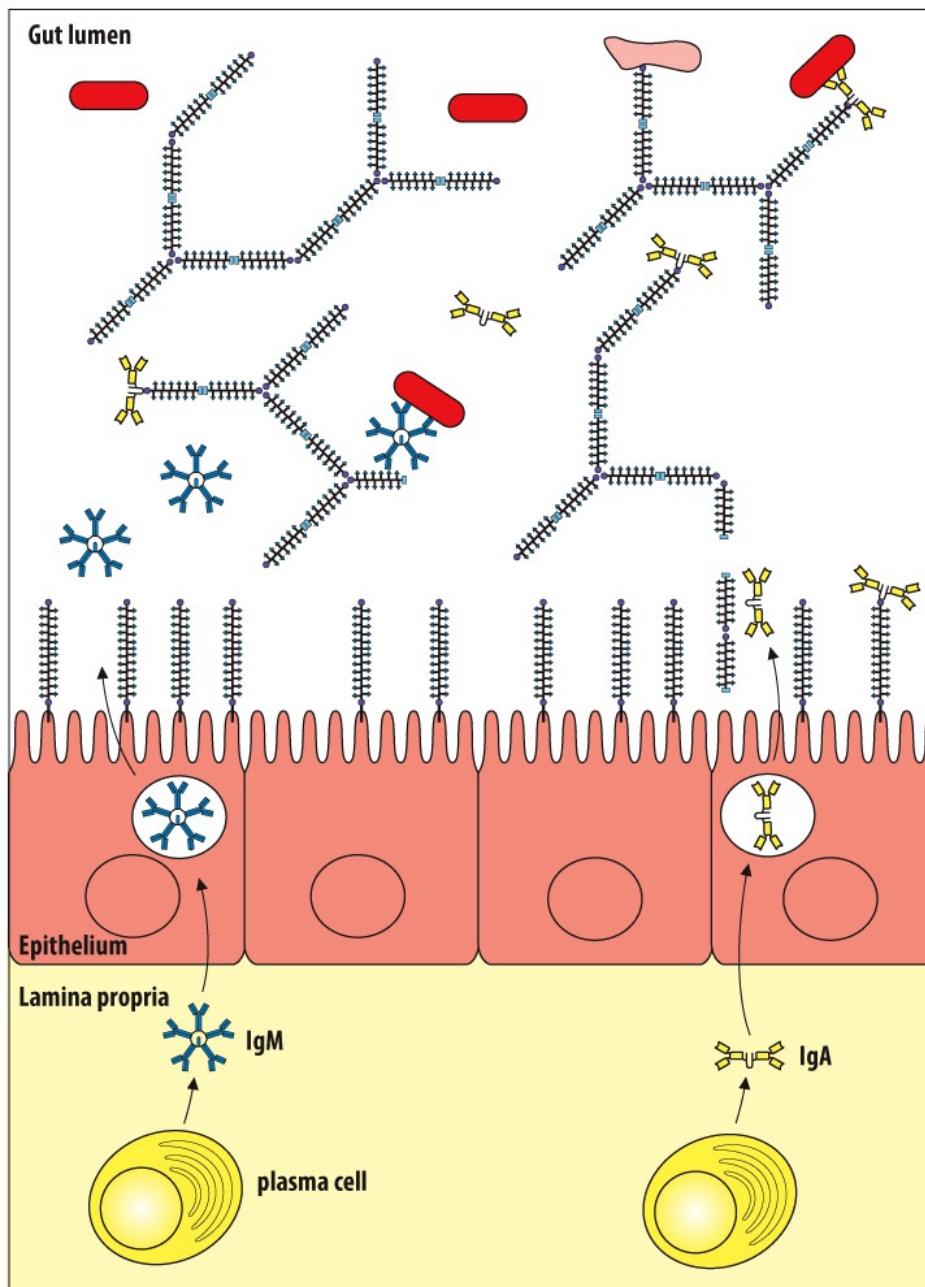


The avidity of IgM interaction is very high!

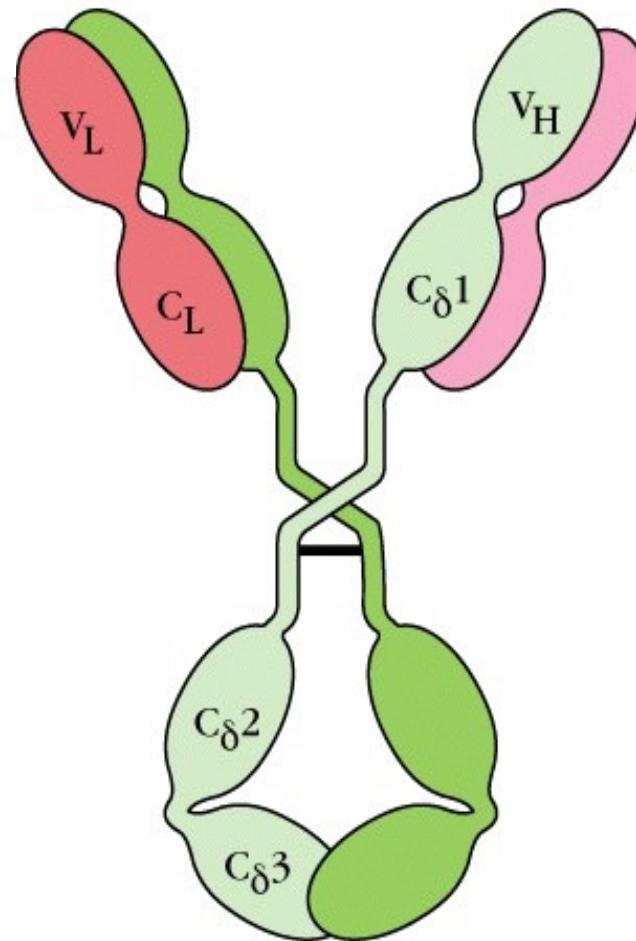
Affinity
≠
Avidity



Secretory IgM protects together with IgA mucosal surfaces from microbial invasion

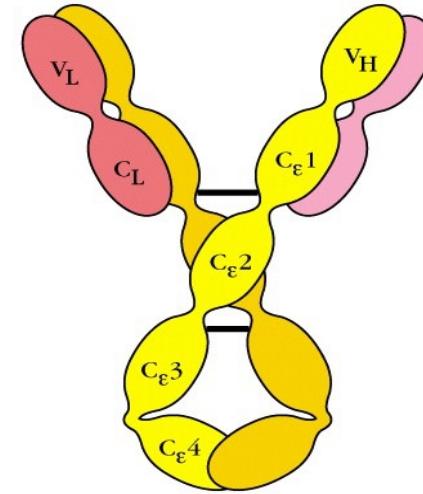


IgD



- E' co-espressa con le IgM sulla membrana dei linfociti B naive
- Non è nota la funzione delle IgD rilasciate in forma secreta

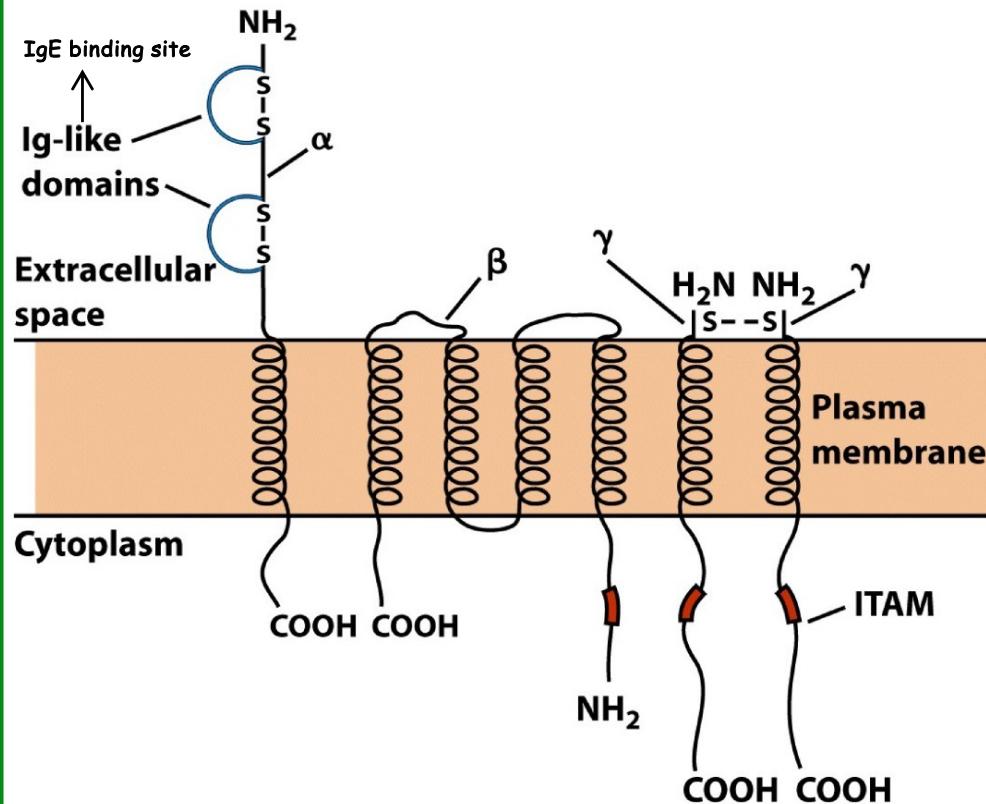
IgE



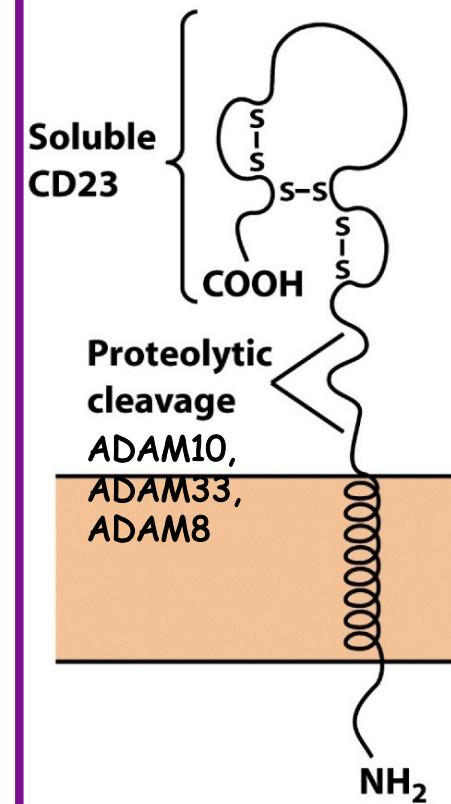
- **Proteggono nei confronti di infezioni parassitarie (elminti)**
 - Legano recettori Fcε espressi sui mastociti e granulociti

I recettori Fc per le IgE

Fc ϵ RI: High-affinity IgE receptor



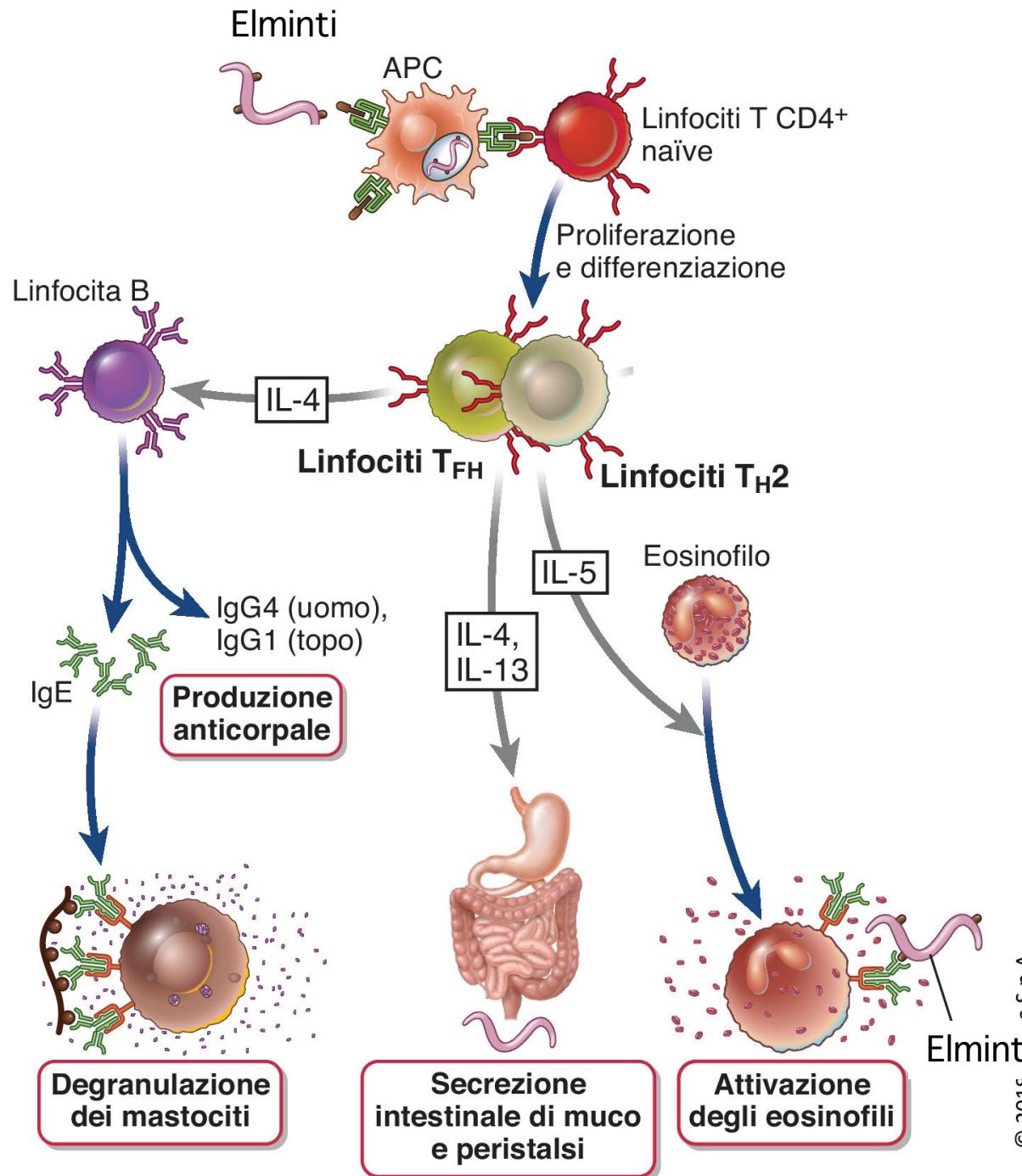
Fc ϵ RII (CD23): Low-affinity IgE receptor



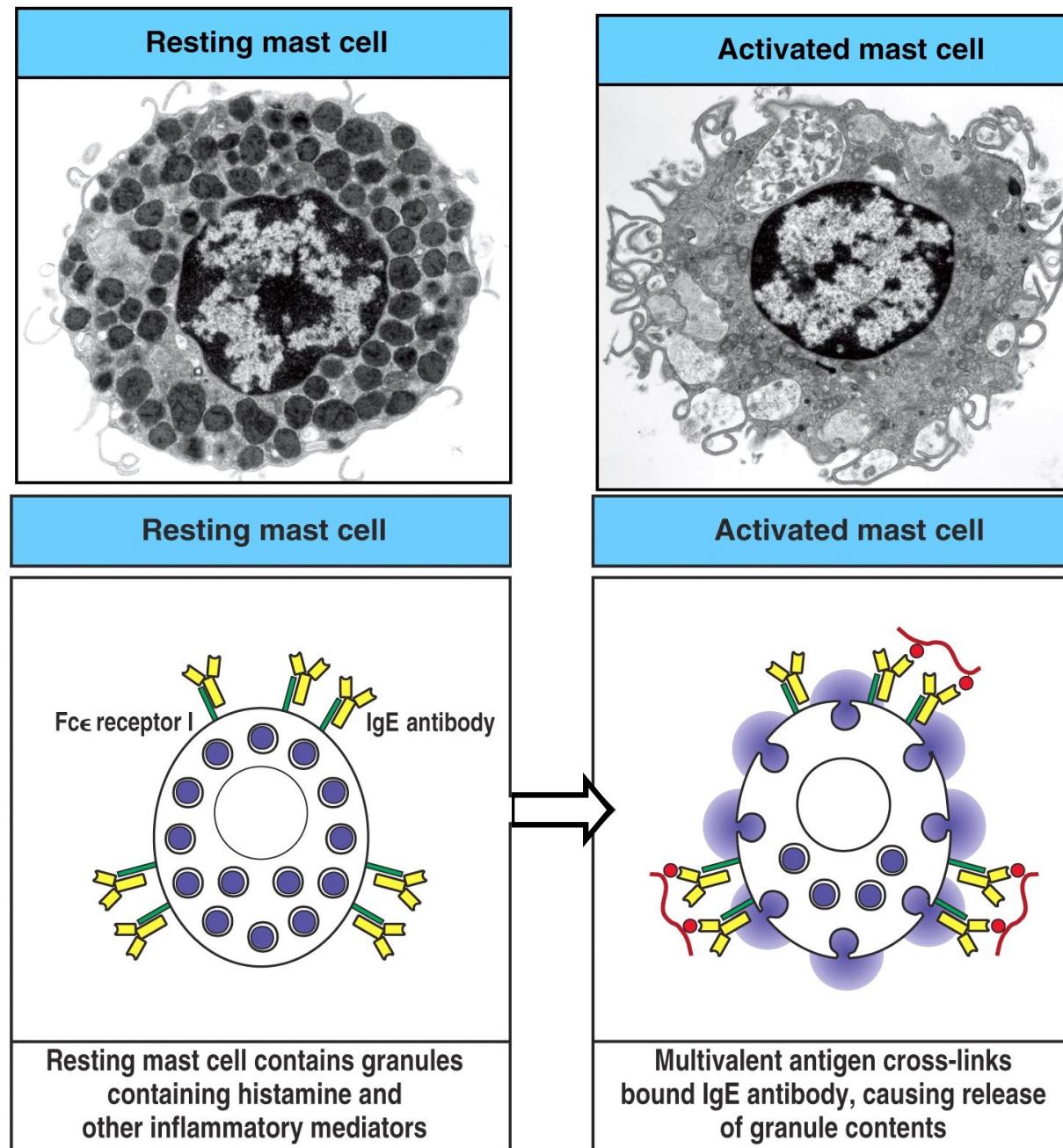
Mastociti, granulociti basofili,
cellule di Langerhans,
monociti attivati
eosinofili (bassi livelli)

Granulociti **eosinofili**,
altre cellule (linfociti B)

Effector functions in helmint infections

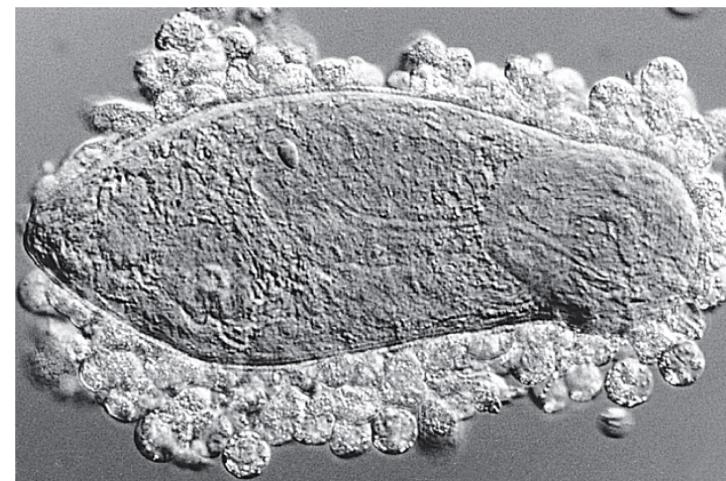
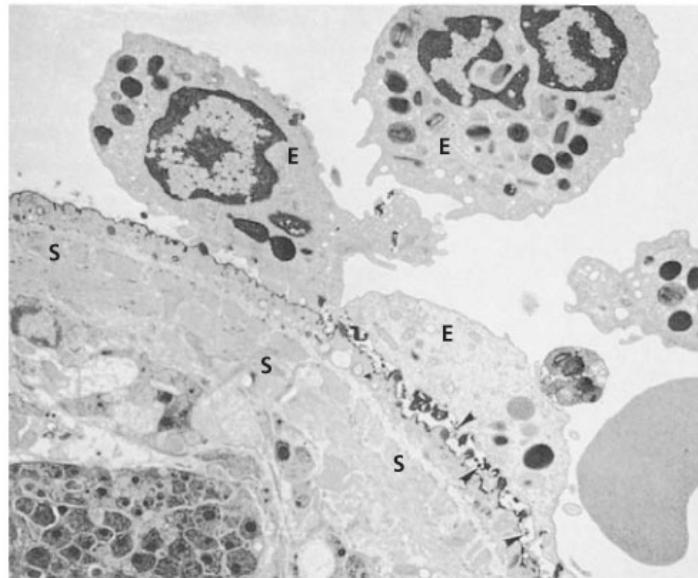
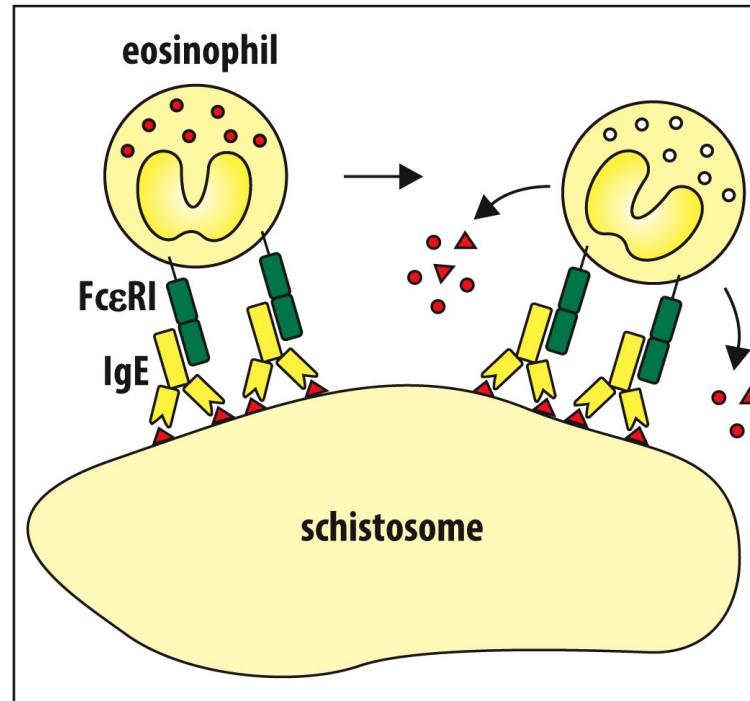


IgE antibody cross-linking on mast cell leads to a rapid release of pro-inflammatory mediators

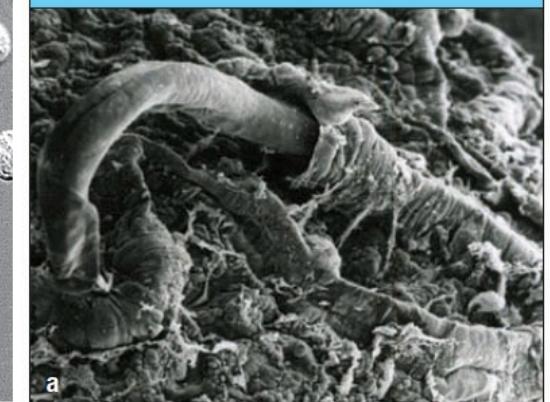


IgE provides a mechanism for the rapid ejection of parasites

I RECETTORI Fc ϵ ESPRESSI SUGLI EOSINOFILI PROMUOVONO LA CITOTOSSICITA' CELLULARE ANTICORPO DIPENDENTE (ADCC)



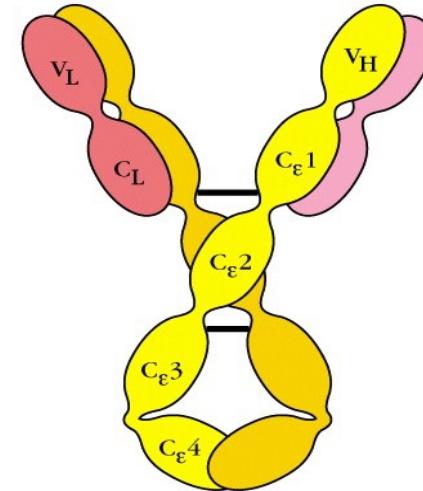
The whipworm *Trichuris trichiura* embeds in the surface epithelium of the colon, leaving its posterior free in the lumen



LE PRINCIPALI FUNZIONI EFFETTRICI DEGLI ANTICORPI

Function	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	-	+++	+++	+++	+++	+++	-
Opsonization	-	-	+++	*	++	+	+	-
Sensitization for killing by NK cells	-	-	++	-	++	-	-	-
Sensitization of mast cells	-	-	+	-	+	-	-	+++
Activation of complement system	+++	-	++	+	+++	-	+	-

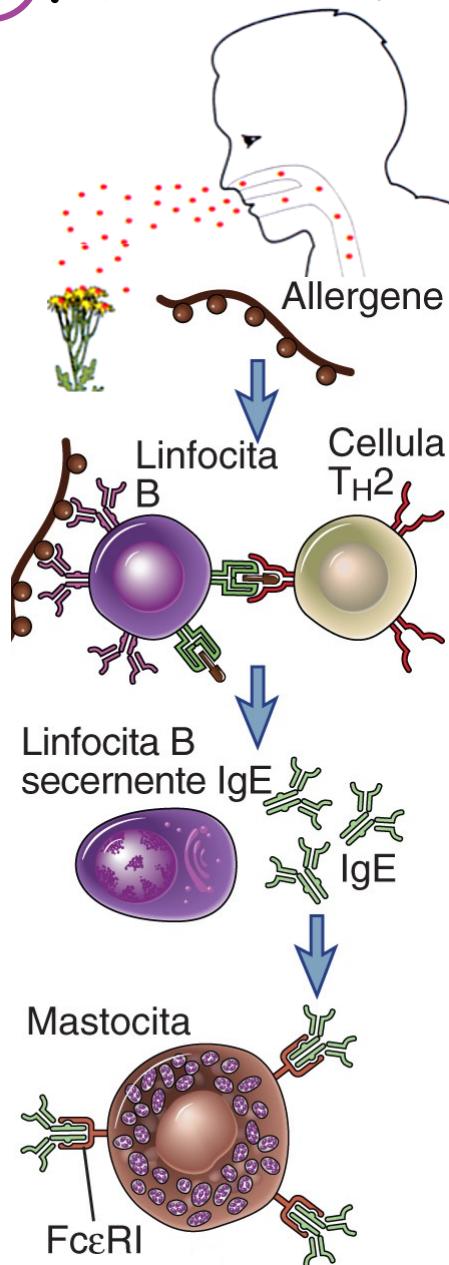
IgE



- Proteggono nei confronti di infezioni parassitarie (Elminti)
 - Legano recettori Fc espressi sui mastociti e granulociti eosinofili
- Mediano le reazioni allergiche

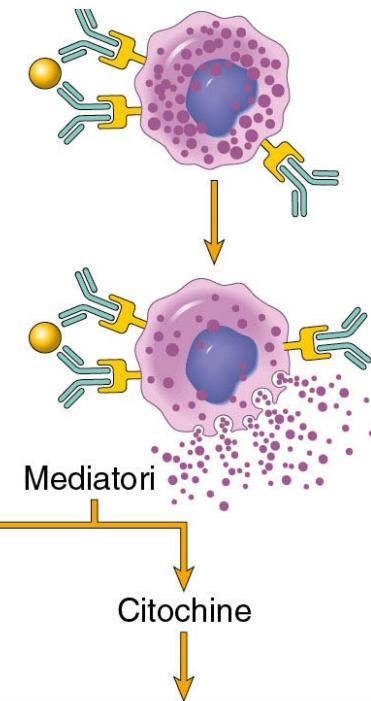
La reazione allergica: una visione integrata

1 fase di sensibilizzazione



2 fase di scatenamento

La seconda esposizione allo stesso allergene attiva il mastocita con conseguente rilascio dei mediatori proinfiammatori



Amine vasoattive,
mediatori lipidici

Reazione di ipersensibilità
immediata (entro pochi minuti
dall'esposizione all'antigene)

Citochine

Reazione tardiva
(2-8 ore dopo l'esposizione
ripetuta all'allergene)