

BIOQ GRACIELA R SVIBEL DE MIZDRAJI

# **INMUNOLOGÍA CLÍNICA**

## **2009**



# Activación de los macrófagos

Producida  
por  
macrófagos y  
células T

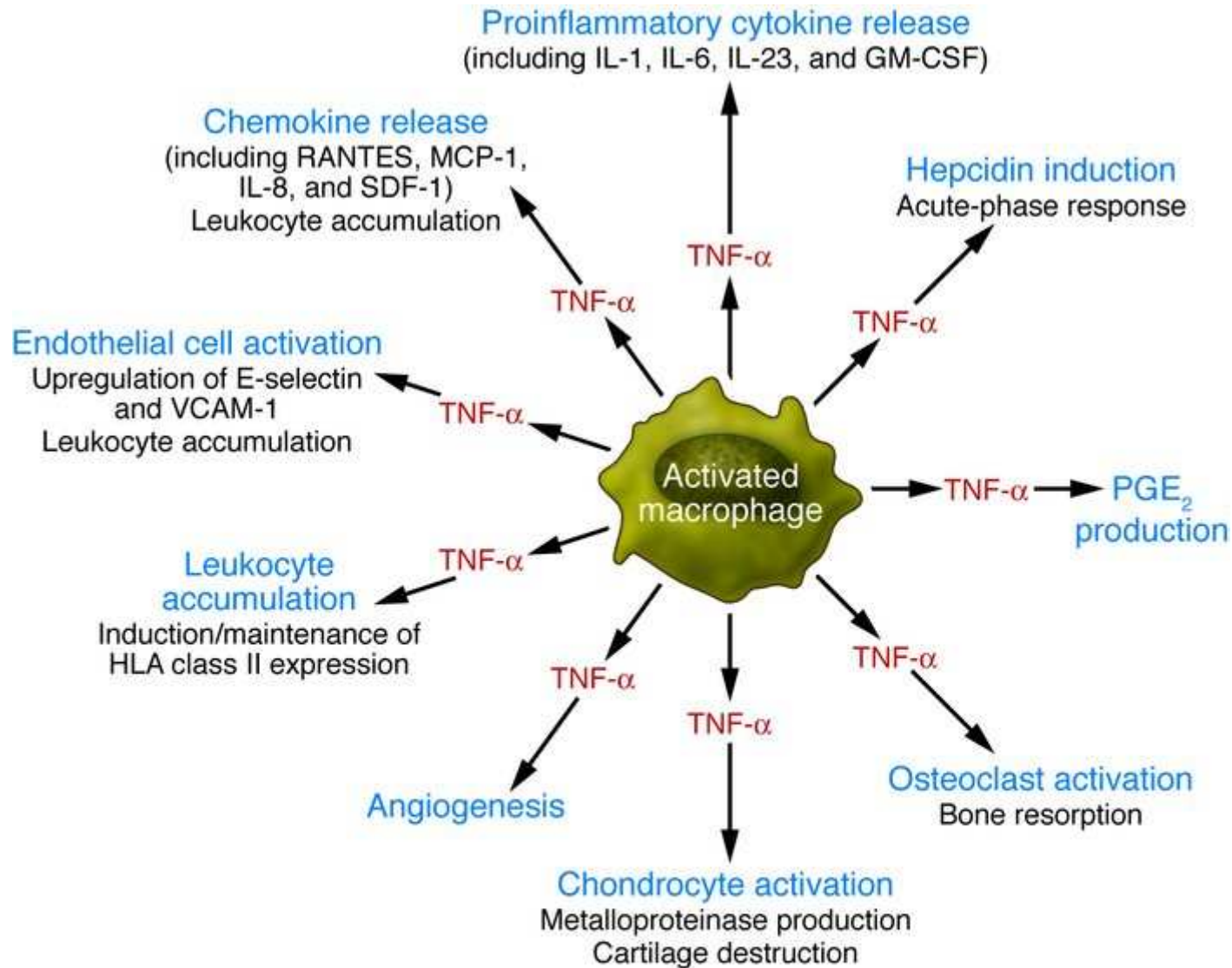
## Primera citocina liberada en el sitio de inflamación

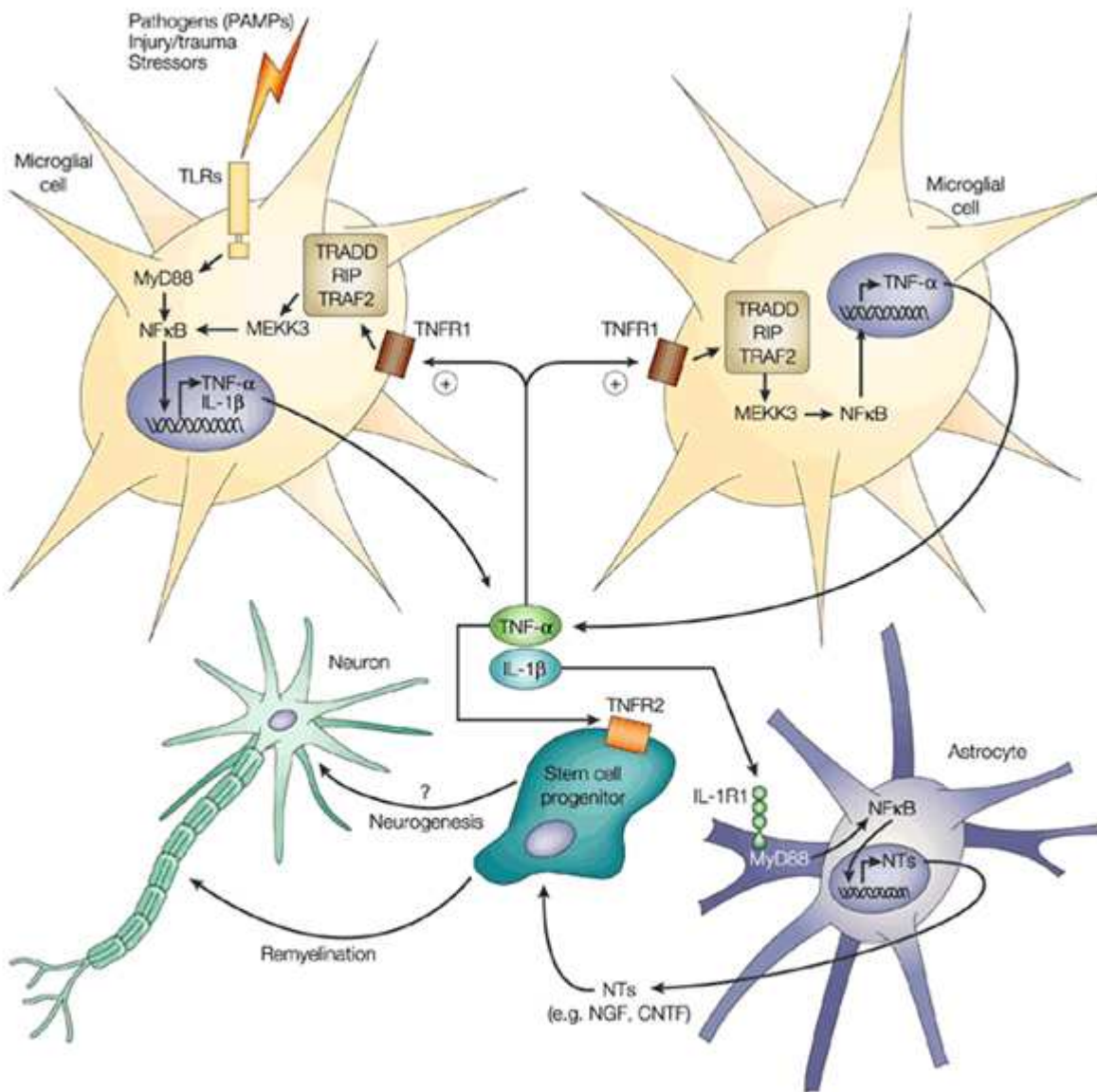
**TNF -  $\alpha$**

- Inductora de mediadores de inflamación, leucotrienes y PAF
- Activadora del endotelio vascular, expresión de moléculas de adhesión
- Pirógeno endógeno directo y por inducción de IL-1
- Aumenta la actividad de osteoclastos, resorción ósea

**Induce síntesis de IL-1 e IL-6**

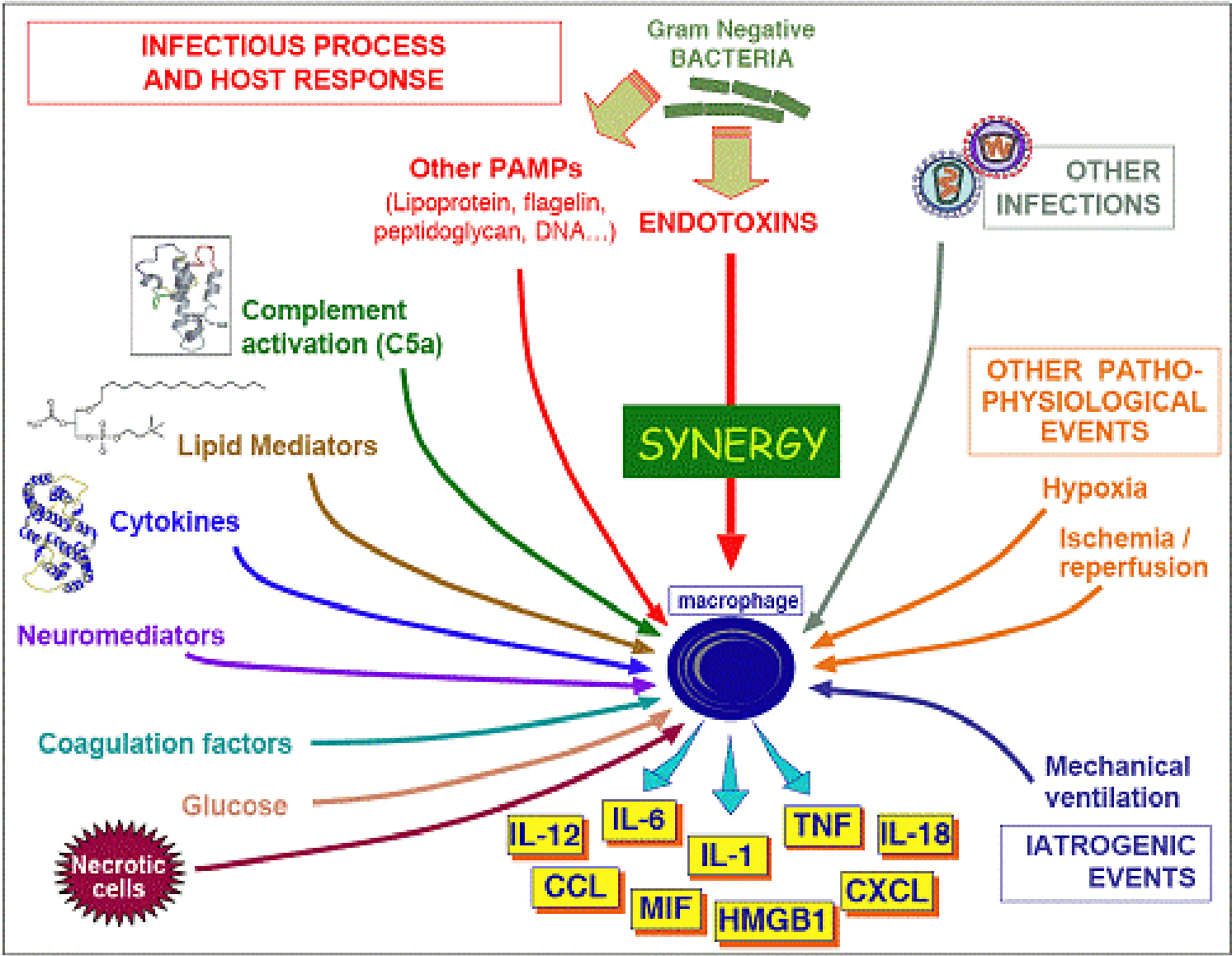
**Induce la síntesis de proteínas de fase aguda**

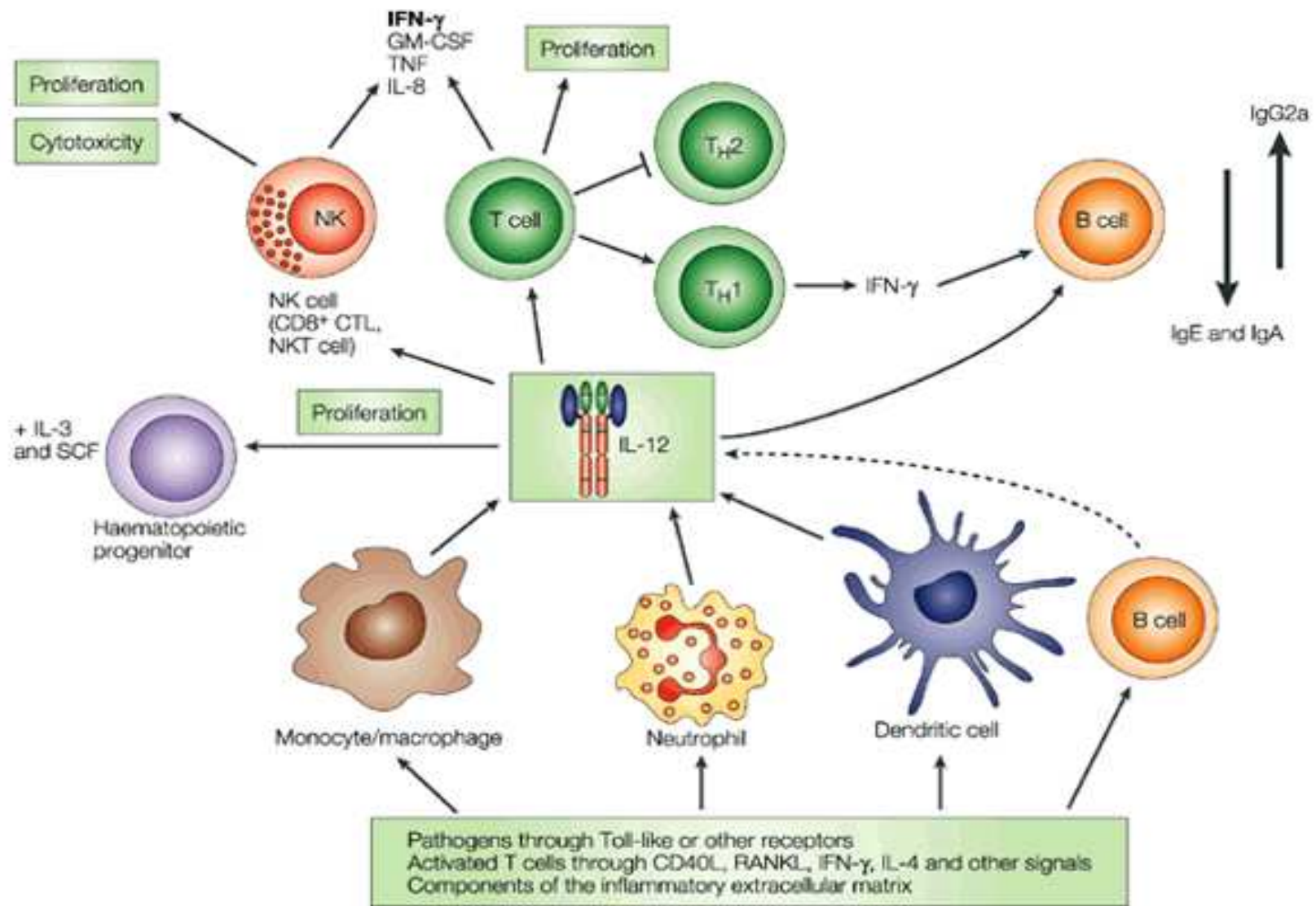


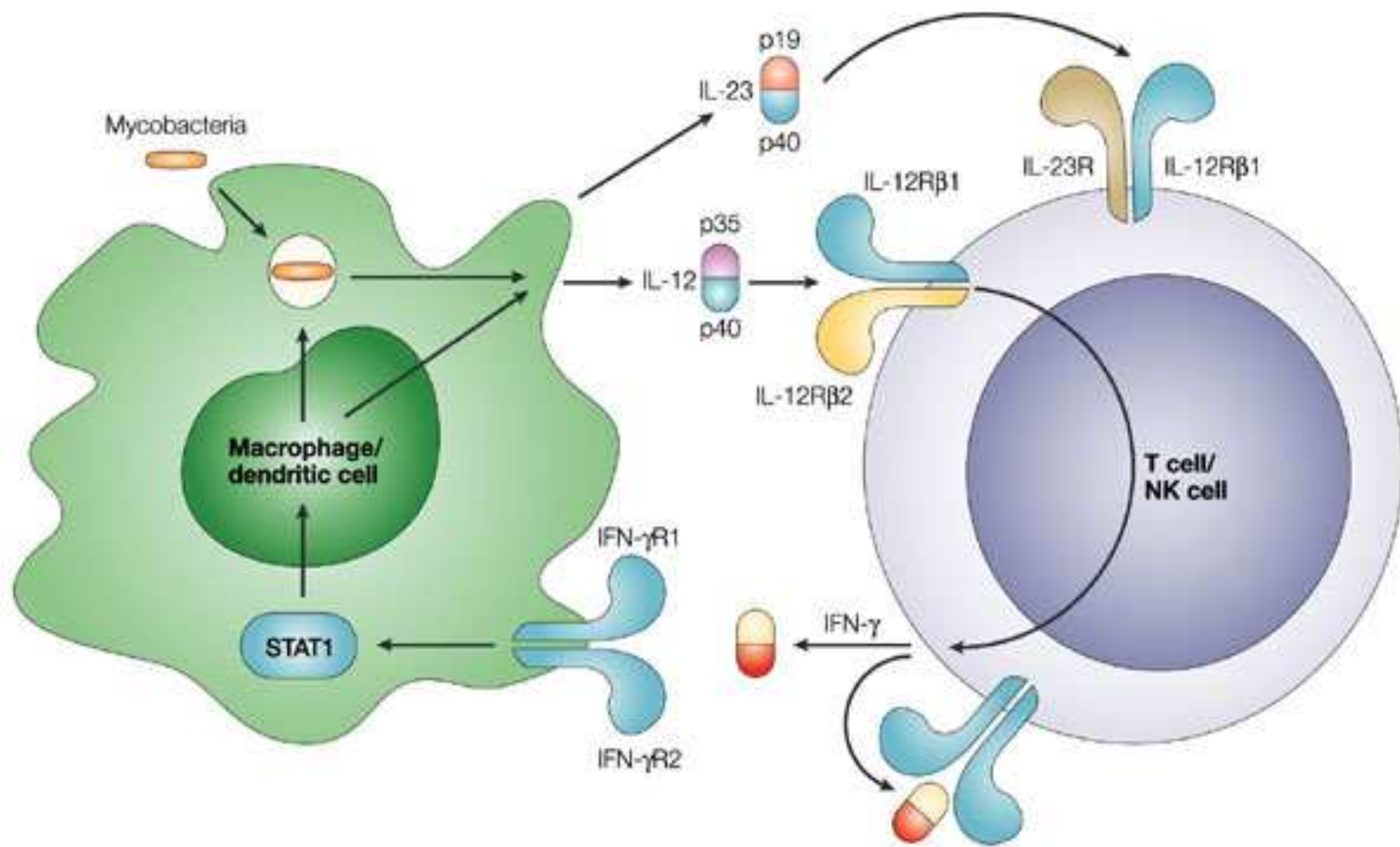


neurotrophins (NTs):  
 nerve growth factor (NGF),  
 ciliary neurotrophic factor (CNTF).

MEKK3, mitogen-activated protein  
 kinase kinase kinase 3;  
 NFB, nuclear factor B;  
 PAMPs, pathogen-associated  
 molecular patterns;  
 RIP, receptor-interacting protein;  
 TRADD, TNFR1-associated protein  
 with death domain;  
 TRAF, TNFR-associated factor.







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# Defensa Innata



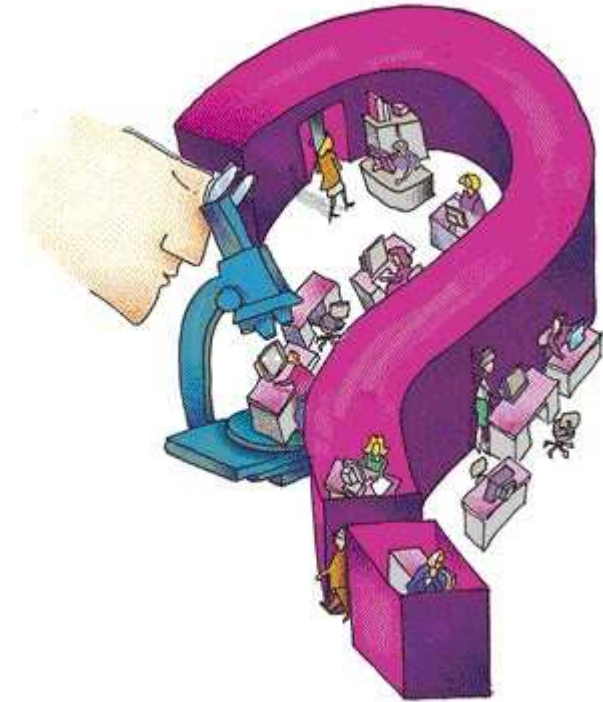
- Primera línea de defensa ante cualquier microorganismo exógeno.
- No es específica
- **Barreras mecánicas, químicas y microbiológicas.**
- *Células de la respuesta inmune.*
- **Moléculas solubles y receptores de membrana.**



**¿Cómo unos pocos receptores del sistema inmune innato pueden reconocer tantos microorganismos?**

# Receptores y Targets del reconocimiento inmune

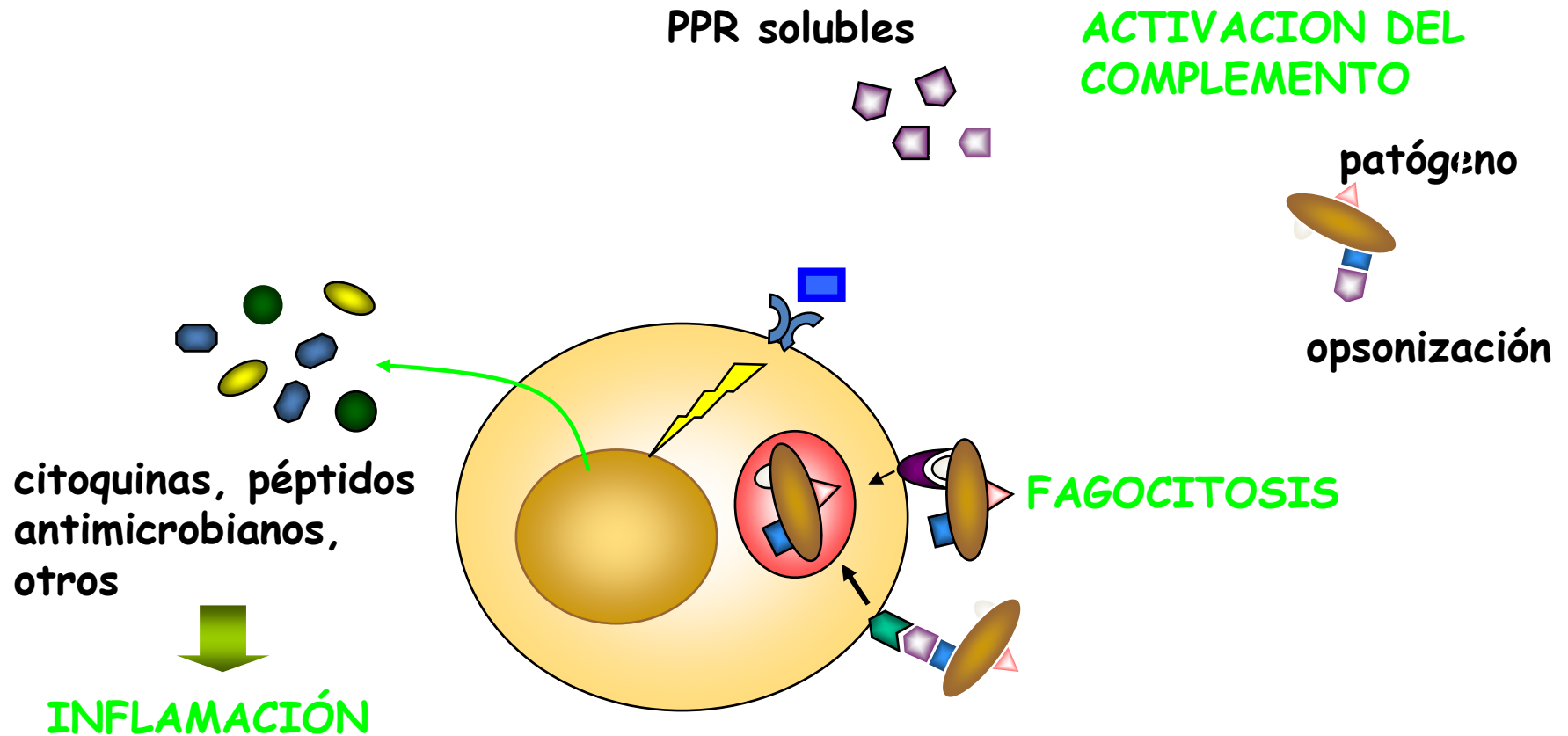
- Los targets de reconocimiento representan patrones moleculares, denominados PAMPs: **Pathogen-Associated Molecular**
- El huésped ha desarrollado un grupo de receptores que específicamente reconocen los PAMPs y se denominan PRRs: **Pattern Recognition Receptors.**



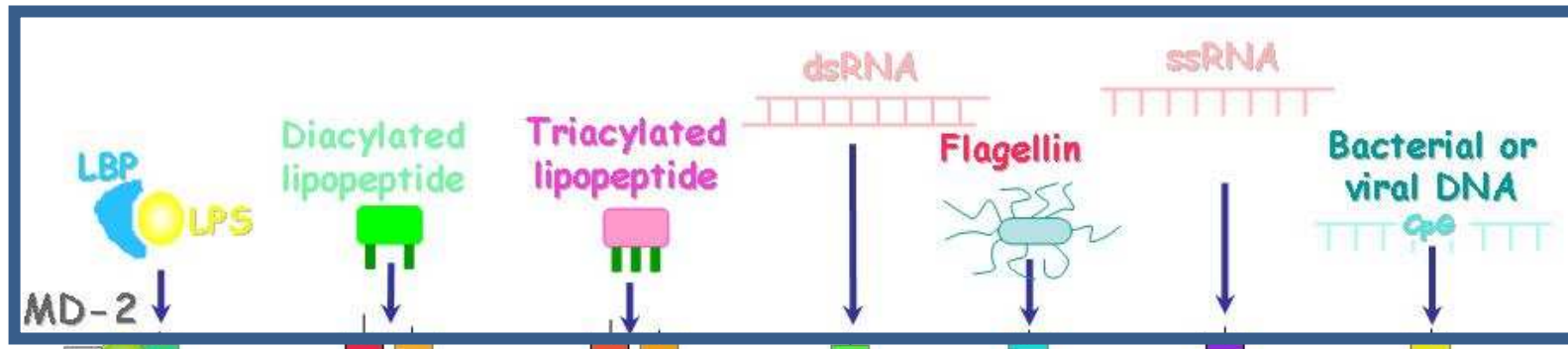
# PAMPs

- Son esenciales para la supervivencia o virulencia del microorganismo;
- se encuentran en los microorganismos pero no en la célula huésped;
- son compartidos por clases enteras de microorganismos....
- **representan estructuras invariantes compartidas por un gran grupo de microorganismos.**

# El reconocimiento de PAMPs por receptores PRR desencadena diferentes efectos



# PAMPs- PRRs



## Pathogen-associated molecular patterns (PAMPS)

- Conserved microbial molecules shared by many pathogens

- Include:

Bacterial lipopolysaccharides  
 Peptidoglycan  
 Zymosan  
 Flagellin  
 Unmethylated CpG DNA

## Pattern Recognition Receptors (PRR)

- Include:

Toll like receptors

Receptors for apoptotic cells (scavenger receptors)

Receptors for opsonins

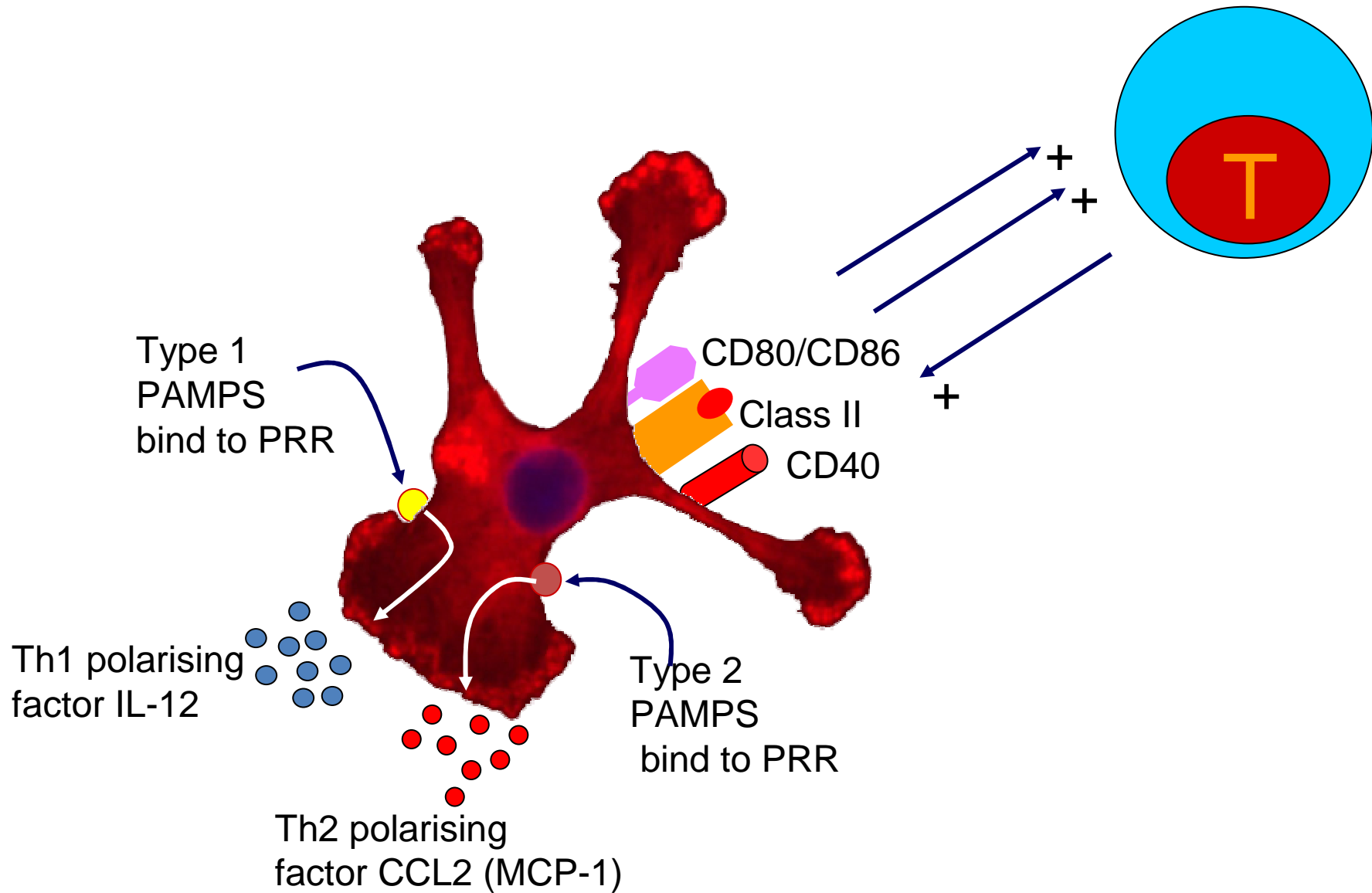
Receptors for coagulation and complement proteins

## Patrones microbianos

### Molecules Recognized by Innate Immunity

Molecular component	Type of organism
<b>Nucleic acids</b>	
double-stranded RNA (dsRNA)	viruses
CpG-containing DNA (CpG DNA)	bacteria, fungi, viruses
<b>Cell wall components</b>	
lipopolysaccharide	Gram-negative bacteria
lipoteichoic acids	Gram-positive bacteria
peptidoglycan	bacteria
flagellin	Gram-negative bacteria
lipoproteins	bacteria
mannose-, fucose-rich polysaccharides	bacteria, fungi
$\beta$ -glucans	bacteria, fungi
<b>Membrane properties</b>	
exposed acidic phospholipids	bacteria, fungi (apoptotic cells)
<b>Biosynthetic components</b>	
<i>N</i> -formylated methionyl peptides	bacteria

# POLARIZACIÓN DE LA DC POR PAMPs TIPO 1 Y TIPO 2





# PAMPs TIPO 1 y TLR

Peptidoglycan (Gram + bacteria)

Lipoproteins

Lipoarabinomannan (Mycobacteria)

LPS (Leptospira)

LPS (Porphyromonas)

Glycophosphatidylinositol - (*T. Cruzi*)

Zymosan (Yeast)

dsDNA

LPS

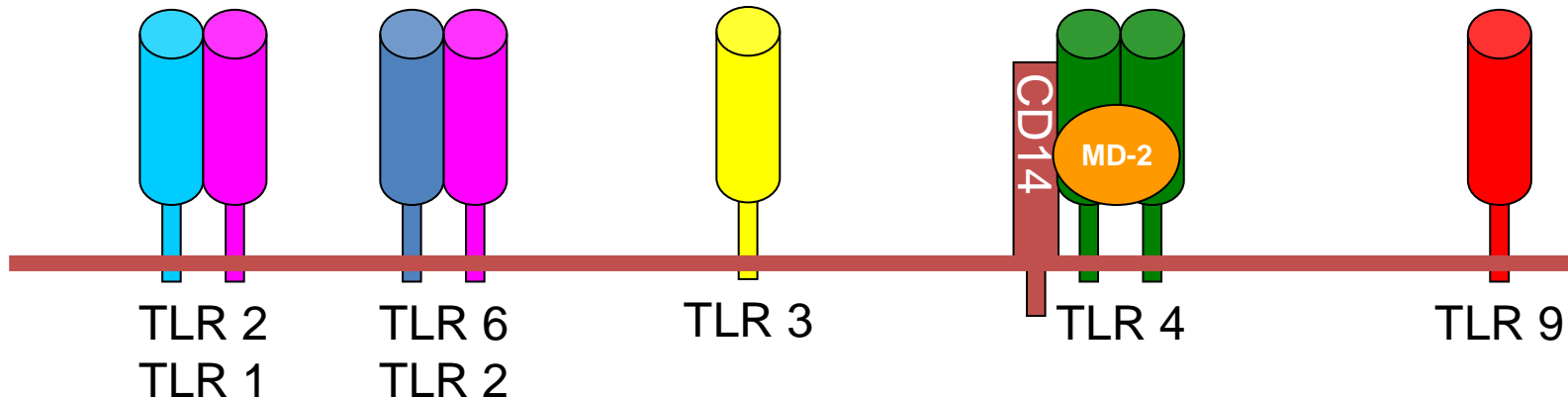
Lipotechoic acid -

(Gram + bacteria)

RSV F protein

Unmethylated

CpG DNA



Low level IL-12p70

Some ligands

induce IL-10

or IL-12p35

High

IL-12p70

IFN- $\alpha$

High

IL-12p70

High

IL-12p70

IFN- $\alpha$

# PAMPs TIPO 2 y PRR

? ?



# PATRONES MOLECULARES ENDÓGENOS

## Endogenous molecular patterns

- Include:

- Heat shock proteins

- (HSP60 HSP70 GP96)

- Extracellular matrix proteins

- (hyaluronan, fibronectin, fibrinogen)

- Immune complexes

- Surfactant protein A

- Necrotic cell components

## Pattern Recognition Receptors (PRR)

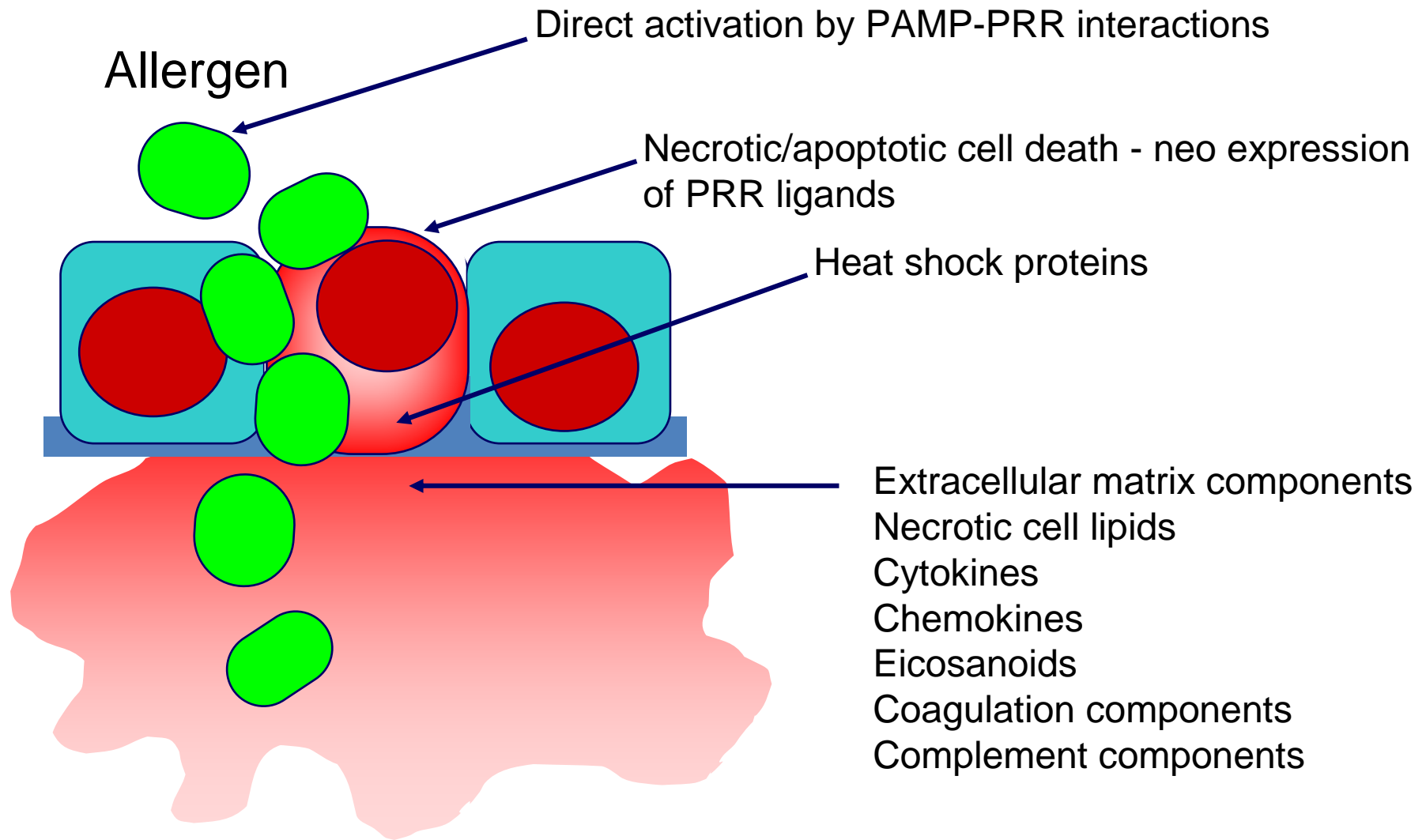
- Include:

- Toll like receptors

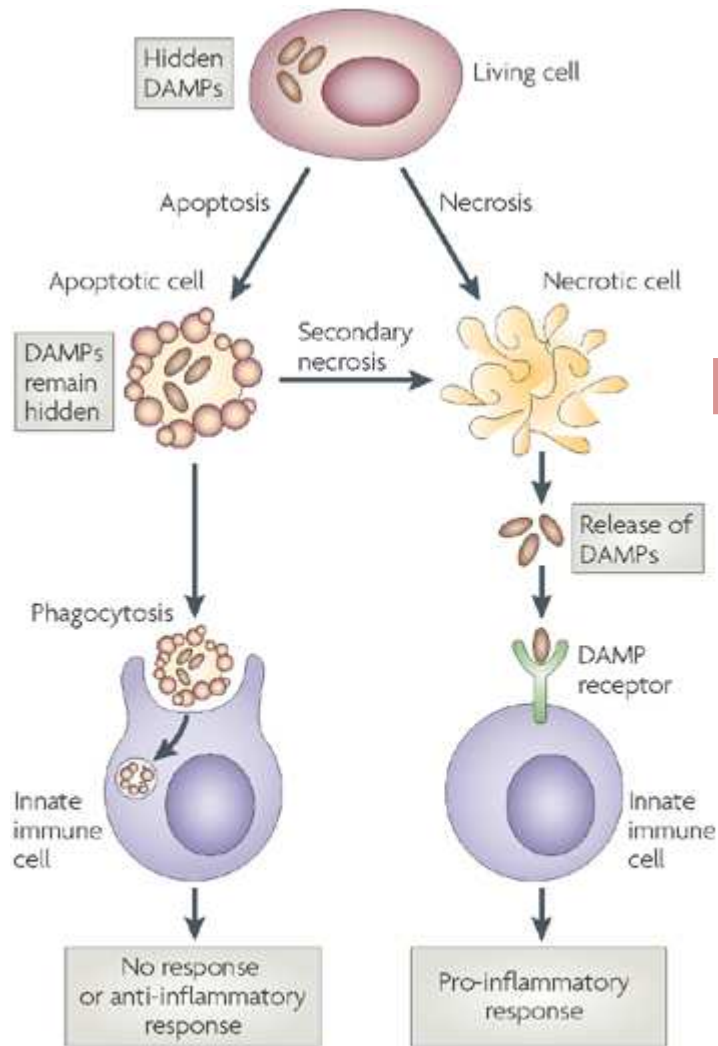
- Receptors for apoptotic cells (scavenger receptors)

- Receptors for opsonins

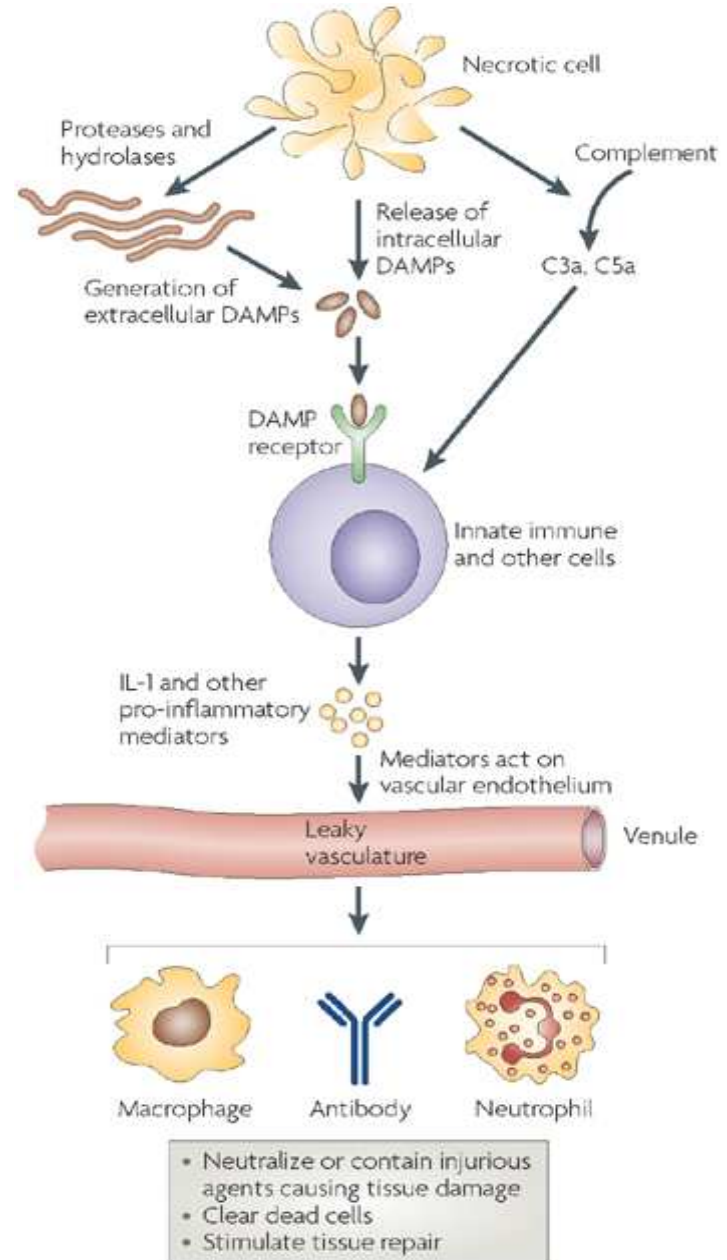
- Receptors for coagulation and complement proteins



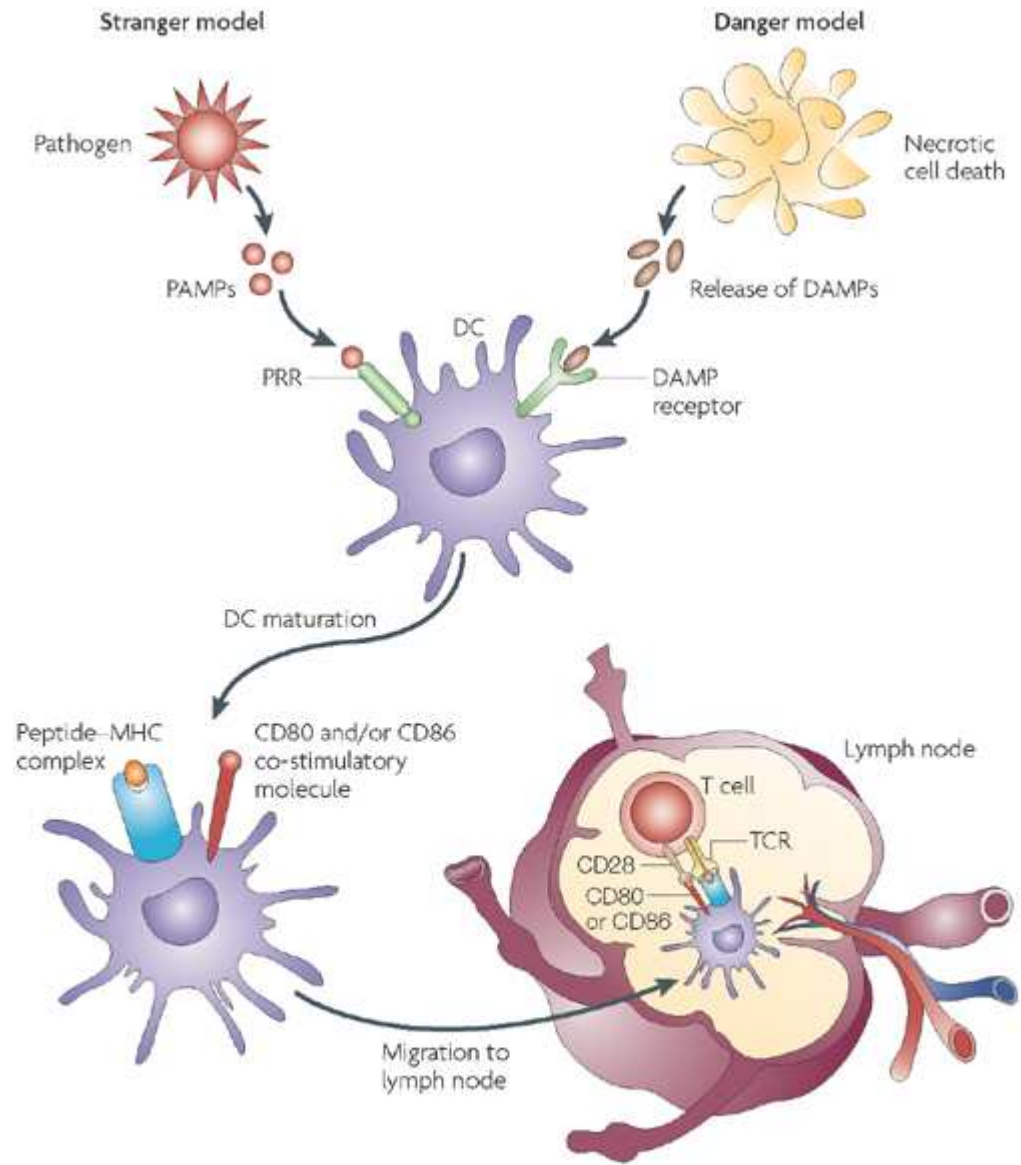
Activates the expression of costimulatory molecules on DC



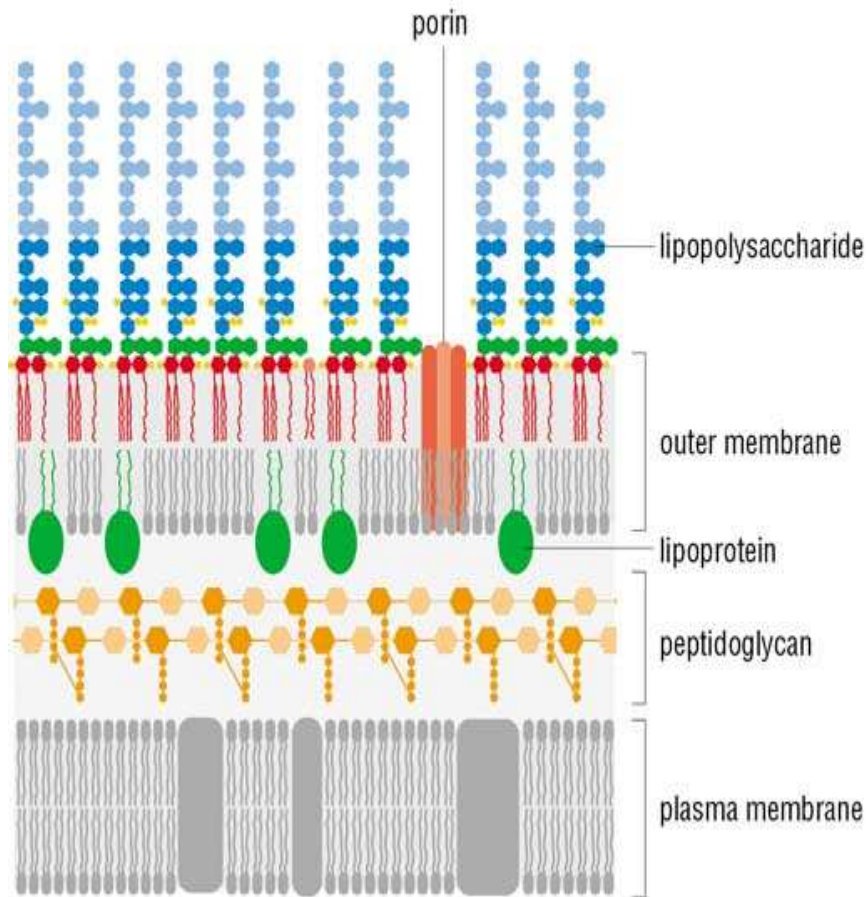
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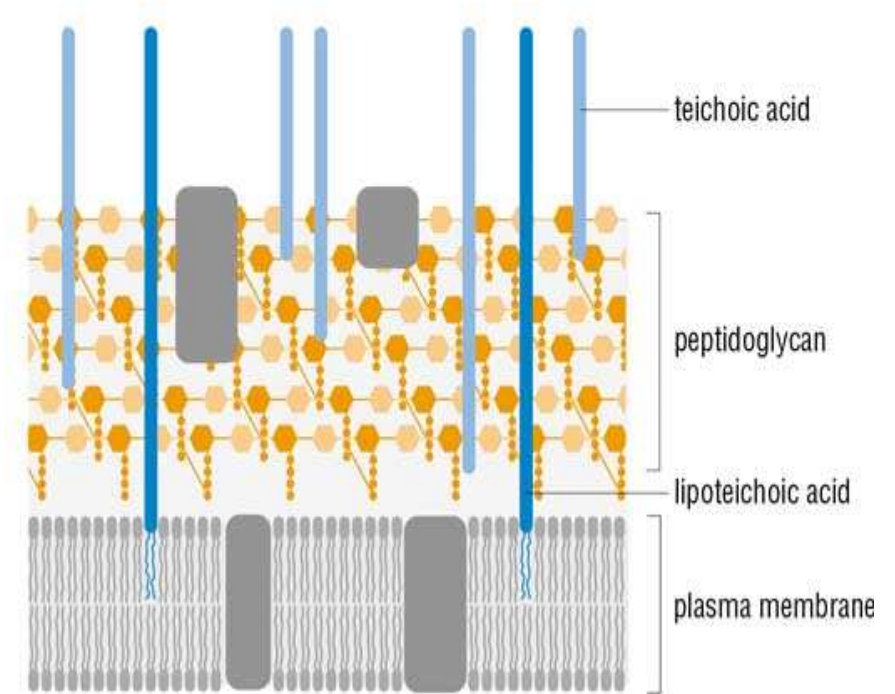


# ESTRUCTURA DE LA PARED BACTERIANA

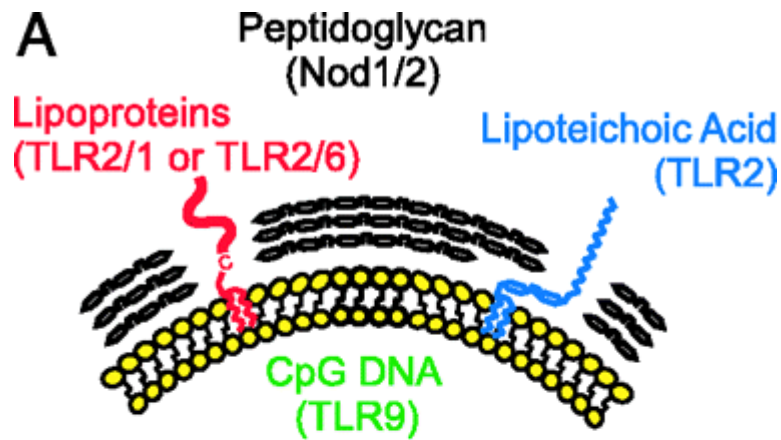


**Gram-negative bacterial cell wall**

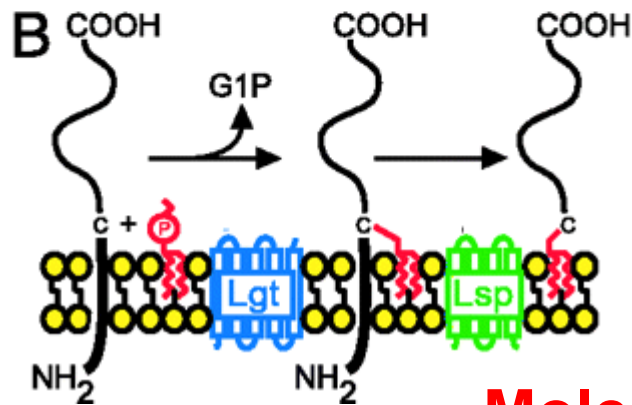
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**Gram-positive bacterial cell wall**

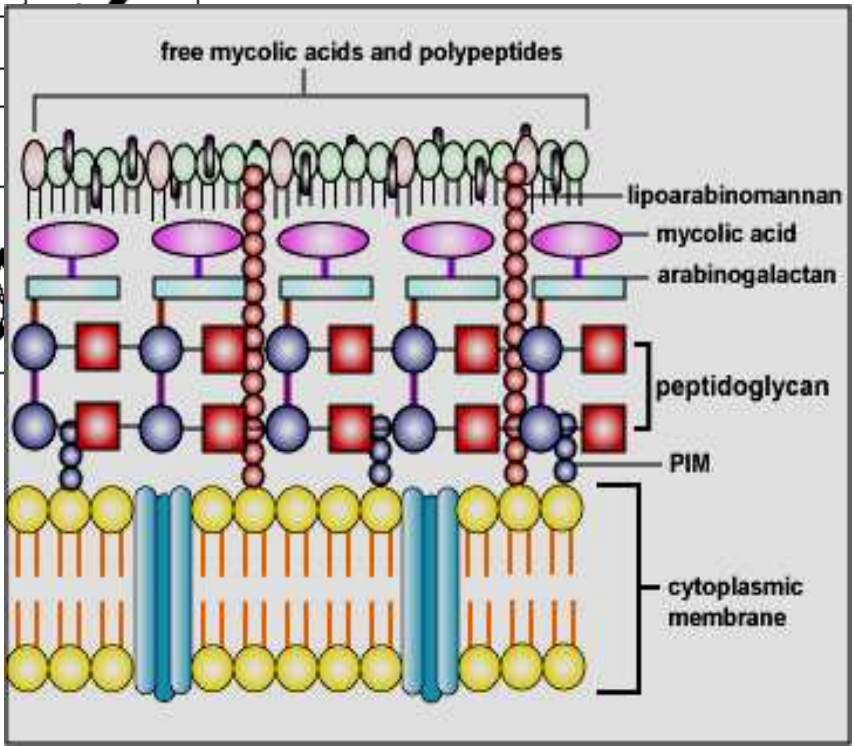
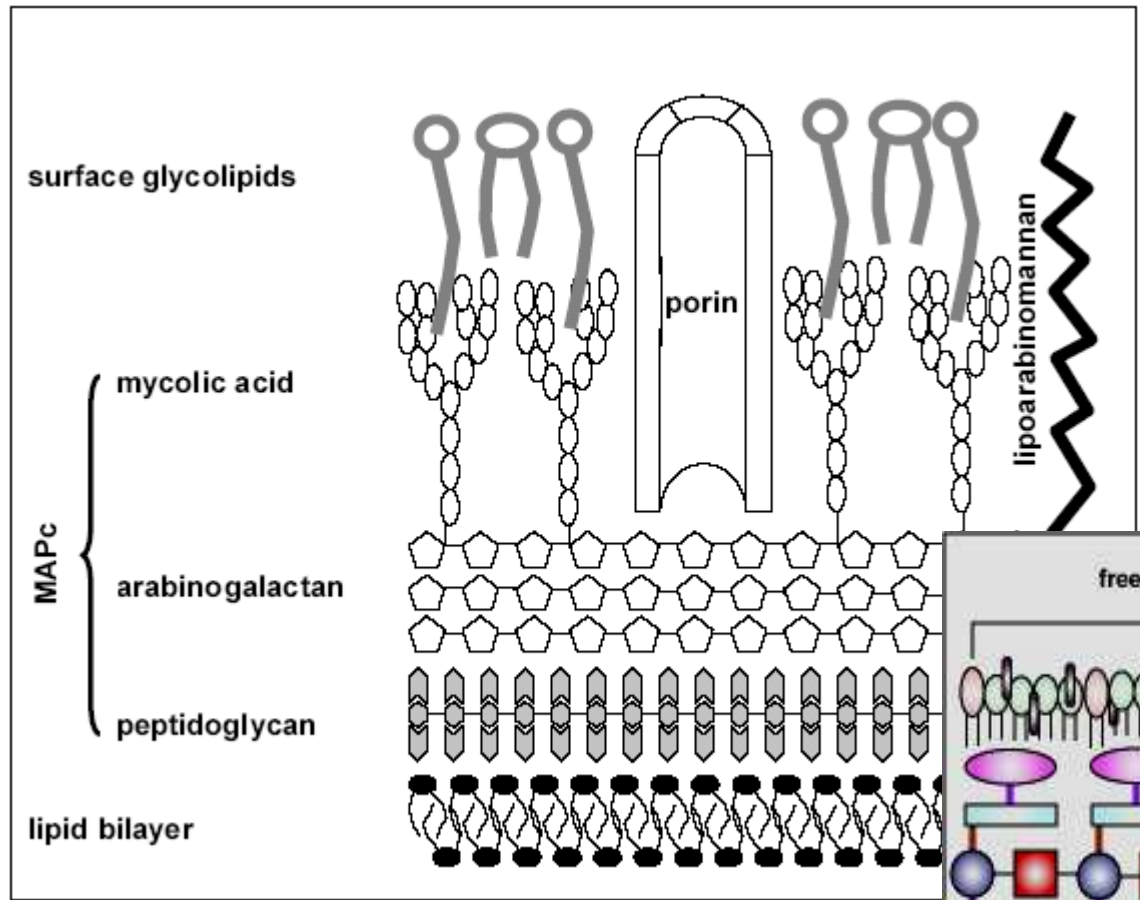


# Staphylococcus aureus



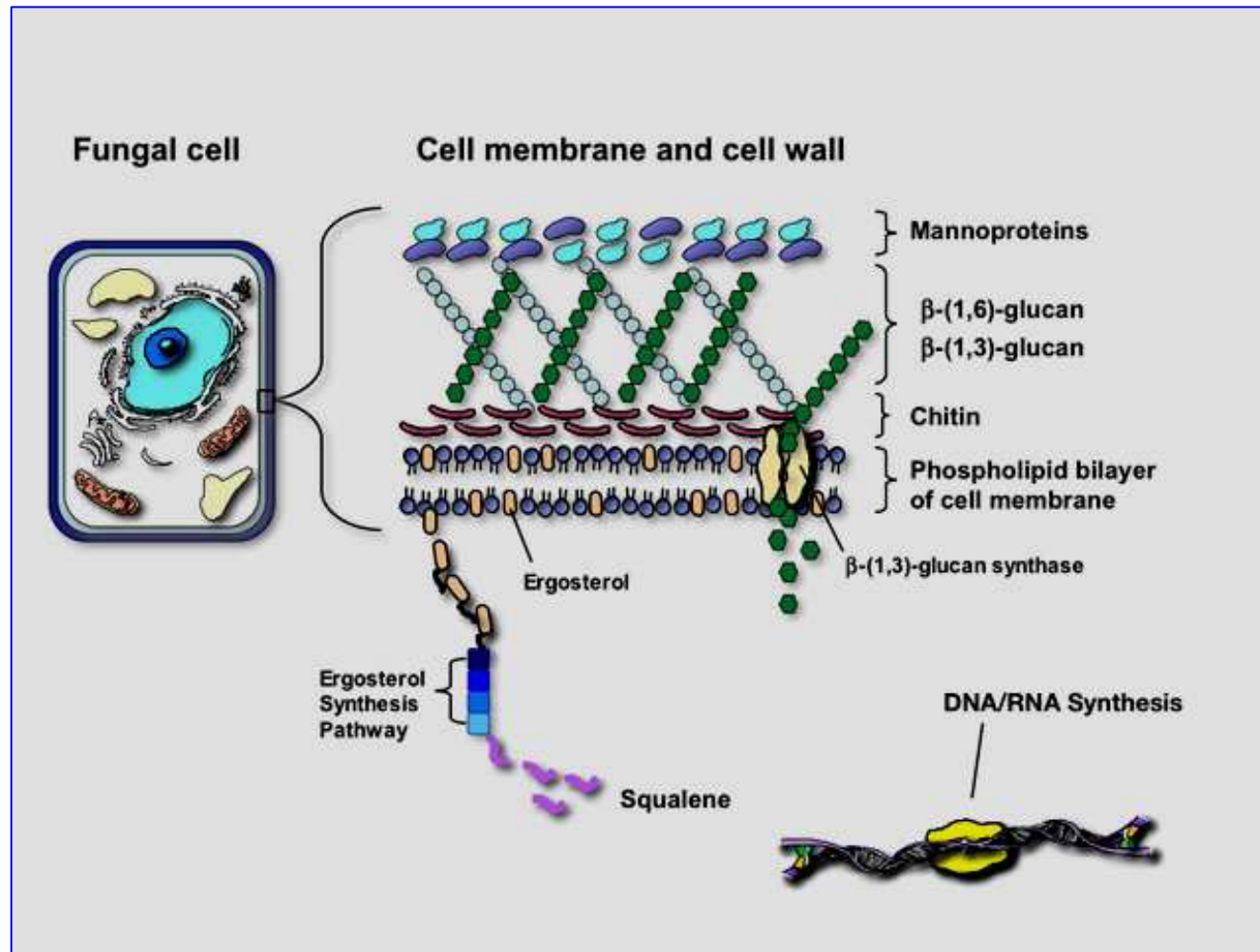
**Molecules of Gram-positive bacteria** recognized by the innate immune system. (A) Schematic representation of proposed bacterial PAMPs to known TLRs and Nod1/2. The plasma membrane and peptidoglycan are depicted in yellow and black, respectively.

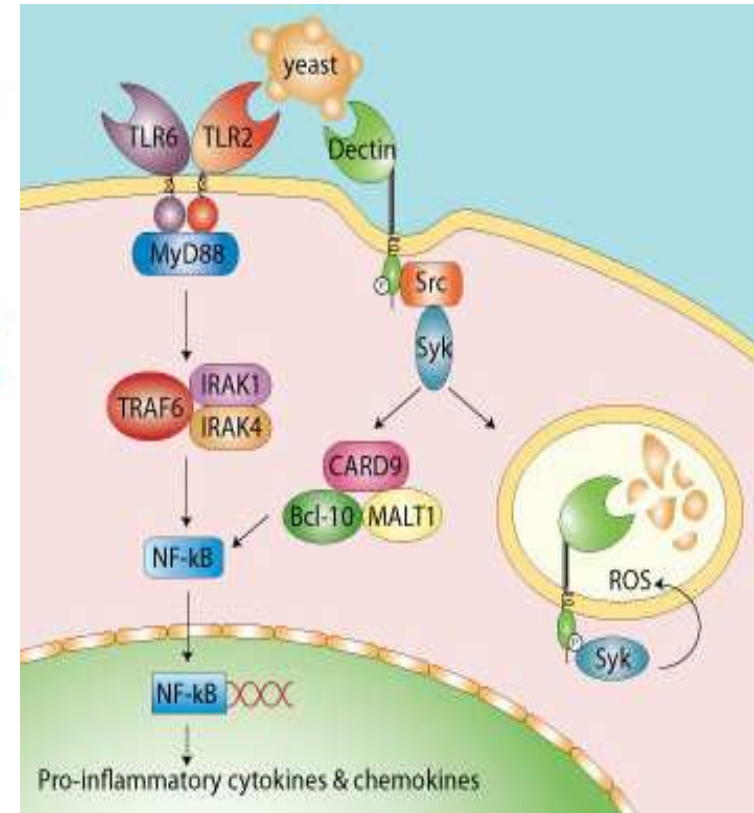
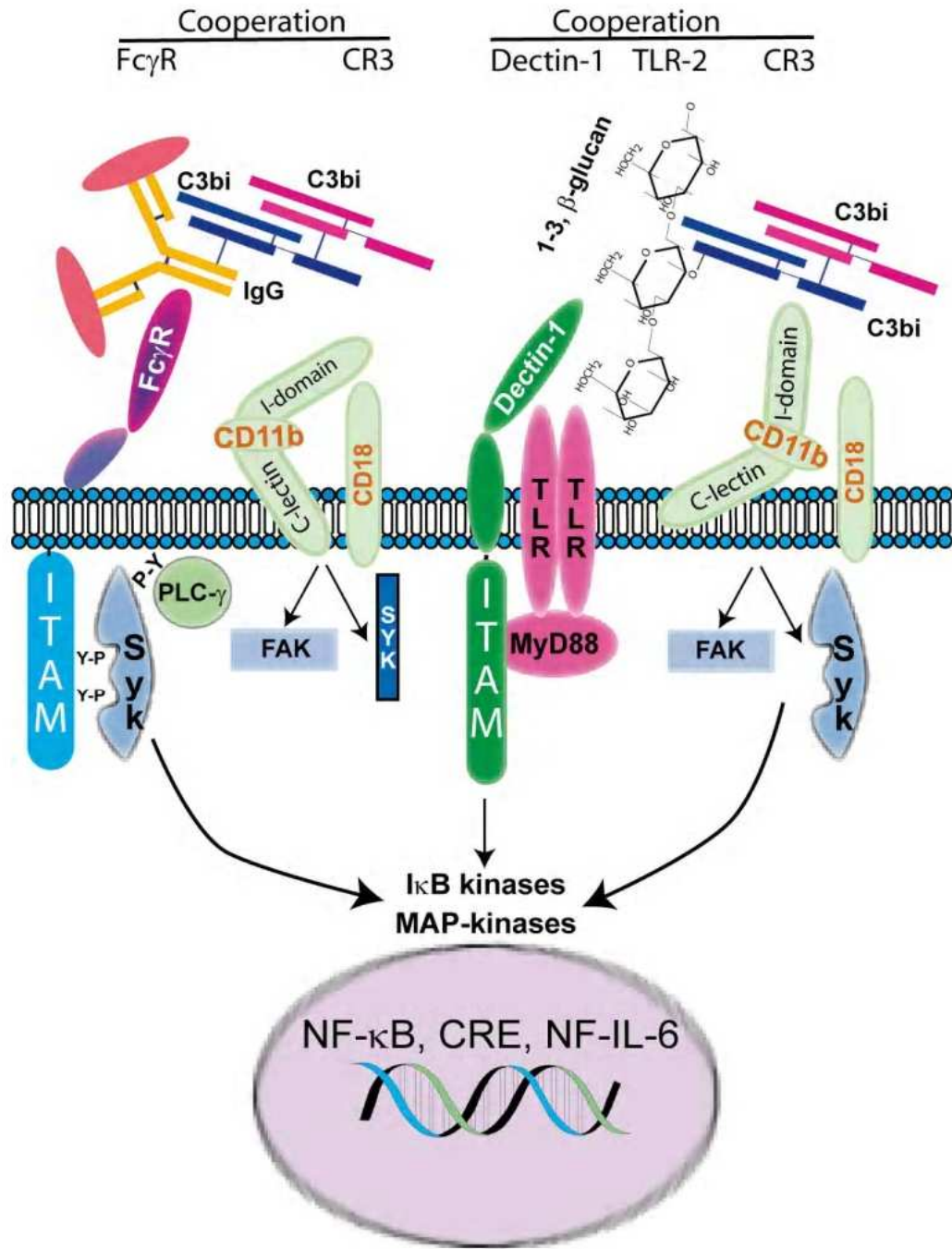




**Micobacterias**

# Hongos





# **RECEPTORES DE LA INMUNIDAD INNATA**

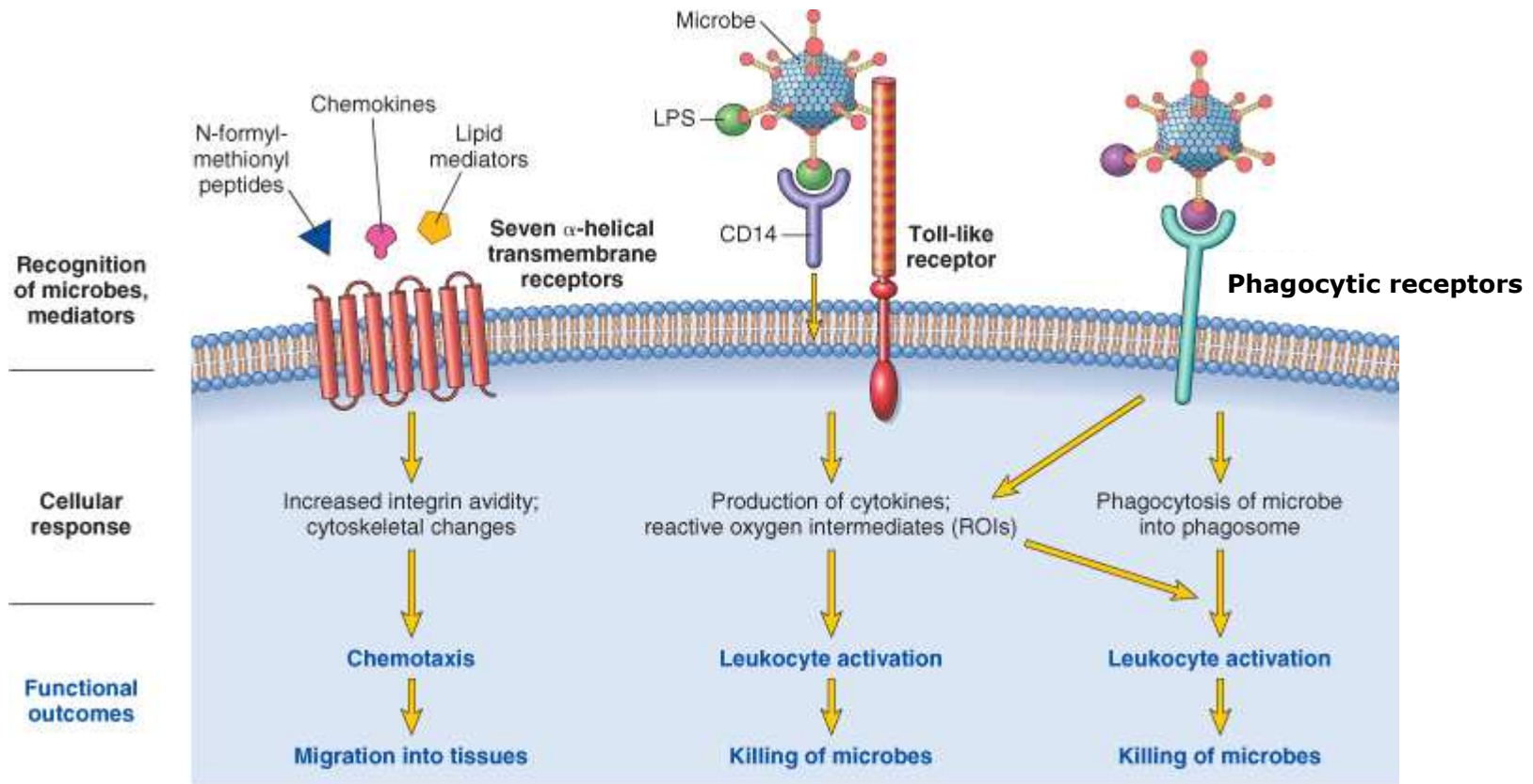
	<b>Inmunidad innata</b>	<b>Inmunidad adaptativa</b>
<b>Especificidad</b>	Para estructuras compartidas por clases de microorganismos (PAMPs)	Para detalle estructural de moléculas microbianas (antígenos); puede reconocer antígenos no microbianos.
<b>Receptores</b>	Codificados en la línea germinal; diversidad limitada	Codificado por genes producidos por la recombinación somática de segmentos génicos; mayor diversidad
<b>Distribución de receptores</b>	No clonal: receptores idénticos en todas las células de la misma estirpe	Clonal: clones de linfocitos con distintas especificidades expresan receptores diferentes
<b>Discriminación entre propio y no propio</b>	Si; no se reconocen las células del huésped o pueden expresar moléculas que evitan reacciones inmunitarias innatas	Si; basado en la selección contra los linfocitos autorreactivos; puede ser imperfecta...

# PRRs

- Son proteínas expresadas por células del SI que reconocen **moléculas** asociadas con microorganismos patógenos (**PAMPs**) o al stress celular (**DAMPs**: danger-associated molecular patterns).
- **Teniendo en cuenta su FUNCIÓN se clasifican en:**
  - ✓ **PRRs de señalización:** a través de NFκB y vía de MAP- kinasa con la consiguiente secreción de citocinas pro-inflamatorias y moléculas co-estimuladoras; incluye los TLR asociados a membrana y los NOD-like receptors, citoplasmáticos.
  - ✓ **PRRs endocíticos:** responsables de la fagocitosis de los microorganismos, sin inducir señalización intracelular. Incluyen el receptor de manosa (MR) de macrófagos y células dendríticas, receptor de glucano (GR), scavenger receptors (SR) de todos los fagocitos.

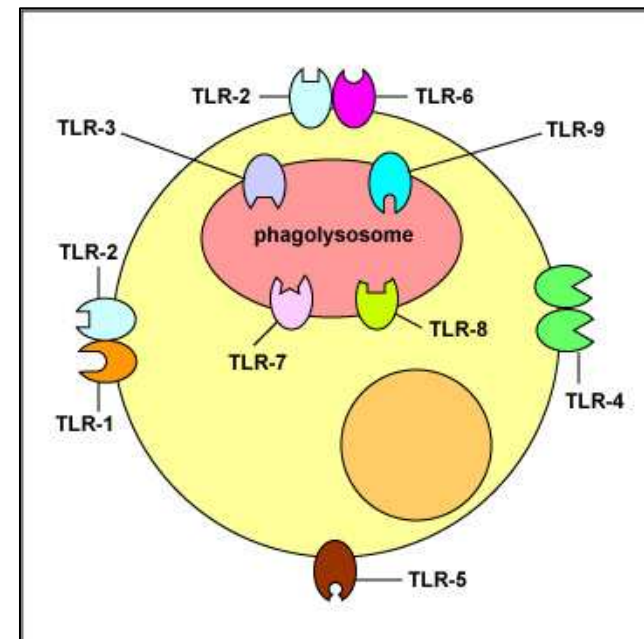
SEÑALIZAN

INDUCEN  
ENDOCITOSIS

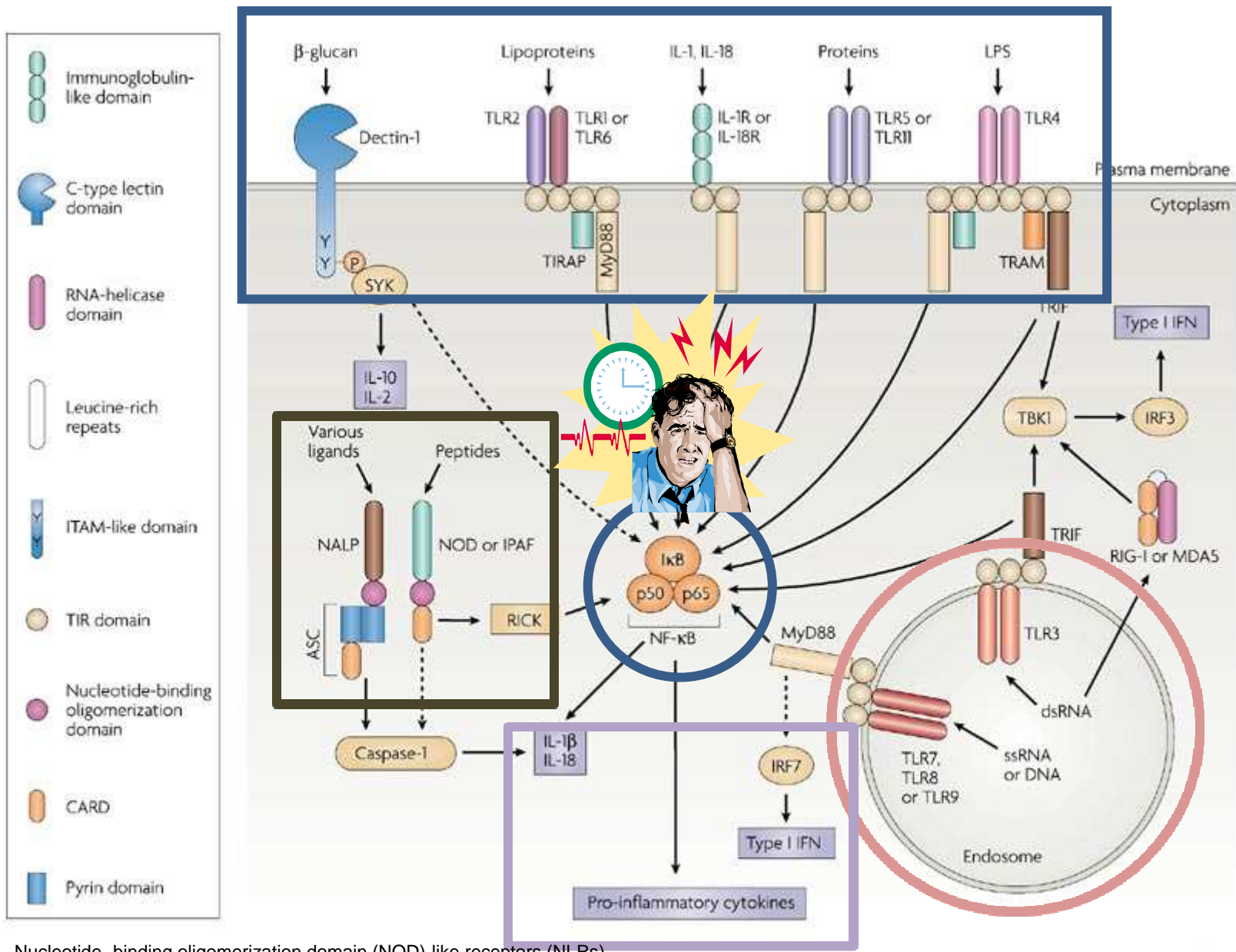


# PRR (PATTERNS RECOGNITION RECEPTORS), según su LOCALIZACIÓN, pueden estar.....

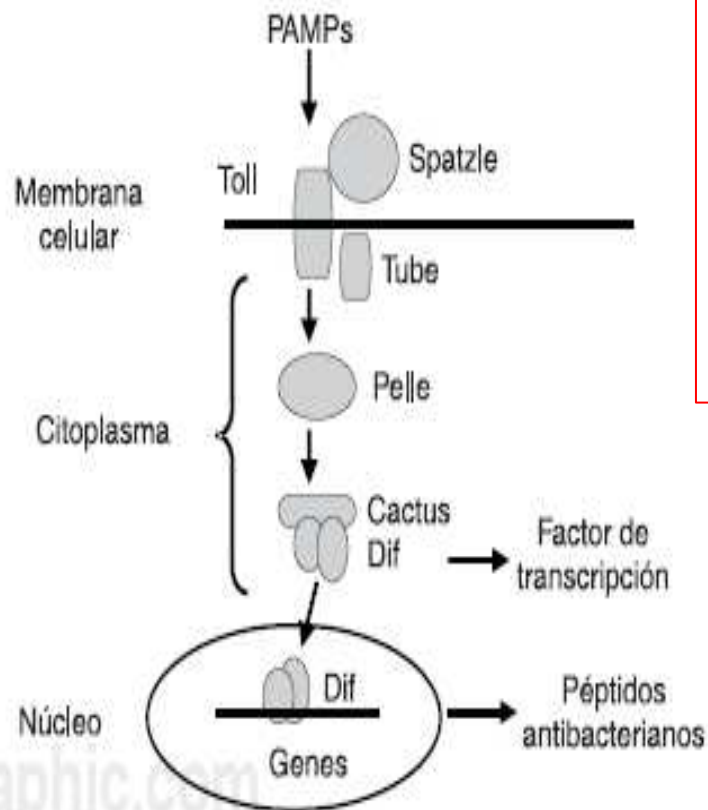
- EXPRESADOS EN LA MEMBRANA CELULAR: TLR 1,2,4,5,6,10,11; CLR, SCAVENGER RECEPTORS
- EXPRESADOS EN LA MEMBRANA DEL ENDOSOMA: TLR 3,7,8,9
- EXPRESADOS EN EL CITOPLASMA: NOD (Nucleotide-binding oligomerization domain) like receptors (NLR)
- SECRETADOS: MBL, PCR, SAP, SP-A Y SP-D







Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs),



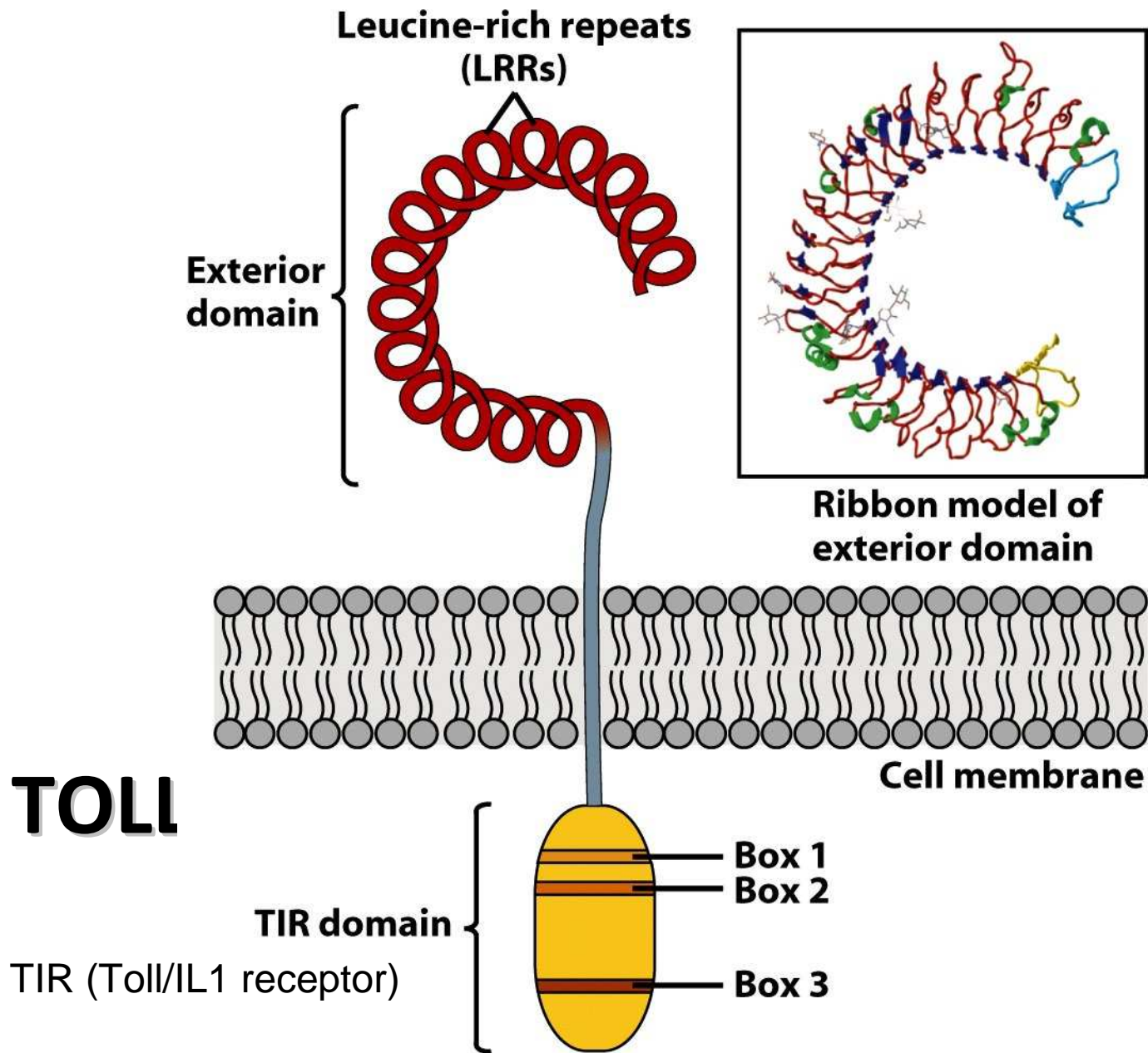
PAMPs: Pathogen Associated Molecular Patterns.

Dif: Complejo dorsal-factor de inmunidad semejante a dorsal.

Figura 1. Representación esquemática de la inmunidad innata mediada por receptores Toll en *Drosophila melanogaster*.

Los receptores Toll son una familia de proteínas transmembrana con un dominio extracelular caracterizado por repeticiones de leucina (LRR: Leucine-Rich Repeat) y un dominio intracelular homólogo al receptor de interleucina 1 de los mamíferos, cuya función es el reconocimiento de los PAMPs. En *Drosophila* los receptores Toll inducen la activación de genes que inducen la síntesis de péptidos antibacterianos como la atocina y antifúngicos como la drosomicina. Se han descrito en la mosca de la fruta, nueve proteínas semejantes a Toll, pero sólo dos de éstas intervienen en la respuesta inmune: Toll y 18-Wheeler<sup>(13-15)</sup>.

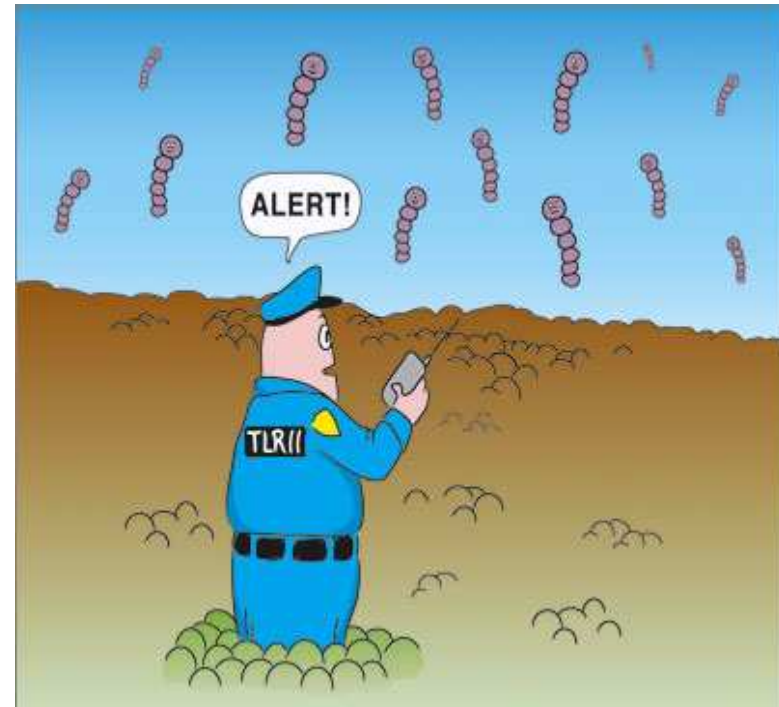
## TLR EN LA DROSOPHILA MELANOGASTER

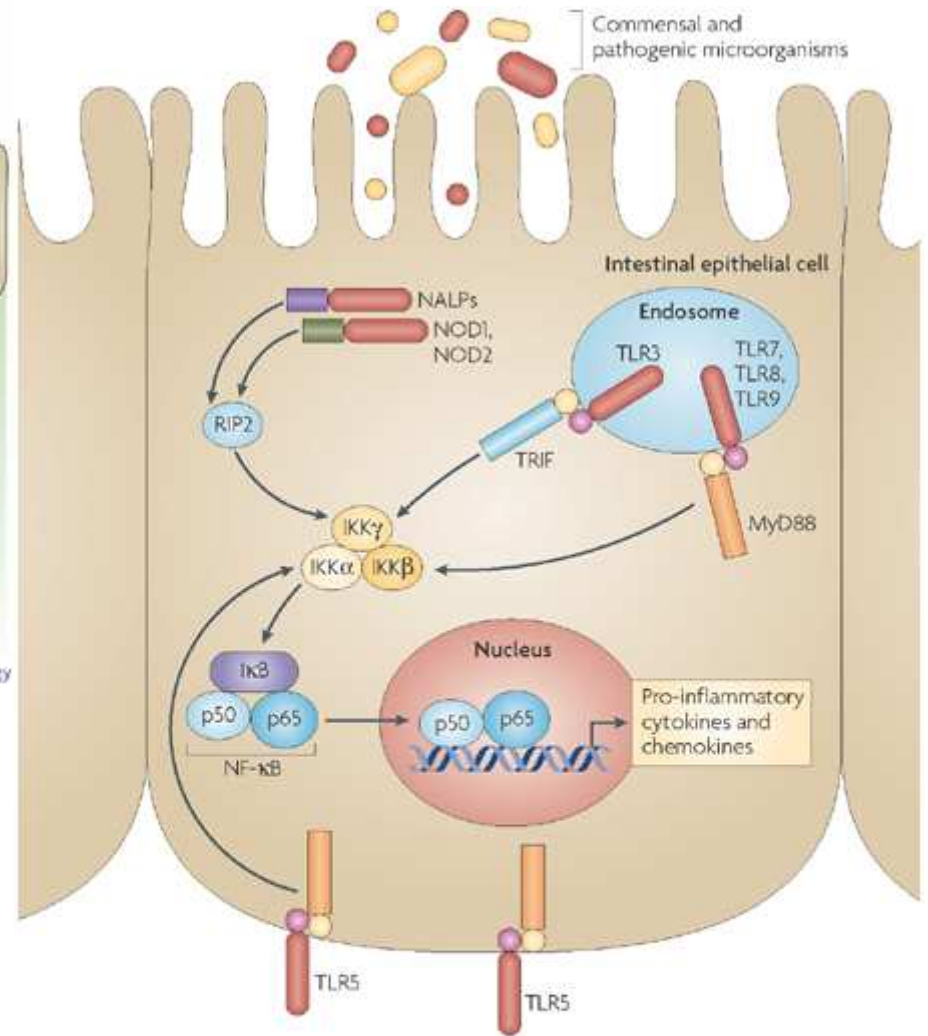
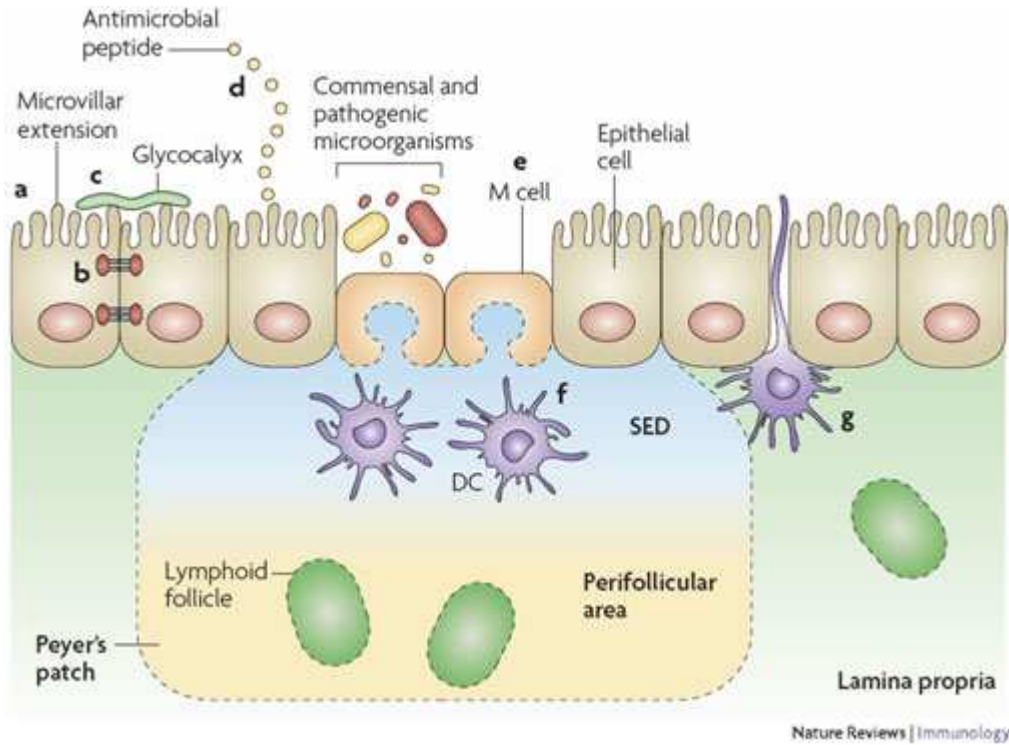


**Figure 3-10**  
*Kuby IMMUNOLOGY, Sixth Edition*  
 © 2007 W.H. Freeman and Company

- Son receptores específicos que pertenecen al sistema inmune innato
- Se encuentran en:
  - ✓ células epiteliales del intestino
  - ✓ IEL
  - ✓ células del mesénquima subepitelial
  - ✓ macrófagos
  - ✓ células dendríticas
- Reconocen ligandos secretados por las bacterias comensales : lipopolisacáridos y ácidos lipoteicoicos
- Pueden funcionar mediante el reconocimiento continuo de bacterias comensales
- Su activación controla los genes y los procesos que promueven la **homeostasis** del epitelio intestinal confiriendo así un efecto protector a estas células e induciendo la reparación del tejido

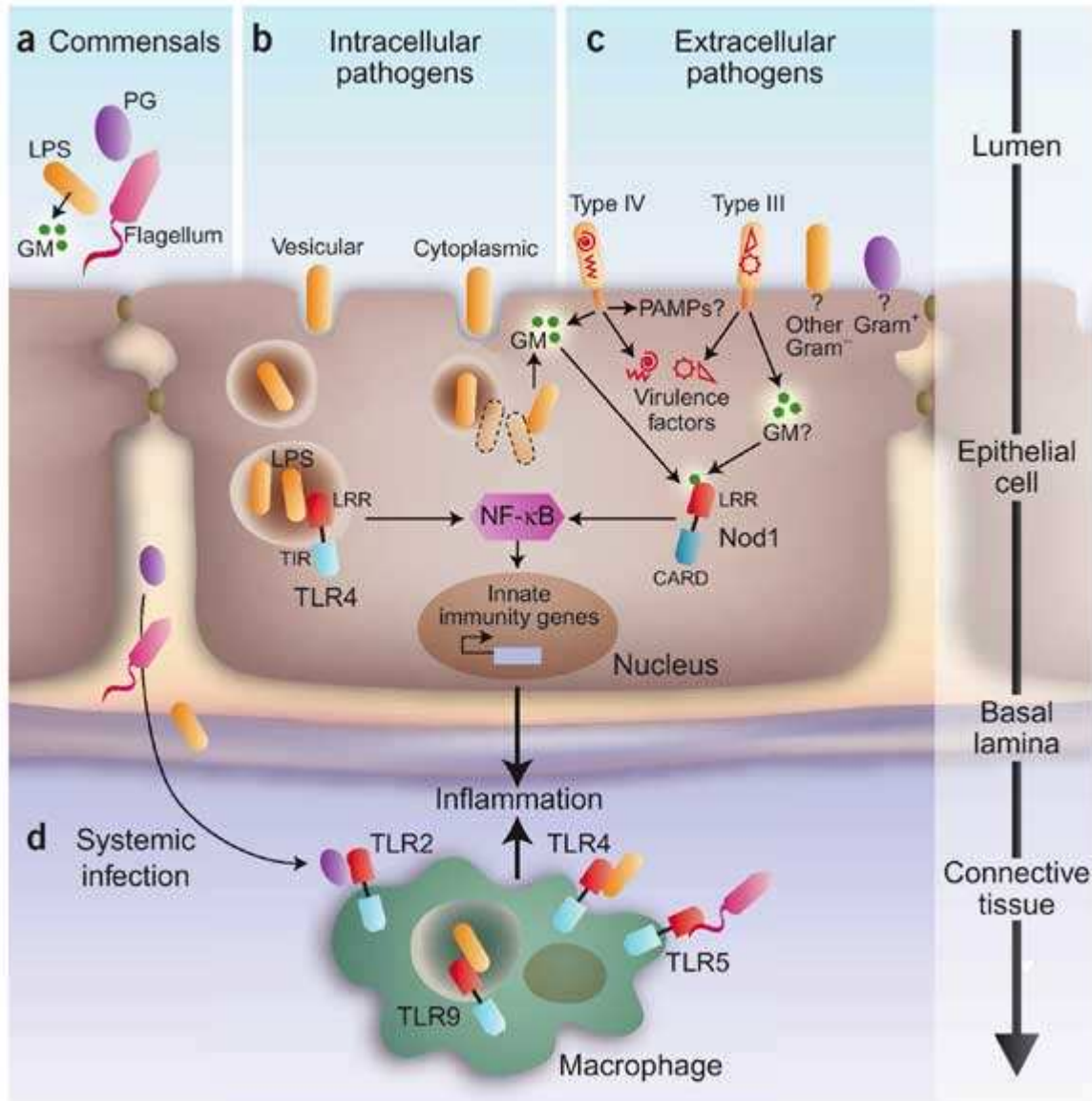
## TLRs (toll-like receptors)





**BARRERA MUCOSA EPITELIAL**

# Reconocimiento de los microorganismos por células epiteliales

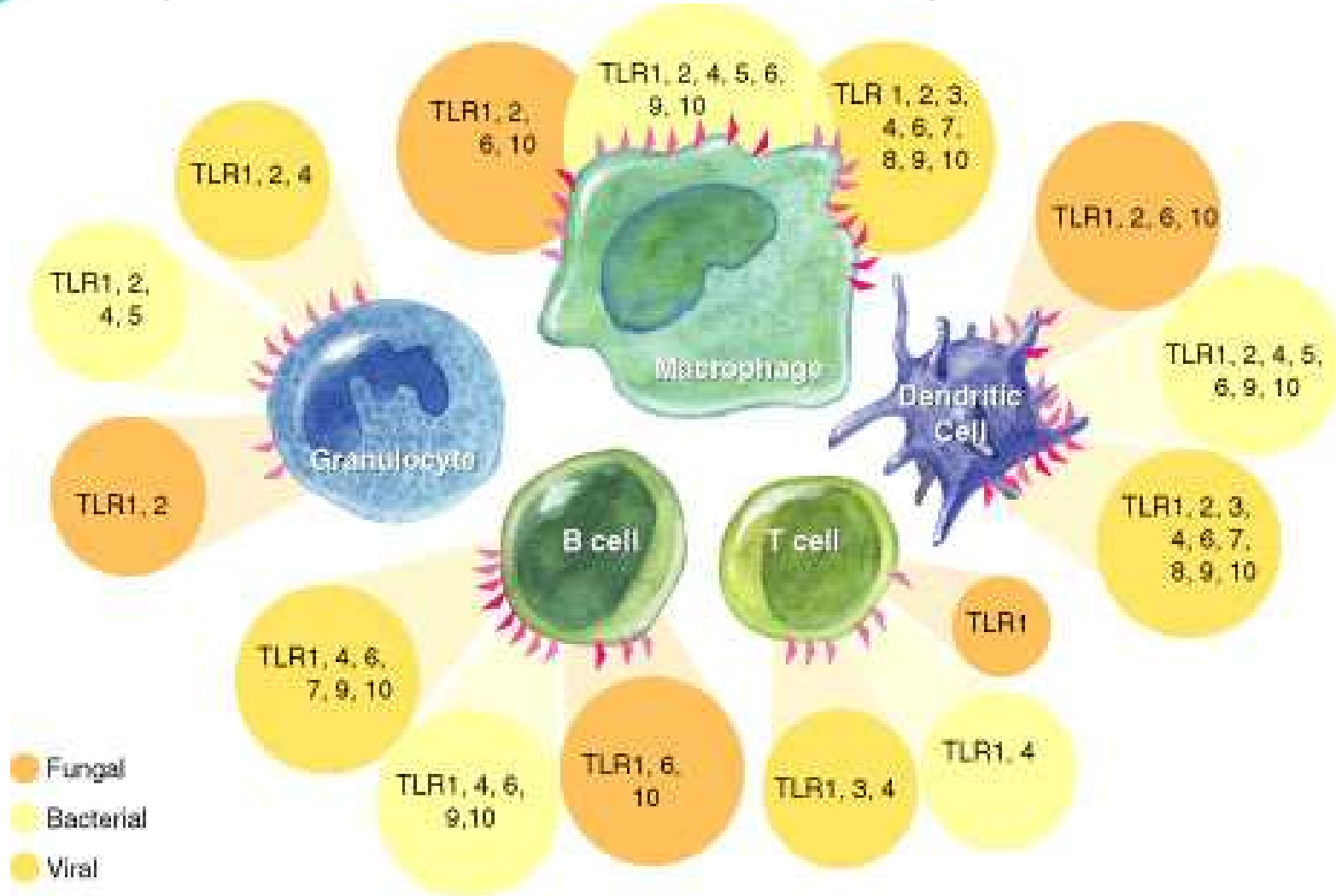


Nature Immunology 5, 1099 - 1100 (2004)

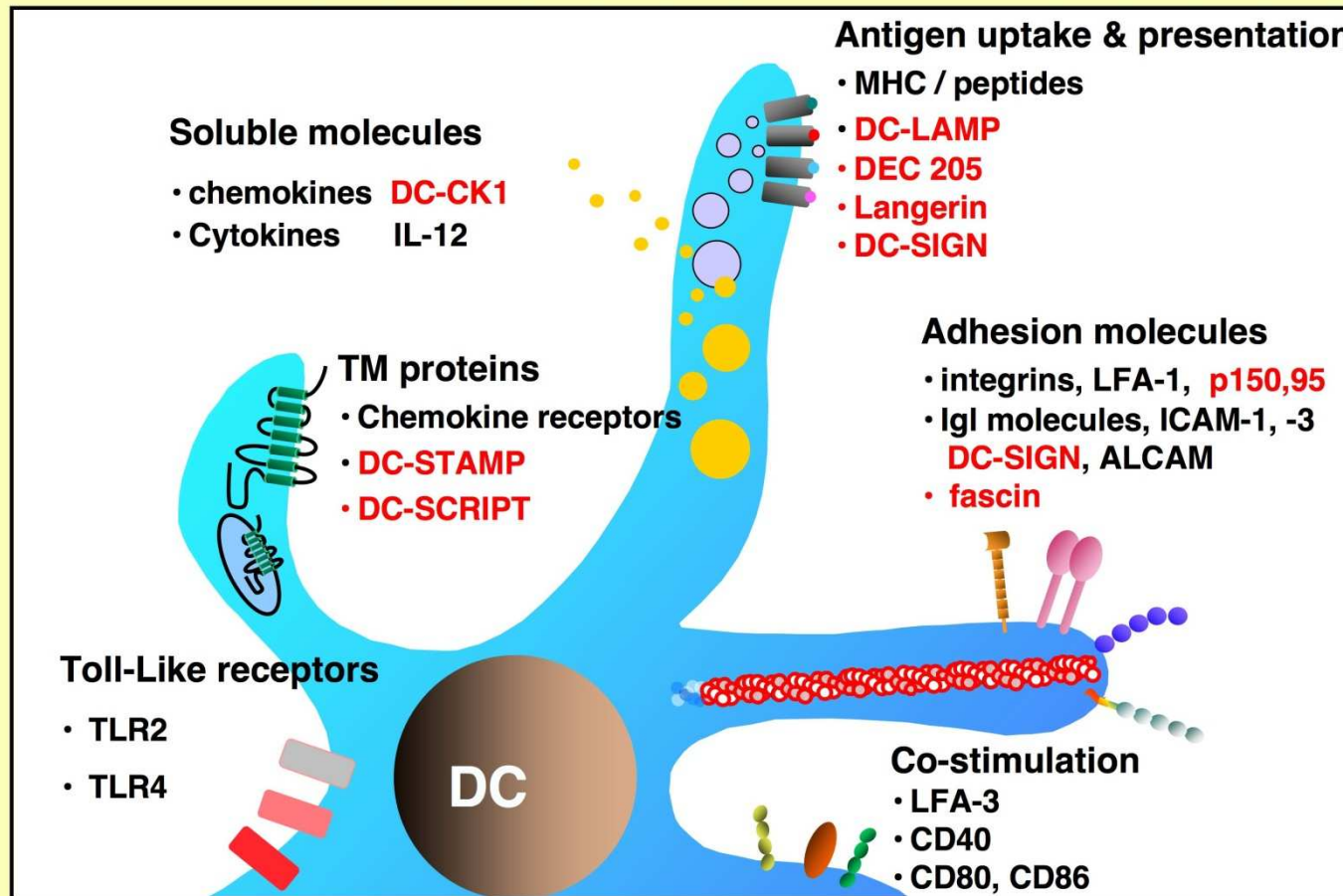
(a) Commensal bacteria are tolerated in the lumen because epithelial cells do not have receptors recognizing their associated PAMPs (peptidoglycan (PG), GM-tri-DAP muropeptide (GM), LPS and flagellin) on their apical surface. (b) Intracellular Gram-negative bacteria replicating in vesicles are recognized by TLR4-LPS interaction. Intracytoplasmic Gram-negative bacteria release GM-tri-DAP muropeptide and activate Nod1. (c) Extracellular pathogens with a type IV secretion system translocate virulence factors and GM-tri-DAP muropeptide in the cytoplasm of epithelial cells, activating Nod1. Other Gram-negative pathogens with a type III secretion system might translocate GM-tri-DAP muropeptide or other PAMPs in the host cell. The detection mechanism for other extracellular Gram-negative and Gram-positive pathogens that lack a secretion system is unknown. Engagement of TLR4 and Nod1 leads to NF-κB activation and inflammation. (d) All bacteria that cross the epithelium and establish a systemic infection are recognized by immune cells such as macrophages, which express both intracellular and plasma membrane-associated TLRs. LRR, leucine-rich repeat recognition domain; TIR, Toll-interleukin 1 receptor domain; CARD, caspase activation and recruitment domain.



## Expresión de TLR en distintos tipos celulares



# Molecules expressed by DC

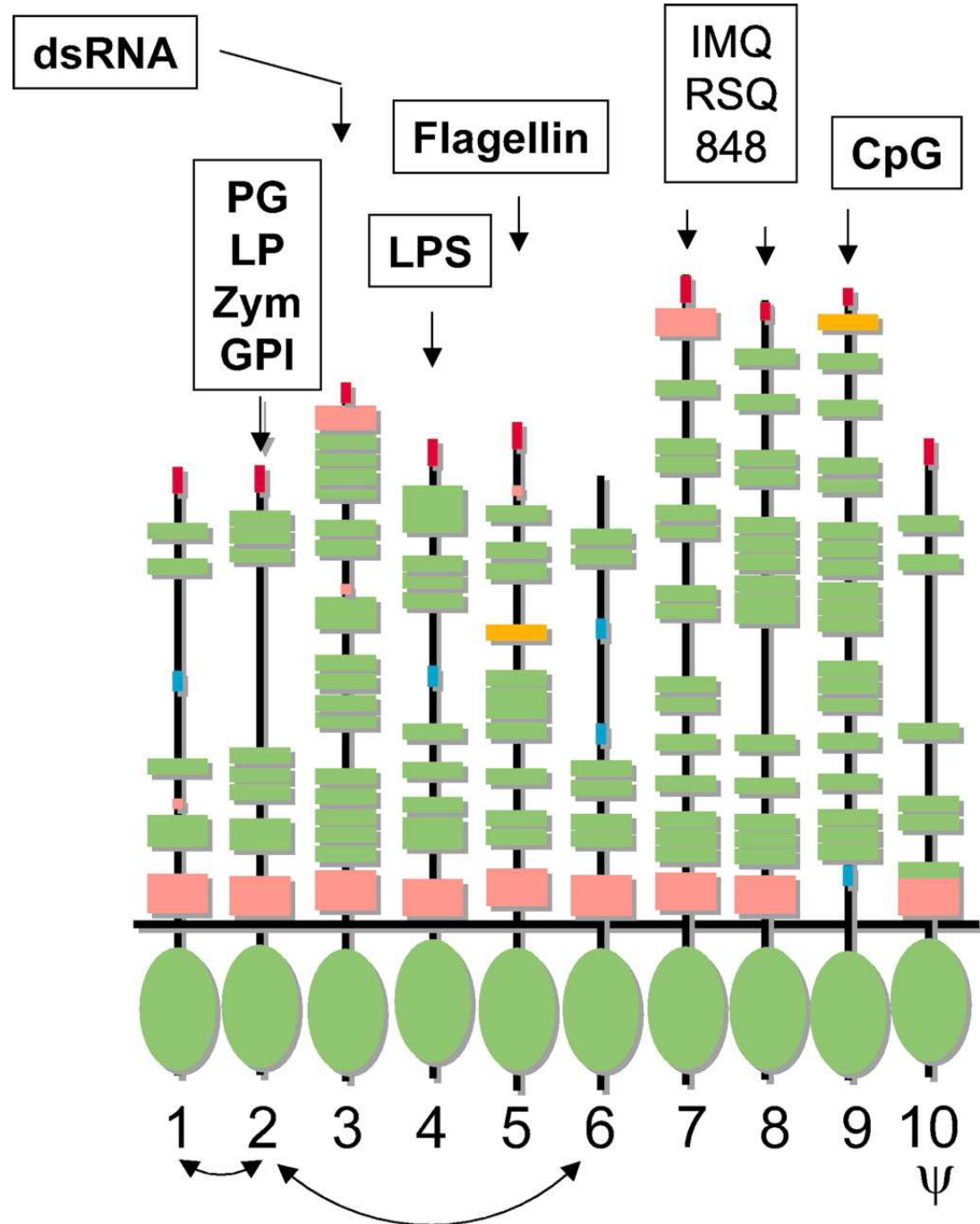




# TOLL LIKE RECEPTORS

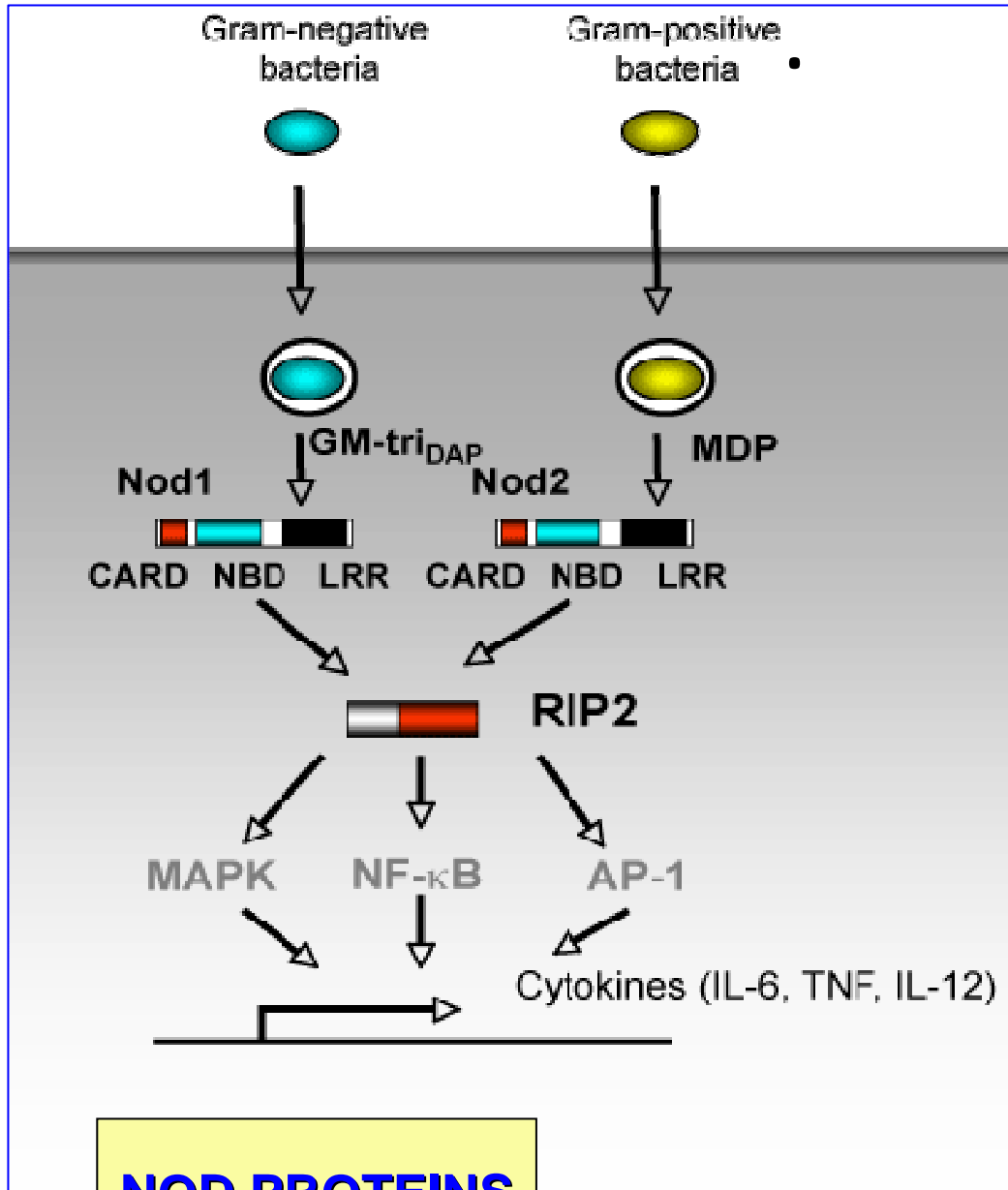
## Mammalian Toll-Like Receptors and their Ligands

TLR2+TLR1	bacterial lipoproteins
TLR2+TLR6	bacterial lipoproteins, lipoteichoic acid, yeast cell wall mannans
TLR2+?	GPI anchors (parasites), bacterial porins, HMGB1
TLR3	dsRNA
TLR4	LPS, HSPs, HMGB1, some viral proteins
TLR5	bacterial flagellin
TLR7	ssRNA (viral)
TLR8	ssRNA (viral)
TLR9	CpG-containing DNA (viral and bacterial)
TLR10	unknown
TLR11	<i>Toxoplasma</i> profilin
TLR12	unknown
TLR13	unknown



Pathogen	Toll-like receptor (TLR)	TLR ligand
<i>Mycobacterium tuberculosis</i>	TLR2	Lipoarabinomannan
	TLR4	Phosphatidylinositol mannosides
	TLR9	DNA
<i>Salmonella typhimurium</i>	TLR2	Bacterial lipoprotein
	TLR4	Lipopolysaccharide
	TLR5	Flagellin
<i>Neisseria meningitidis</i>	TLR2	Porin
	TLR4	Lipopolysaccharide
	TLR9	DNA
<i>Haemophilus influenzae</i>	TLR2	Lipoprotein
	TLR4	Lipopolysaccharide
<i>Candida albicans</i>	TLR2	Phospholipomannan
	TLR4	Mannan
	TLR9	DNA
Murine cytomegalovirus	TLR2	Viral protein
	TLR3	Double-stranded RNA
	TLR9	DNA
Herpes simplex virus	TLR2	Viral protein
	TLR3	Double-stranded RNA
	TLR9	DNA
Influenza virus	TLR7, TLR8	Single-stranded RNA
	TLR3	Double-stranded RNA
	TLR4	Not determined
Respiratory syncytial virus	TLR3	Double-stranded RNA
	TLR4	Envelope F protein
<i>Trypanosoma cruzi</i>	TLR2	Glycosylphosphatidylinositol anchor
	TLR4	Glycoinositolphospholipid-ceramides
	TLR9	DNA
<i>Toxoplasma gondii</i>	TLR2	Glycosylphosphatidylinositol anchor?
	TLR11	Profilin

## PAMPs expresados por distintos microorganismos y su interacción con distintos TLRs



**NOD PROTEINS**

**Dominio de oligomerización de la unión a nucleótidos (NOD)**

Los NOD-1 y NOD-2 son proteínas citosólicas que responden a la degradación de péptidoglicanos.

Nod1 detects a small moiety of peptidoglycan, termed GM-triDAP which exists in Gram-negative bacteria.

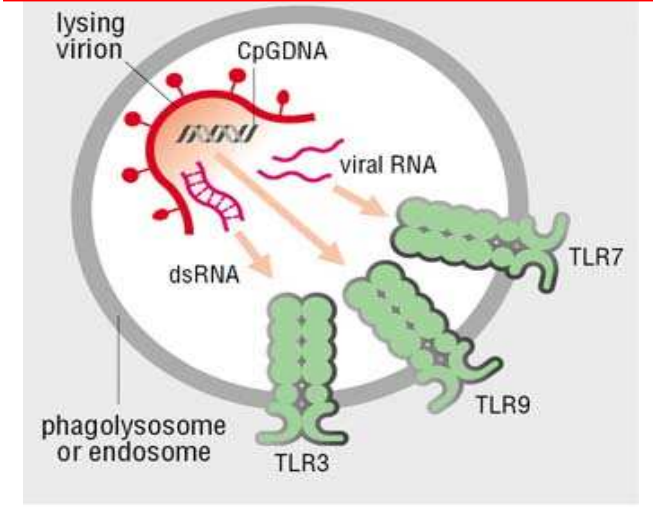
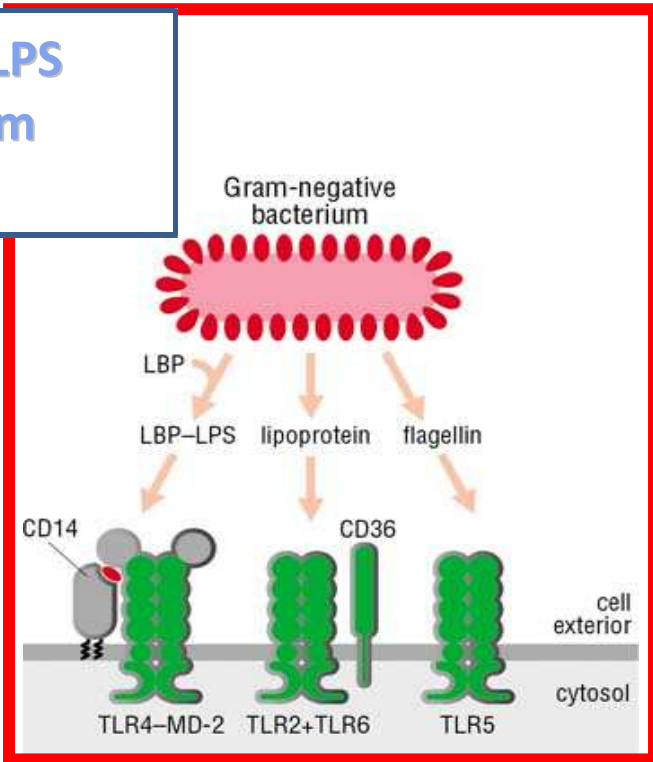
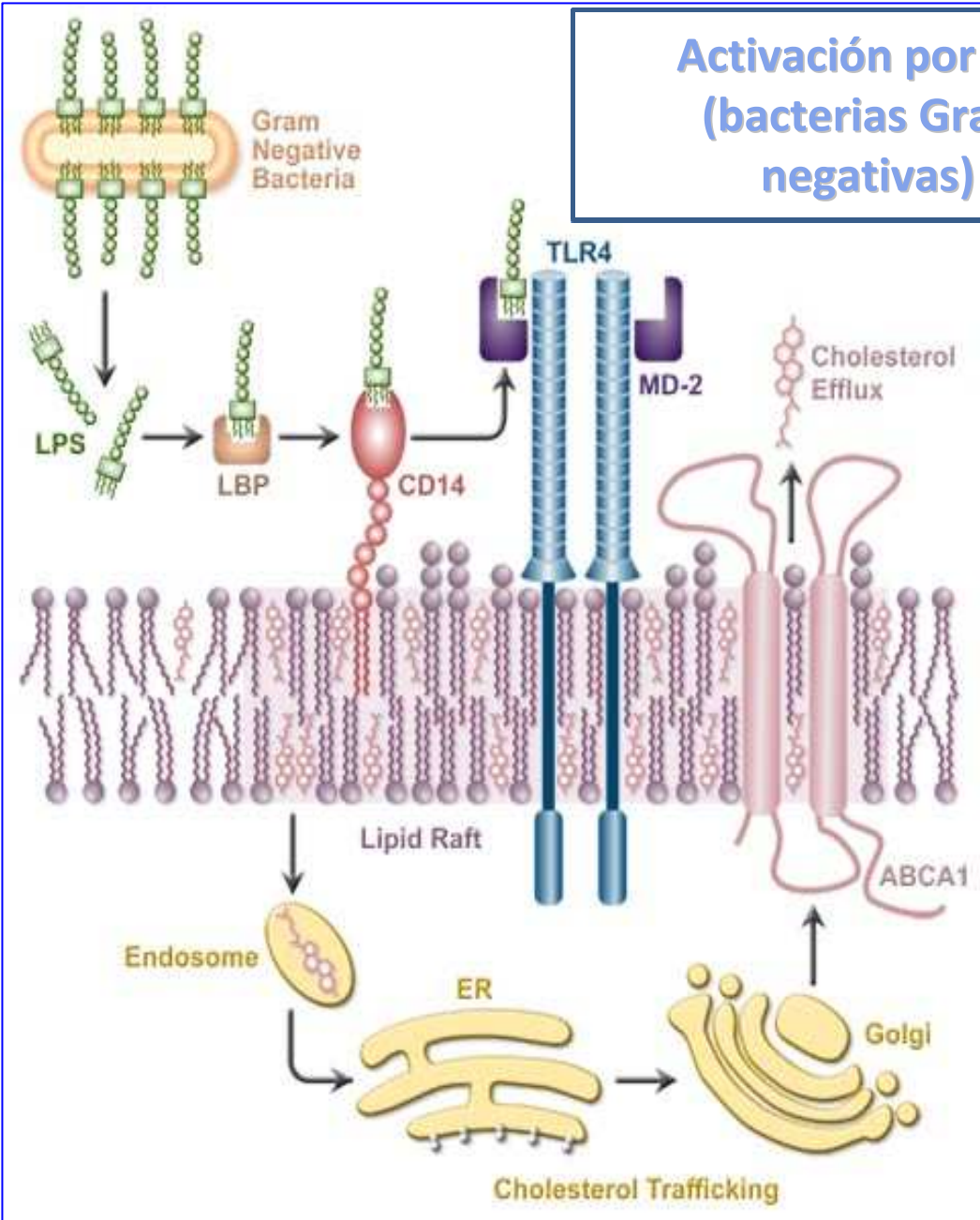
Nod2 detect another moiety of peptidoglycan termed muramyl dipeptide (MDP).

Los queratinocitos expresan NOD1 y NOD2, los cuales al ser estimulados por peptidoglicanos producen IL-6.

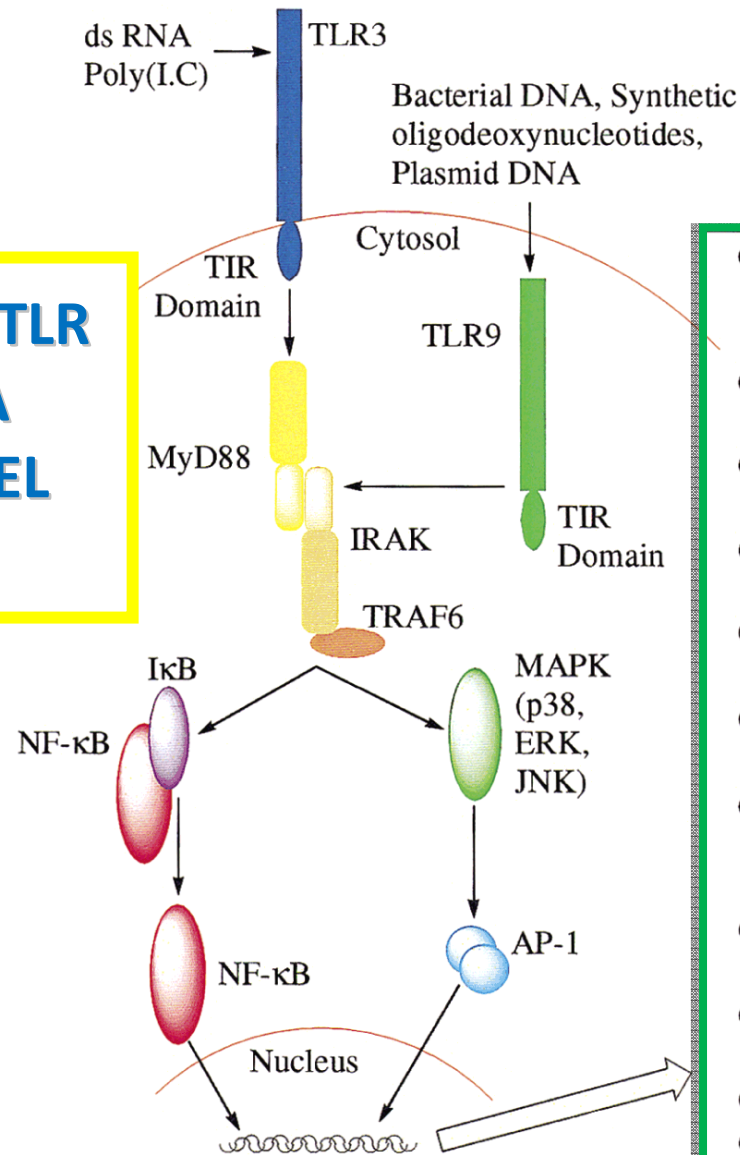
- Mammalian NBD-LRRs (NLRs) detect pathogens such as bacteria and activate host defense mechanisms. Each member can detect a specific strain or molecular pattern in bacteria. There are two distinct downstream signaling pathways resulting in two different outcomes: the activation of inflammatory cytokine genes such as interleukins (IL) -6, -12 or TNF- $\alpha$  or the programmed cell death and IL-1 secretion by activation of Caspase-1.
- Nod1 and Nod2 belong to the CARD-NBD-LRR subfamily that detects parts of peptidoglycan, a bacterial cell wall component.
- **Nod1 detects a small moiety of peptidoglycan, termed GM-triDAP which exists in Gram-negative bacteria.**
- **Nod2 detect another moiety of peptidoglycan termed muramyl dipeptide (MDP).**
- After recognition of bacteria, Nod1 and Nod2 activate a downstream kinase, Rip2, resulting in the activation of immune response genes such as inflammatory cytokines. (Figure 1).
- Some members of the NBD-LRR proteins activate a specific protease, Caspase-1, upon bacterial recognition. For example, one of NBD-LRR protein, NAIP, activates Caspase-1 upon recognition of an intracellular pathogen, ***Legionella pneumophila***. Caspase-1 exists as an inactive form (pro-Caspase-1) before activation. Upon its activation, Caspase-1 cleaves itself and becomes an active tetrameric complex. Active Caspase-1 induces cell death to inhibit proliferation of bacteria and also induces maturation of IL-1, an important pro-inflammatory cytokine.

# **ACTIVACIÓN CELULAR**

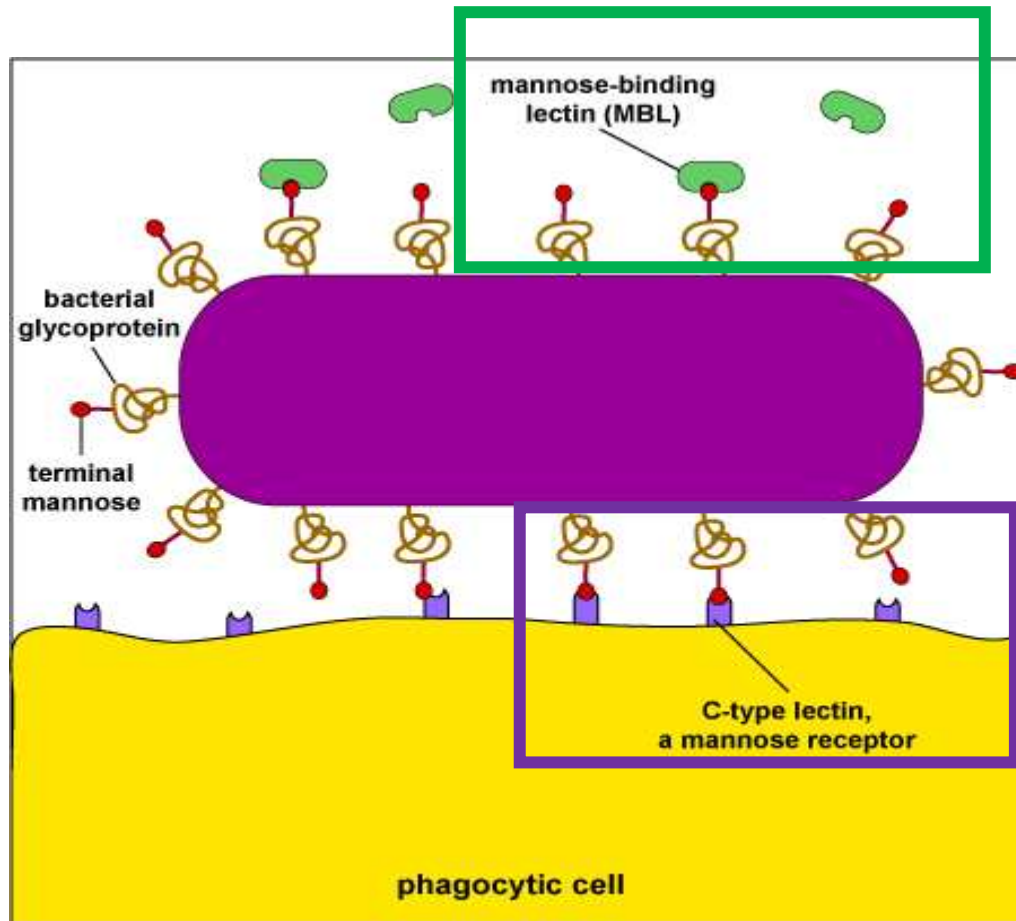
# Activación por LPS (bacterias Gram negativas)



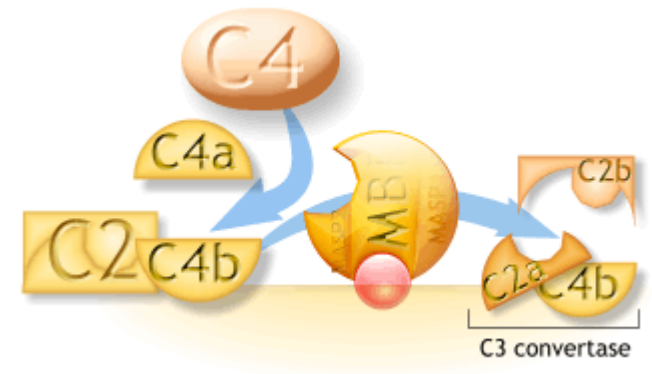
## ACTIVACIÓN DE TLR ASOCIADOS A MEMBRANA DEL ENDOSOMA



- **IL-12** : IFN- $\gamma$  production by NK and Th1 cells, Th1 cell differentiation, Th2 cell suppression
- **IFN- $\gamma$**  : APC activation, Th1 development, MHC-I upregulation
- **IFN- $\alpha/\beta$**  : MHC-I upregulation, antigen processing and presentation
- **IL-6** : B-cell differentiation, antibody secretion, class switch
- **IL-1** : Adhesion molecule upregulation on endothelial cells, upregulation of IL-6
- **IL-10** : Inhibitor of IL-12 and IFN- $\gamma$  production
- **TNF- $\alpha$**  : Adhesion molecules on endothelial cells, IL-6 upregulation, macrophage activation
- **MHC-I** : Antigen presentation to CD8+ cells
- **CD40** : Co-stimulatory signal, IL-12 secretion
- **CD86** : Co-stimulatory signal
- **CD69** : Co-stimulatory signal



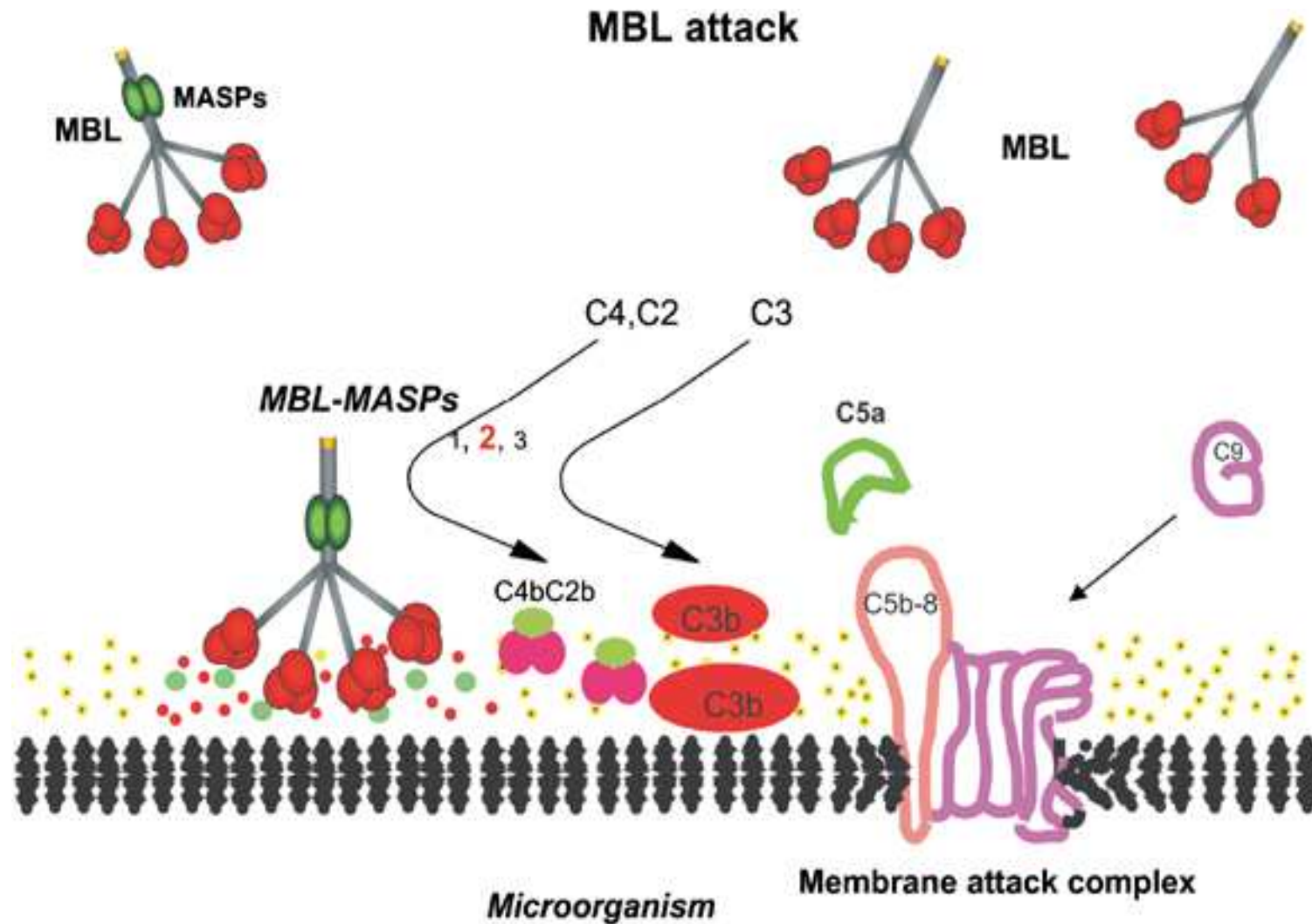
PRR SECRETADO



PRR ENDOCÍTICO

Mannose-rich glycans are short carbohydrate chains with the sugar mannose or fructose as the terminal sugar. They are commonly found in microbial glycoproteins and glycolipids but are rare in those of humans. (Human glycoproteins and glycolipids typically have terminal N-acetylglucosamine and sialic acid groups.) C-type lectins, found on the surface of phagocytes, are endocytic pattern recognition receptors that bind to mannose-rich glycans in order to attach microbes to phagocytes. **Mannose-binding lectin (MBL), also known as mannan-binding protein, is a soluble pattern recognition receptor in plasma and tissue fluid that binds to mannose-rich glycans on microbes in order to activate the lectin complement pathway.**





**MBL binds to repetitive mannose and/or N-acetylglucosamine residues on microorganisms, leading to opsonization and activation of the lectin complement pathway.**

**MBL also interacts with carbohydrates on the glycoprotein (gp)120 of HIV-1. MBL may inhibit DC-SIGN-mediated uptake and spread of HIV.**

# RECEPTORES DE ENDOCITOSIS

## Phagocytic Receptors

Receptor	Type	Expression	Ligands
<b>Innate immune receptors</b>			
mannose receptor	C-type lectin	macrophages, DC	mannans
DEC 205	C-type lectin	DC	mannans
dectin-1	C-type lectin	macrophages	glucose-rich polysaccharides
CD14	leucine-rich repeats	macrophages, neutrophils	apoptotic cells, LPS
MARCO	SR-A	MZ macrophages	bacterial cell walls
scavenger receptor A I	SR-A	macrophages	apoptotic cells; LPS, LTA
CD36	SR-B	macrophages	apoptotic cells; parasitized RBCs
<b>Opsonin receptors</b>			
C1qRp (CD93)	C-type lectin	macrophages	C1q; collectins
CR3 ( $\alpha$ M $\beta$ 2, CD11b/CD18)	integrin	macrophages, neutrophils	iC3b, $\beta$ -glucans, ICAM-1/2
CR4 ( $\alpha$ x $\beta$ 2, CD11c/CD18)	integrin	macrophages, neutrophils, DC	iC3b, fibrinogen
Fc $\gamma$ RI (CD64)	Ig, ITAM	macrophages, neutrophils	IgG, CRP, SAP
Fc $\gamma$ RII (CD32)	Ig, ITAM	macrophages, neutrophils	IgG
Fc $\gamma$ RIII (CD16)	Ig, ITAM	macrophages, neutrophils, NK	IgG, SAP
Fc $\gamma$ RIV	Ig, ITAM	macrophages, neutrophils, DC	IgG2a, IgG2b
Fc $\alpha$ R (CD89)	Ig, ITAM	macrophages, neutrophils, Eos	IgA
integrin $\alpha$ v $\beta$ 3	integrin	macrophages, platelets	thrombospondin-opsionized cells
Mer	RTK	macrophages	apoptotic cells

# Receptores asociados a la fagocitosis: unen e internalizan el patógeno

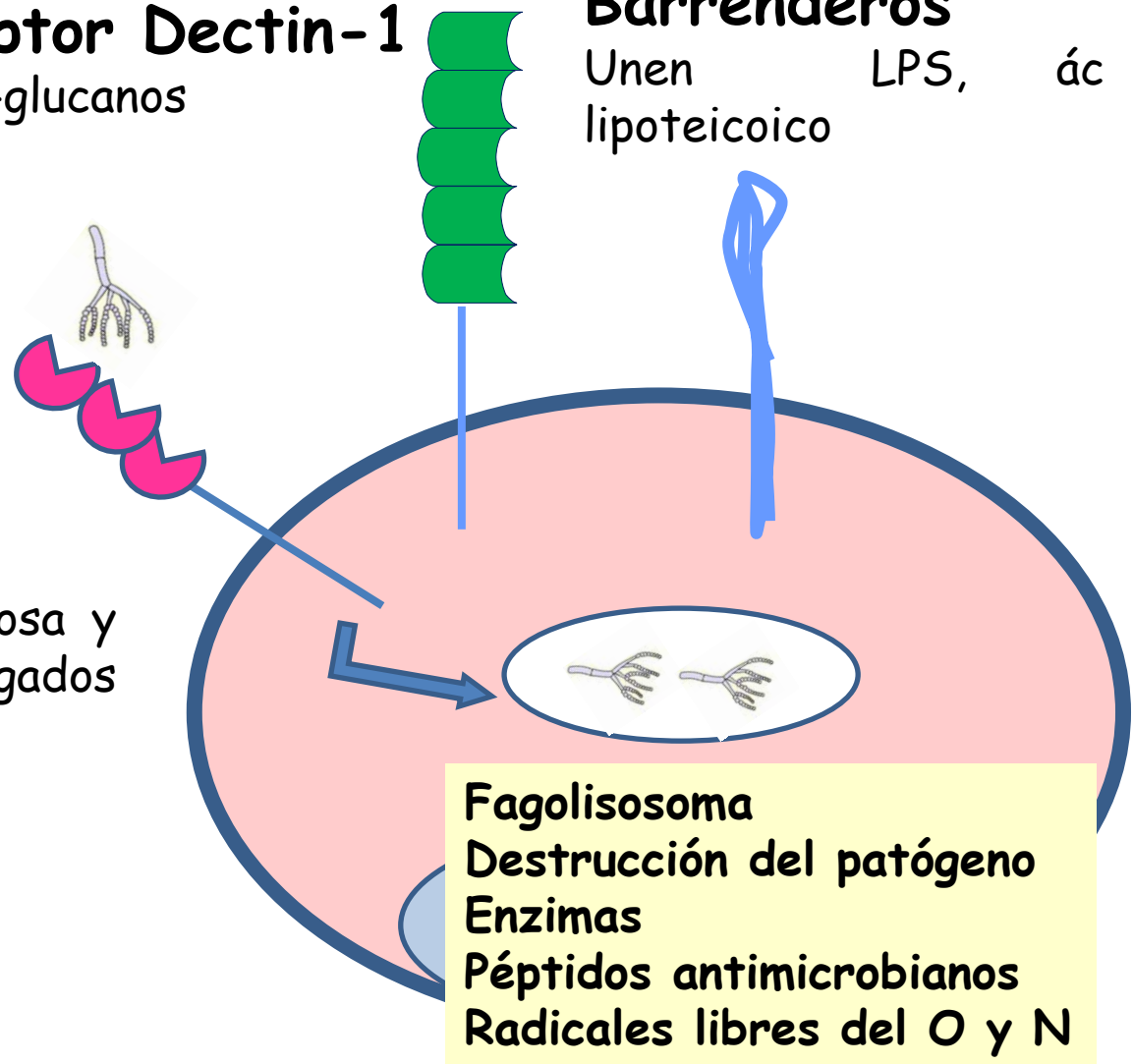
**Receptor Dectin-1**  
Une  $\beta$ -glucanos

**Receptores Barrenderos**

Unen lipoteicoico LPS, ác

**Receptor de manosa**

Reconoce residuos de manosa y fucosa en glicoconjugados expuestos por los patógenos



# **RECEPTORES LECTINA TIPO-C**

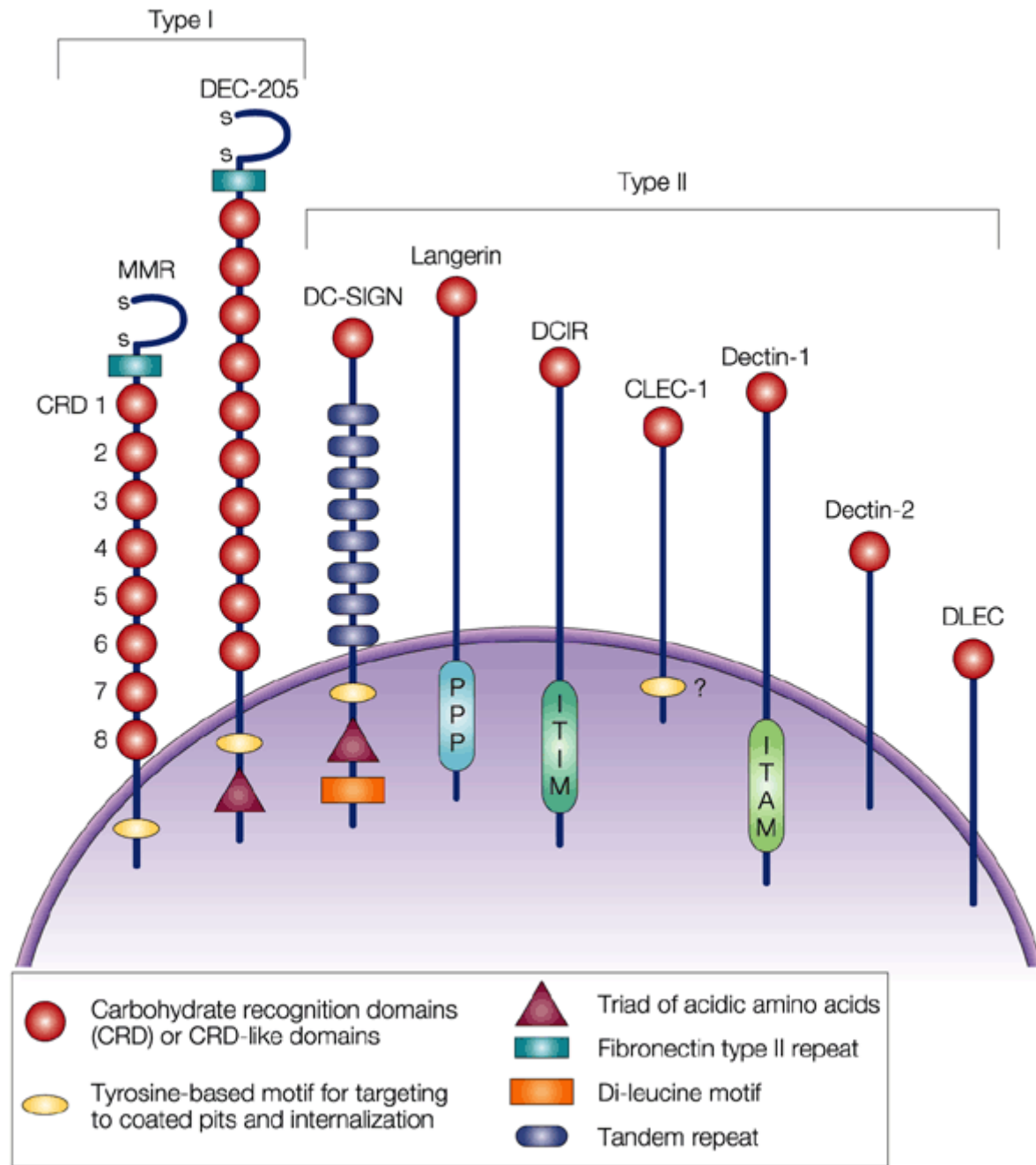


Table 1 | **C-type lectin receptors, pathogen recognition and signalling (part 1)**

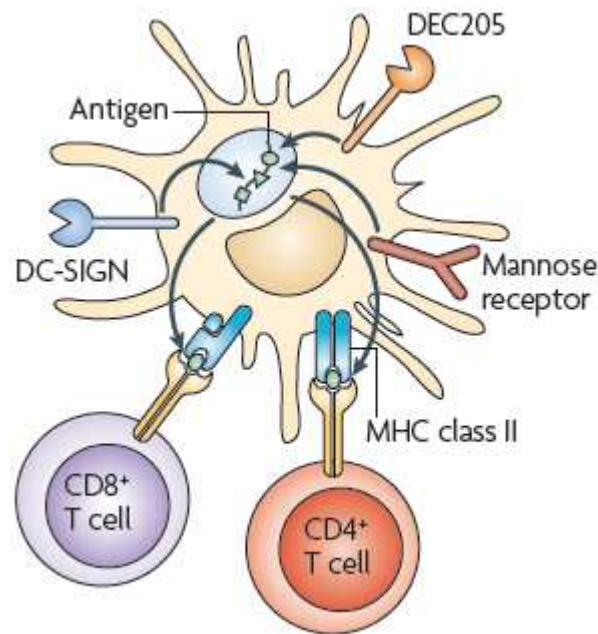
CLR*	Expression	Glycan PAMPs	Pathogenic (exogenous) ligands	Signalling motif or adaptor	Signalling proteins involved	Immunological outcome
<i>Group II CLRs (asialoglycoprotein receptor family)</i>						
DC-SIGN (CD209)	Myeloid DCs	High mannose and fucose (Le <sup>X</sup> , Le <sup>Y</sup> , Le <sup>A</sup> , Le <sup>B</sup> )	<ul style="list-style-type: none"> <li>• <i>Mycobacterium tuberculosis</i>, <i>Mycobacterium leprae</i>, BCG, <i>Lactobacilli</i> spp. and <i>Helicobacter pylori</i></li> <li>• HIV-1, measles virus, dengue virus, SARS coronavirus and filoviruses</li> <li>• <i>Candida albicans</i></li> <li>• <i>Leishmania</i> spp.</li> <li>• Tick <i>Ixodes scapularis</i> saliva protein Salp15, peanut allergen Ara h1 and <i>Schistosoma mansoni</i> soluble egg antigens</li> </ul>	None	LSP1, LARG, RHOA, Ras proteins, RAF1, Src kinases and PAKs	<ul style="list-style-type: none"> <li>• Upregulation of TLR-induced IL-10 production</li> <li>• Induction of T<sub>H</sub>1, T<sub>H</sub>2, T<sub>H</sub>17 and regulatory T cell differentiation<sup>†</sup></li> <li>• Inhibition of T<sub>H</sub>1 cell differentiation<sup>†</sup></li> <li>• Induction of regulatory T cells<sup>†</sup></li> <li>• Antigen presentation</li> </ul>
Langerin (CLEC4K, CD207)	Langerhans cells and dermal DC subset	High mannose, fucose (Le <sup>Y</sup> , Le <sup>B</sup> ) and GlcNAc	<ul style="list-style-type: none"> <li>• <i>M. leprae</i></li> <li>• HIV-1</li> </ul>	Putative proline-rich domain	ND	<ul style="list-style-type: none"> <li>• <i>M. leprae</i> endocytosis and antigen presentation</li> <li>• HIV-1 internalization and degradation</li> </ul>
MGL (CLEC10A, CD301)	Myeloid DCs and macrophages	Terminal GalNAc (Tn antigen, LDN)	<ul style="list-style-type: none"> <li>• Filoviruses</li> <li>• <i>S. mansoni</i></li> </ul>	None	ND	ND
CLEC5A (MDL1)	Monocytes and macrophages	ND	Dengue virus	DAP10 and DAP12	ND	Induction of TNF production

**Group II CLRs (asialoglycoprotein receptor family; dectin 1 subfamily)**

Dectin 1 (CLEC7A)	Myeloid DCs, monocytes, macrophages and B cells	$\beta$ -1,3-glucan	<ul style="list-style-type: none"> <li>• <i>M. tuberculosis</i> and <i>Mycobacterium abscessus</i></li> <li>• <i>C. albicans</i>, <i>Aspergillus fumigatus</i>, <i>Pneumocystis carinii</i>, <i>Penicillium marneffeii</i>, <i>Coccidioides posadasii</i> and <i>Histoplasma capsulatum</i></li> </ul>	YxxL <sup>§</sup>	SYK, PLC $\gamma$ 2, CARD9, BCL-10, MALT1, NIK and RAF1	<ul style="list-style-type: none"> <li>• Induction of T<sub>H</sub>1 and T<sub>H</sub>17 cell differentiation through induction of IL-1<math>\beta</math>, IL-6, IL-12 and IL-23 production</li> <li>• Induction of TNF and CXCL2 production</li> <li>• Phagocytosis</li> <li>• LTB<sub>4</sub> synthesis</li> </ul>
MICL (CLEC12A, DCAL2)	Myeloid DCs, monocytes, macrophages and neutrophils	ND	ND	ITIM	SHP1, SHP2 and ERK	Inhibition of TLR4-induced IL-12 production
CLEC2 (CLEC1B)	Platelets	ND	<ul style="list-style-type: none"> <li>• HIV-1</li> <li>• Snake venom protein rhodocytin</li> </ul>	YxxL <sup>§</sup>	SYK, LAT, RAC1 and PLC $\gamma$ 2	ND
DNGR1 (CLEC9A)	BDCA3 <sup>+</sup> DCs, monocytes and B cells	ND	ND	YxxL <sup>§</sup>	SYK	<ul style="list-style-type: none"> <li>• Induction of TNF production</li> <li>• Antigen cross-presentation</li> </ul>
CLEC12B	Macrophages	ND	ND	ITIM	SHP1 and SHP2	ND

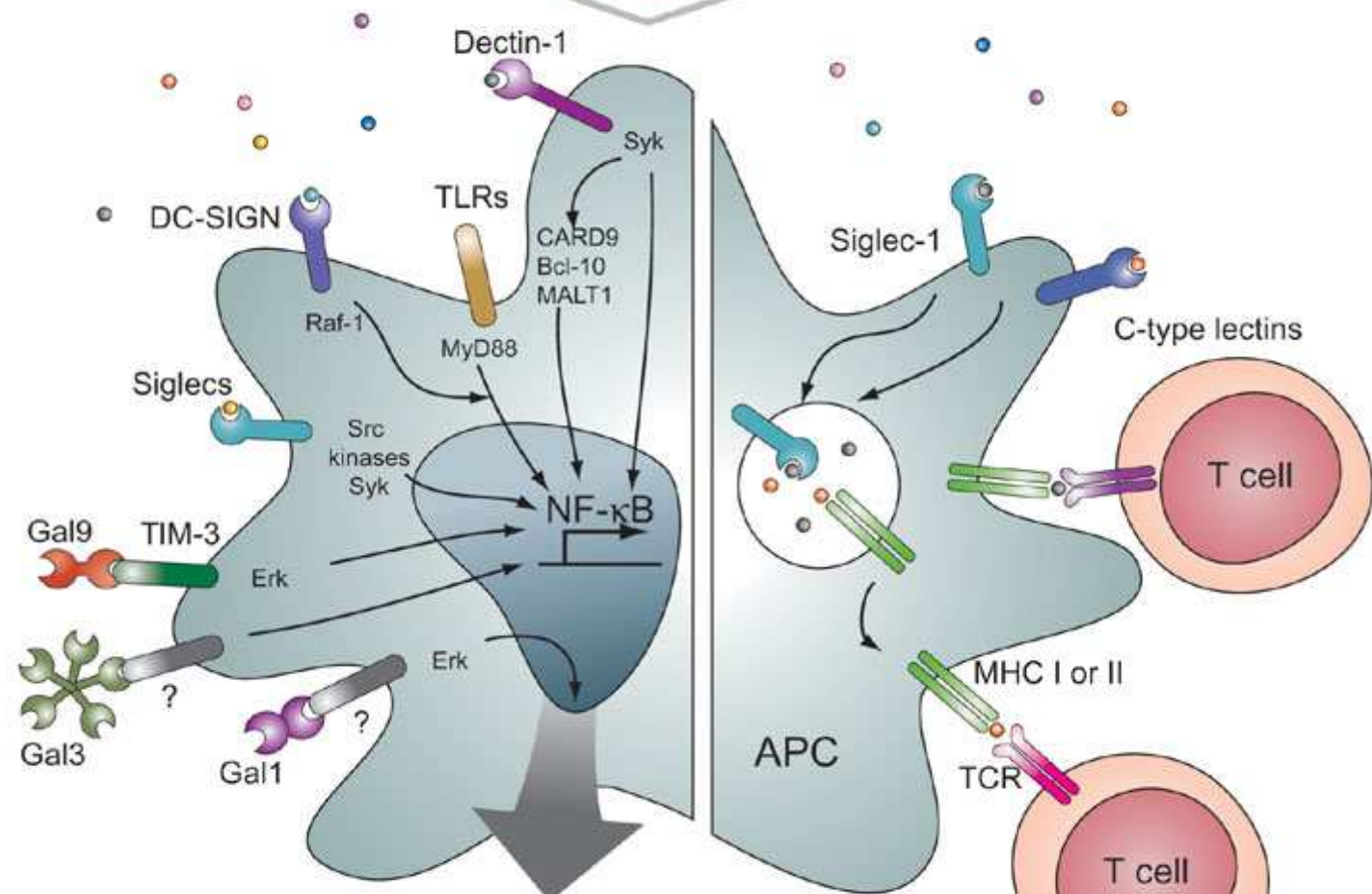
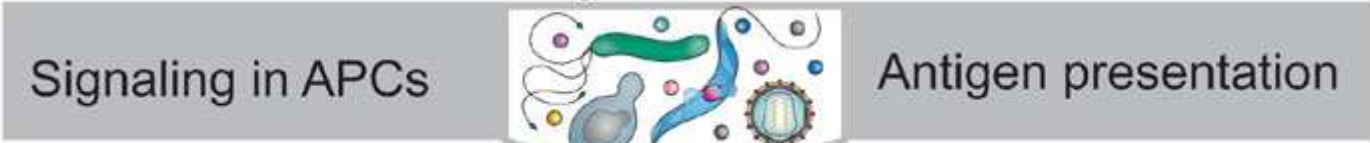
**Group I CLR (mannose receptor family)**

Mannose receptor (CD206)	Myeloid DCs and macrophages	High mannose, fucose and sulphated sugars (sLe <sup>x</sup> )	<ul style="list-style-type: none"> <li>• <i>M. tuberculosis</i>, <i>Mycobacterium kansasii</i>, <i>Francisella tularensis</i>, <i>Klebsiella pneumoniae</i> and <i>Streptococcus pneumoniae</i></li> <li>• HIV-1 and dengue virus</li> <li>• <i>C. albicans</i>, <i>Cryptococcus neoformans</i> and <i>P. carinii</i></li> <li>• <i>Leishmania</i> spp.</li> </ul>	ND	CDC42, RHOB, PAKs and ROCK1	Phagocytosis and antigen presentation
DEC205 (LY75, CD205)	Myeloid DCs	ND	ND	ND	ND	Endocytosis and antigen presentation



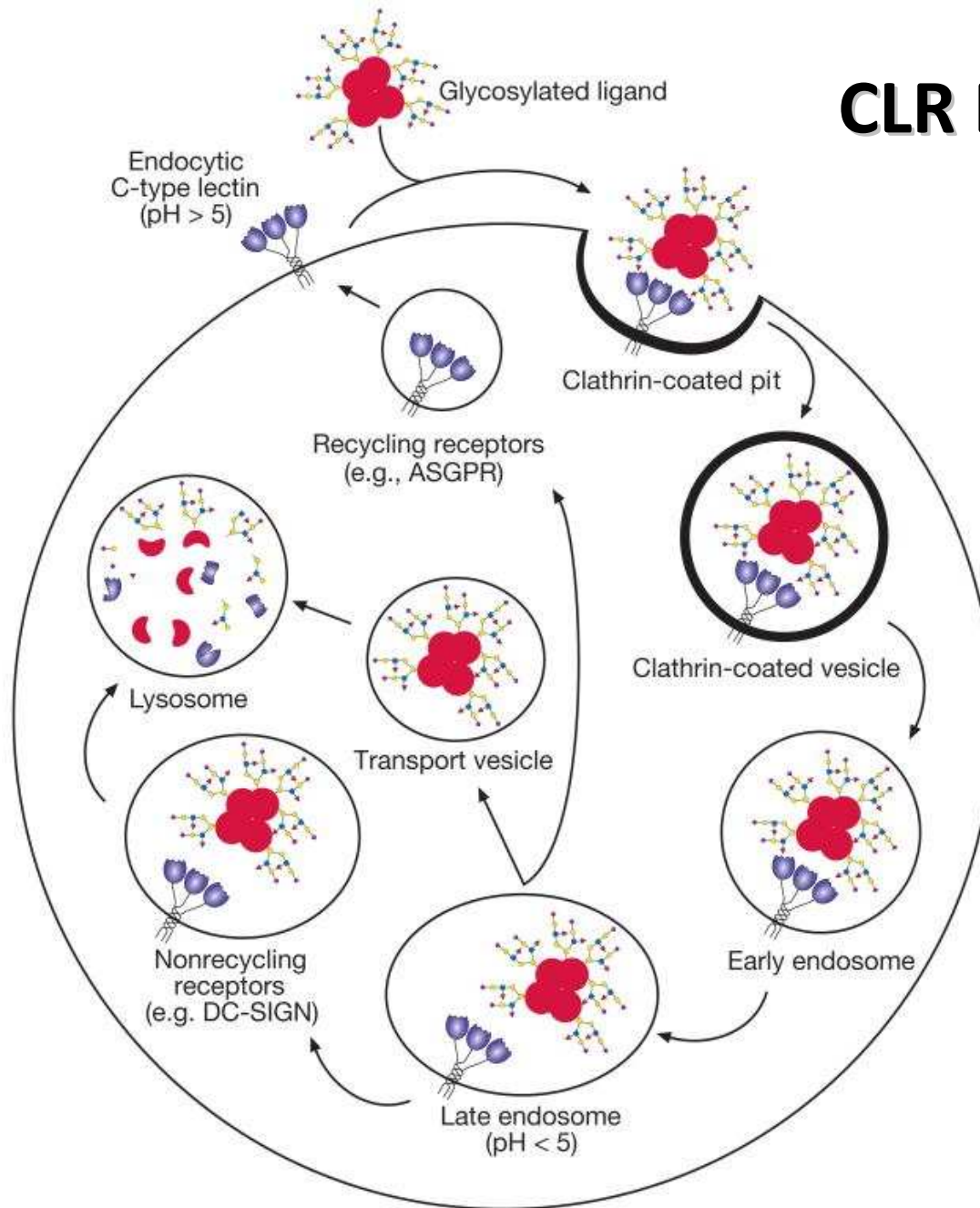


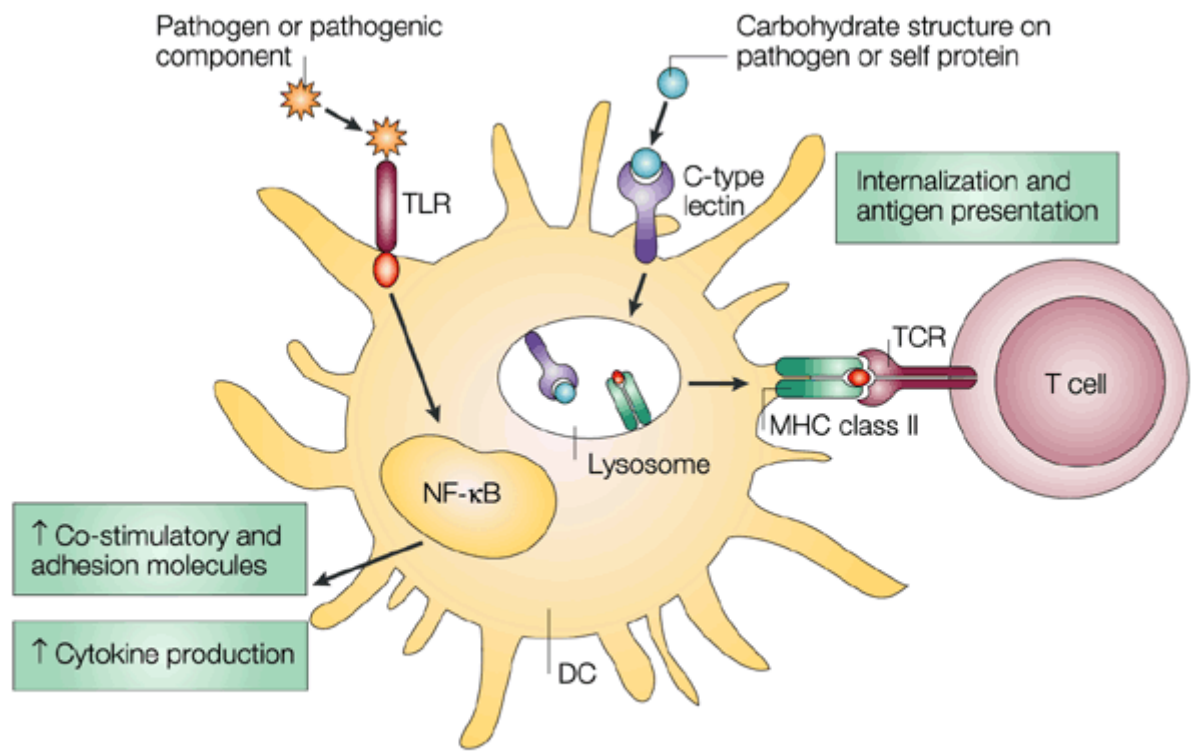
# Pathogenic interaction



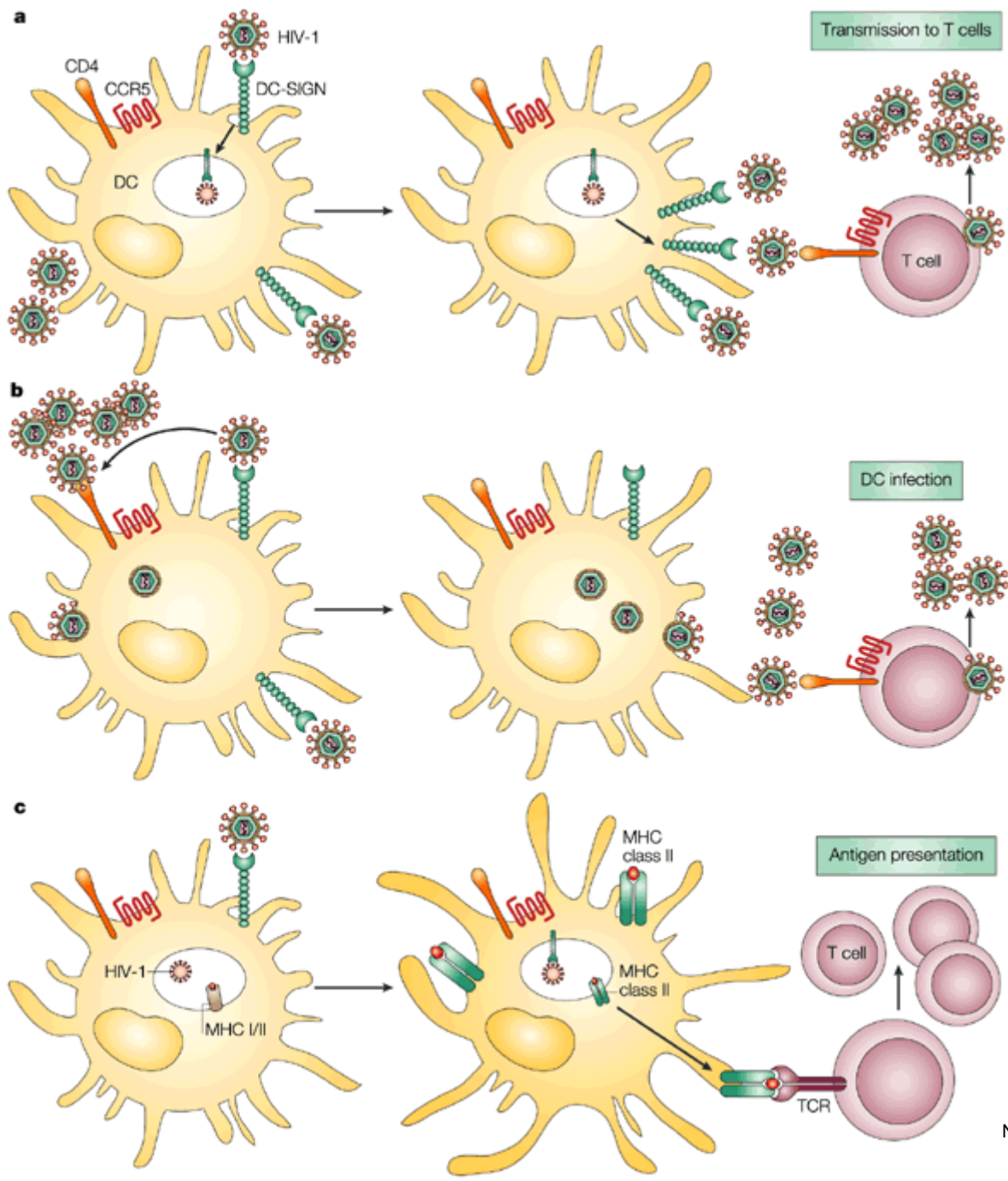
- DC maturation
- Macrophage activation (classical or alternative)
- Cytokine production
- T cell differentiation
- $T_H1$ ,  $T_H2$ ,  $T_H17$

# CLR EN ACCIÓN





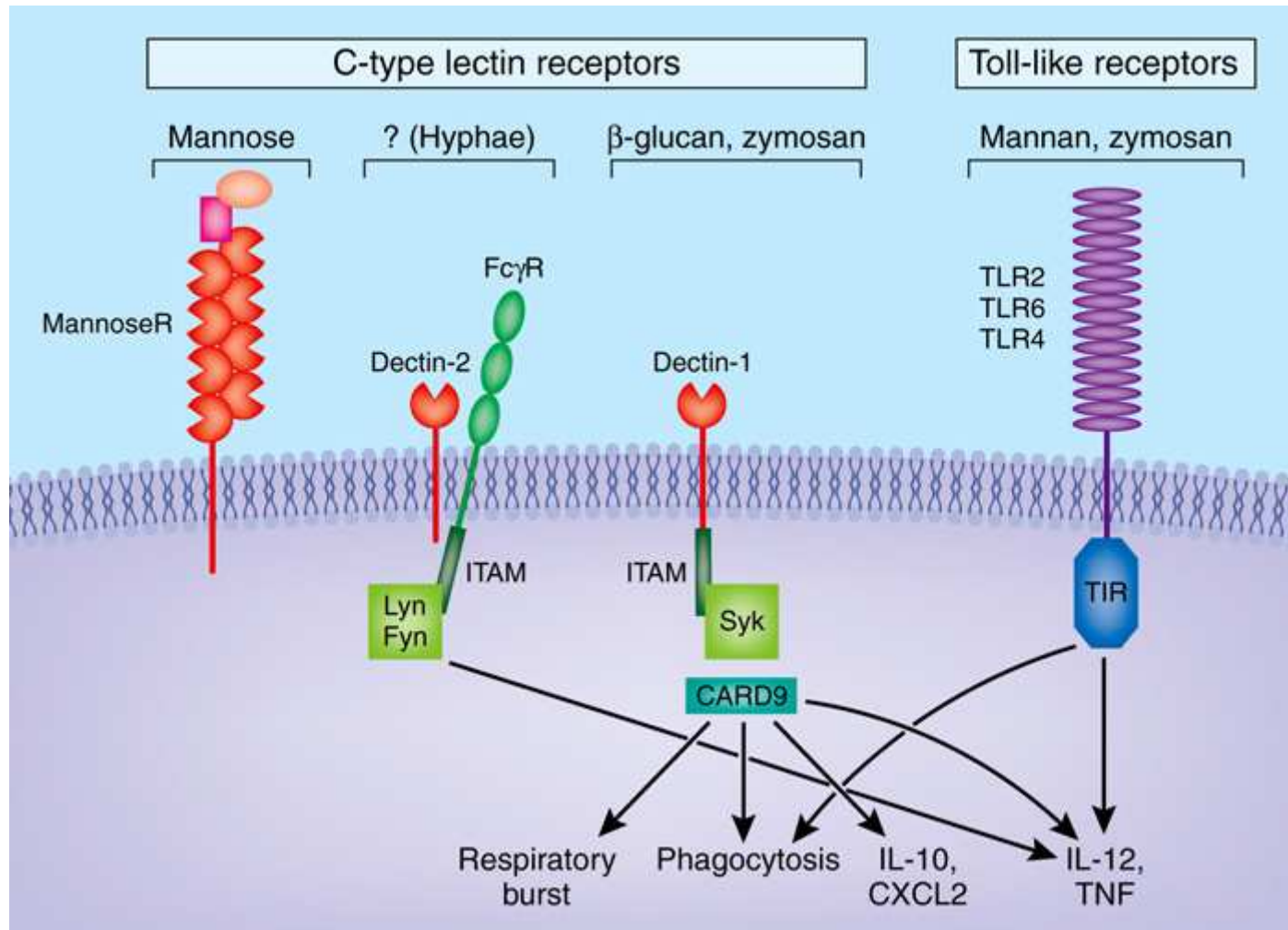
Nature Reviews | Immunology



# DC-SIGN Y HIV

Nature Reviews Immunology 3, 697-709 (September 2003)

- a | DC-SIGN (dendritic cell (DC)-specific intercellular adhesion molecule-grabbing nonintegrin) is expressed by immature DCs in mucosal tissues and lymph nodes, and by DC precursors in the blood. HIV-1 is captured by DC-SIGN that is expressed by DC precursors in the blood after infection or by immature DCs at mucosal entry sites during sexual transmission. DC-SIGN-bound HIV-1 enters the cell, but escapes internalization into lysosomal compartments and recycles back to the cell surface. By hiding intracellularly in DCs, HIV-1 is protected during migration to the lymphoid tissues. On arrival at lymphoid tissues, DCs transmit HIV-1 to CD4<sup>+</sup> T cells in trans, resulting in productive HIV-1 infection of CD4<sup>+</sup> T cells.
- b | High concentrations of HIV-1 allow viral infection of DCs that results in the production of HIV-1 by DCs, which subsequently infect T cells. Sequestration of HIV-1 by DC-SIGN can allow cis-infection of DCs by presenting the infectious virus to CD4 and co-receptors to allow efficient infection of DCs.
- c | C-type lectins function as antigen receptors to internalize antigen into lysosomes to enhance antigen presentation by MHC class I and II molecules. It remains to be determined whether capture of HIV-1 by C-type lectins results in the activation of DCs and presentation of viral antigen by MHC molecules. CCR5, CC chemokine receptor 5; TCR, T-cell receptor.

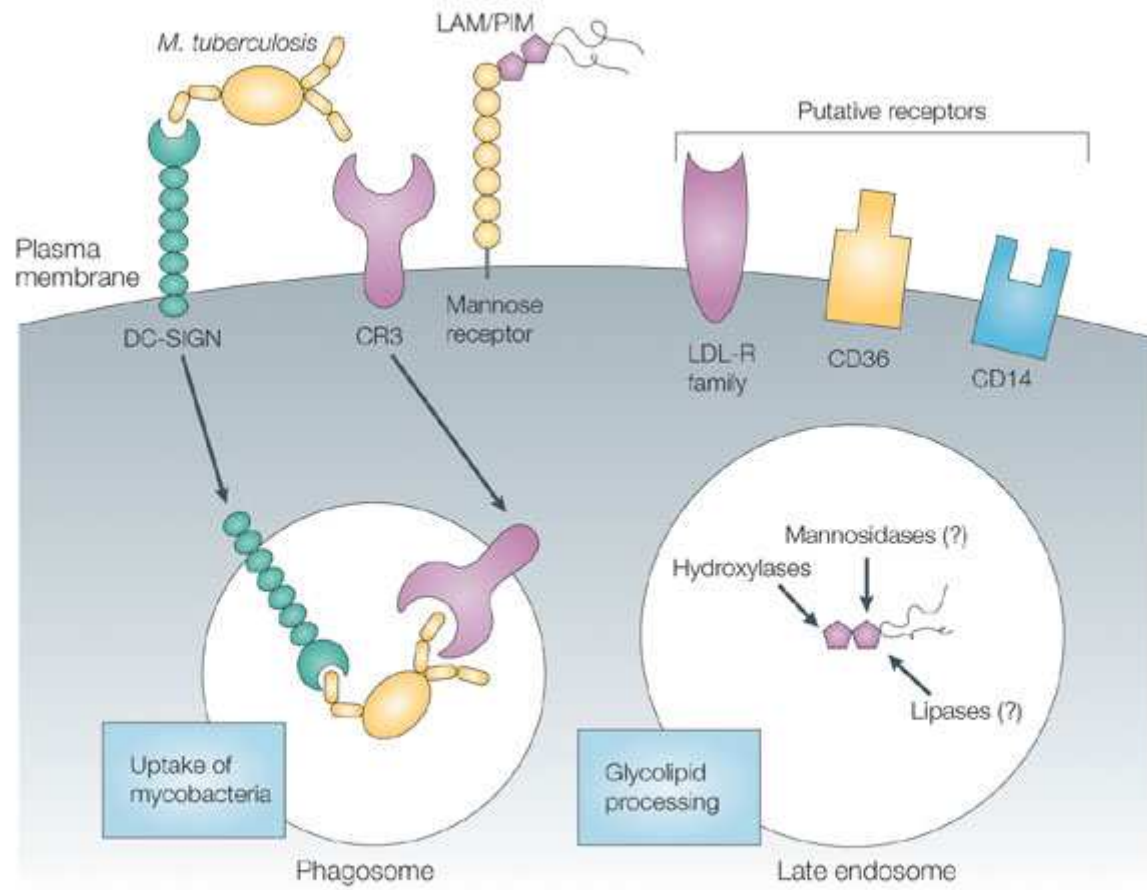


Dectin-1 binds to  $\beta$ -glucan and subsequently signals via Syk and CARD9 to induce cellular responses such as a respiratory burst and cytokine production.

Dectin-2, which interacts with the hyphal form of yeast, associates with the Fc  $\gamma$ -receptor (Fc $\gamma$ R) to trigger cytokine production.

MannoseR, mannose receptor; ITAM, immunoreceptor tyrosine-based activation motif; Lyn and Fyn, kinases; TIR, Toll-IL-1 receptor.

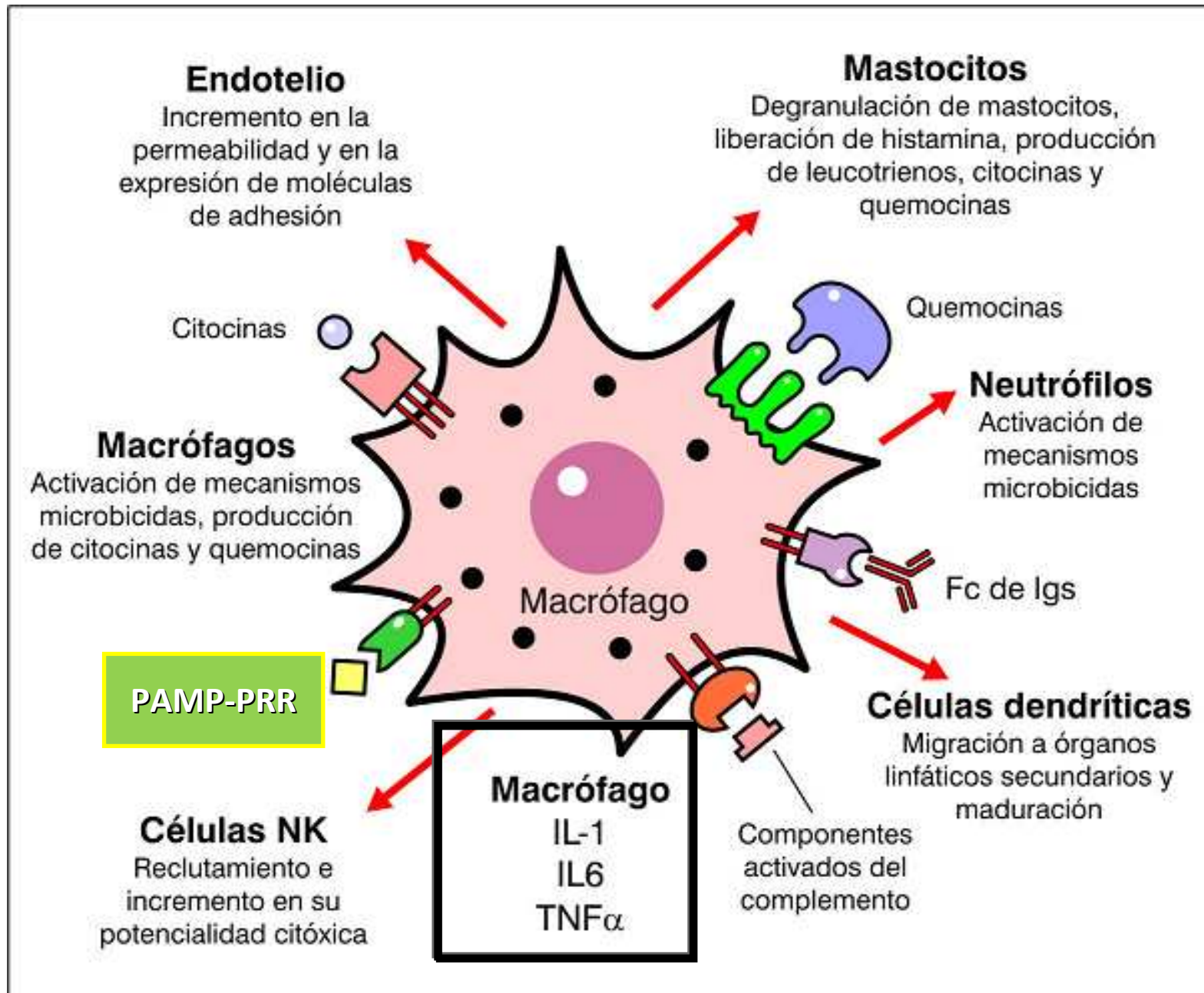
- Dectin-1 is a recently discovered pattern-recognition receptor that plays an important role in antifungal innate immunity. Dectin-1, which is expressed on phagocytes, is a specific receptor for  $\beta$ -glucans[1].
- $\beta$ -Glucans are glucose polymers found in the cell walls of fungi, such as zymosan (a cell wall preparation of *Saccharomyces cerevisiae*) and *Candida albicans*. Dectin-1 binds and internalizes  $\beta$ -glucans and mediates the production of reactive oxygen species (ROS), activation of NF- $\kappa$ B and subsequent secretion of proinflammatory cytokines. Zymosan, which is composed primarily of  $\beta$ -glucan, mannan, mannoprotein and chitin, induces immune responses that are both Dectin-1 and TLR2-dependent[2]. However, it is now clear that its  $\beta$ -glucan moiety triggers NF- $\kappa$ B activation only through Dectin-1 as treatment with hot alkali or organic solvents abrogates the TLR2-dependent response.
- Dectin-1 is a type II transmembrane protein with a C-type lectin-like carbohydrate recognition domain (CRD) connected by a stalk to the transmembrane region, followed by a cytoplasmic tail containing an immunoreceptor tyrosinase-based activation motif (ITAM). Dectin-1 binds specifically to  $\beta$ -1,3 glucans and induces its own signaling pathway[4, 5]. After binding to its ligand, Dectin-1 is phosphorylated by a non-receptor tyrosinase kinase Src. Syk is then activated and induces the CARD9-Bcl10-Malt1 complex. This complex mediates the activation of NF- $\kappa$ B and the production of proinflammatory cytokines. Recent data suggest that Dectin-1 and TLR2/TLR6 signalings combine to enhance the responses triggered by each receptor.
- $\beta$ -Glucans display various biological activities, including anti-tumor and anti-infective activities, that depend on their physicochemical properties. Further studies are needed to clarify the specificity of both Dectin-1 and  $\beta$ -glucans, thereby allowing to elucidate the immunomodulatory activities of  $\beta$ -Glucans.

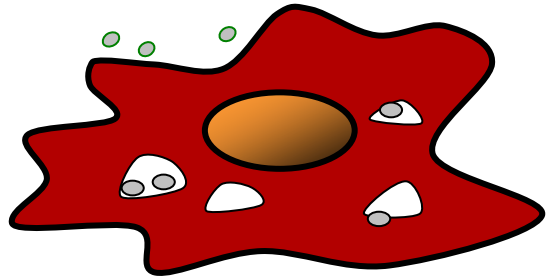


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# **Componentes humorales de la respuesta innata**





In situ, el macrófago residente fagocita y degrada las bacterias, es activado por moléculas del patógeno y secreta citocinas

**IL-1**

Activa el endotelio, linfocitos, destrucción local de tejidos, incrementa el acceso de células efectoras



**Fiebre**

**IL-8**

Quimiotaxina para leucocitos, incrementa el acceso de células efectoras, activa la unión por integrinas

**TNF- $\alpha$**

Activa endotelio vascular, incrementa permeabilidad, conduce a edema



**Fiebre, shock**

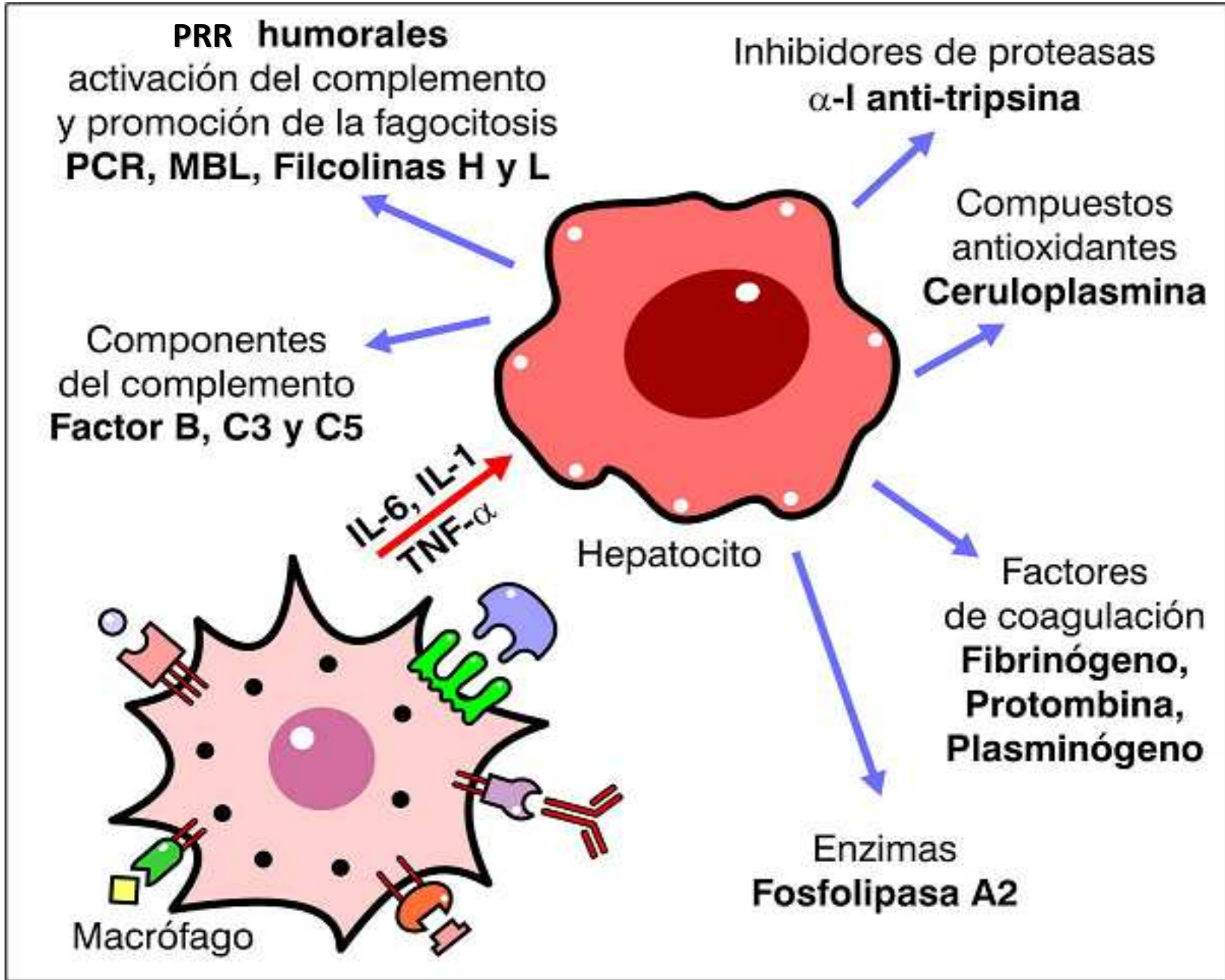
**IL-6**

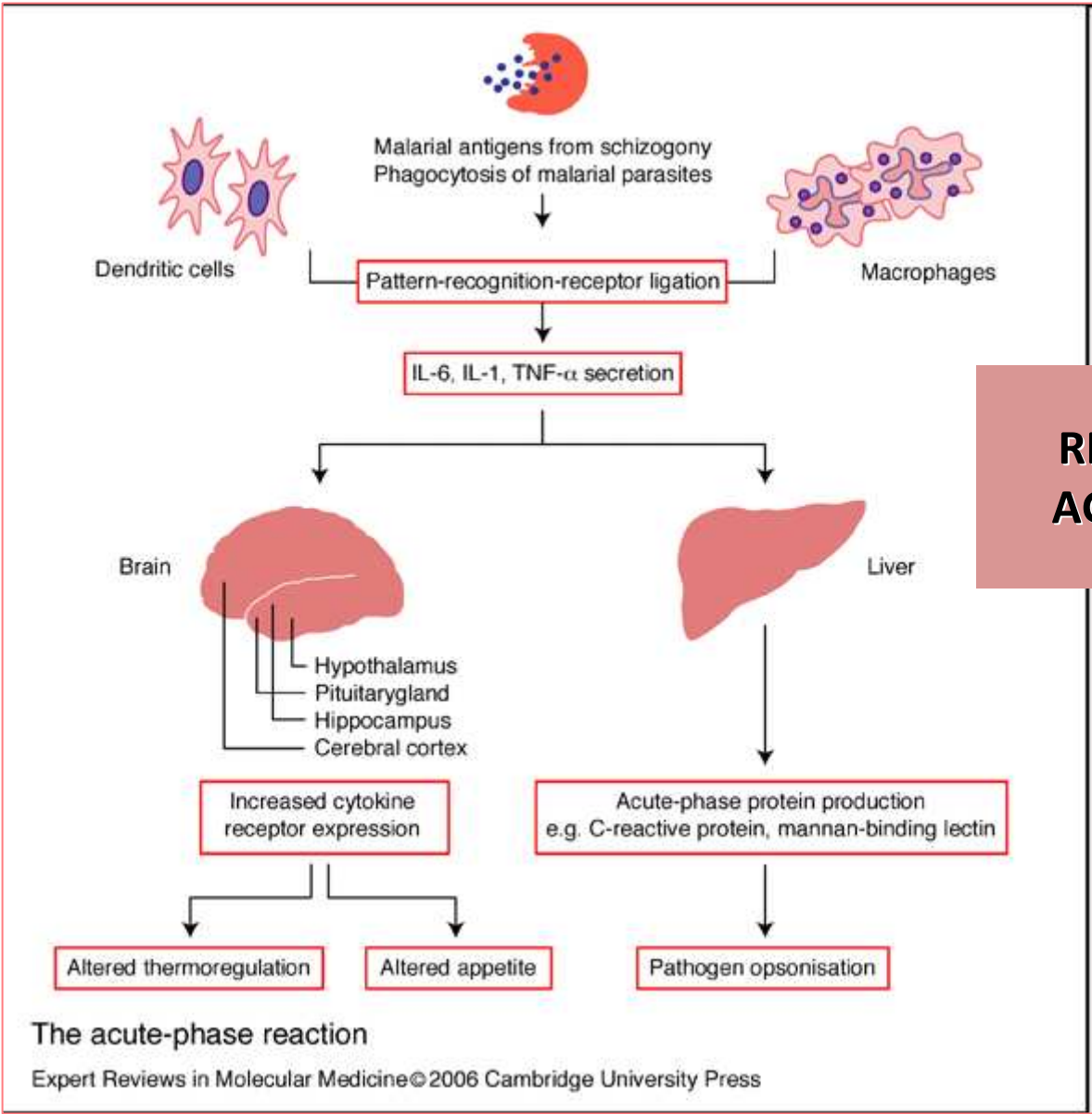
Activa linfocitos, aumenta producción de anticuerpos

**IL-12**

Activa células NK, induce la diferenciación de las células CD4 a células Th1

**REACTANTES DE FASE AGUDA**





**REACTANTES DE FASE AGUDA Y PALUDISMO**

## PROTEÍNAS DE FASE AGUDA - FUNCIONES

### Acciones

Reclutamiento de células y proteínas plasmáticas

Eliminación de restos celulares e inmunocomplejos

Reparación de la herida, del daño tisular

Limitación de la extensión del daño. Bloqueo de hidrolasas de macrófagos y neutrófilos infiltrados.

Recuperación de hierro. Impedir a las bacterias el acceso al hierro del grupo hemo.

**Proteínas del sistema complemento**

**Proteínas de la coagulación**

**Inhibidores de proteasas**

**Proteínas de unión a metales**

# Otros fenómenos de fase aguda

---

## Neuroendocrine changes

- Fever, somnolence, and anorexia
- Increased secretion of corticotropin-releasing hormone, corticotropin, and cortisol
- Increased secretion of arginine vasopressin
- Decreased production of insulin-like growth factor I
- Increased adrenal secretion of catecholamines

## Hematopoietic changes

- Anemia of chronic disease
- Leukocytosis
- Thrombocytosis

## Metabolic changes

- Loss of muscle and negative nitrogen balance
- Decreased gluconeogenesis
- Osteoporosis
- Increased hepatic lipogenesis
- Increased lipolysis in adipose tissue
- Decreased lipoprotein lipase activity in muscle and adipose tissue
- Cachexia

## Hepatic changes

- Increased metallothionein, inducible nitric oxide synthase, heme oxygenase, manganese superoxide dismutase, and tissue inhibitor of metalloproteinase-1
- Decreased phosphoenolpyruvate carboxykinase activity

## Changes in nonprotein plasma constituents

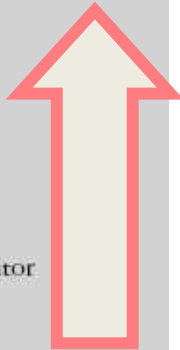
- Hypoalbuminemia, hypoferrremia, and hypercupremia
  - Increased plasma retinol and glutathione concentrations
-



# Proteínas de fase aguda

## Proteins whose plasma concentrations increase

- Complement system
  - C3
  - C4
  - C9
- Factor B
- C1 inhibitor
- C4b-binding protein
- Mannose-binding lectin
- Coagulation and fibrinolytic system
  - Fibrinogen
  - Plasminogen
  - Tissue plasminogen activator
  - Urokinase
  - Protein S
  - Vitronectin
  - Plasminogen-activator inhibitor 1
- Antiproteases
  - $\alpha_1$ -Protease inhibitor
  - $\alpha_1$ -Antichymotrypsin
  - Pancreatic secretory trypsin inhibitor
  - Inter- $\alpha$ -trypsin inhibitors
- Transport proteins
  - Ceruloplasmin
  - Haptoglobin
  - Hemopexin
- Participants in inflammatory responses
  - Secreted phospholipase A<sub>2</sub>
  - Lipopolysaccharide-binding protein
  - Interleukin-1-receptor antagonist
  - Granulocyte colony-stimulating factor
- Others
  - C-reactive protein
  - Serum amyloid A
  - $\alpha_1$ -Acid glycoprotein
  - Fibronectin
  - Ferritin
  - Angiotensinogen



## Proteins whose plasma concentrations decrease

- Albumin
- Transferrin
- Transthyretin
- $\alpha_2$ -HS glycoprotein
- Alpha-fetoprotein
- Thyroxine-binding globulin
- Insulin-like growth factor I
- Factor XII

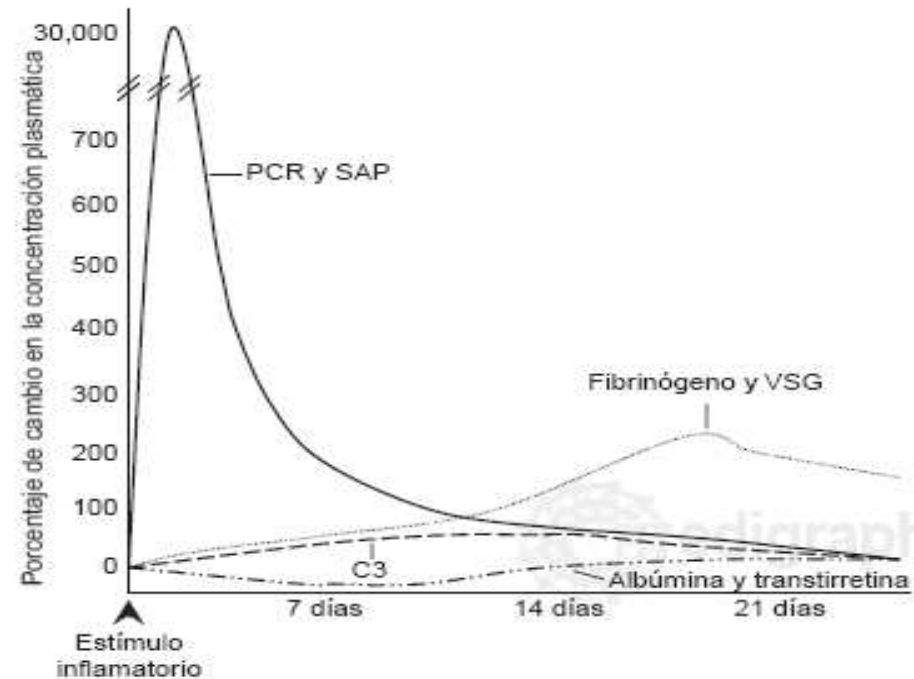


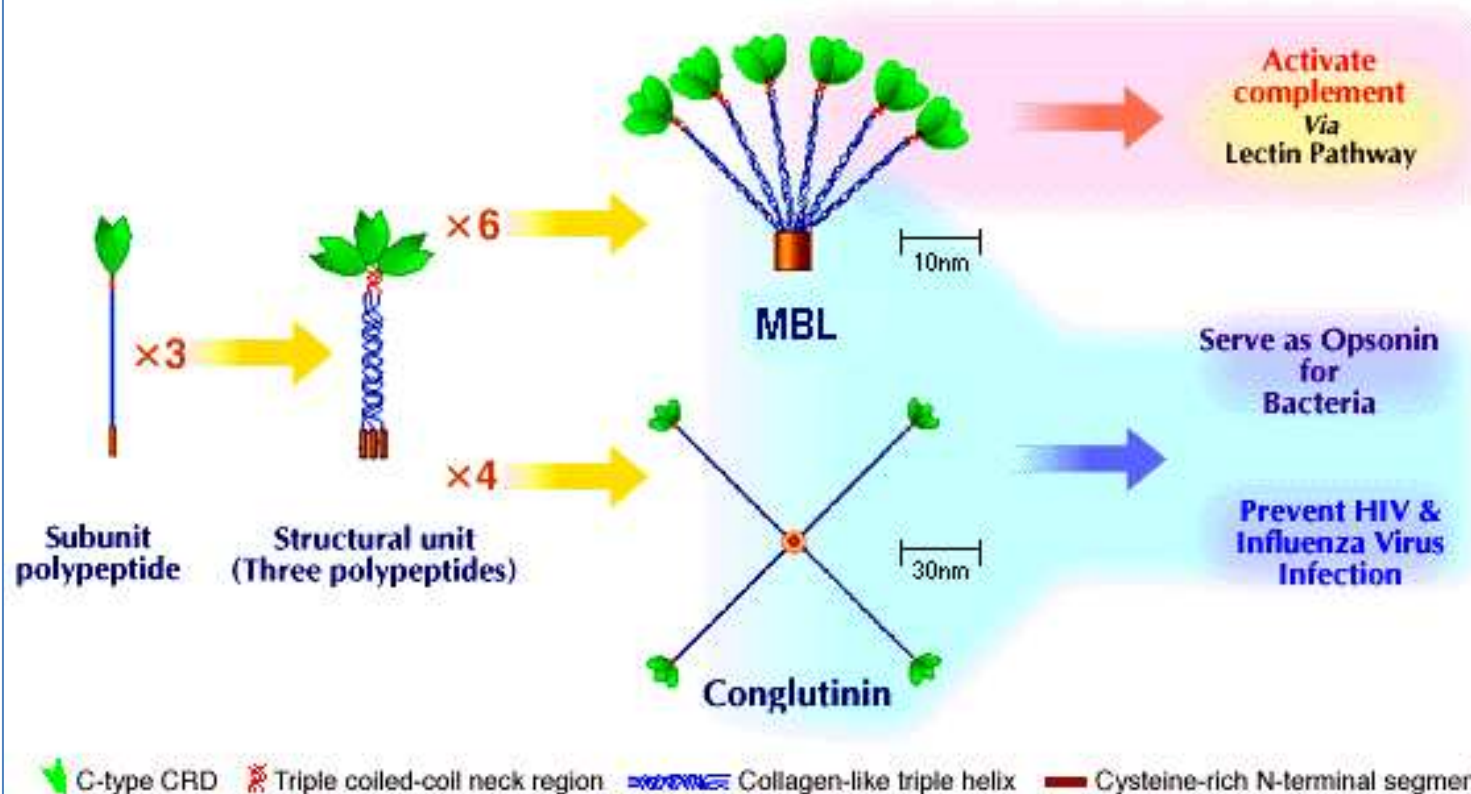
Fig. 1. Porcentaje de cambio de diferentes fenómenos de fase aguda a través del tiempo en respuesta a un estímulo inflamatorio. PCR, proteína C reactiva; SAP, proteína amiloide sérica A; VSG, velocidad de sedimentación globular.

**COLLAGEN-LIKE LECTIN :**  
**COLECTINAS**

- Son un subgrupo de lectinas animales tipo-C ( $\text{Ca}^{2+}$  dependientes) caracterizadas por la presencia de secuencias tipo colágeno.
- Incluyen tres proteínas séricas:
  - 1. Lectina ligadora de manano (Mannan-binding protein -MBL),**
  2. Conglutinina bovina y colectina bovina 43 (CL-43),
  - 3. Dos proteínas surfactantes pulmonares (SP-A and SP-D).**
- Se unen a residuos terminales de azúcares no reductores manosa, N acetil-glucosamina, fucosa y glucosa. SP-D se une a maltosa.
- **Juegan un papel importante en la inmunidad innata sin involucrar a los anticuerpos.**

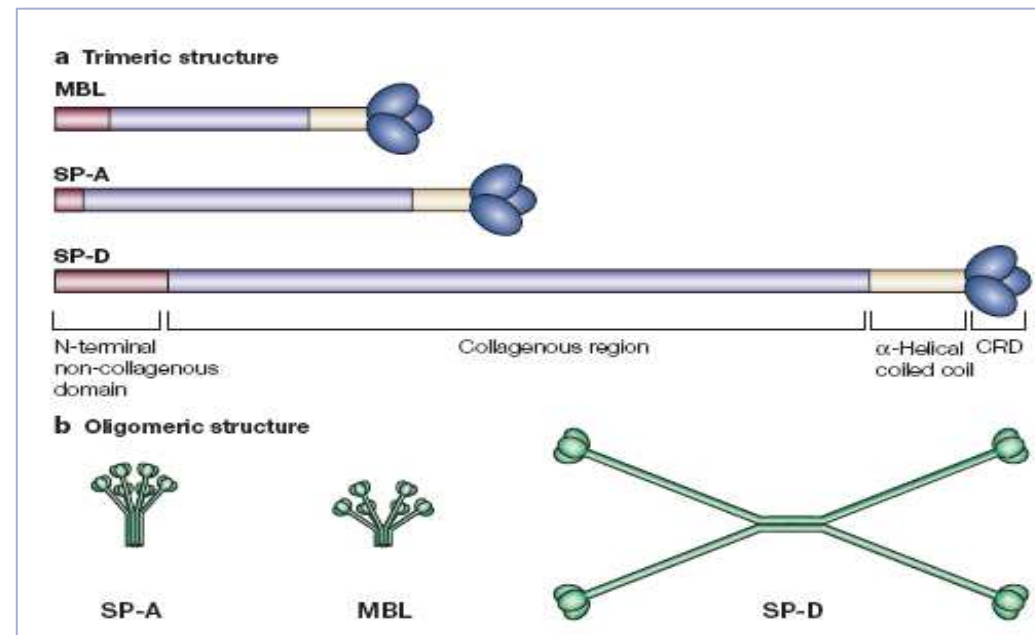
# COLECTINAS

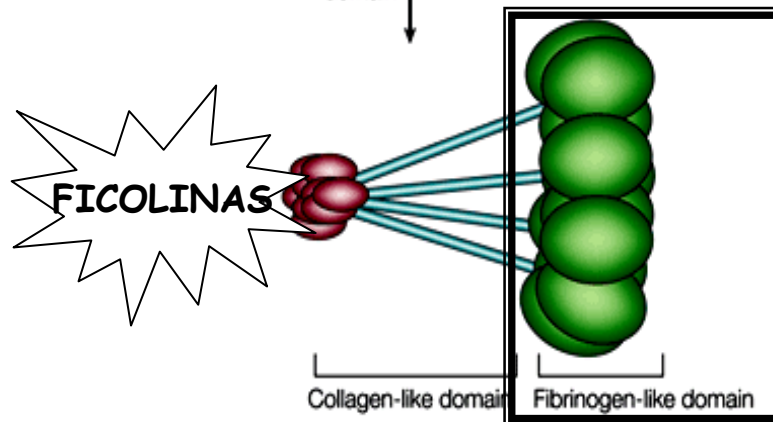
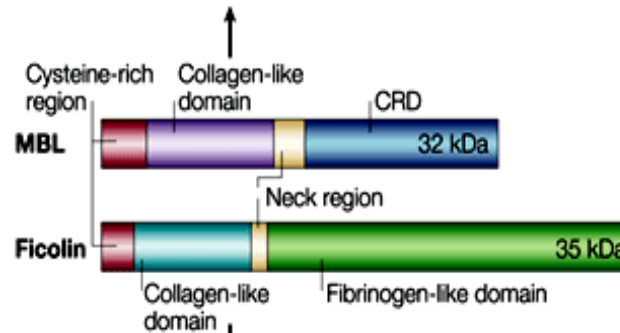
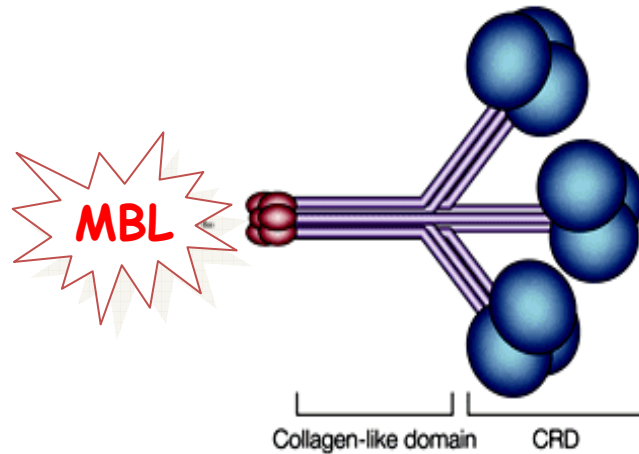
## Molecular structures and biological activities of collectins (MBL and conglutinin)



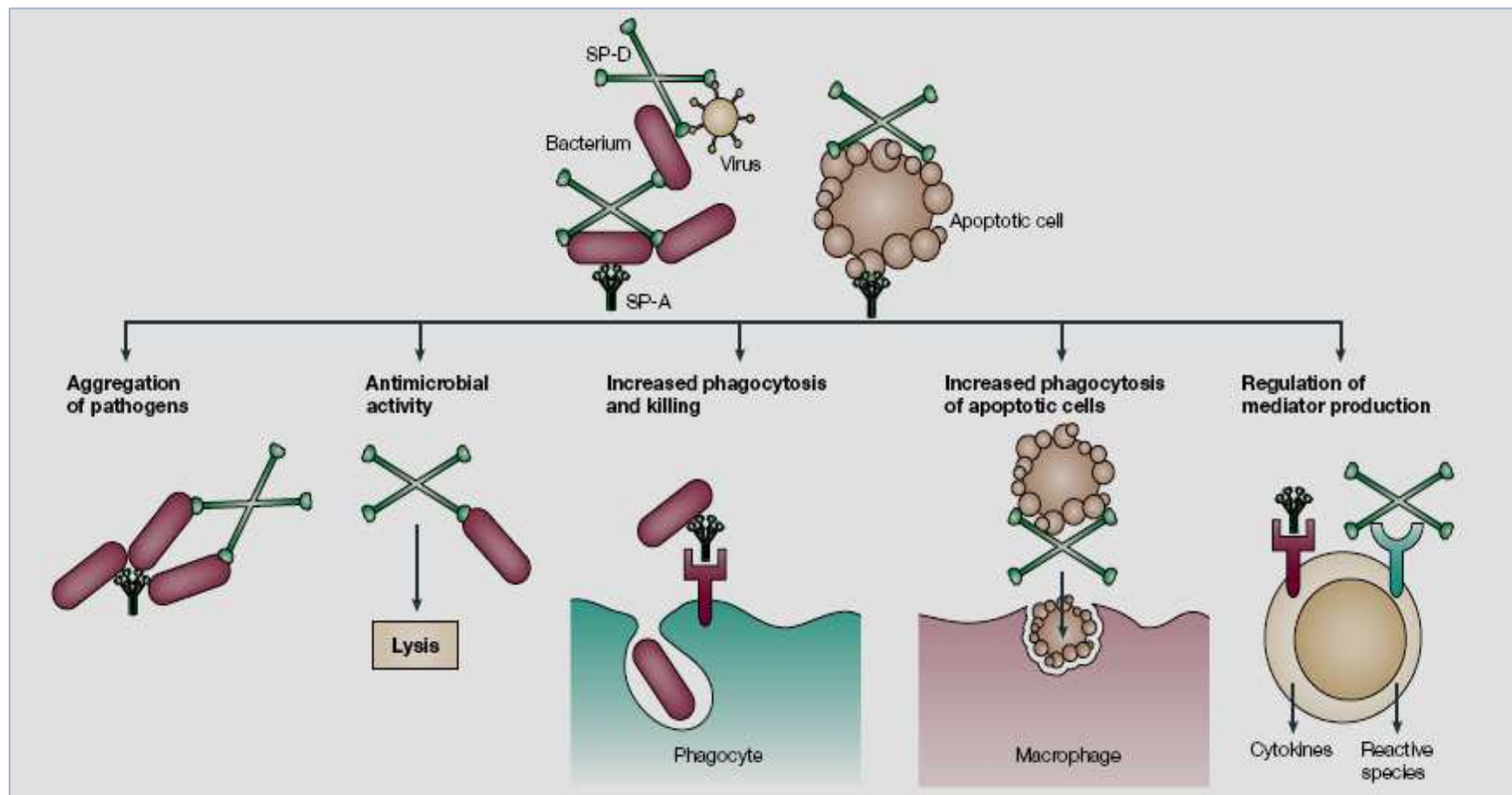
# ¿Dónde se sintetizan?

- MBL, conglutinina, y CL-43 son sintetizadas en el hígado y secretadas a la circulación.
- SP-A y SP-D son principalmente sintetizadas por las células alveolares pulmonares de tipo II y células Claras y secretadas al espacio alveolar.

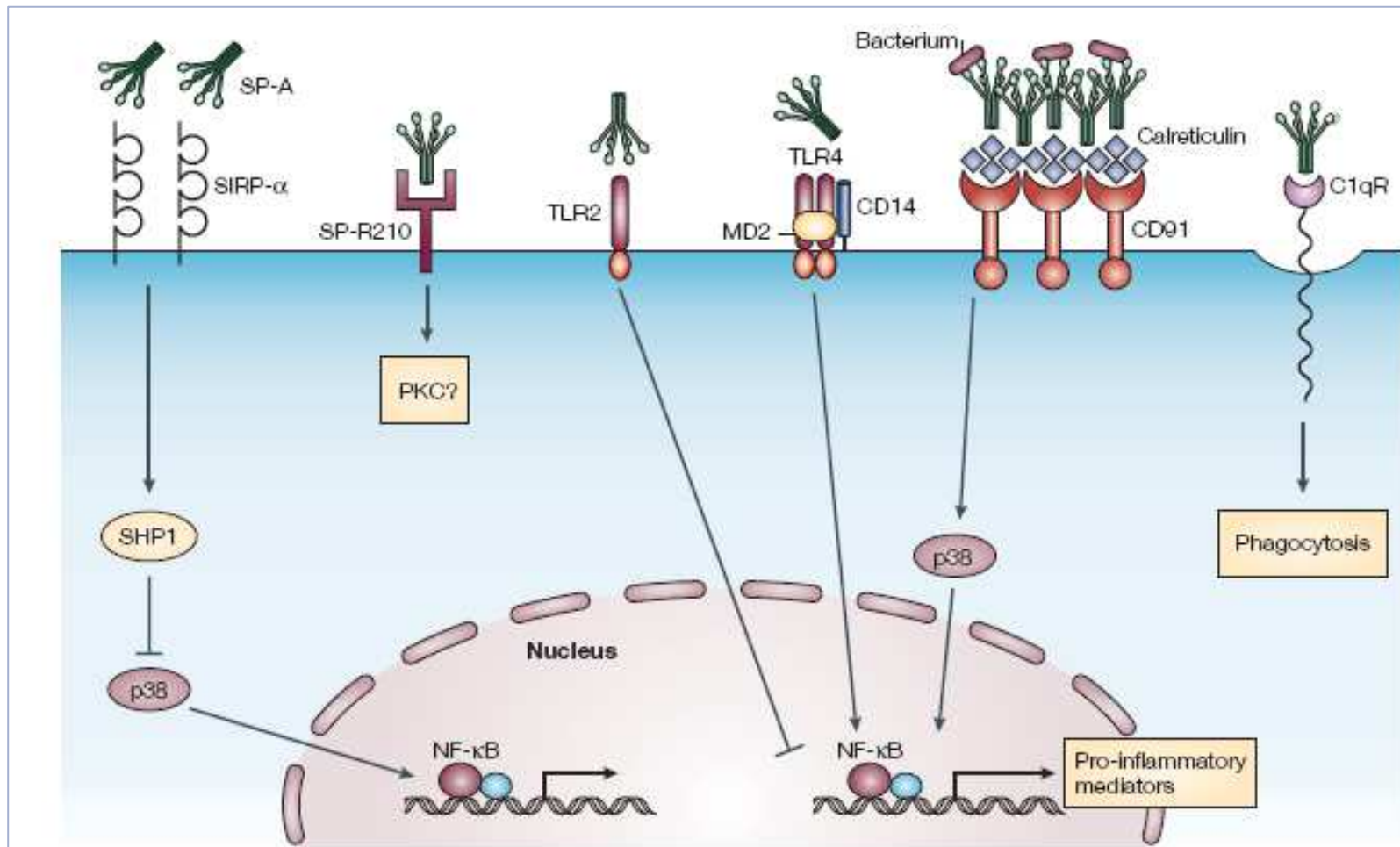




- Es capaz de activar el complemento por la vía de la lectina o bien opsonizar bacterias e inducir fagocitosis a través del C1qR
- La concentración de MBL incrementa significativamente después del nacimiento y sus niveles varían entre los individuos adultos saludables.
- Los niveles aumentan después de una infección y permanecen elevados durante largos períodos, comparado con otros reactantes de fase aguda.



## FUNCIONES DE LAS SP-A y SP-D



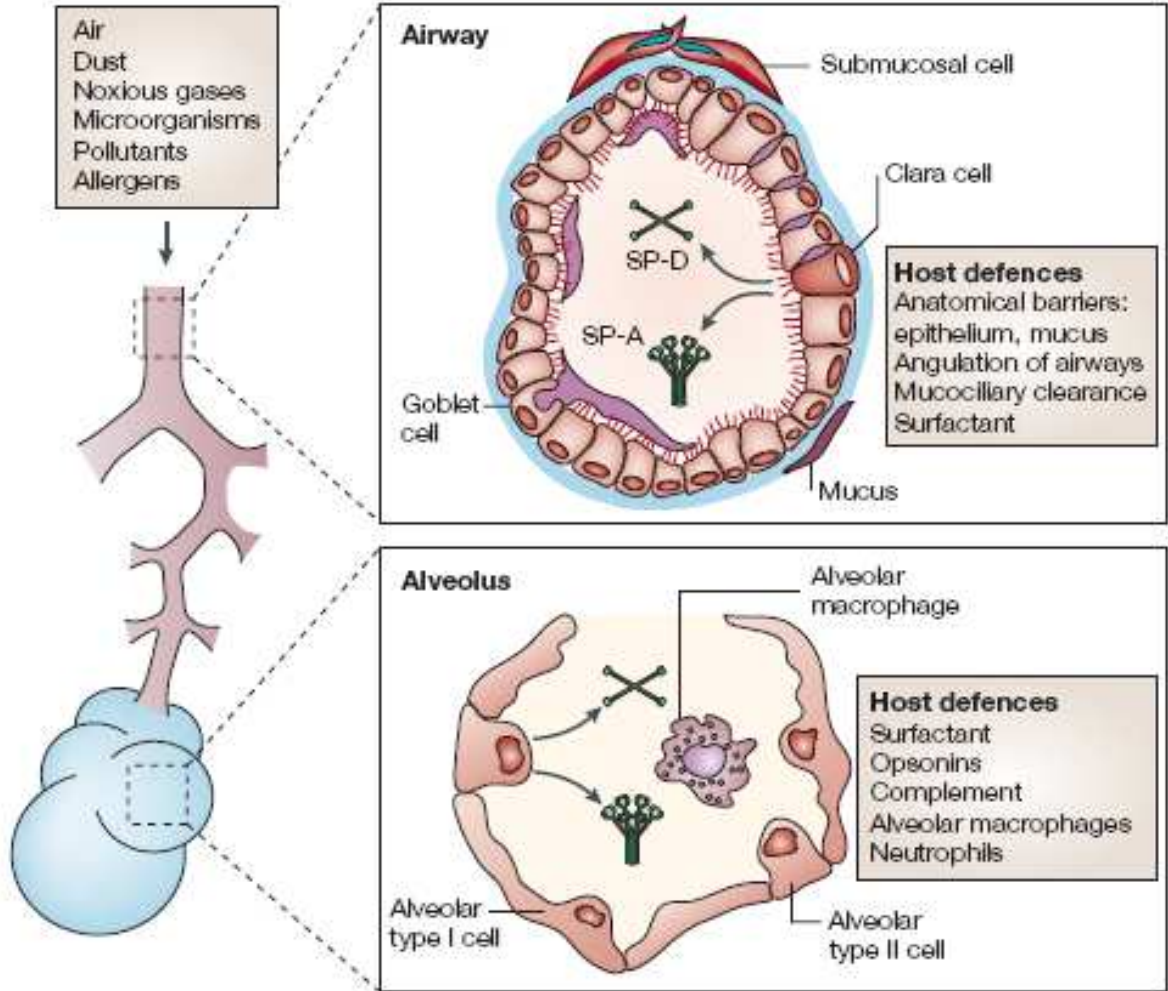
**Receptores de SP-A**



Table 1 | **Interactions of SP-A and SP-D with various microorganisms\***

Microorganism	SP-A binding	SP-D binding
<i>Aspergillus fumigatus</i>	+	+
Bacillus Calmette-Guérin	+	?
<i>Cryptococcus neoformans</i>	+	+
<i>Escherichia coli</i>	+	+
<i>Haemophilus influenzae</i>	+	+
<i>Klebsiella pneumoniae</i>	+	+
<i>Mycobacterium tuberculosis</i>	+	+
<i>Pneumocystis jirovecii</i> <sup>†</sup>	+	+
<i>Pseudomonas aeruginosa</i>	+	+
<i>Staphylococcus aureus</i>	+	+
Group A <i>Streptococcus</i>	+	?
Group B <i>Streptococcus</i>	+	+
<i>Streptococcus pneumoniae</i>	+	+
Cytomegalovirus	+	?
Herpes simplex virus	+	?
Influenza A virus	+	+
Respiratory syncytial virus	+	+
Rotavirus	?	+

# Mecanismos de defensa en el pulmón



# **PROTEÍNA C REACTIVA**

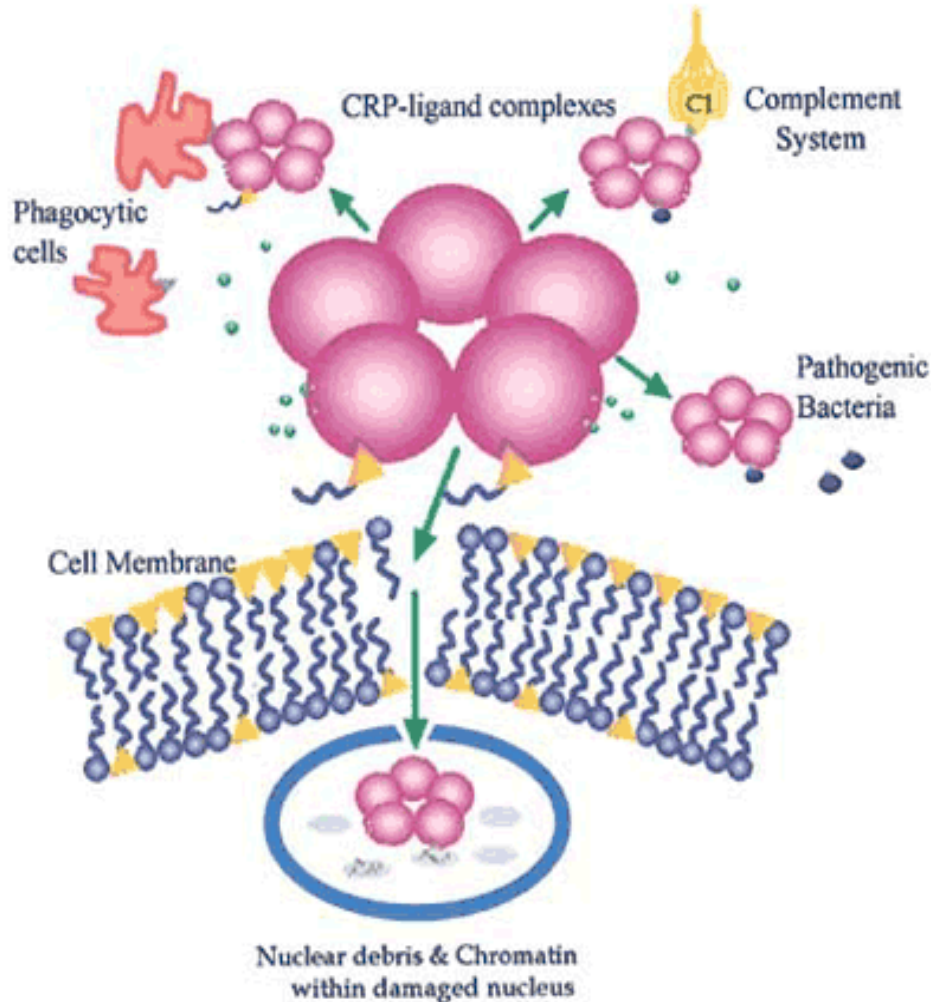
La proteína C reactiva fue descubierta en 1930 por William Tillet y Thomas Francis. Vieron, que **sueros de pacientes con neumonía por neumococo coprecipitaban con el polisacárido C de la pared celular de los neumococos.** Debido a esta reacción se bautizó este factor como "proteína C reactiva" (PCR).

- **LOFSTROM** demostró su presencia tanto en las situaciones inflamatorias crónicas y agudas con lo que PCR se convirtió en una **PROTEÍNA DE FASE AGUDA NO ESPECÍFICA.**

# Proteína C reactiva

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www.medscape.com



Source: Adv Neonatal Care © 2003 W. B. Saunders

- Pertenece a la familia de las **PENTRAXINAS**: compuesta por cinco subunidades idénticas unidas no covalentemente.

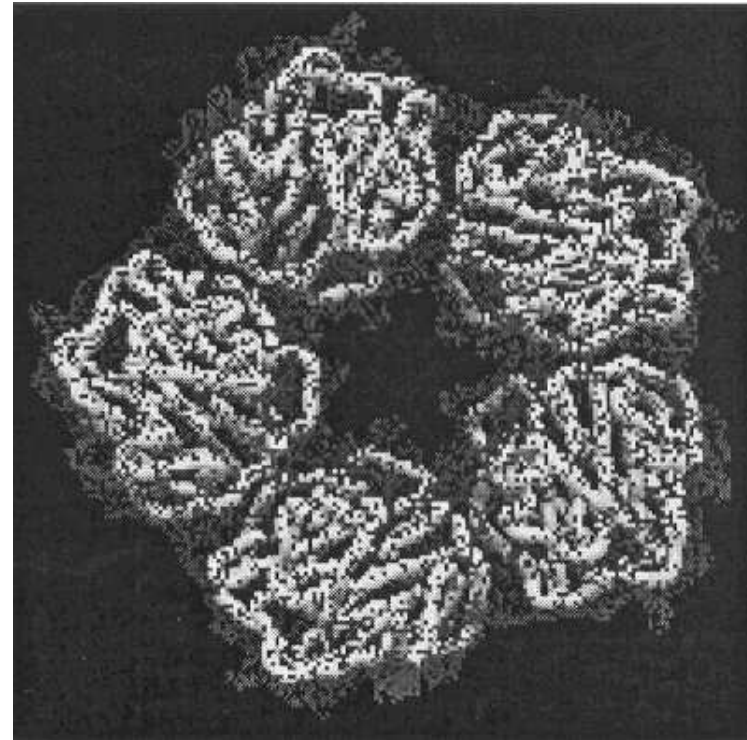
Tiene alta afinidad por residuos **fosfocolina** (PC), presentes en polisacáridos de virus, bacterias, hongos y parásitos

La PC también se expresa en las membranas celulares de células necróticas o apoptóticas.....

La PCR luego de interactuar con el patógeno puede ser reconocida por el **RFc $\gamma$** , expresado por macrófagos y PMNs.....

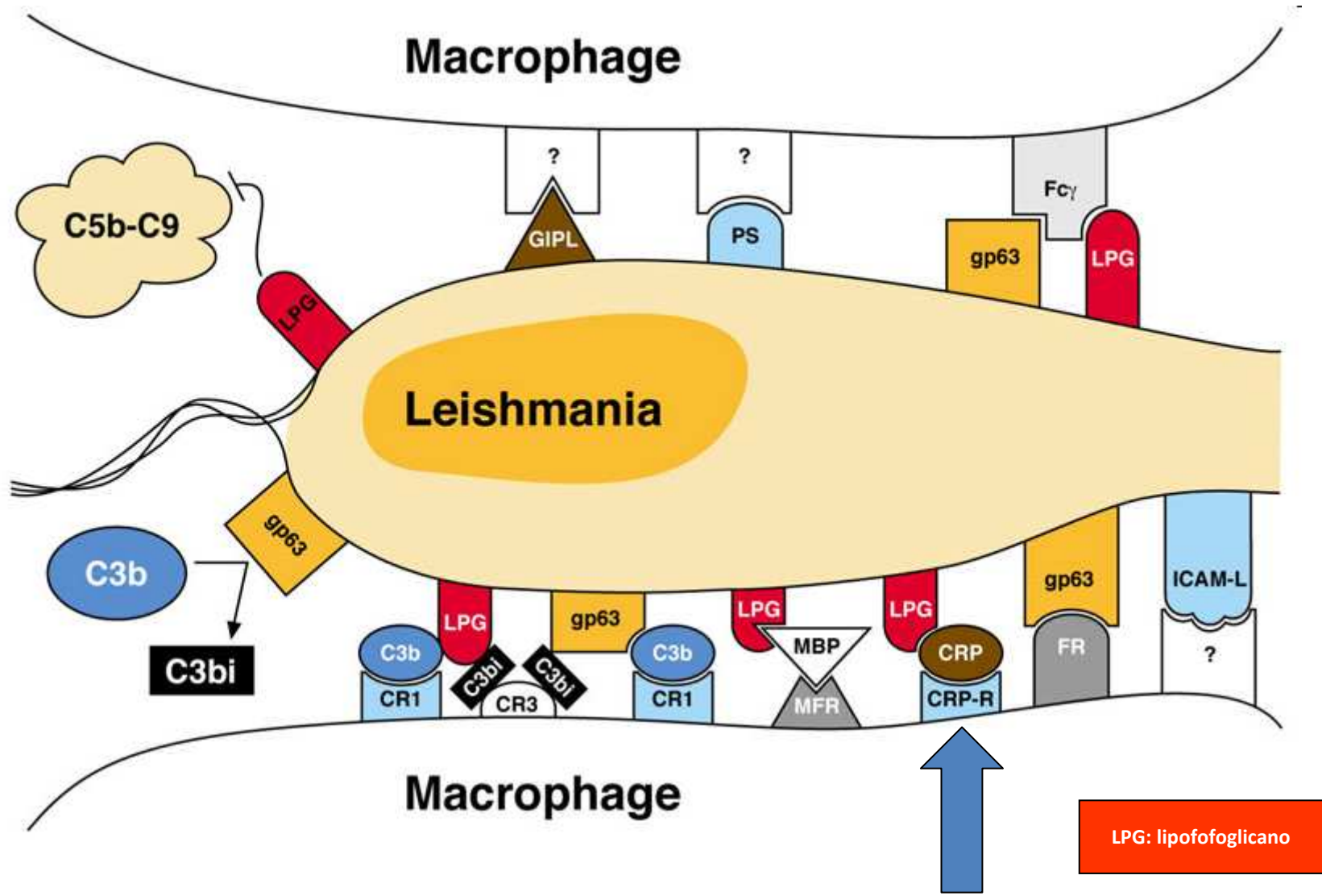
# PCR

- La PCR es una  $\beta$  globulina de fase aguda con una masa molecular de 118.000 daltons.
- Es una proteína no glicosilada de simetría cíclica.
- Estable en suero >20 años a -70 °C
- Niveles elevados de PCR en suero o plasma los encontramos como respuesta no específica a infecciones, inflamaciones no infecciosas, AR ,ECV ...



- **Encontramos:**
  - Respuesta intensa (>1 mg/dL) en :
    - Infección: bacterias > hongos > virus  
( meningitis, neumonía,...)
    - Inflamación aguda ( AR , pancreatitis )
    - Tumores sólidos, traumatismos, cirugía
  - Respuesta moderada (<1 mg/dL) en :
    - Leucemias
    - Autoinmunidad: DM, C.ulcerosa/Crohn, ...
- La PCR es sintetizada en el hígado y normalmente sólo se detectan trazas en circulación.
- **Cuando se produce algún daño al tejido:**
  - Aumento detectable a las 6-8 horas
  - Pico en 48 horas
  - Descenso a partir de las 48 horas

# Leishmaniasis y PCR





## Nuevas pistas sobre las causas de la obesidad

**Un estudio demuestra que la Proteína C reactiva, marcador de inflamación y lesión tisular, podría estar implicada en el desarrollo de la obesidad**

La obesidad, auténtica epidemia del siglo XXI, afecta a 300 millones de personas en todo el mundo y no sólo se limita a los países más industrializados, ya que se calcula que 115 millones de personas en países en vías de desarrollo padecen enfermedades relacionadas con el exceso de peso. En la actualidad se están desarrollando numerosas investigaciones con el fin de encontrar respuestas a los mecanismos por los que se produce la obesidad. **En esta línea, hace unos años se demostró el papel que ejerce la hormona leptina en el control del apetito.**





- **La leptina** se segrega con el aumento de la grasa corporal -cuanta más grasa más leptina-, y su misión consiste en bloquear los receptores de las neuronas del centro del hambre en el hipotálamo, de modo que el cerebro envíe la orden de comer menos. Curiosamente, y aunque las personas obesas producen más cantidad de leptina que las delgadas, por un mecanismo hasta ahora desconocido, son resistentes a sus efectos.
- Investigadores de la Universidad de Pittsburgh (Estados Unidos) han descubierto que **la proteína C-reactiva (PCR) bloquea la hormona leptina**. Los resultados, publicados recientemente en la revista Nature Medicine, contribuyen a explicar el mecanismo por el cual se produce la obesidad y podría explicar en parte por qué muchas personas obesas tienen problemas para perder peso. Según Allan Zhao, autor del estudio, se sabe que la PCR bloquea la leptina e impide el envío de sus señales. Pero todavía se desconoce cómo, aunque se apunta que algunos factores que dificultan la circulación sanguínea pueden inhibir la acción de la leptina por la obstrucción del paso de la sangre.

## Fibra y Proteína C Reactiva

Una dieta rica en fibra ayuda a controlar los niveles de Proteína C Reactiva, marcador inflamatorio que distintos estudios han asociado a un mayor riesgo cardiovascular.

Un estudio publicado en el "American Journal of Clinical Nutrition", firmado por investigadores de la Universidad de Massachusetts (Estados Unidos), realizado con 524 voluntarios sanos, indica que aquellos que consumían más fibra en la dieta son los que presentaban niveles más bajos de proteína C reactiva.

Los resultados apoyan la recomendación general de que los adultos consuman a través de la dieta de 20 a 35 gramos de fibra cada día.



# Valores de referencia

10-50 mg/dl	INFLAMACIÓN LEVE O INFECCIÓN VIRAL
50-200 mg/dl	INFLAMACIÓN ACTIVA O INFECCIÓN BACTERIANA
>200 mg/dl	INFECCIÓN SEVERA O TRAUMA
<3-5 mg/dl	ESTRATIFICACIÓN DE RIESGO CARDIOVASCULAR

Ledue, Rifai, Clinical Chemistry 2003. Rifai, Ridker, Clinical Chemistry 2001

## TÉCNICAS DE ANÁLISIS

INICIALMENTE



AGLUTINACIÓN SEMICUANTITATIVA CON  
LATEX

POSTERIORMENTE



NEFELOMETRÍA Y TURBIDIMETRÍA

ACTUALMENTE

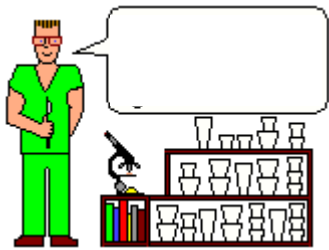


USO DE ANTICUERPOS MARCADOS CON  
FLUORESCENCIA, LUMINISCENCIA O  
RADIOACTIVIDAD

TURBIDIMETRÍA CON PARTÍCULAS DE  
LÁTEX

**Tabla II.** Ventajas y desventajas como reactante de fase aguda entre la velocidad de sedimentación globular (VSG) y la proteína C reactiva (PCR).

	VSG	PCR
Ventajas	<ul style="list-style-type: none"> <li>• Barata</li> </ul>	<ul style="list-style-type: none"> <li>• Rápida respuesta ante estímulos inflamatorios</li> <li>• Refleja directamente el valor de una proteína de fase aguda</li> <li>• La cuantificación es precisa y reproducible</li> <li>• Se puede determinar en suero almacenado</li> </ul>
Desventajas	<ul style="list-style-type: none"> <li>• Se altera por la edad y el género</li> <li>• Se altera por anemia o poliglobulia</li> <li>• Refleja el nivel de muchas proteínas plasmáticas</li> <li>• Responde lentamente ante estímulos inflamatorios</li> <li>• Requiere muestras frescas</li> </ul>	<ul style="list-style-type: none"> <li>• Costosa</li> <li>• En algunas enfermedades autoinmunes inflamatorias (lupus y esclerodermia) no muestra incremento</li> </ul>



**Tabla III.** Utilidad clínica de la medición de la PCR.

---

Prueba de escrutinio para diferenciar enfermedades orgánicas

- Fibromialgia vs condiciones inflamatorias autoinmunes

Evaluación de la actividad de la enfermedad en condiciones inflamatorias

- Artritis idiopática juvenil
- Artritis reumatoide
- Espondilitis anquilosante
- Enfermedad de Reiter
- Artritis psoriásica
- Vasculitis sistémicas
- Enfermedad de Crohn
- Fiebre reumática
- Fiebres periódicas
- Pancreatitis aguda

Diagnóstico y seguimiento de enfermedades infecciosas

- Endocarditis infecciosa
- Sepsis neonatal y meningitis
- Infección intercurrente en lupus eritematoso sistémico
- Infección intercurrente en leucemias en tratamiento
- Complicaciones post-operatorias incluyendo infección y tromboembolismo

Graduación pronóstica en enfermedades cardiovasculares

- Infarto agudo de miocardio
- Angina inestable
- Evento vascular cerebral

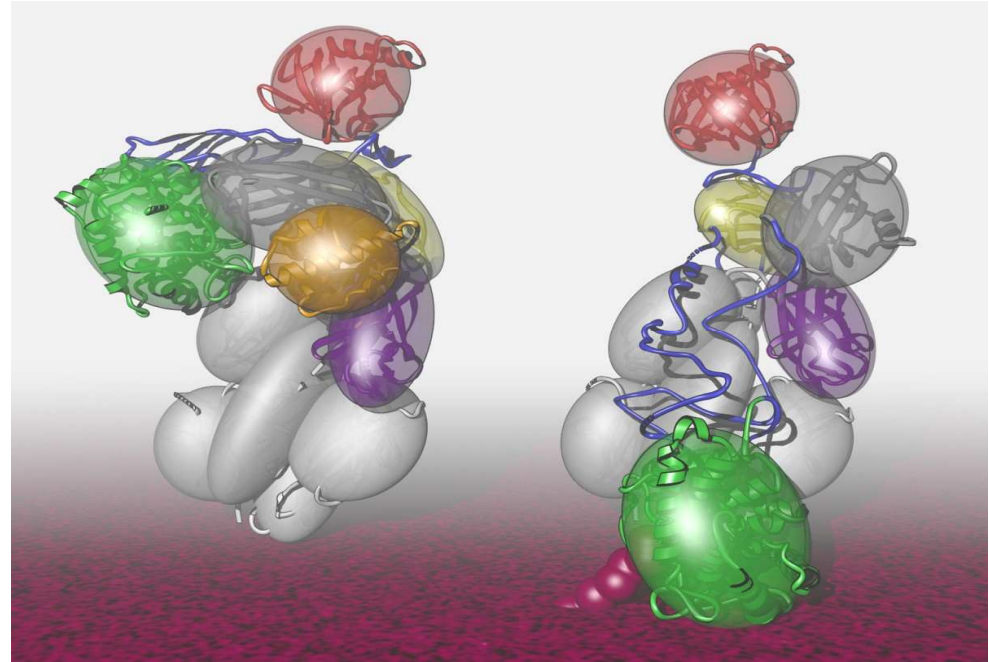
Diagnóstico diferencial de enfermedades inflamatorias

- Lupus eritematoso sistémico vs artritis reumatoide
  - Enfermedad de Crohn vs colitis ulcerativa
-

# Serum amilod A (SSA)

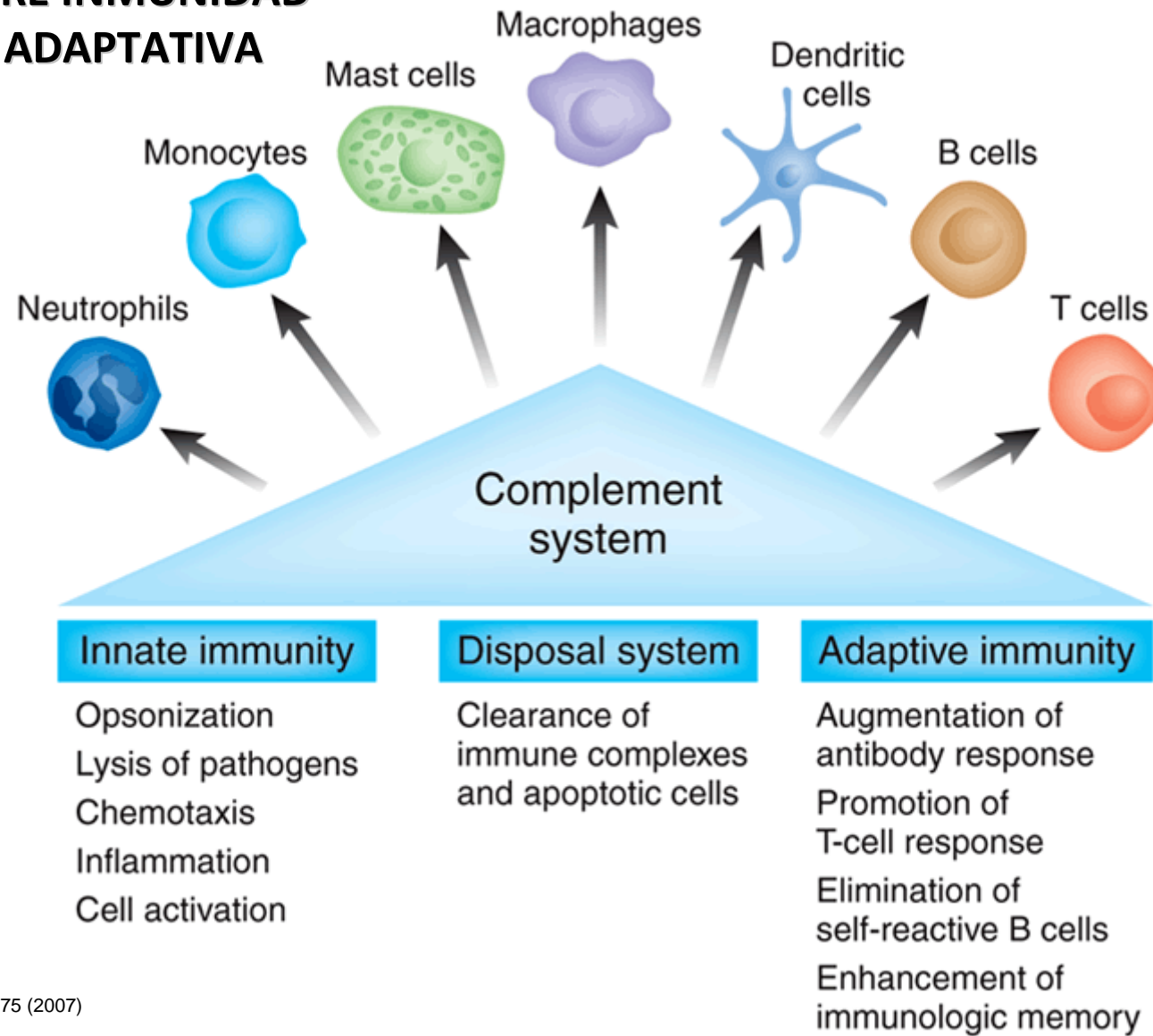
- **El amiloide sérico A**, está constituido por una familia de apolipoproteínas que rápidamente se liga a las HDL después de su síntesis y es potencialmente influido por el metabolismo del colesterol durante el estado inflamatorio.
- Causa adhesión y quimiotaxis de los fagocitos y linfocitos, y **puede contribuir a la inflamación del proceso aterosclerótico en las arterias coronarias, por incremento de la oxidación de las LDL.**
- La síntesis de SAA y el aumento de sus concentraciones séricas ocurre rápidamente tras un estímulo agudo, pudiendo alcanzar 10,000 veces sus valores normales en algunos pacientes con enfermedad inflamatoria aguda.
- Las concentraciones de SAA en el pico de fase aguda suelen oscilar entre 1-5 mg/ml . Este hecho sugiere que la determinación de las concentraciones circulantes de SAA podrían ser clínicamente útiles para monitorizar las reacciones de fase aguda
- Se ha observado que las concentraciones de SAA se correlacionan estrechamente con las concentraciones de la proteína C reactiva (PCR).



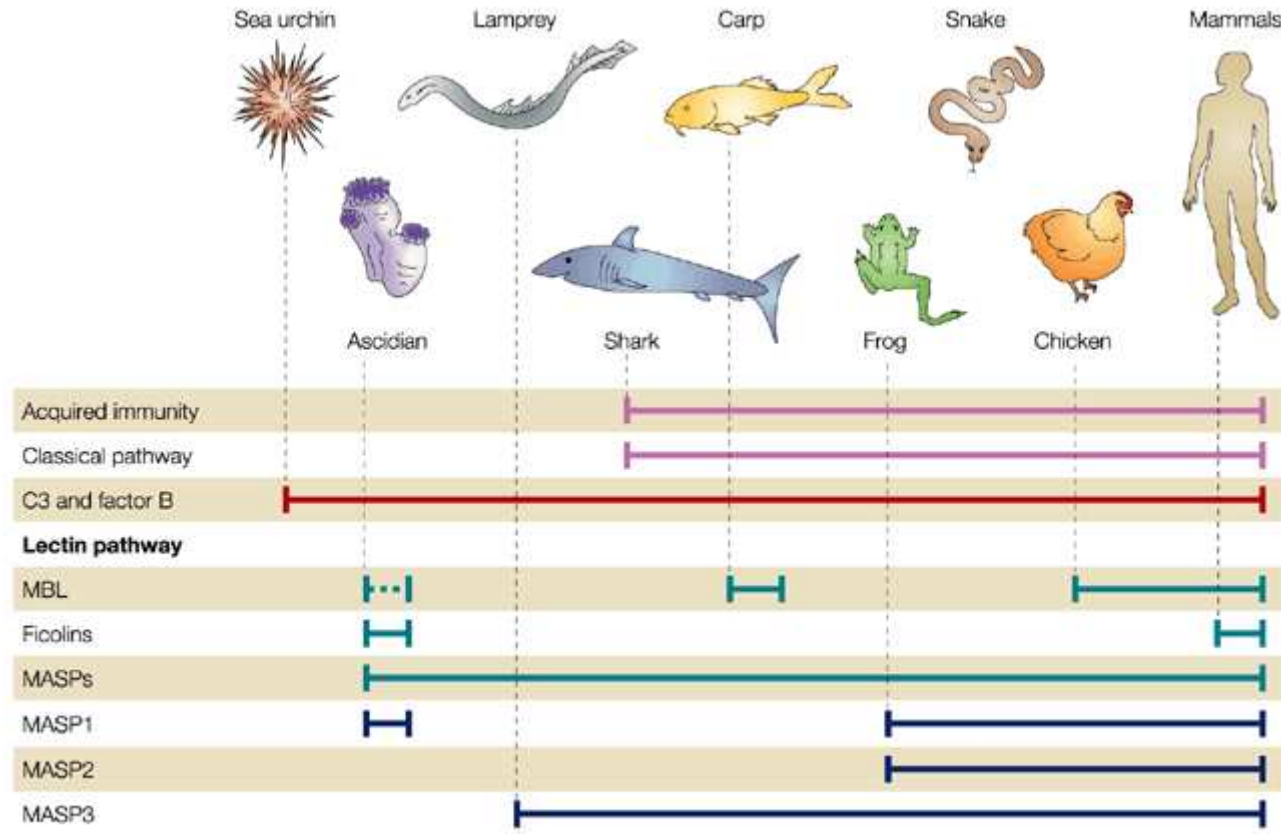


# SISTEMA DEL COMPLEMENTO

# PUENTE ENTRE INMUNIDAD INNATA Y ADAPTATIVA



Katie Ris-Vicari

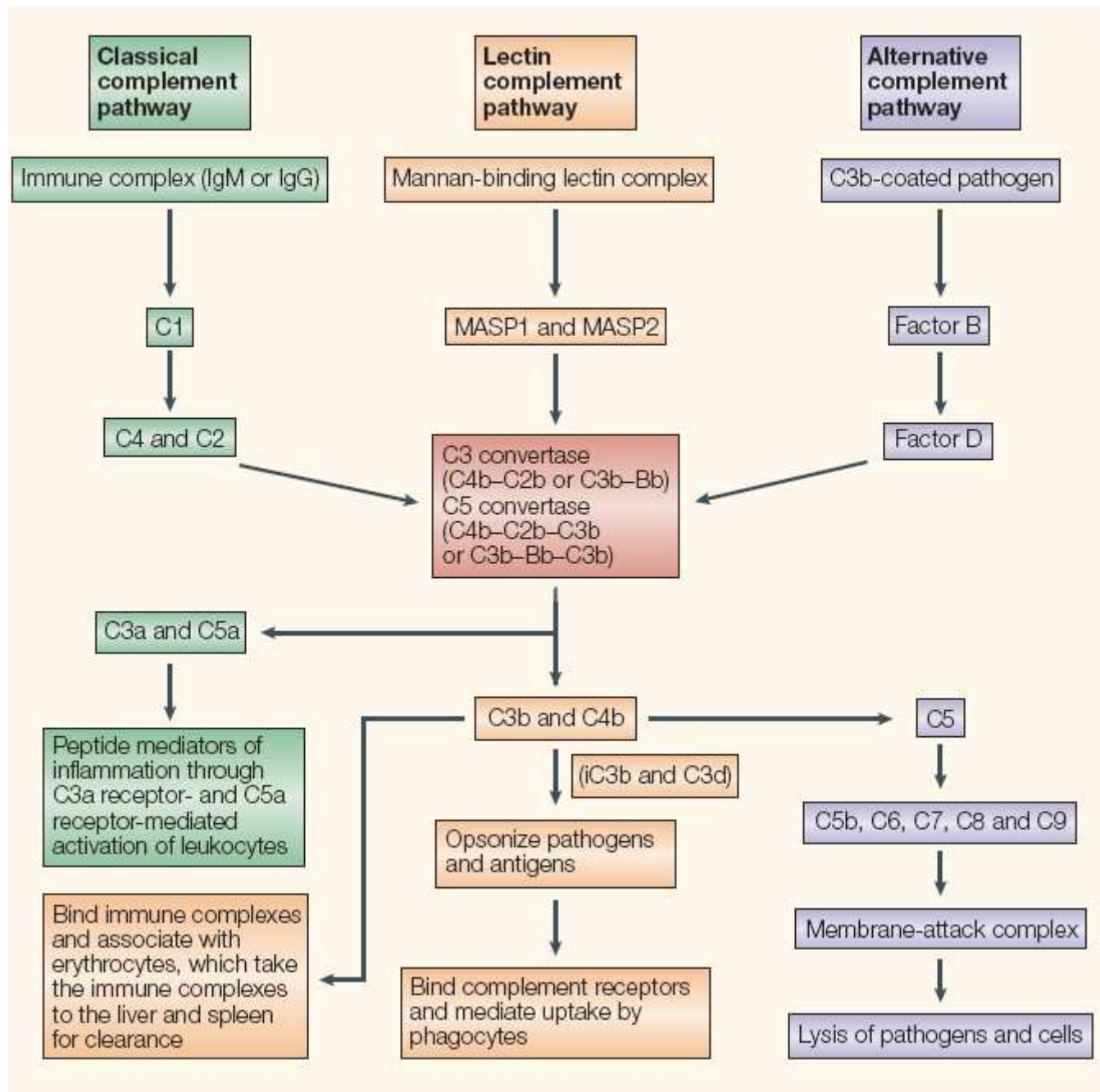


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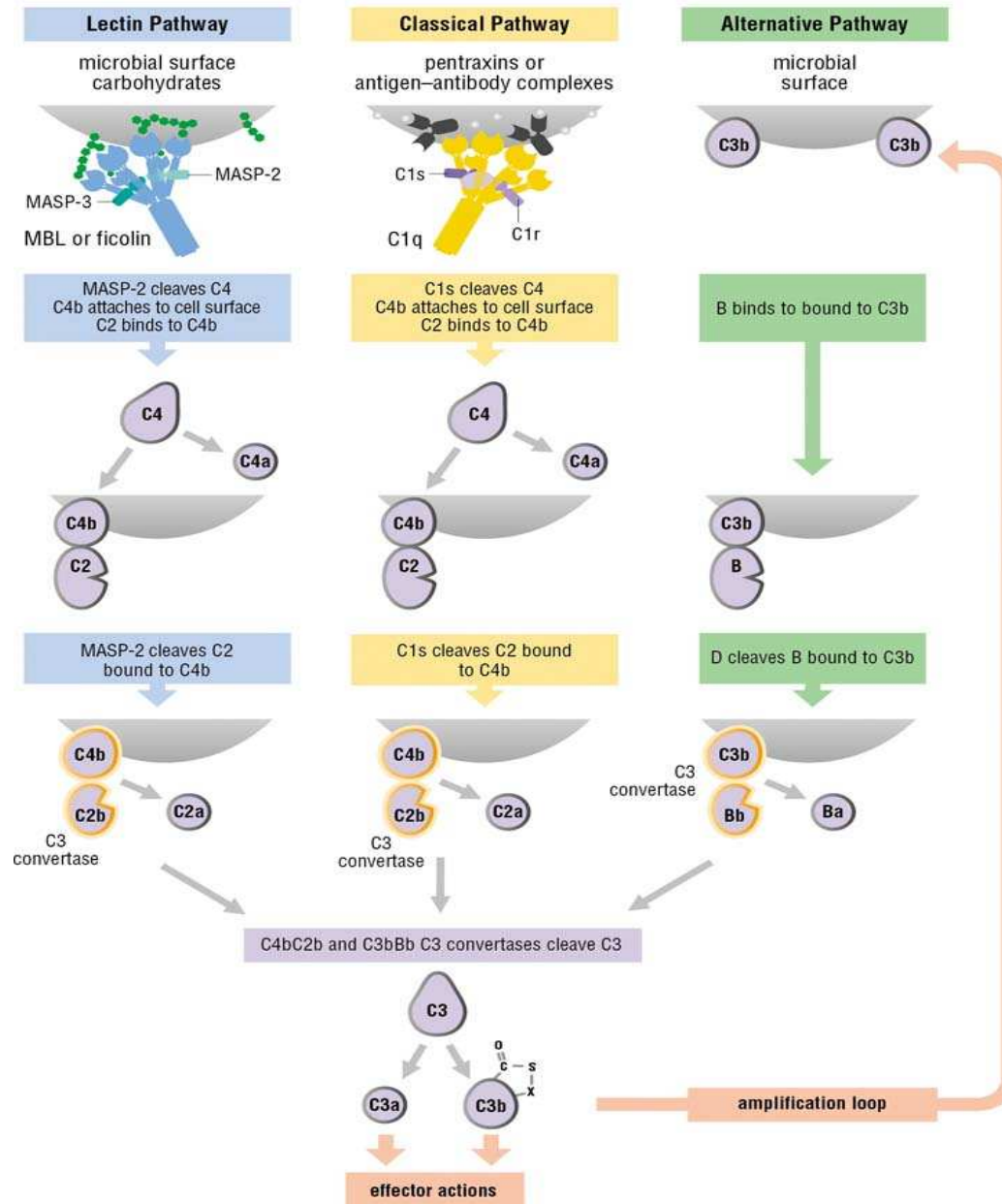
# FILOGENIA DEL SISTEMA DEL COMPLEMENTO

# COMPONENTES Y RECEPTORES DEL COMPLEMENTO

Major Complement Components and Receptors				
Component	Role in Complement Cascade and Actions			
C1qrs	binding to IgG or IgM or pentraxins and activation of classical pathway			
MASP-1, -2, -3	proteases activated by MBL or ficolins to initiate lectin pathway			
C2, C4	cleavage by C1s or MASP-2 generates C4b, which attaches to membranes, and C2b, which, associated with C4b forms the classical C3 convertase, the protease domain of which is contained in C2b			
C3	cleaved by C3 convertases. C3b attaches to particles and promotes phagocytic uptake; C3b combines with factor B to form alternative pathway C3 convertase; C3b acts together with C3 convertases to form C5 convertases; C3a induces inflammation			
factors B, D	B combines with C3b and is cleaved by D to yield the alternative pathway C3 convertase (C3bBb). The protease domain of this convertase is contained within the Bb subunit			
properdin	binds to C3bBb (alternative pathway C3 convertase), stabilizing it			
C5	cleaved by C5 convertase; C5b directs assembly of membrane-attack complex; C5a is proinflammatory peptide, neutrophil chemoattractant			
C6, C7, C8, C9	associate with C5b and are assembled into the membrane attack complex, which forms pores in membranes. Multiple copies of C9 are incorporated into the pore structure			
iC3b	cleavage fragment of C3b that opsonizes microbes, inducing phagocytosis			
C3d	cleavage fragment of C3b that binds to CR2 and promotes antibody production by B cells			
Receptor	Type	Ligand	Cells Expressing	Main Functions
CR1 (CD35)	SCR	C3b	B, E, M, N, Eos, FDC	promote cleavage of C3b and C4b, inactivate C3 convertases, limit alternative pathway, promote clearance of immune complexes
CR2 (CD21)	SCR	iC3b, C3d	B, FDC	promote antibody production
CR3 (CD11b/CD18)	integrin	iC3b, ICAM-1, $\beta$ -glucans	NK, M, N, FDC, Ma	phagocytosis
CR4 (CD11c/CD18)	integrin	iC3b, fibronectin	NK, M, N, DC, Ma	phagocytosis
CR1g	Ig	C3b, iC3b	Kupffer	phagocytosis of particles in blood
C3aR	GPCR	C3a	Ba, Ma, SM	induce inflammation, smooth muscle contraction
C5aR (CD88)	GPCR	C5a	Ba, EC, Ma, M, N, SM	induce inflammation, chemotaxis, increase vascular permeability
C1qRp (CD93)	C-type lectin	C1q, MBL, SP-A	EC, M, N	phagocytosis
CD91, calreticulin	–	C1q, MBL, SP-A	M, NHC	phagocytosis of apoptotic cells

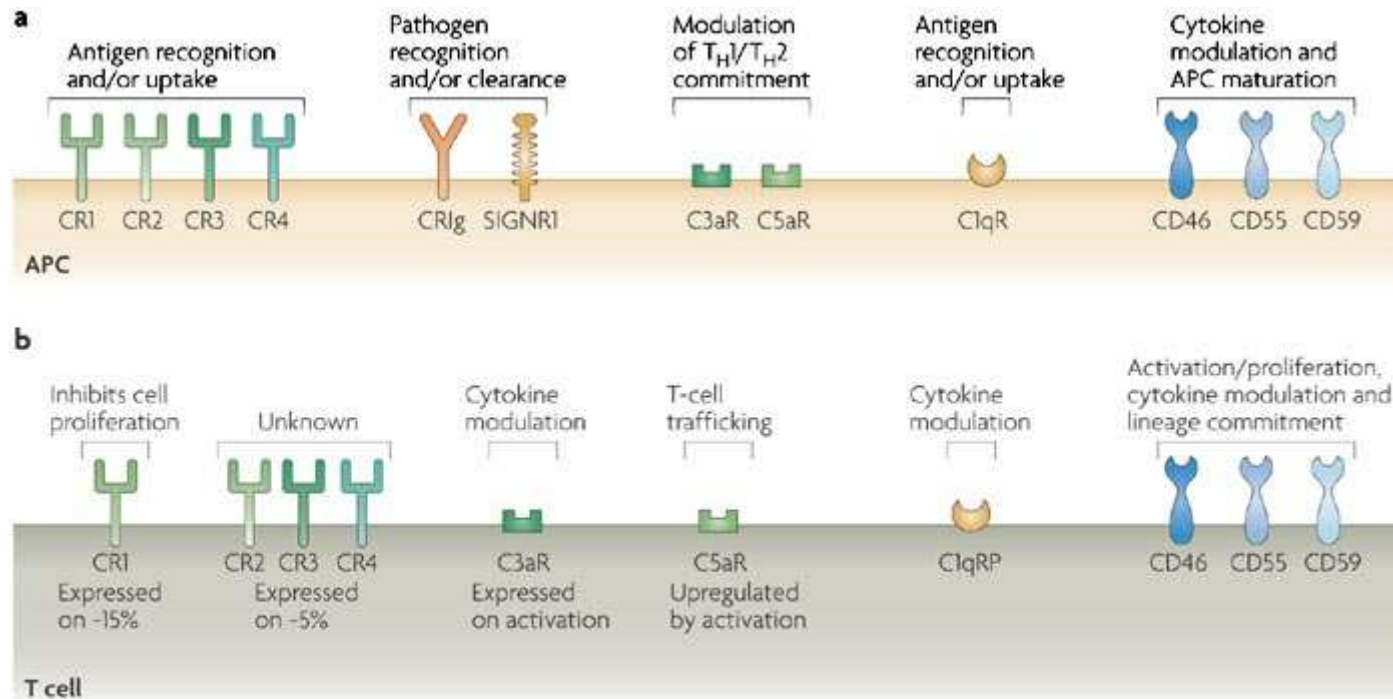


# VÍAS DE ACTIVACIÓN DEL COMPLEMENTO



# **RECEPTORES DEL COMPLEMENTO**

# EXPRESIÓN DE RECEPTORES DE COMPLEMENTO EN DISTINTOS TIPOS CELULARES

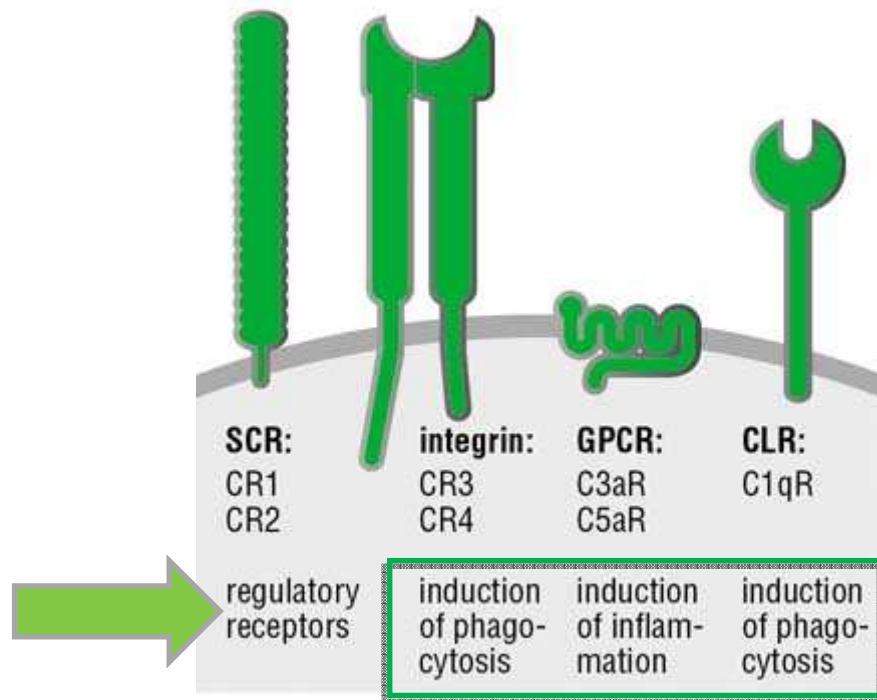


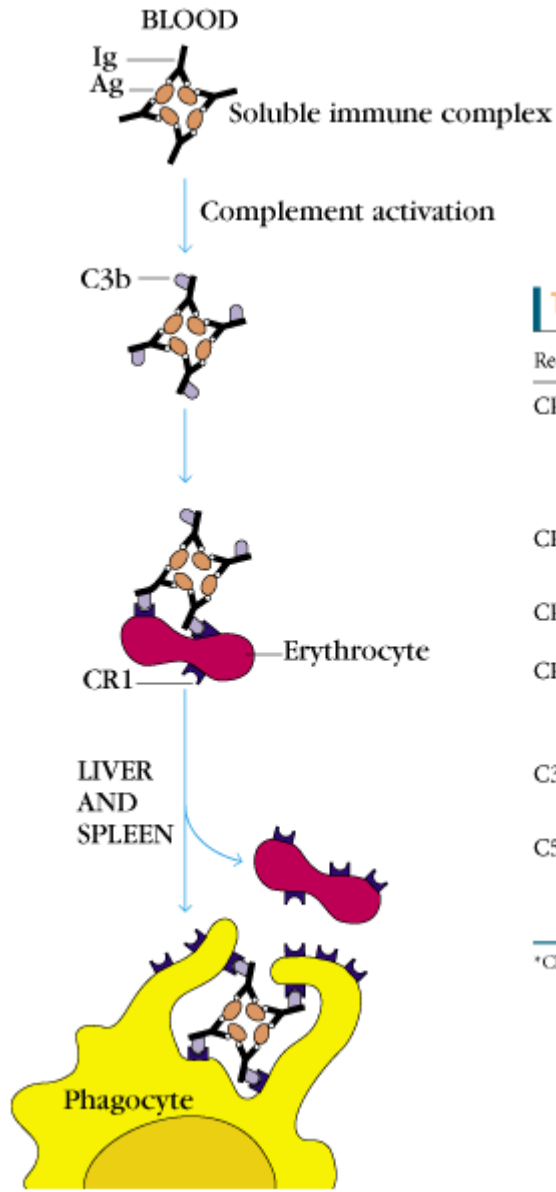
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CR1g, complement receptor of the immunoglobulin superfamily;  
SIGNR1, a mouse homologue of dendritic-cell-specific ICAM3-grabbing non-integrin (DC-SIGN)



## FUNCIONES DE LOS RECEPTORES DEL COMPLEMENTO



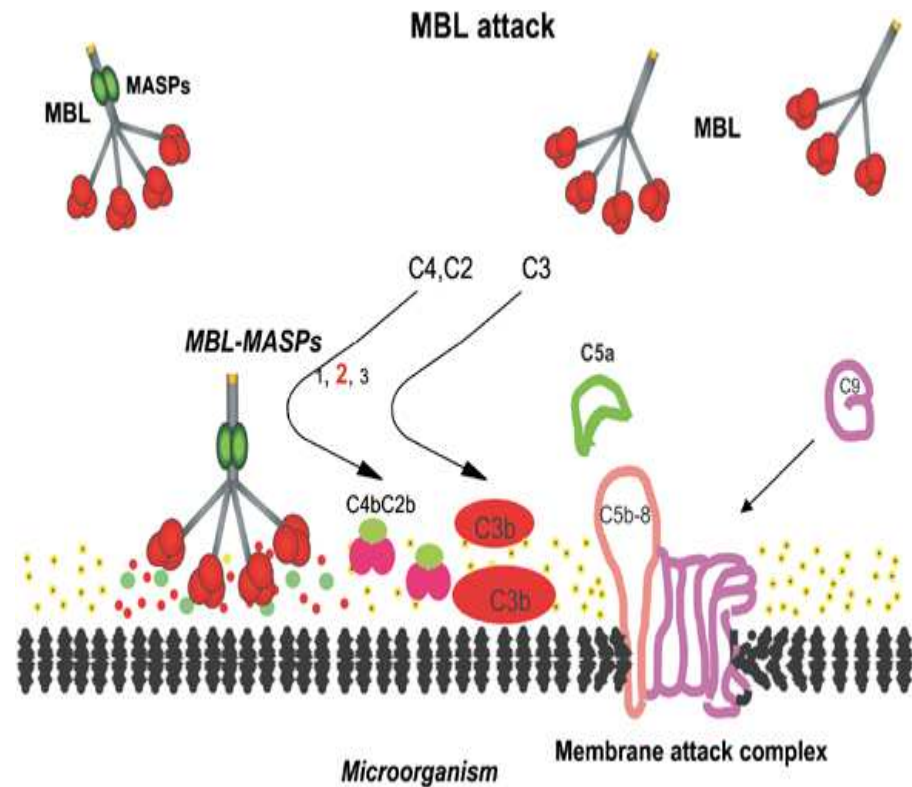
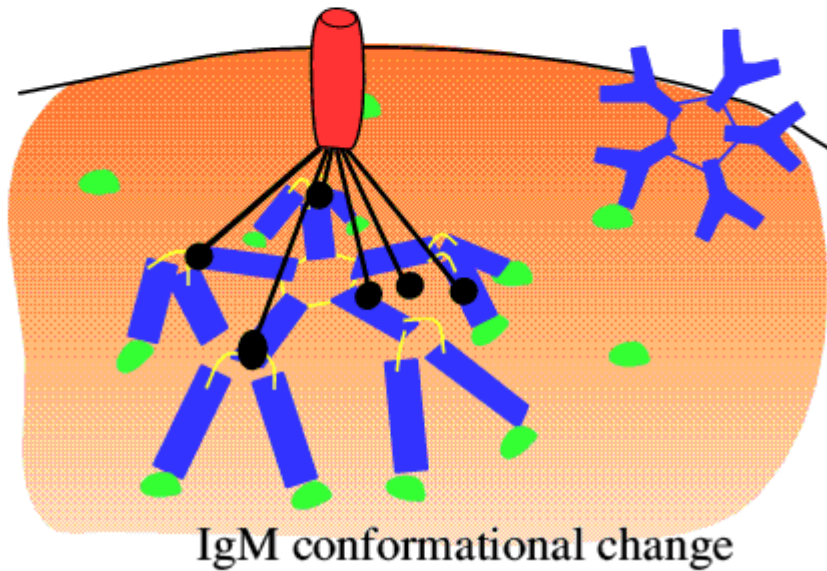
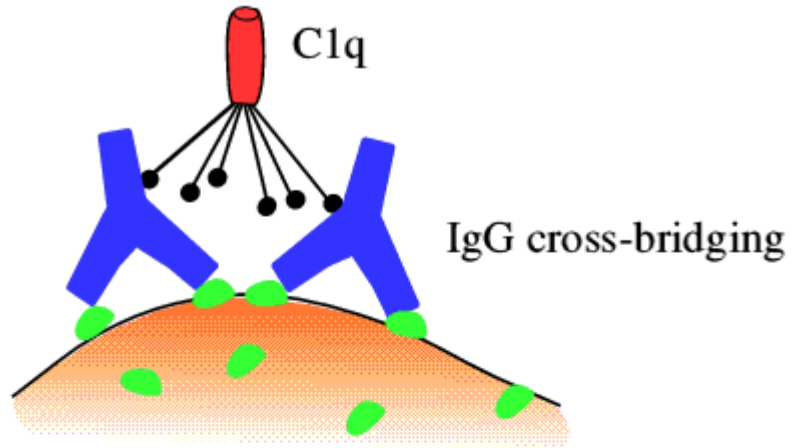


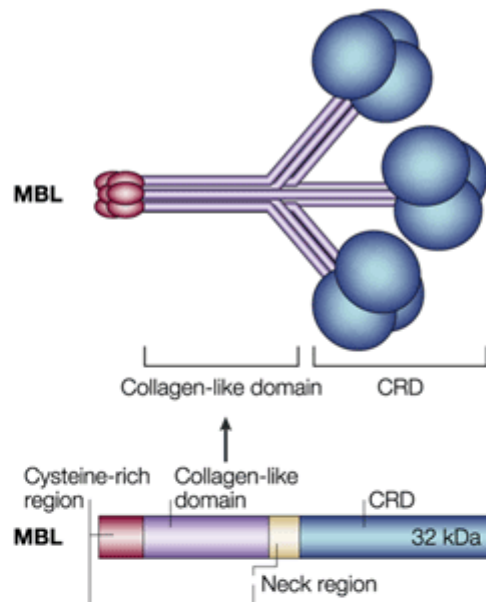
**TABLE 13-8 COMPLEMENT-BINDING RECEPTORS**

Receptor	Major ligands	Activity	Cellular distribution
CR1 (CD35)	C3b, C4b	Blocks formation of C3 convertase; binds immune complexes to cells	Erythrocytes, neutrophils, monocytes, macrophages, eosinophils, follicular dendritic cells, B cells, some T cells
CR2 (CD21)	C3d, C3dg,* iC3b	Part of B-cell coreceptor; binds Epstein-Barr virus	B cells, some T cells
CR3 (CD11b/18)	iC3b	Bind cell-adhesion molecules on neutrophils, facilitating their extravasation; bind immune complexes, enhancing their phagocytosis	Monocytes, macrophages, neutrophils, natural killer cells, some T cells
CR4 (CD11c/18)			
C3a/C4a receptor	C3a, C4a	Induces degranulation of mast cells and basophils	Mast cells, basophils, granulocytes
C5a receptor	C5a	Induces degranulation of mast cells and basophils	Mast cells, basophils, granulocytes, monocytes, macrophages, platelets, endothelial cells

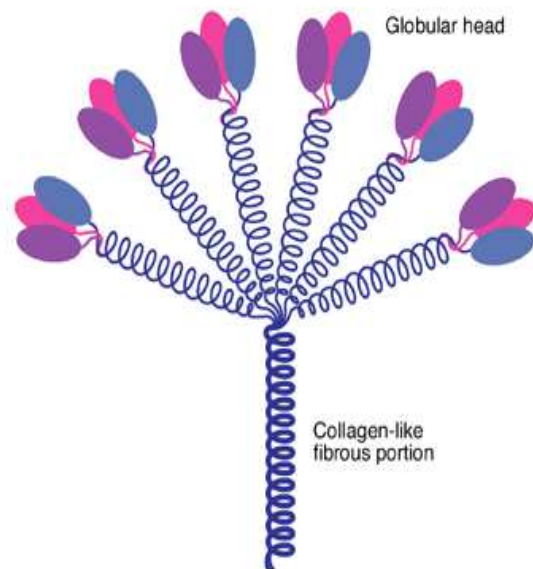
\*Cleavage of C3dg by serum proteases generates C3d and C3g.

# Activación de la VÍA CLÁSICA/MBL





## MBL/C1Q



Globular head binds to:

- antibody-antigen complexes
- damaged neurons
- myelin debris
- amyloid fibrils
- apoptotic cells
- pathogens

Collagen-like portion binds to:

- C1q receptors and C1q-binding molecules to promote phagocytosis
- serine proteases C1r and C1s to initiate activation of the classical pathway
- serum amyloid P and C-reactive protein to initiate activation of the classical pathway

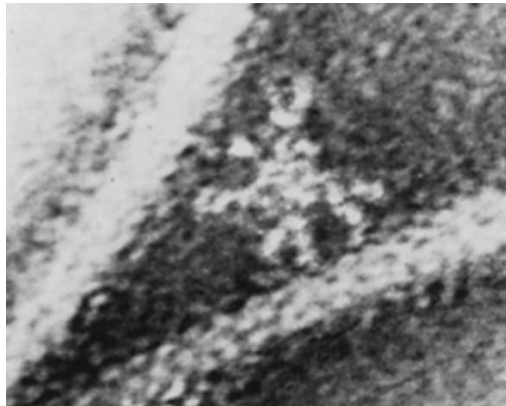
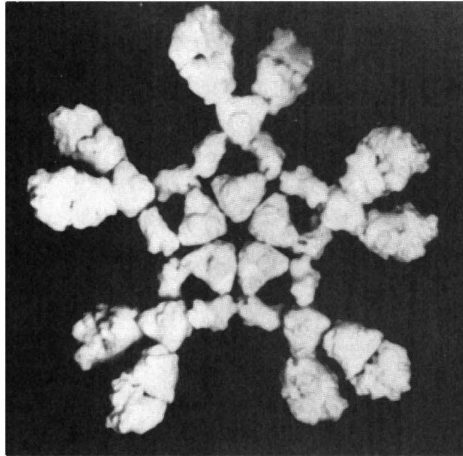
### Structure and interactions of C1q

Expert Reviews in Molecular Medicine © 2003 Cambridge University Press

# IgM

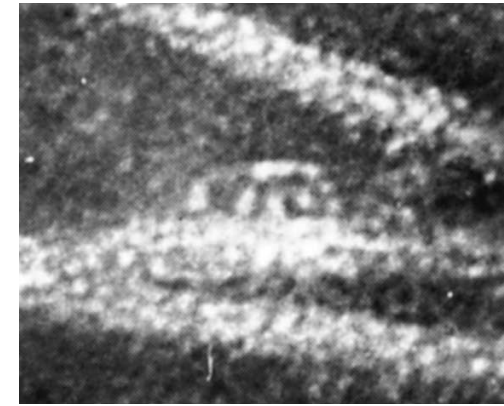
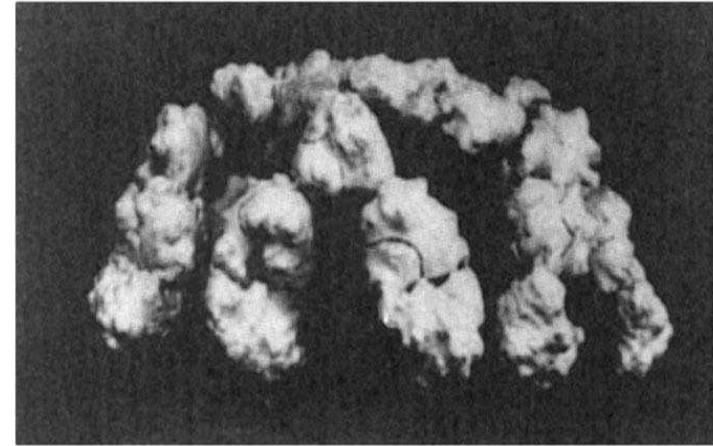
<b>Heavy chain:</b>	<i><math>\mu</math> - <math>M\mu</math></i>
<b>Half-life:</b>	<i>5 to 10 days</i>
<b>% of Ig in serum:</b>	<i>10</i>
<b>Serum level (mgml<sup>-1</sup>):</b>	<i>0.25 - 3.1</i>
<b>Complement activation:</b>	<i>++++ by <b>classical pathway</b></i>
<b>Interactions with cells:</b>	<i>Phagocytes via C3b receptors Epithelial cells via polymeric Ig receptor</i>
<b>Transplacental transfer:</b>	<i>No</i>
<b>Affinity for antigen:</b>	<i>Monomeric IgM - low affinity - valency of 2 Pentameric IgM - high avidity - valency of 10</i>

## CAMBIO CONFORMACIONAL DE IgM INDUCIDO POR EL ANTÍGENO



Planar or 'Starfish' conformation found in solution.

Does not fix complement



Staple or 'crab' conformation of IgM  
Conformation change induced by

binding to antigen.

Efficient at fixing complement

# IgG

<b>Heavy chains:</b>	$\gamma 1 \ \gamma 2 \ \gamma 3 \ \gamma 4$ - Gamma 1 - 4			
<b>Half-life:</b>	<i>IgG1</i>	21 - 24 days	<i>IgG2</i>	21 - 24 days
	<i>IgG3</i>	7 - 8 days	<i>IgG4</i>	21 - 24 days
<b>Serum level (mgml<sup>-1</sup>):</b>	<i>IgG1</i>	5 - 12	<i>IgG2</i>	2 - 6
	<i>IgG3</i>	0.5 - 1	<i>IgG4</i>	0.2 - 1
<b>% of Ig in serum:</b>	<i>IgG1</i>	45 - 53	<i>IgG2</i>	11 - 15
	<i>IgG3</i>	3 - 6	<i>IgG4</i>	1 - 4
<b>Complement activation:</b>	<b>IgG1</b>	+++	<b>IgG2</b>	+
	<b>IgG3</b>	+++++	<i>IgG4</i>	No
<b>Interactions with cells:</b>	<i>All subclasses via IgG receptors on macrophages and phagocytes</i>			
<b>Transplacental transfer:</b>	<i>IgG1</i>	++	<i>IgG2</i>	+
	<i>IgG3</i>	++	<i>IgG4</i>	++

**The neonatal Fc $\gamma$  receptor may be responsible!**

# IgA

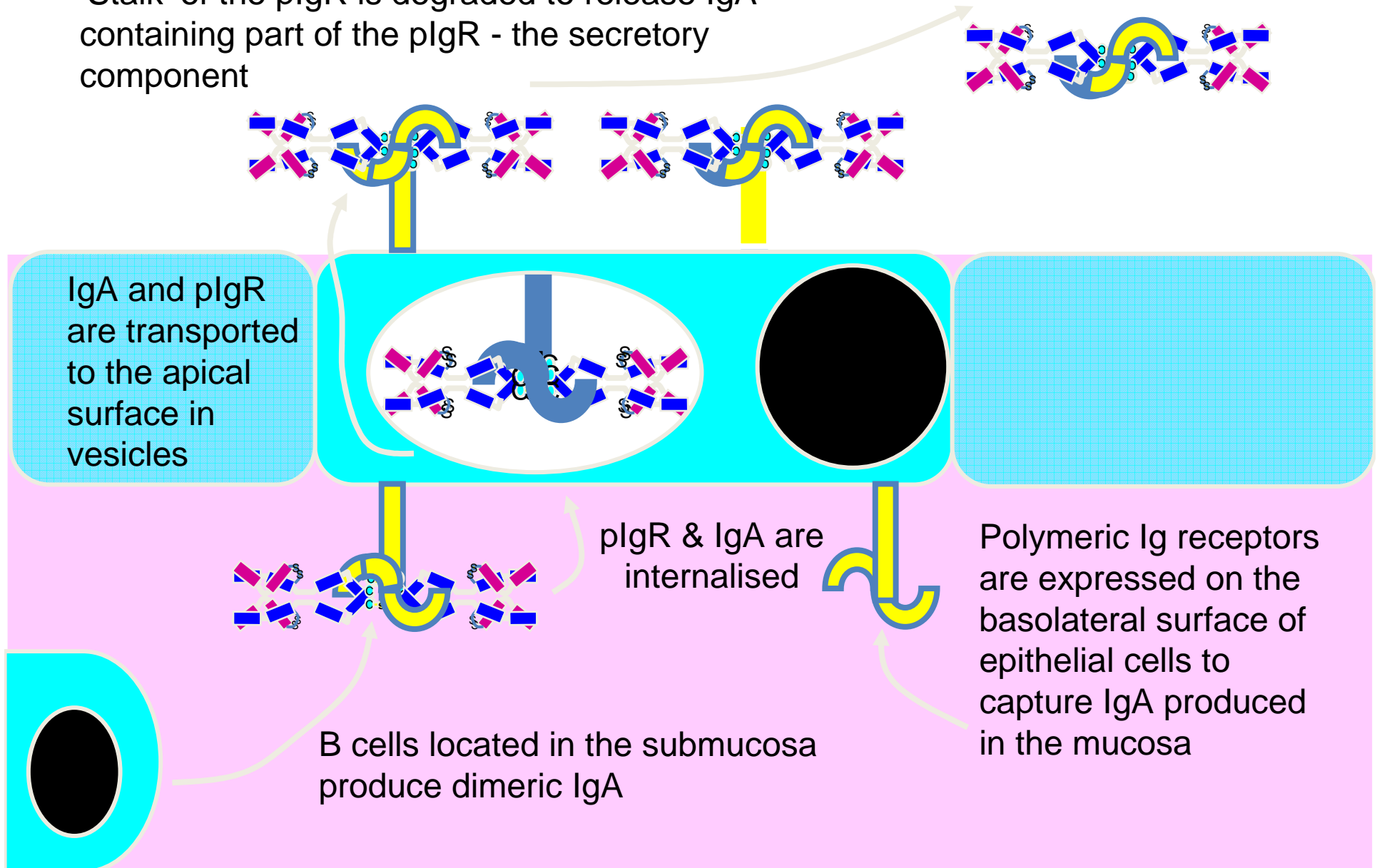
<b>Heavy chains:</b>	<i><math>\alpha 1</math> or <math>\alpha 2</math> - Alpha 1 or 2</i>
<b>Half-life:</b>	<i>IgA1: 5 - 7 days IgA2: 4 - 6 days</i>
<b>Serum levels (mgml<sup>-1</sup>):</b>	<i>IgA1: 1.4 - 4.2 IgA2: 0.2 - 0.5</i>
<b>% of Ig in serum:</b>	<i>IgA1 11 - 14 IgA2 1 - 4</i>
<b>Complement activation:</b>	<i>IgA1 - <b>by alternative and lectin pathway</b> IgA2 - No</i>
<b>Interactions with cells:</b>	<i>Epithelial cells by pIgR Phagocytes by IgA receptor</i>
<b>Transplacental transfer:</b>	<i>No</i>

To reduce vulnerability to microbial proteases the hinge region of IgA2 is truncated, and in IgA1 the hinge is heavily glycosylated.



# IgA SECRETORIA

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



# LIGANDOS de MBL

Table 1 Some clinically relevant microorganisms recognized by MBL

Bacteria	Viruses	Fungi	Protozoa
<i>Staphylococcus aureus</i>	HIV-1 and 2	<i>Aspergillus fumigatus</i>	<i>Plasmodium falciparum</i>
<i>Streptococcus pneumoniae</i>	Herpes simplex 2	<i>Candida albicans</i>	<i>Cryptosporidium parvum</i>
<i>Streptococcus pyogenes</i>	Influenza A	<i>Cryptococcus neoformans</i>	<i>Trypanosoma cruzi</i>
<i>Enterococcus spp.</i>	Hepatitis B virus	<i>Saccharomyces cerevisiae</i>	
<i>Listeria monocytogenes</i>	Hepatitis C virus		
<i>Haemophilus influenzae</i>			
<i>Neisseria meningitidis</i>			
<i>Neisseria gonorrhoeae</i>			
<i>Escherichia coli</i>			
<i>Klebsiella spp.</i>			
<i>Pseudomonas aeruginosa</i>			
<i>Salmonella montevideo</i>			
<i>Salmonella typhimurium</i>			
<i>H. pylori</i>			
<i>Chlamydia trachomatis</i>			
<i>Chlamydia pneumoniae</i>			
<i>Propionibacterium acnes</i>			
<i>Mycobacterium avium</i>			
<i>Mycobacterium tuberculosis</i>			
<i>Mycobacterium leprae</i>			
<i>Leishmania chagasi</i>			

Table 1

## The endogenous ligands of MBL.

### Endogenous ligands of MBL

#### Dying cells

- Apoptosis
- Necrosis

#### Ischemic tissues

- Myocardial reperfusion injury
- Renal reperfusion injury
- Gastrointestinal reperfusion injury

#### Anoxic endothelial cells

- Endothelial oxidative stress

#### Transformed cells

- Colon adenocarcinoma
- Colorectal carcinoma

#### Immunoglobulins

- Agalactosyl IgG
- Dimeric/polymeric IgA
- IgM

#### Nucleic acids

- DNA
- RNA

#### Phospholipids

- Phosphatidylserine
- Phosphatidylinositol
- Phosphatidylcholine

#### Zinc metalloproteases

- Meprin  $\alpha$  and  $\beta$

# **ACTIVACIÓN DE LA VÍA ALTERNA**

## ACTIVACIÓN DE LA VÍA ALTERNATIVA

- Se inicia con **C3b** generado por las vías anteriores o por hidrólisis espontánea del agua (Tickover).
  - **Se une a patógenos y partículas de origen microbiano** (antígeno O, ácido teicoico, zimosán, tripanosomas).
  - **Se une a no patógenos:** IgG<sub>4</sub>, IgA, IgE humanos en complejos, factor veneno de cobra, polímeros aniónicos, carbohidratos puros...
- La **PROPERDINA** es sintetizada por los monocitos, macrófagos, **PMNs**, Eo, Ba, Células T, precursores de MO.
  - **C5a** induce la liberación de P por los PMNs, TCD4 y macrófagos.
  - En la **inflamación crónica**, el INF- $\gamma$  liberado por Th1 inhibe la secreción de P por el macrófago, y en ese momento el TCD4 es el principal secretor de dicho factor.

**TABLE 13-3 INITIATORS OF THE ALTERNATIVE PATHWAY OF COMPLEMENT ACTIVATION**

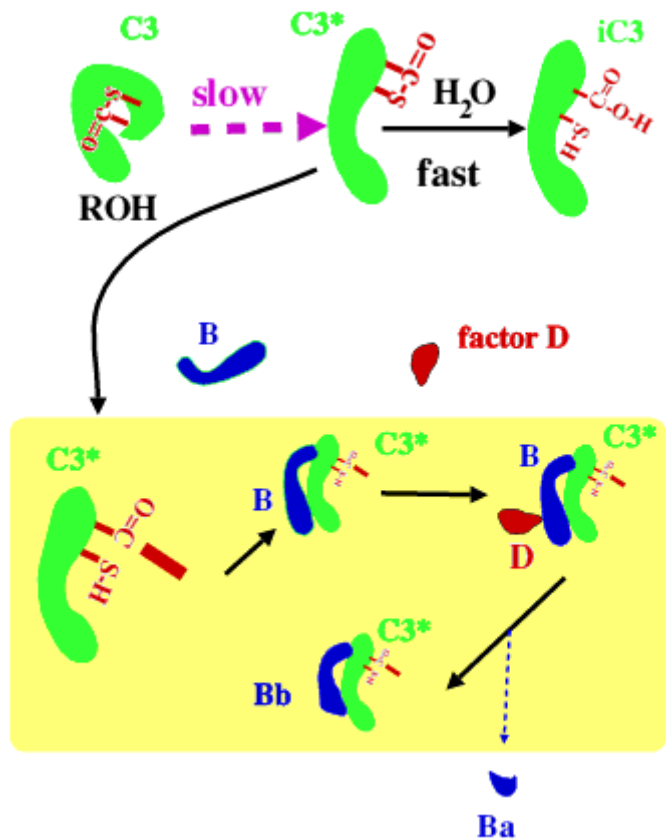
Pathogens and particles of microbial origin
Many strains of gram-negative bacteria
Lipopolysaccharides from gram-negative bacteria
Many strains of gram-positive bacteria
Teichoic acid from gram-positive cell walls
Fungal and yeast cell walls (zymosan)
Some viruses and virus-infected cells
Some tumor cells (Raji)
Parasites (trypanosomes)
Nonpathogens
Human IgG, IgA, and IgE in complexes
Rabbit and guinea pig IgG in complexes
Cobra venom factor
Heterologous erythrocytes (rabbit, mouse, chicken)
Anionic polymers (dextran sulfate)
Pure carbohydrates (agarose, inulin)

SOURCE: Adapted from MK Pangburn, 1986, in *Immunobiology of the Complement System*, Academic Press.

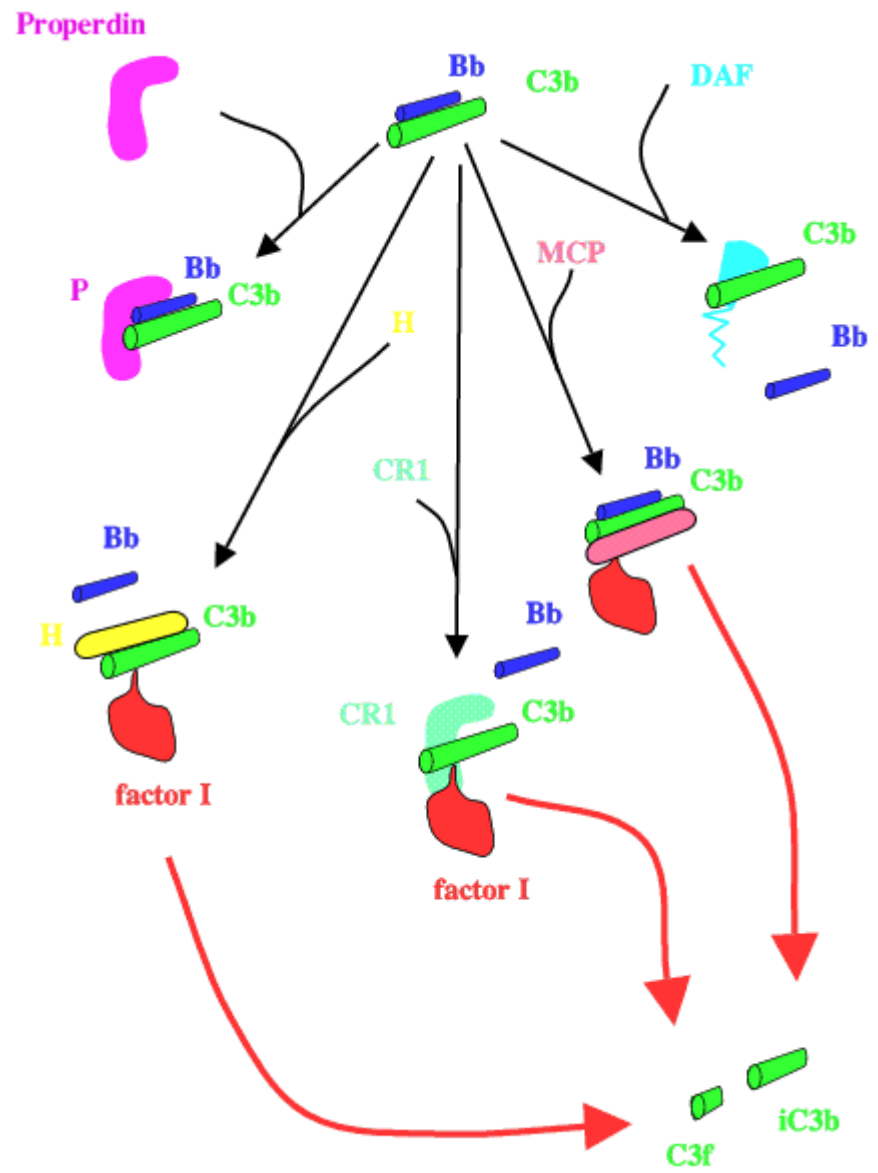
**TABLE 13-4 COMPLEMENT COMPONENTS IN THE FORMATION OF C3 AND C5 CONVERTASES**

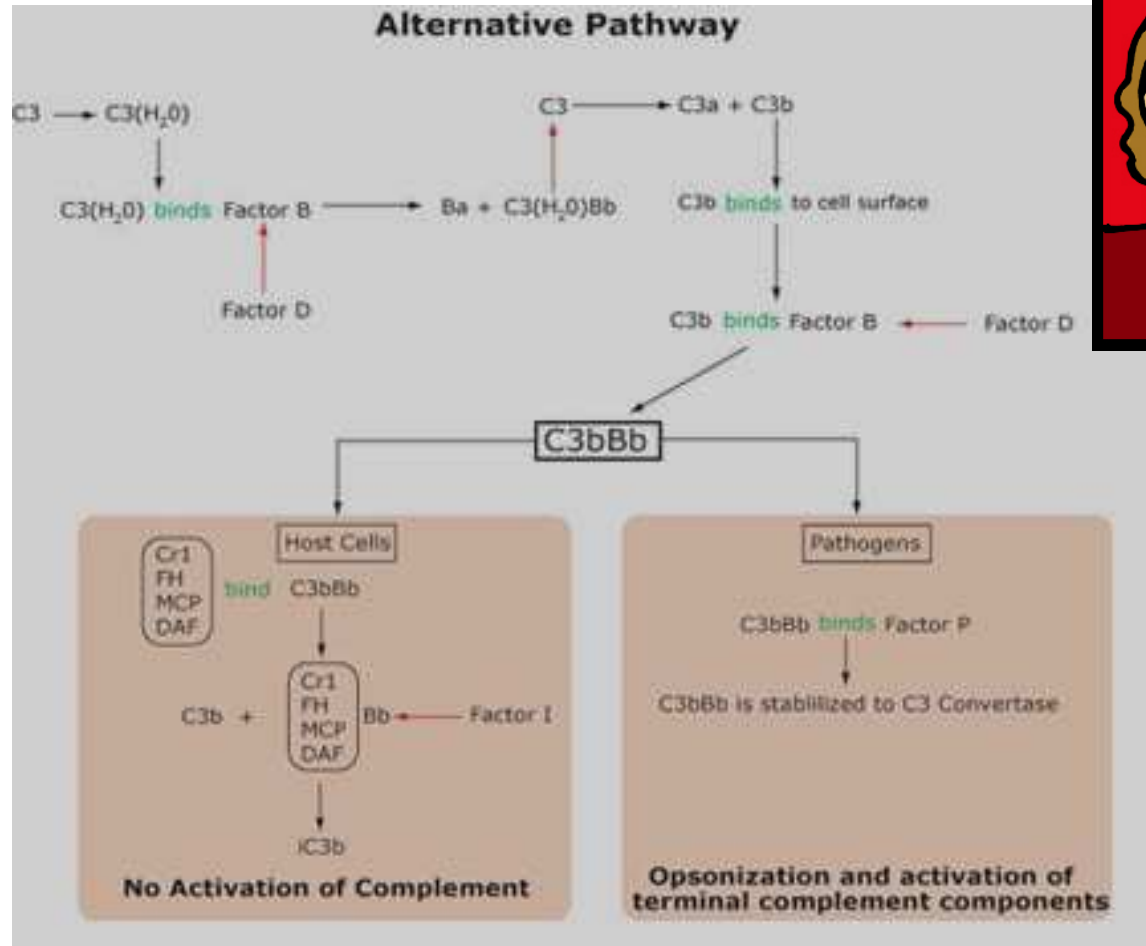
	Classical pathway	Lectin pathway	Alternative pathway
Precursor proteins	C4 + C2	C4 + C2	C3 + factor B
Activating protease	C1s	MASP	Factor D
C3 convertase	C4b2a	C4b2a	C3bBb
C5 convertase	C4b2a3b	C4b2a3b	C3bBb3b
C5-binding component	C3b	C3b	C3b

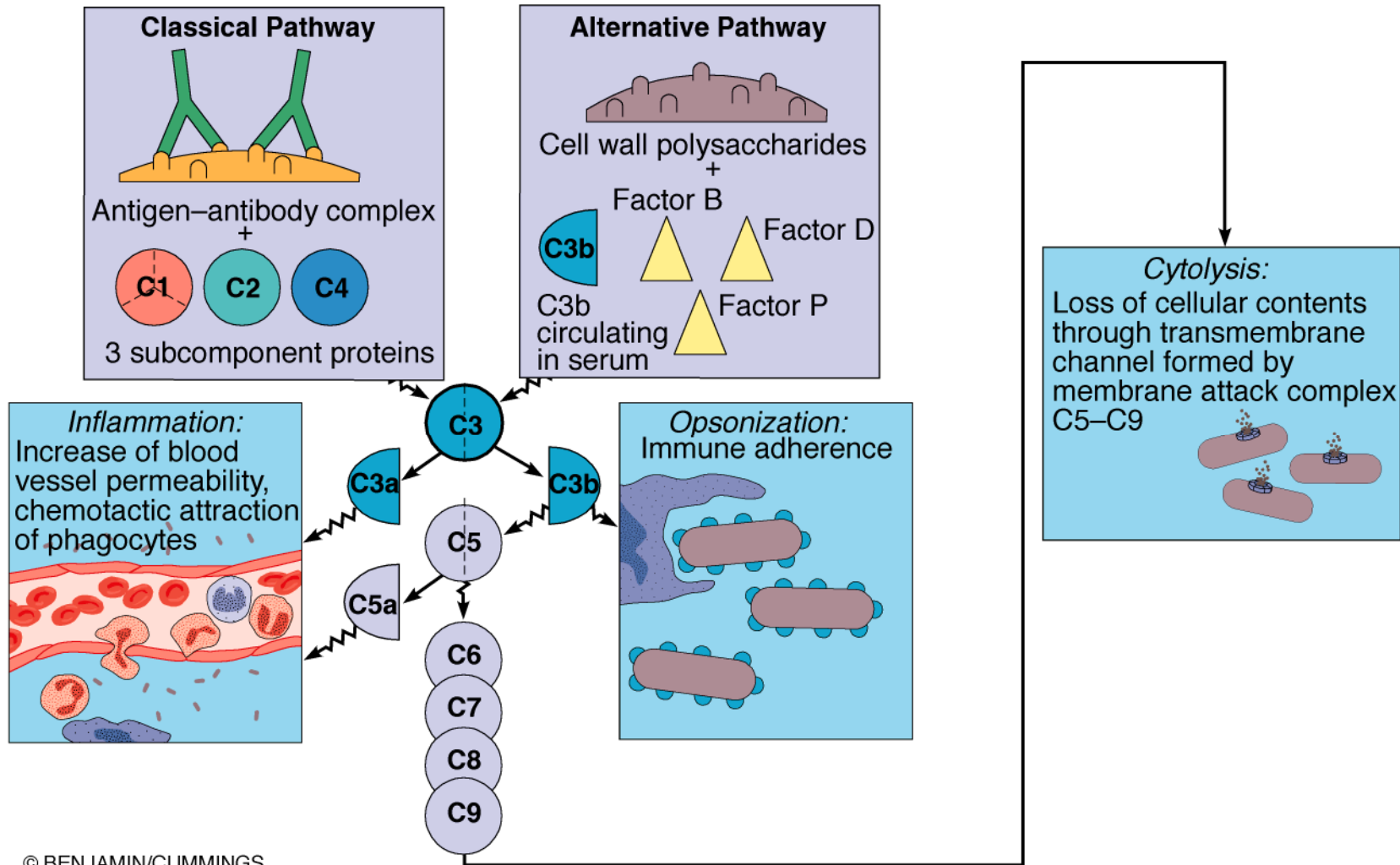
LA NUEVA NOMENCLATURA ESTABLECE QUE C2a ES EL PÉPTIDO QUE SE LIBERA y C2b el que forma la C3 CONVERTASA y C5 CONVERTASA.



**Vía alterna**









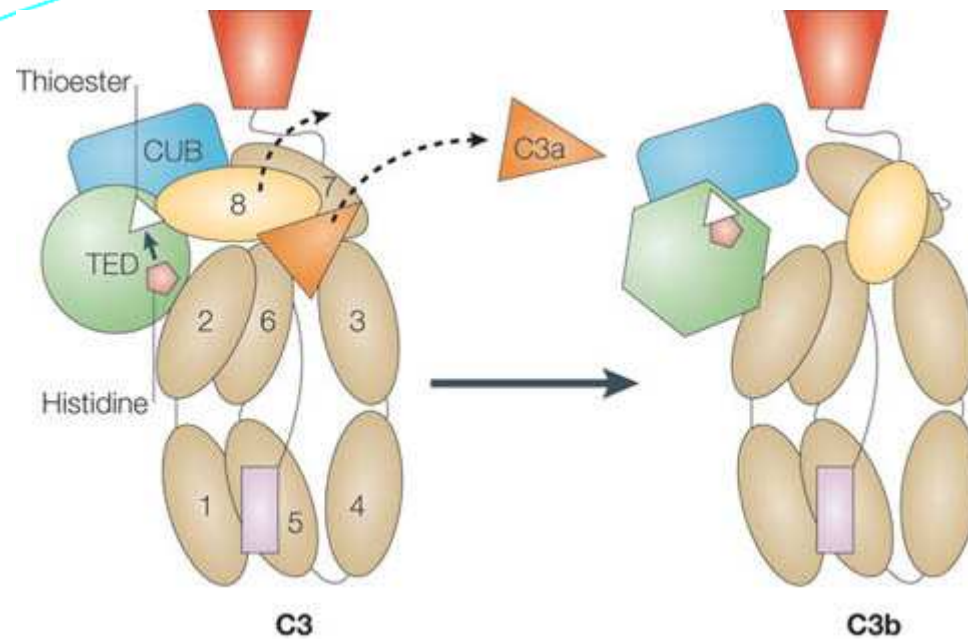
# **PÉPTIDOS DE LA ACTIVACIÓN DEL COMPLEMENTO**

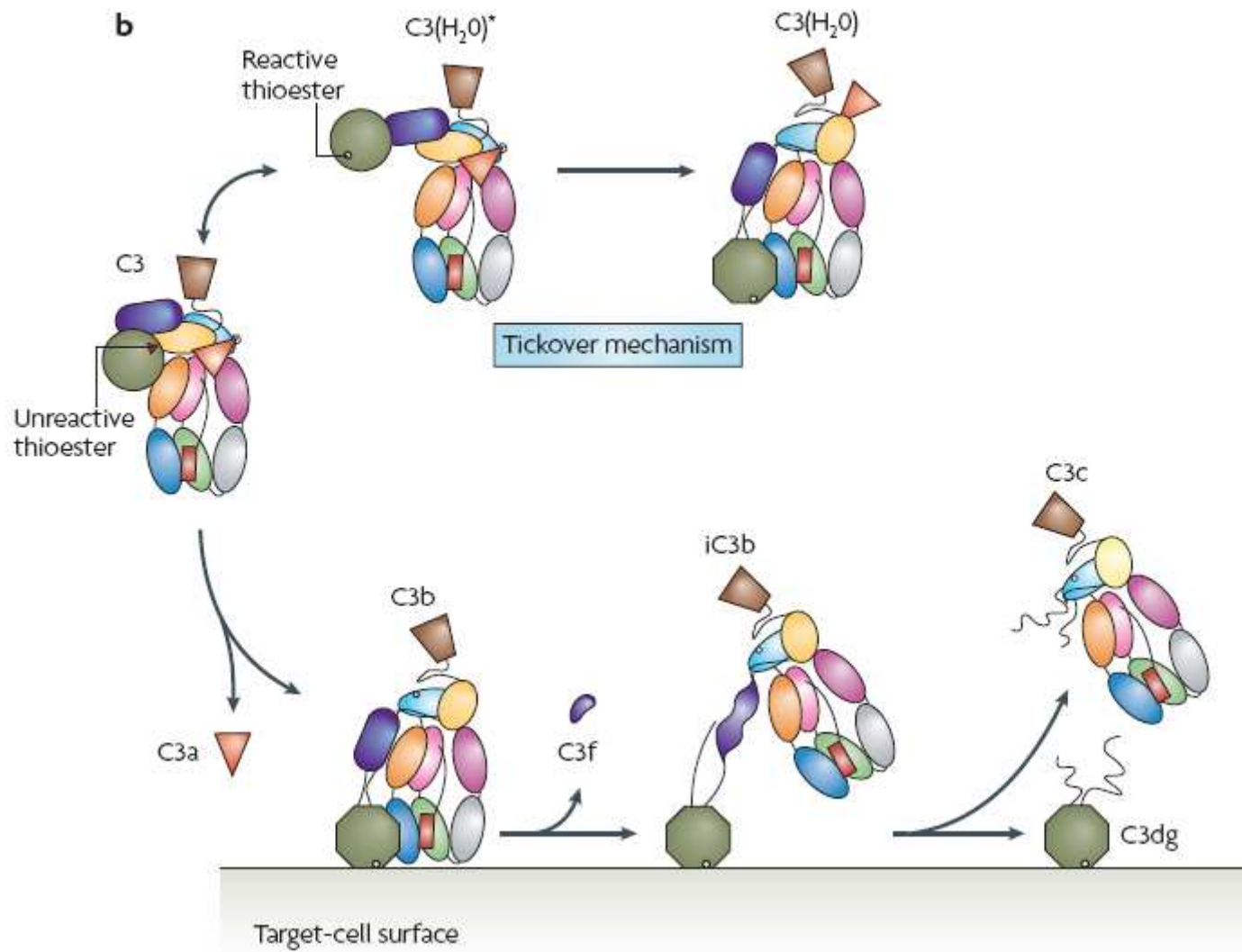
This is a small soluble fragment  
It leads to increased vascular permeability  
and recruitment and activation of phagocytes.  
of the microorganisms by PMN and macrophages.

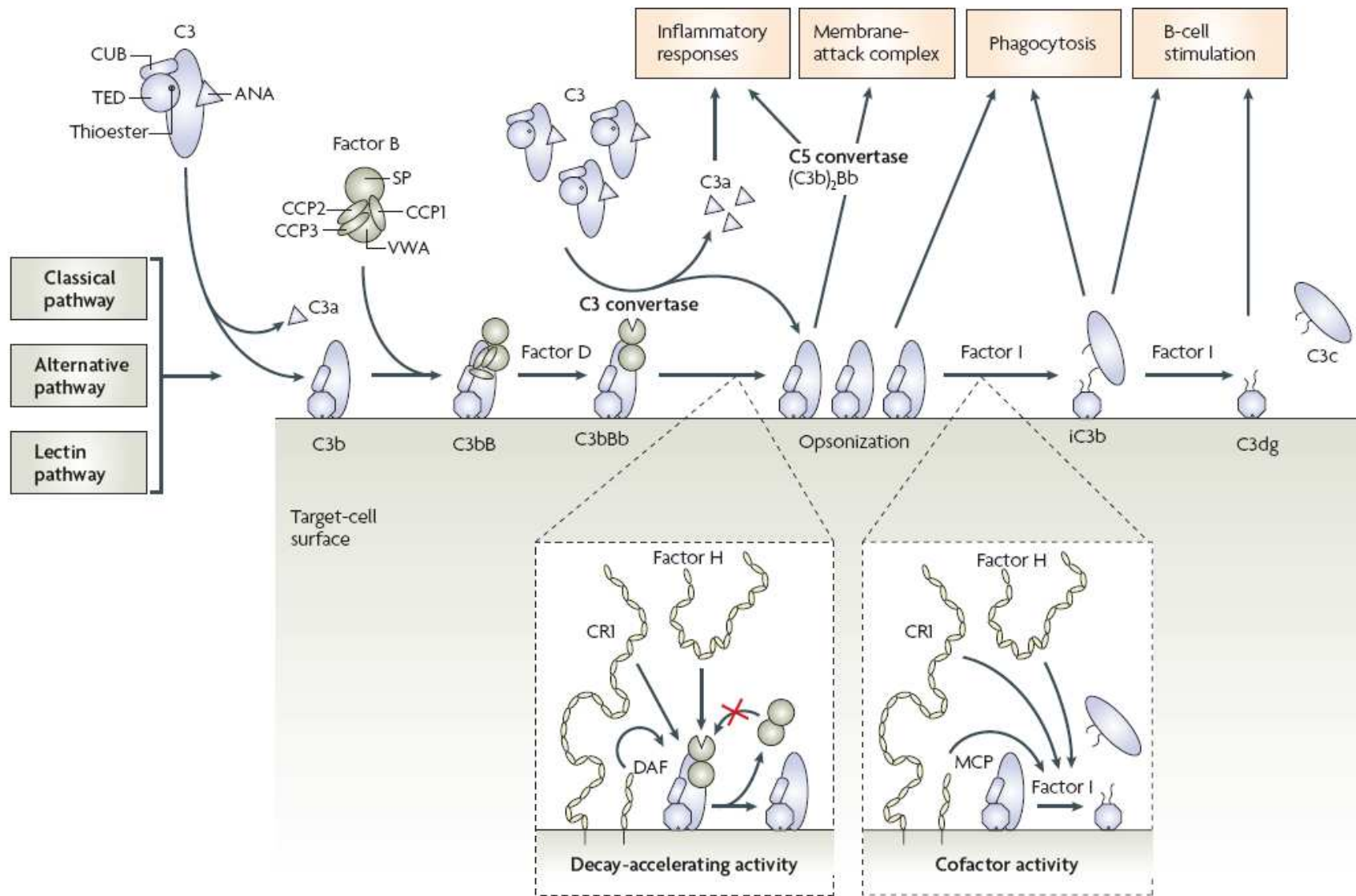


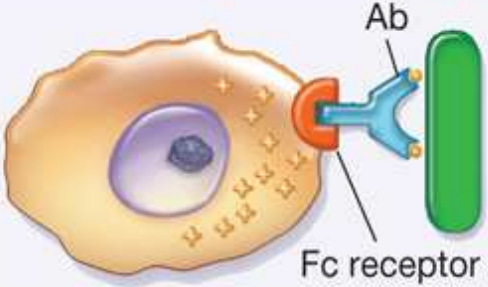
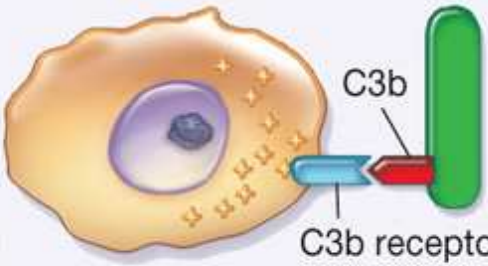

This is the large fragment and contains the now highly reactive thiolester.  
It acts mainly by covalently tagging bacteria etc .  
The C3b tag enhances the recognition, phagocytosis and killing  
of the microorganisms by PMN and macrophages.

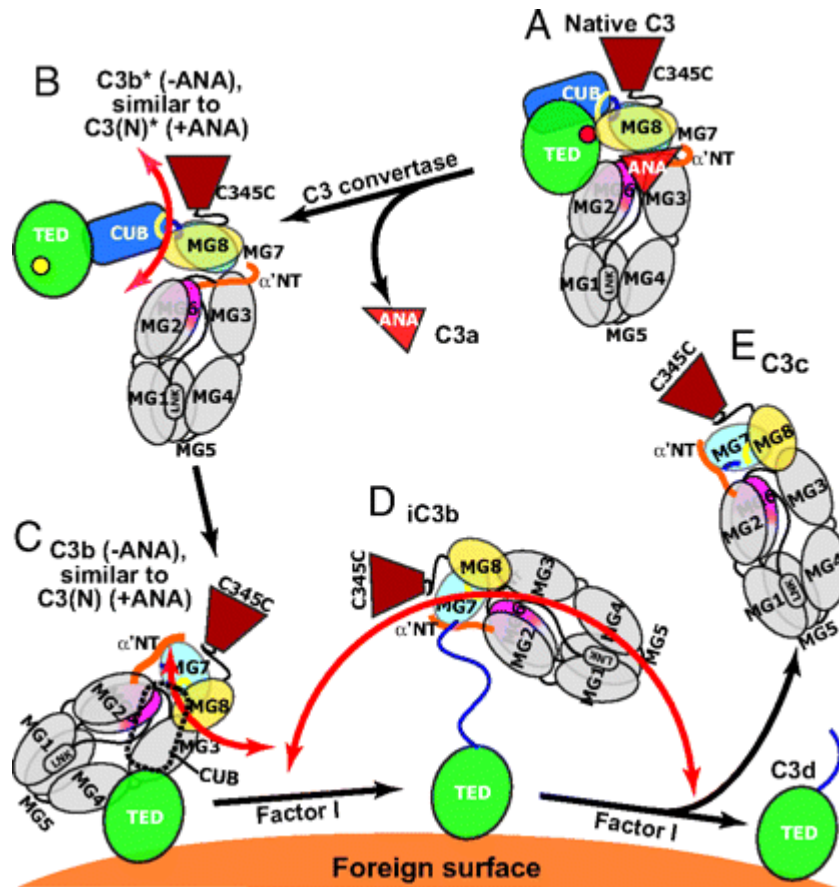
**This tagging is called opsonisation.**





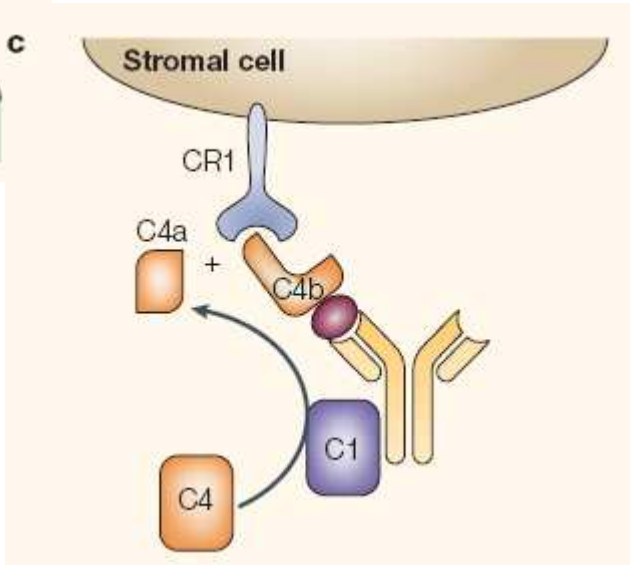
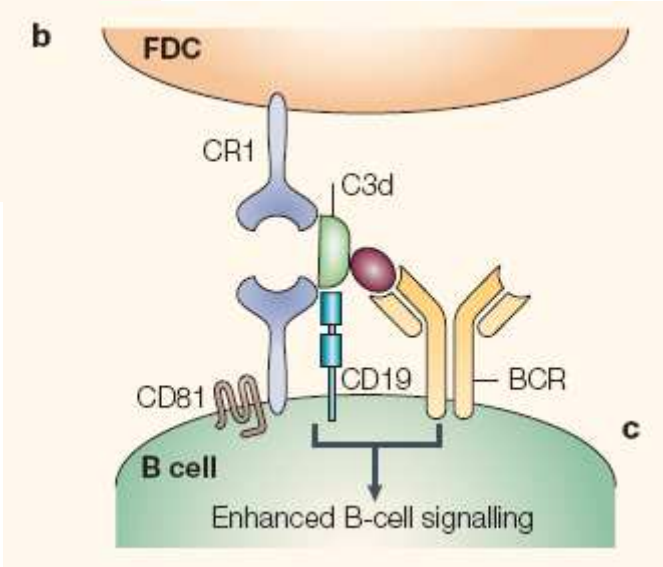
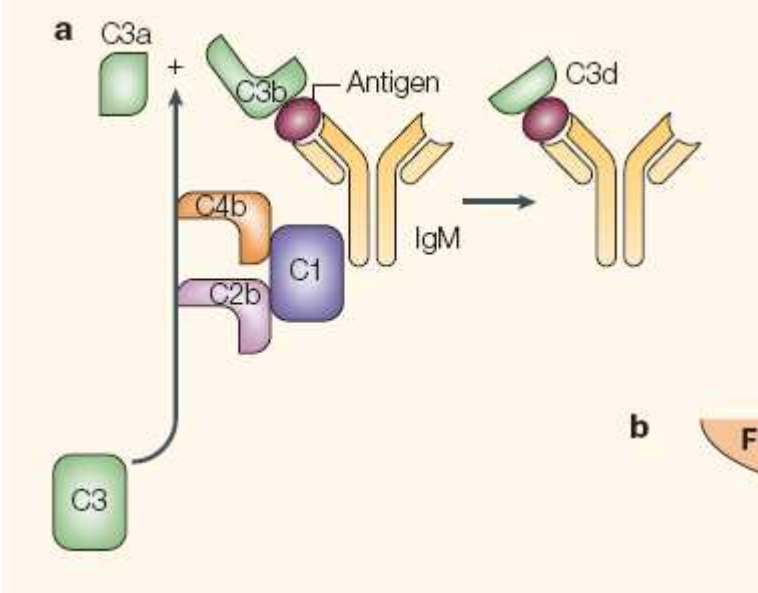


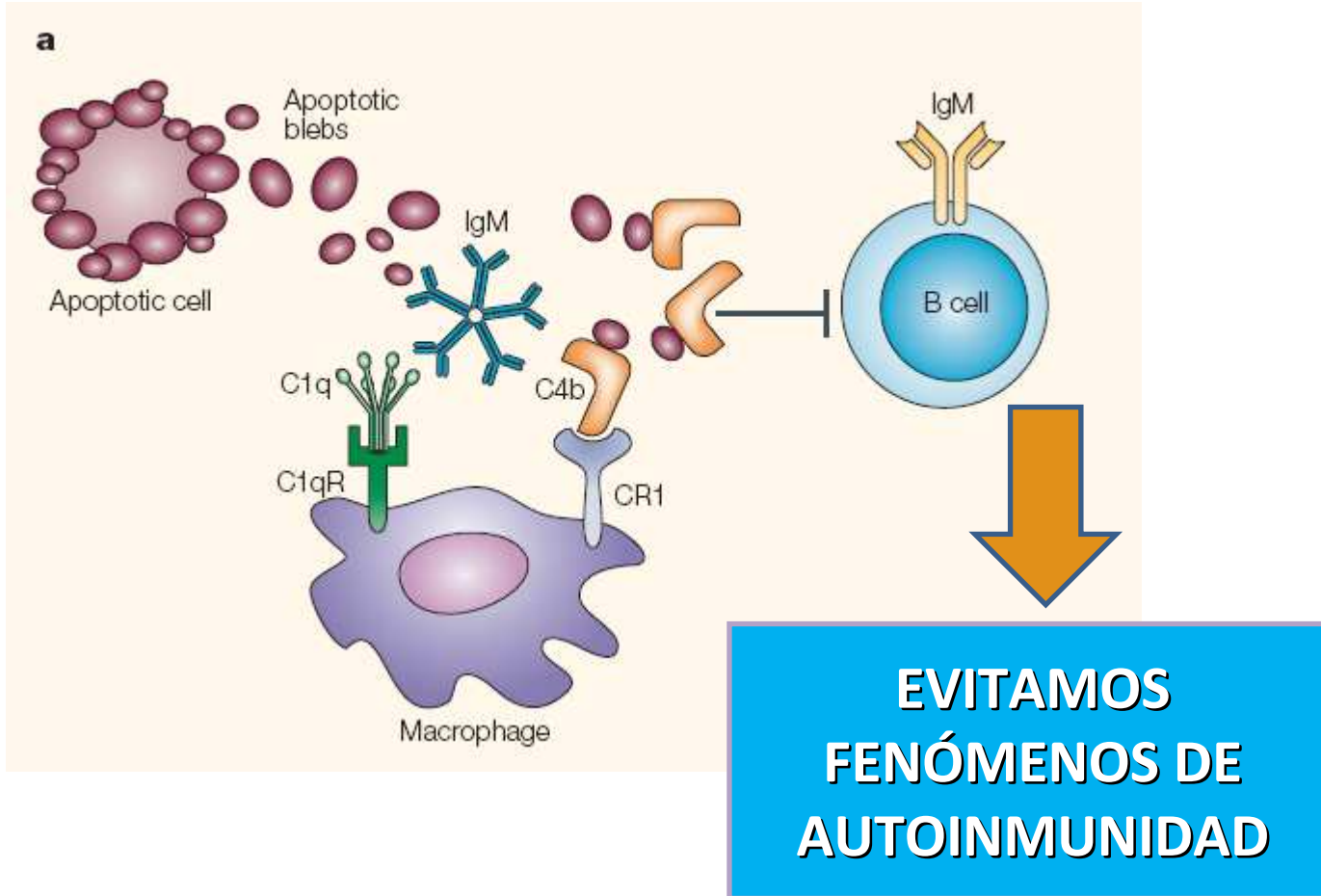
Phagocytic cell	Degree of binding	Opsonin
<p>(a)</p>  <p>Ab</p> <p>Fc receptor</p>	+	Antibody
<p>(b)</p>  <p>C3b</p> <p>C3b receptor</p>	++	Complement C3b
<p>(c)</p> 	++++	Antibody and complement C3b



Schematic diagram of domain rearrangements and flexibility in C3 products. The positions of domains are schematically represented. The CUB domain in C3(N) and C3b is shown with a dashed outline, because it did not provide clear density in EM class averages. The unfolded N-terminal portion of the CUB segment in iC3b is shown as a blue line. The range of mobility of the key ring moiety relative to the TED is represented with orange arrows. (N) represents nucleophiles added to the thioester including  $H_2O$  and  $CH_3NH_2$ .

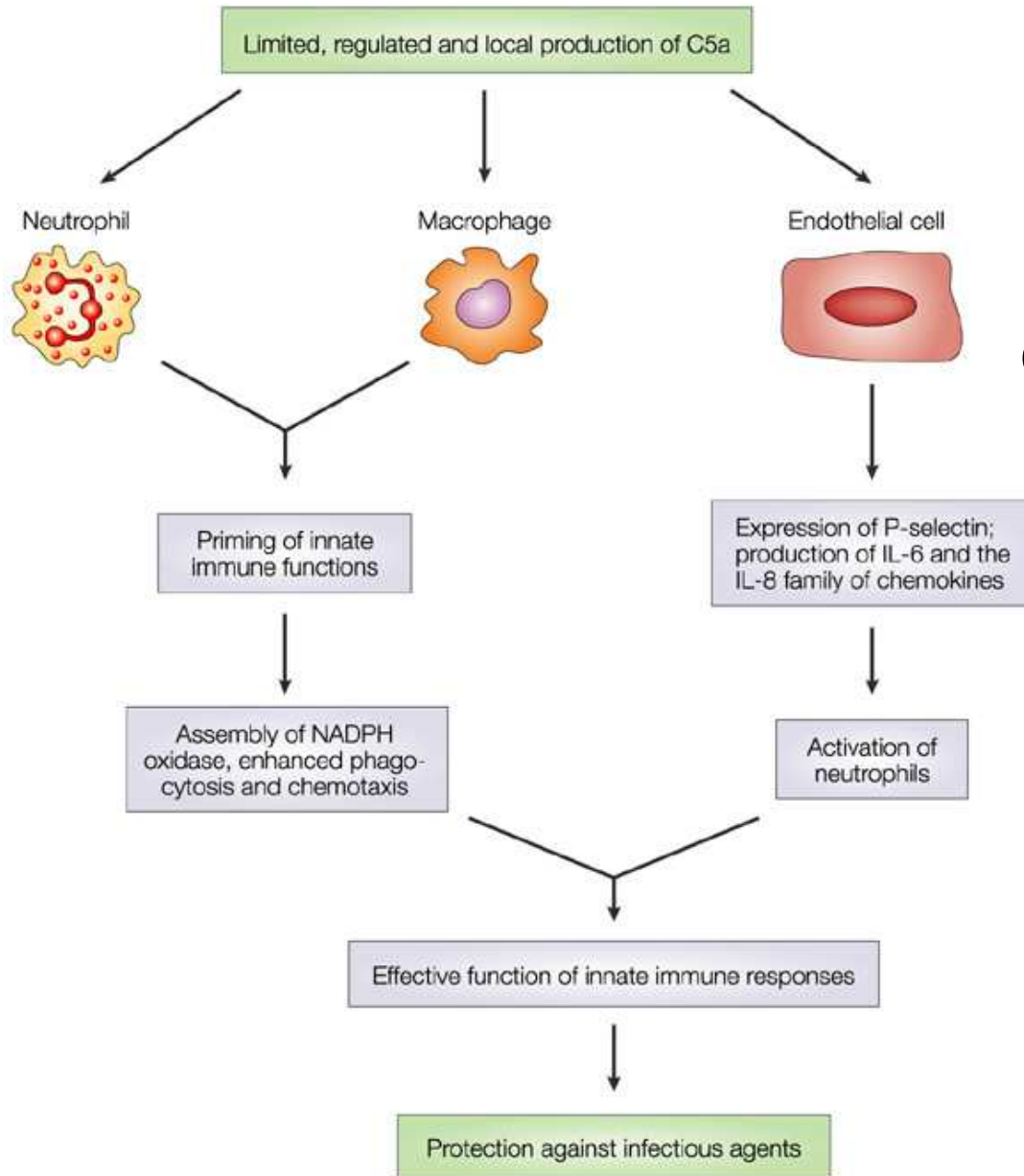
# FUNCIÓN DE PÉPTIDOS DERIVADOS DE LA ACTIVACIÓN DEL COMPLEMENTO





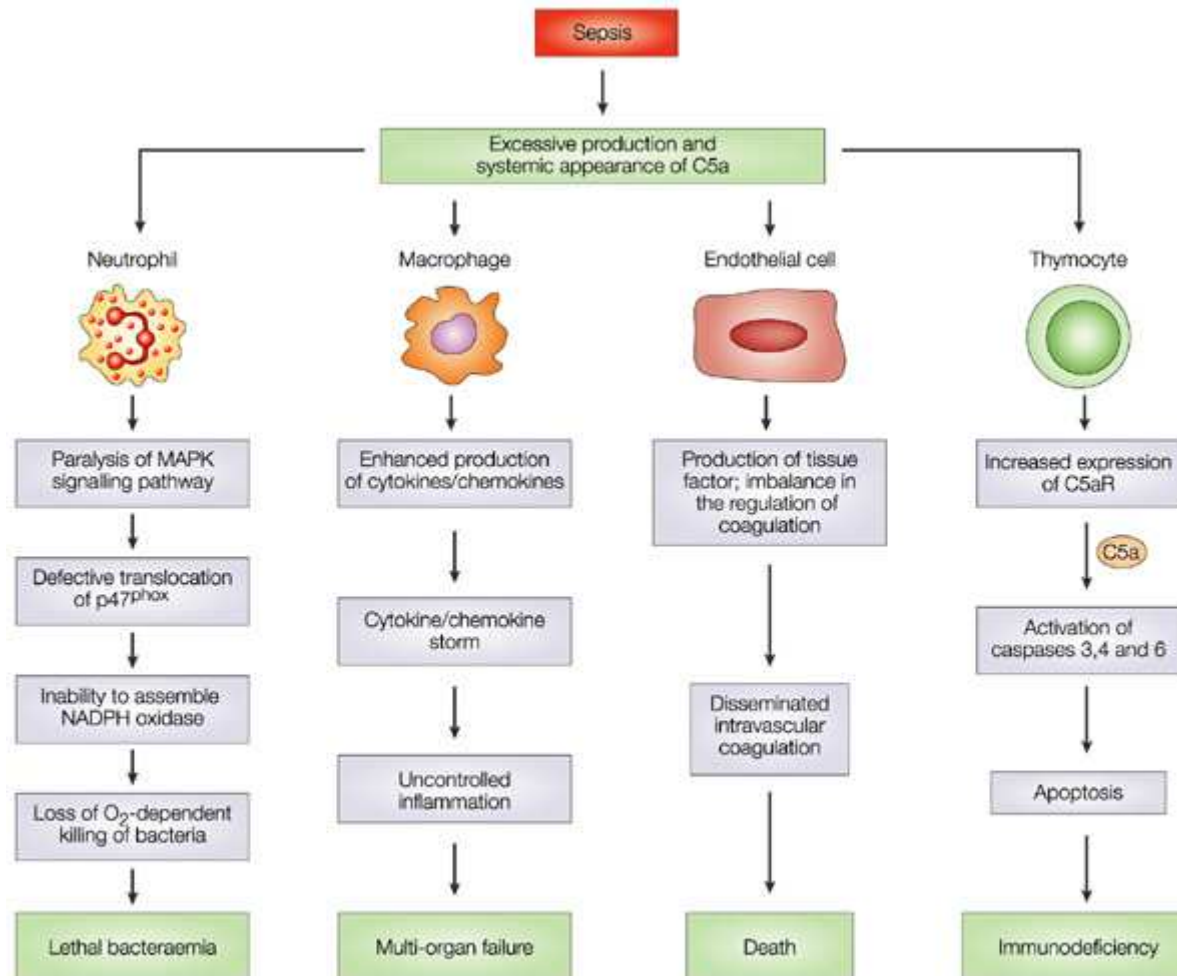


# ROL DEL C5a en condiciones fisiológicas

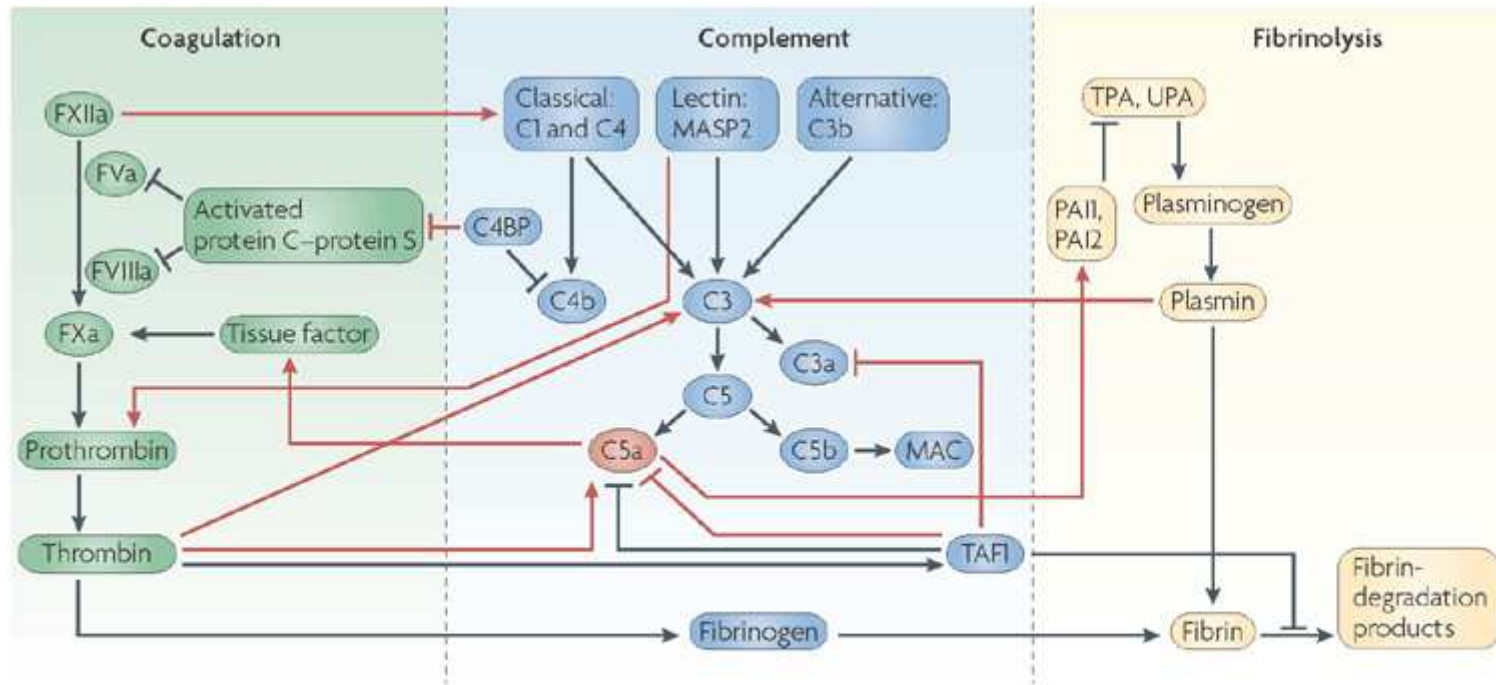


Nature Reviews Immunology 4, 133-142 (February 2004)

# ROL DEL C5a en la SEPSIS



# CROSS-TALK ENTRE SISTEMA DE COAGULACIÓN, COMPLEMENTO Y FIBRINÓLISIS



Nature Reviews | Immunology

# Efectos biológicos de productos del complemento

Effect	Complement product mediating*
Cell lysis	C5b–9, the membrane-attack complex (MAC)
Inflammatory response	
Degranulation of mast cells and basophils†	C3a, C4a, and C5a (anaphylatoxins)
Degranulation of eosinophils	C3a, C5a
Extravasation and chemotaxis of leukocytes at inflammatory site	C3a, <b>C5a</b> , C5b67
Aggregation of platelets	C3a, C5a
Inhibition of monocyte/macrophage migration and induction of their spreading	Bb
Release of neutrophils from bone marrow	C3c
Release of hydrolytic enzymes from neutrophils	C5a
Increased expression of complement receptors type 1 and 3 (CR1 and CR3) on neutrophils	C5a
Opsonization of particulate antigens, increasing their phagocytosis	<b>C3b</b> , C4b, iC3b
Viral neutralization	C3b, C5b–9 (MAC)
Solubilization and clearance of immune complexes	C3b

\*Boldfaced component is most important in mediating indicated effect.

†Degranulation leads to release of histamine and other mediators that induce contraction of smooth muscle and increased permeability of vessels.

# **REGULACIÓN DE LA ACTIVACIÓN DEL COMPLEMENTO**

# REGULACIÓN DE LA ACTIVACIÓN DEL COMPLEMENTO

(b) Regulation, deactivation and inhibition of the complement cascade on host cells by natural regulators and complement-specific therapeutics. Several pathogenic processes and diseases are the result of an erroneous activation or insufficient downregulation of the complement cascade. Under normal conditions, any host-associated C3 convertase (C3bBb) undergoes an accelerated decay mediated by complement receptor 1 (CR1), decay accelerating factor (DAF), C4b-binding protein (C4BP) or factor H (fH). C3b is degraded to inactive iC3b by factor I in a reaction that requires as cofactor CR1, fH, C4BP or membrane cofactor protein (MCP). In addition, CD59 prevents the formation of the MAC. Some of the therapeutic interventions focus on increasing this downregulation by using soluble forms of these regulators (that is, sCR1, sDAF, sMCP, sCD59). Other approaches involve the substitution of the natural C1 inhibitor (C1-INH), the inhibition of the central conversion of C3 to C3b and C3a (compstatin), blockage of C5 or C5a by antibodies, and the suppression of anaphylatoxic signaling by C5a receptor antagonists. For clarity, only the regulation of the alternative pathway, which may contribute up to 80% of all complement activity, is shown here. The C3 convertase of the classical pathway (C4bC2a), as well as the C5 convertases (C4bC2aC3b and C3bC3bBb), can be modulated by the same regulators and drug compounds.

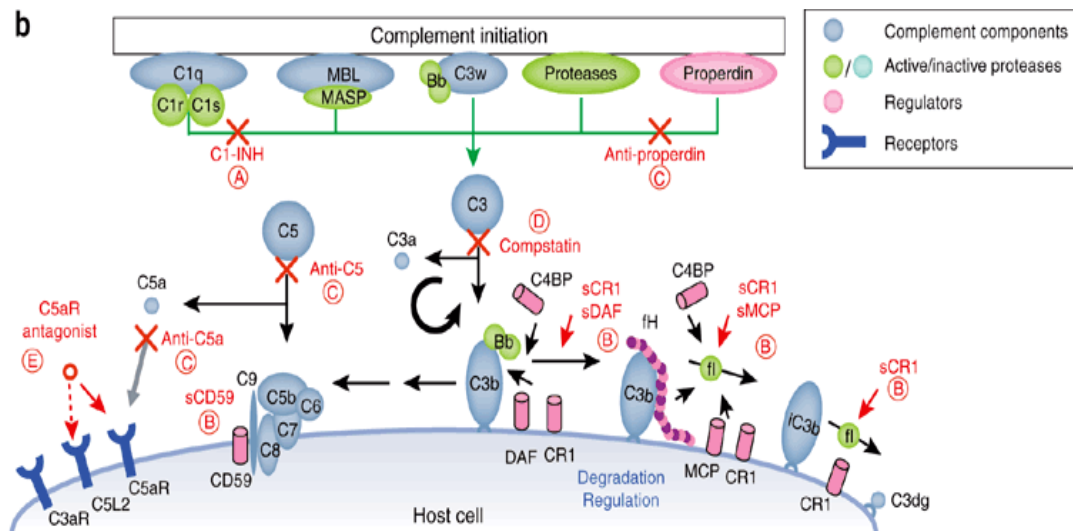


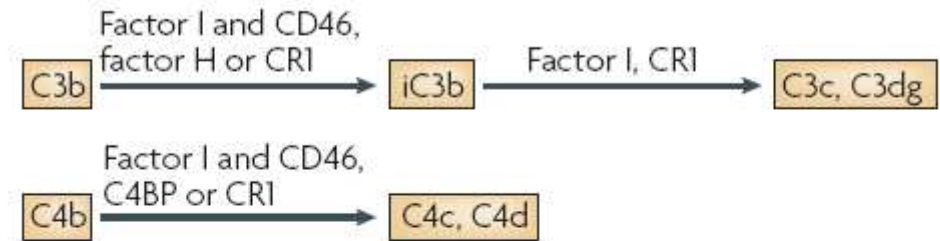
Table 2 | Complement proteins and ligands

Name	CD number(s)	Ligand(s)
<i>Complement receptors</i>		
CR1	CD35	C3b, C4b
CR2	CD21	C3d
CR3	CD18/CD11b	iC3b
CR4	CD18/CD11c	iC3b
<i>Complement regulators</i>		
MCP	CD46	C3b, C4b
DAF	CD55	Convertases, CD97
MAC inhibitor	CD59	C5b–C9
<i>Anaphylatoxin receptors</i>		
C3aR	None	C3a
C5aR	CD88	C5a
<i>Others</i>		
C1qR	CD93	C1q
CR1g	None	C3b, iC3b
SIGNR1	CD209	C1q

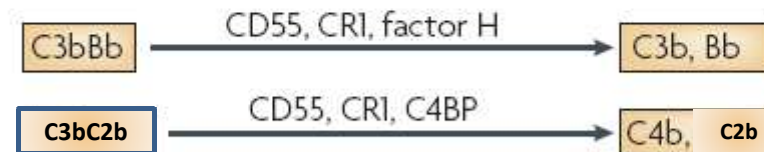
C3b, complement component 3b; C3aR, C3a receptor; CR, complement receptor; CR1g, complement receptor of the immunoglobulin superfamily; DAF, decay-accelerating factor; iC3b, inactivated form of C3b; MAC, membrane-attack complex; MCP, membrane cofactor protein; SIGNR1, a mouse homologue of dendritic-cell-specific ICAM3-grabbing non-integrin (DC-SIGN).

## b Regulation

### Cofactor activity

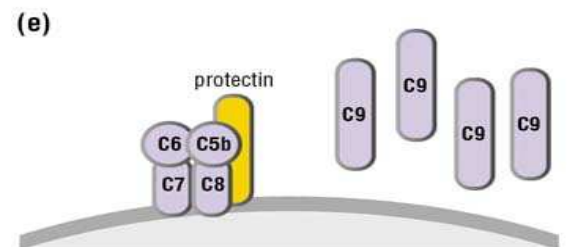
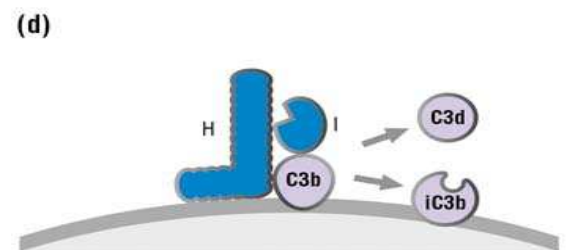
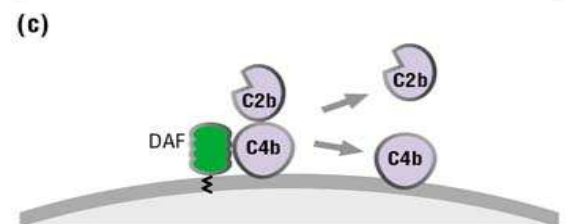
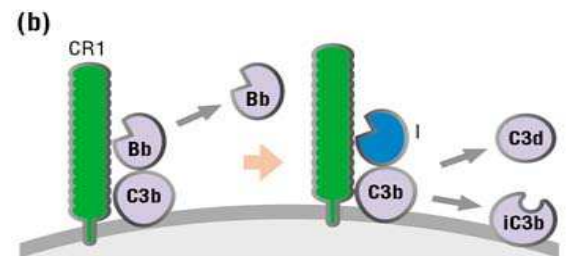
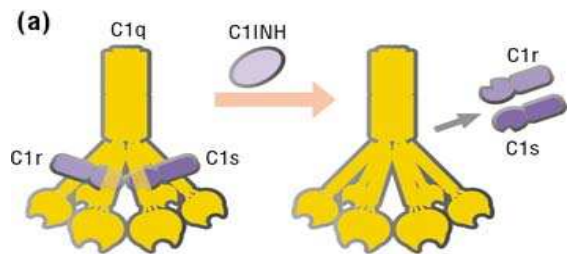


### Decay-accelerating activity for C3 convertases



### Inhibition of lysis





**TABLE 13-6 PROTEINS THAT REGULATE COMPLEMENT SYSTEM**

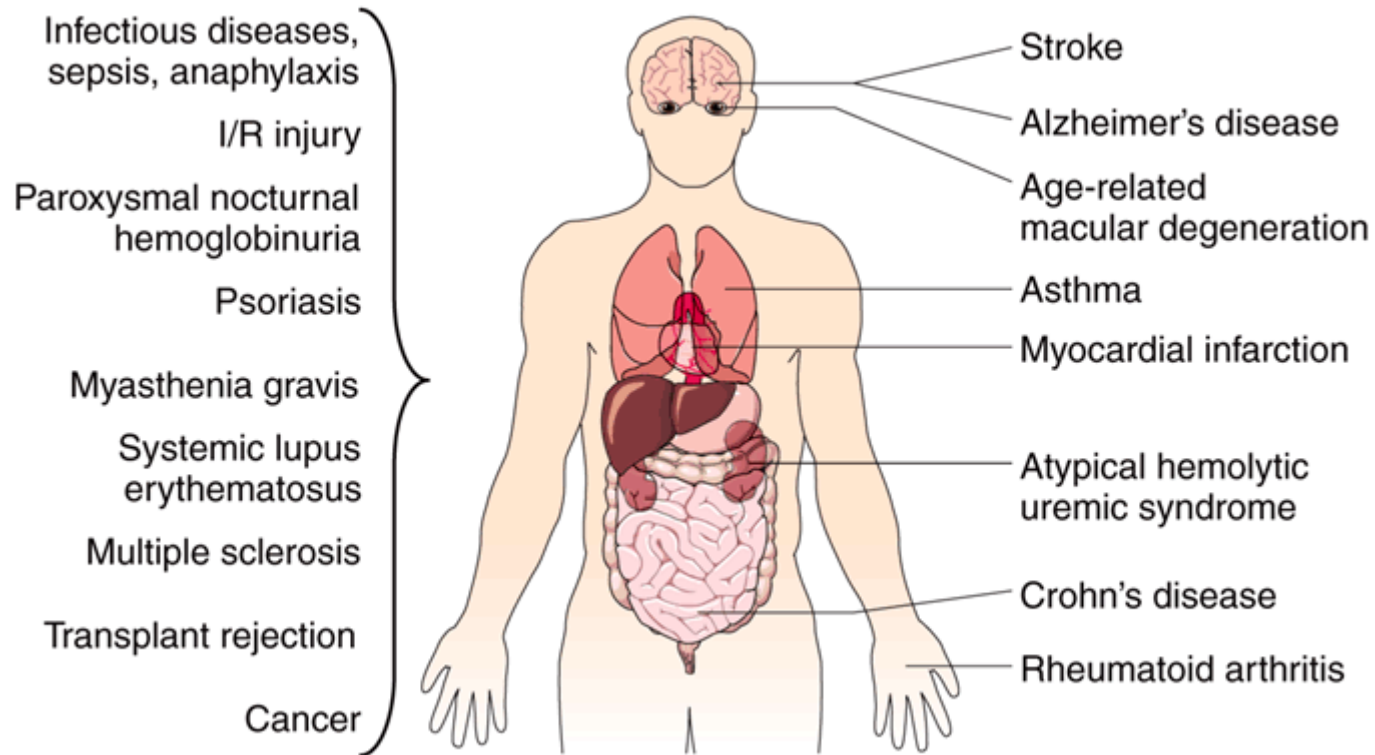
Protein	Type of protein	Pathway affected	Immunologic function
C1 inhibitor (C1Inh)	Soluble	Classical	Serine protease inhibitor: causes C1r <sub>2</sub> s <sub>2</sub> to dissociate from C1q
C4b-binding protein (C4bBP)*	Soluble	Classical and lectin	Blocks formation of C3 convertase by binding C4b; cofactor for cleavage of C4b by factor I
Factor H*	Soluble	Alternative	Blocks formation of C3 convertase by binding C3b; cofactor for cleavage of C3b by factor I
Complement-receptor type 1 (CR1)* Membrane-cofactor protein (MCP)*	Membrane bound	Classical, alternative, and lectin	Block formation of C3 convertase by binding C4b or C3b; cofactor for factor I-catalyzed cleavage of C4b or C3b
Decay-accelerating factor (DAF)*			Accelerates dissociation of C4b <sub>2</sub> a and C3bBb (classical and alternative C3 convertases)
Factor I	Soluble	Classical, alternative, and lectin	Serine protease: cleaves C4b or C3b using C4bBP, CR1, factor H, DAF, or MCP as cofactor
S protein	Soluble	Terminal	Binds soluble C5b67 and prevents its insertion into cell membrane
Homologous restriction factor (HRF) Membrane inhibitor of reactive lysis (MIRL)	Membrane bound	Terminal	Bind to C5b678 on autologous cells, blocking binding of C9
Anaphylatoxin inactivator			Inactivates anaphylatoxin activity of C3a, C4a, and C5a by carboxypeptidase N removal of C-terminal Arg

\*An RCA (regulator of complement activation) protein. In humans, all RCA proteins are encoded on chromosome 1 and contain short consensus repeats.





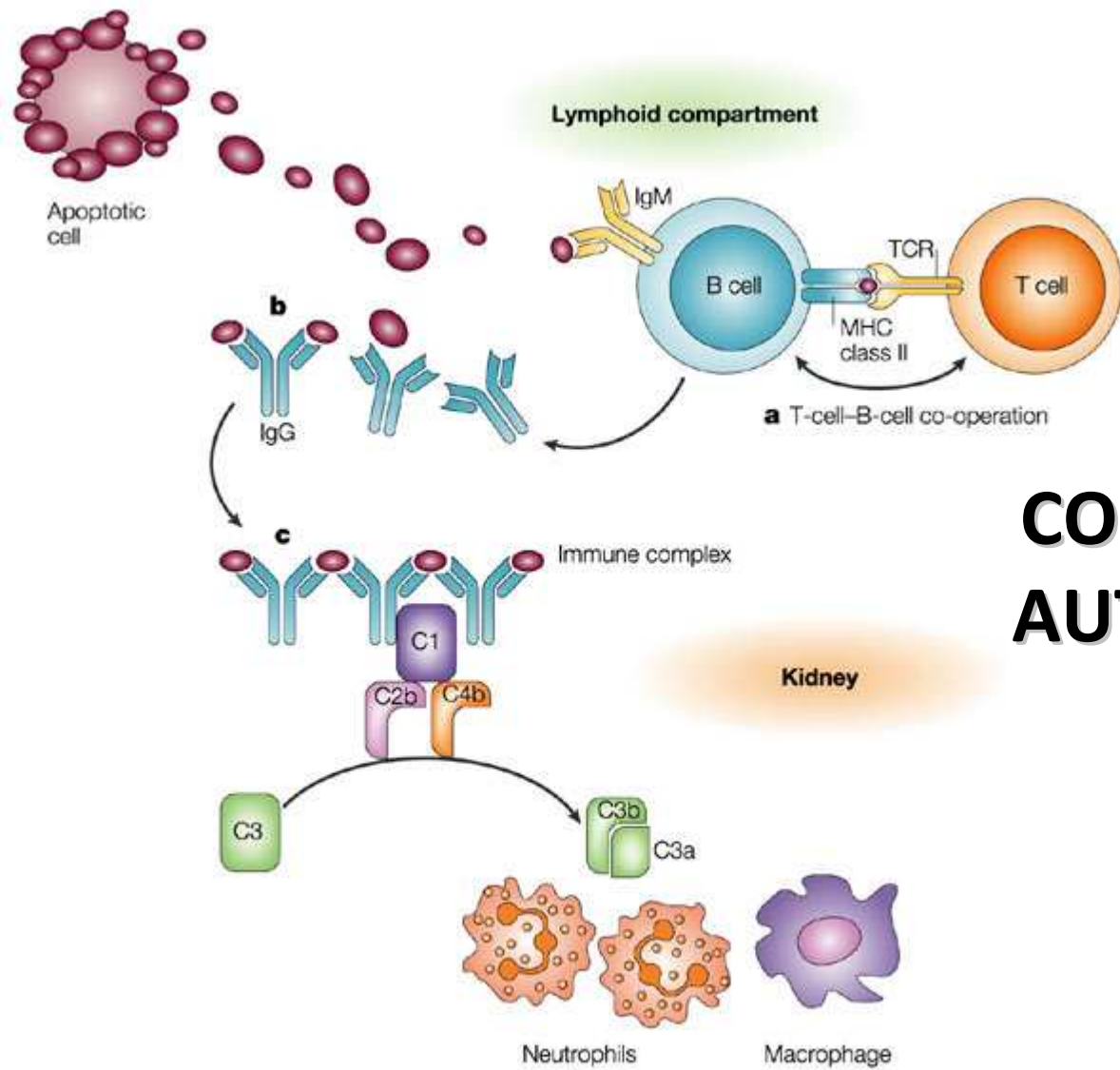
# EL COMPLEMENTO Y LA ENFERMEDAD



# Paradoja

**La activación del complemento juega un rol crítico en el proceso inflamatorio y en el daño tisular en el LES...**

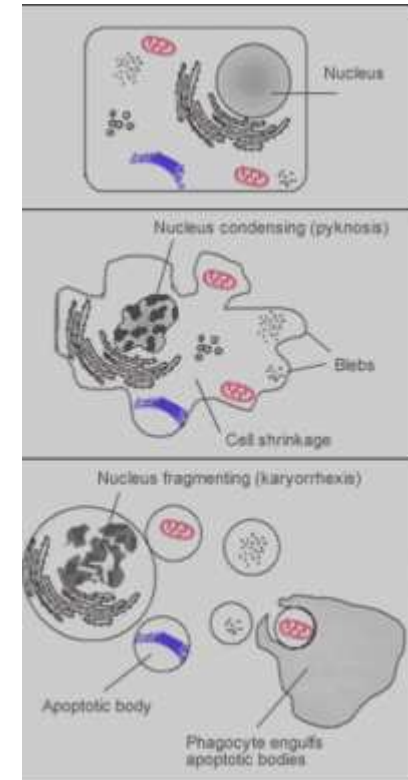
**Pero la deficiencia de complemento causa LES.....**



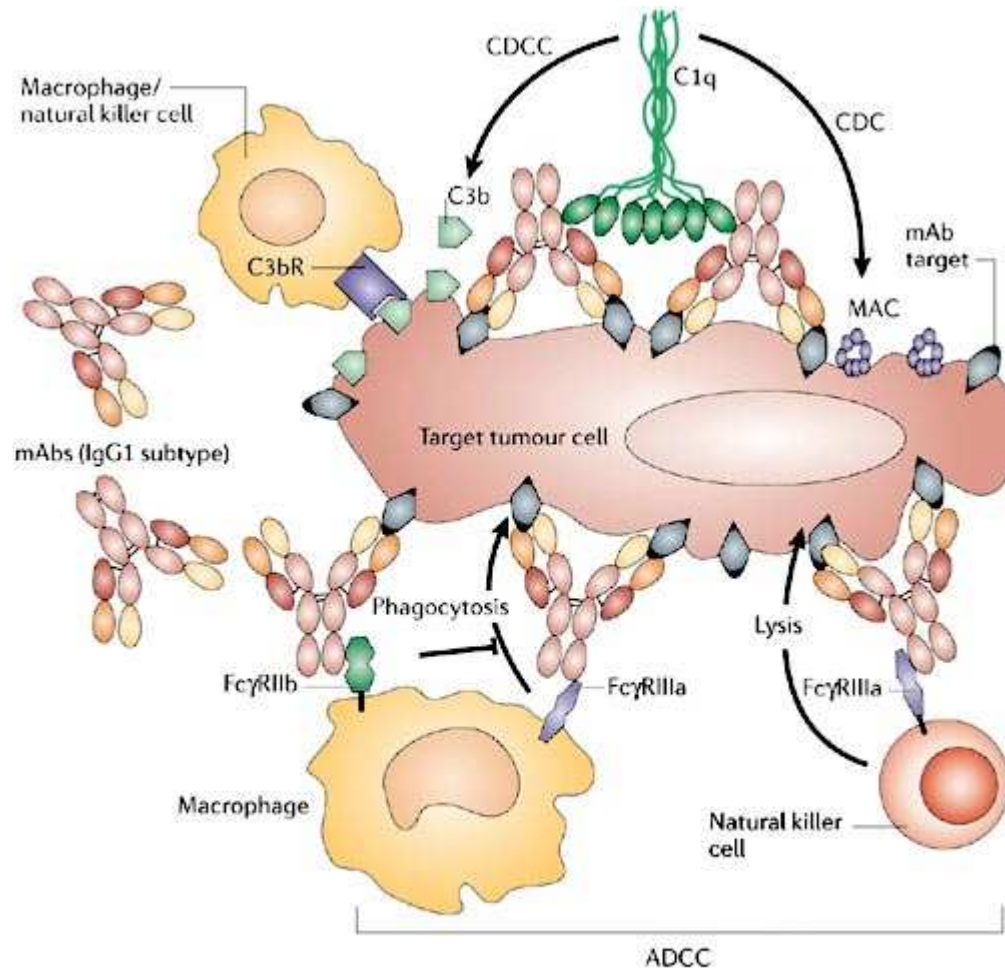
# COMPLEMENTO Y AUTOINMUNIDAD

# Posibles explicaciones

1. C1q depura ICs
2. C1q se une y depura *apoptotic blebs* (células apoptóticas con ampollas de nucleosomas; fuente de autoantígenos)
3. Ausencia de C1q favorece infecciones que podrían disparar respuestas autoinmunes.



# SISTEMA DEL COMPLEMENTO Y TUMORES



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Following the binding of monoclonal antibodies (mAbs) to a specific target on a tumour cell, C1q complement factor interacts with the C<sub>H</sub>2 constant region of the mAb, which leads to the activation of a proteolytic cascade of the complement classical pathway and consequently induces the formation of a membrane-attack complex (MAC) for the lysis of tumour cells; this effect is termed complement-dependent cytotoxicity (CDC). C3b, which is generated during this cascade reaction, functions as an [opsonin](#) to facilitate phagocytosis and cytolysis through its interaction with the C3b receptor (C3bR) on a macrophage or natural killer (NK) cell; this activity is termed [complement-dependent cell-mediated cytotoxicity](#) (CDCC). In addition, mAb-binding to tumour cells induces antibody-dependent cellular cytotoxicity (ADCC); immune-effector cells such as macrophages and NK cells are recruited and interact with the C<sub>H</sub>3 region of the mAbs through FcγRIIIa expressed by both effector cells. Then, mAb-coated tumour cells are phagocytosed by macrophages or undergo cytolysis by NK cells. On the other hand, there is a negative regulation to modulate the cytotoxic response against tumours through FcγRIIb, which is expressed on the cell surface of macrophages. Immunoglobulin G1 (IgG1) and IgG3 can activate the classical complement pathway and interact with Fcγ receptors more potently than IgG2 or IgG4. **In particular, IgG4 cannot activate the classical complement pathway.**

# DEFICIENCIAS Y ENFERMEDADES

Componente	Enfermedad	Mecanismo
<b>MBL</b>	Susceptibilidad a infecciones bacterianas en niños e inmunosuprimidos	Inhabilidad para iniciar la vía de MBL

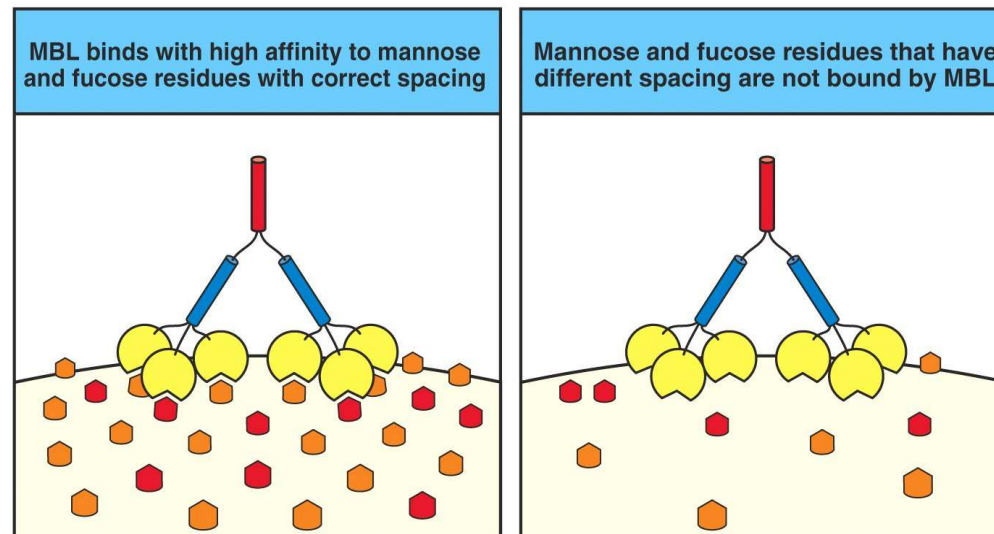
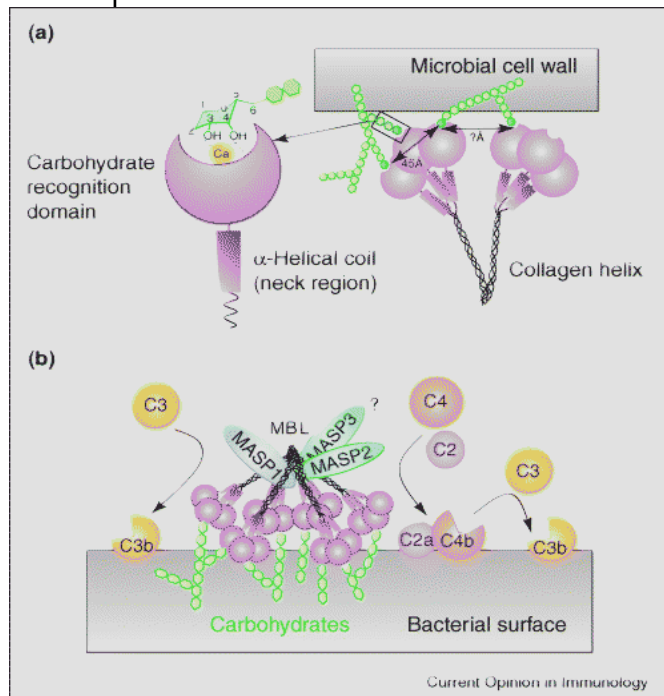


Figure 2-11 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

## DEFICIENCIA DE MBL E INFECCIONES

**Table 2. Clinical associations between mannose-binding lectin (MBL) and malignancy-associated neutropenia, susceptibility to meningococcal infection, and invasive pneumococcal infection.**

Clinical event	Observation	OR	P	Reference
Malignancy-associated neutropenia				
Invasive bacterial infection after chemotherapy	MBL level of <1000 $\mu\text{g}/\text{mL}$ was associated with invasive infection	...	.0001	[42]
Febrile neutropenia	Median duration of episodes was 20.5 and 10.0 days for $A/O^a$ and $A/A^b$ respectively	...	.014	[43]
Infection after allogeneic hematopoietic stem cell transplantation	Infective episodes increased among $A/O$ recipients (76%), compared with $A/A$ recipients (44%)	4.1	.002	[44]
	Rate of infection was 41% when $HYA$ promoter haplotype was present and 81% when it was absent	0.16	.0001	[44]
Susceptibility to meningococcal infection	In a hospital-based study, 7.7% of $O/O$ patients <sup>c</sup> were susceptible to infection, compared with 1.5% of control subjects	6.5	.0006	[26]
	In a community-based study, 8.3% of $O/O$ patients were susceptible to infection, compared with 2.7% of control subjects	4.5	.06	[26]
Invasive pneumococcal infection	12% of 229 patients were homozygous for MBL variants, compared with 5% of 353 controls	2.59	.002	[48]
	7% of 140 patients were homozygous for MBL variants, compared with 3% of 250 controls	...	NS	[49]

Eisen, DP and Minchinton RM, 2003, *Clin Inf Dis* 37:1496.

# DEFICIENCIA DE MBL E INFECCIONES

**Table 3. Clinical associations between mannose-binding lectin (MBL) and viral, parasitic, and fungal infection.**

Clinical condition or outcome	Observation	P
Viral infection		
HIV		
Susceptibility to HIV infection	Susceptibility was 8% among HIV-infected <i>O/O</i> patients, vs. 0.8% among healthy control subjects and 0% among high-risk control subjects	.005; .05
	<i>H</i> promoter allele frequency was 0.48 among HIV-uninfected subjects who were perinatally exposed to HIV and 0.31 for HIV-infected patients	.0214
	MBL was undetectable in 12.1% HIV-infected patients, compared with 3.5% of HIV-uninfected subjects	.009
Disease progression	Duration of survival for patients with variant alleles and those with wild-type alleles, 11 vs. 18 months	.007
	<i>B</i> variant alleles were present in 52% of children with rapidly progressing disease, compared with 18.5% of those with slow progression	.011 <sup>a</sup>
	<i>HH</i> promoter allele (high MBL) was present in 23% of patients with rapidly progressing disease, compared with 5% of those with slow progression	.0194
Chronic hepatitis B	<i>D</i> variant allele was present in 27% of white patients with hepatitis B and in 4% of healthy uninfected controls	.0004
	<i>B</i> variant allele was present in 44% of Chinese patients with cirrhosis and in 23% of healthy uninfected controls	.007
Outcome of fulminant hepatic failure	<i>B</i> variant allele was present in 40% of nonsurvivors and in 13% of survivors	.043
	<i>H</i> promoter allele was present in 39.5% of nonsurvivors and in 70% of survivors	.0005
Chronic hepatitis C		
IFN responsiveness	<i>LX</i> promoter or <i>B</i> variant allele was present in 60.7% of unresponsive patients and in 38.5% of responsive patients	.008
Disease status	<i>B</i> variant allele was present in all patients with progressive disease and in 76% of those with wild-type MBL alleles	.04
Herpes simplex virus-associated Mollaret meningitis	<i>D</i> variant allele was present in 20% of patients and in 6.8% of control subjects	.04
Parasitic infection		
<i>Plasmodium falciparum</i>		
	<i>B</i> and <i>C</i> variant alleles were more common in patients from Gabon with severe malaria	.04
	<i>C</i> variant allele was not associated with malaria in patients from The Gambia	...
Susceptibility to <i>Cryptosporidium parvum</i> infection	MBL variant alleles were more common among HIV-infected patients	.02
Fungal infection: chronic necrotizing pulmonary aspergillosis	MBL variant alleles were present in 70% of patients and in 26% of control subjects	.004

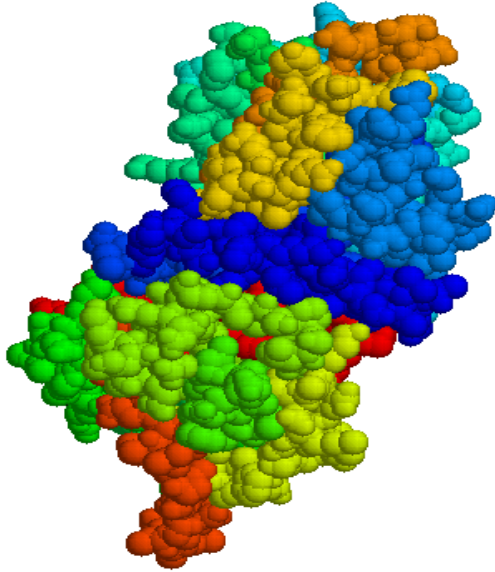
Eisen, DP and Minchinton RM, 2003, *Clin Inf Dis* 37:1496.



# DEFICIENCIAS Y ENFERMEDADES

Componente	Enfermedad	Mecanismo
<b>Factores B or D</b>	Susceptibilidad a infecciones por bacterias piógenas	Insuficiente opsonización de las bacterias
<b>C3</b>	Susceptibilidad a infecciones bacterianas	Falta de opsonización e inhabilidad para utilizar el MAC
<b>C5, C6, C7 C8, o C9</b>	Susceptibilidad a infecciones por Gram negativos	Inhabilidad para atacar la membrana externa de las bacterias Gram negativas

## Deficiencia C1-inhibitor : angioedema hereditario



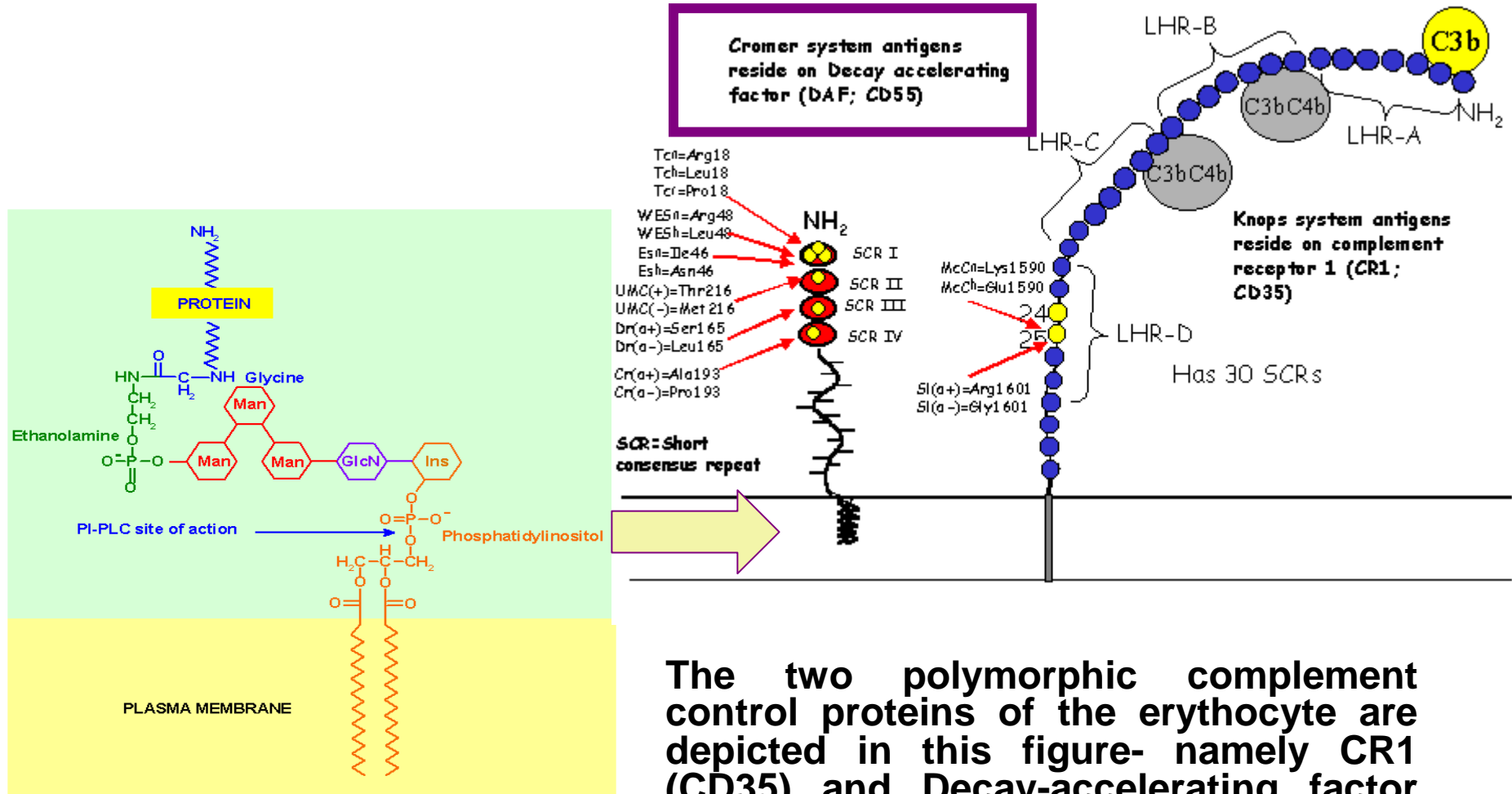
En el angioedema, la extravasación se localiza en planos más profundos (tejido celular subcutáneo), por lo que su principal manifestación es la tumefacción o "hinchazón". Normalmente no produce prurito y afecta preferentemente a la cara, extremidades y área genital.



## Hemoglobinuria Paroxística Nocturna

..... descrita por primera vez en 1882.

Llamada así porque la *hemólisis intravascular*, ocurre en crisis y por la noche, durante el sueño, después de situaciones desencadenantes, infecciones, esfuerzo físico, vacunaciones, ingesta de ácido acetilsalicílico.



The two polymorphic complement control proteins of the erythrocyte are depicted in this figure- namely CR1 (CD35) and Decay-accelerating factor (DAF, CD55).

## Causas, incidencia y factores de riesgo

- **La hemoglobinuria paroxística nocturna (HPN) es una enfermedad clonal y adquirida causada por una mutación somática en el gen PIG-A que se encuentra en el cromosoma X y codifica una proteína involucrada en la síntesis del glicosilfosfatidilinositol (GPI), el cual le sirve como anclaje a muchas proteínas de la membrana celular.**
- La mutación ocurre en el stem cell hematopoyético y da lugar a una deficiencia parcial o total de la proteína PIG-A con la consecuente alteración en la síntesis del GPI de anclaje; como resultado, una parte de las células sanguíneas serán deficientes de todas las proteínas ligadas al GPI.
- La enfermedad puede afectar a personas de cualquier edad. Aparece en ambos sexos a cualquier edad, pero es algo más frecuente en mujeres entre los 30 y 40 años. Su incidencia es de aproximadamente de 1/100.000
- Se puede confundir con anemia aplásica y convertirse en el síndrome mielodisplásico o leucemia mieloide aguda.
- Varios estudios han demostrado que la inactivación del gen PIG- A por sí sola, no confiere una ventaja proliferativa al stem cell mutado; uno o más factores ambientales externos son necesarios para la expansión de este clon mutado, los cuales ejercen una presión selectiva a favor del clon HPN. La causa por el cual el clon HPN se estimula a proliferar podría ser un daño selectivo a la hematopoyesis normal.

## Síntomas

- Dolor abdominal
- Dolor torácico
- En algunas personas, se pueden formar trombos sanguíneos
- Orina oscura intermitente
- Tendencia al sangrado y a la formación de hematomas
- Cefalea
- Dificultad para respirar

## Signos y exámenes

- Puede existir pancitopenia.
- El color rojo o marrón de la orina indica la descomposición de los glóbulos rojos y que la hemoglobina está siendo liberada en la circulación corporal y finalmente en la orina.

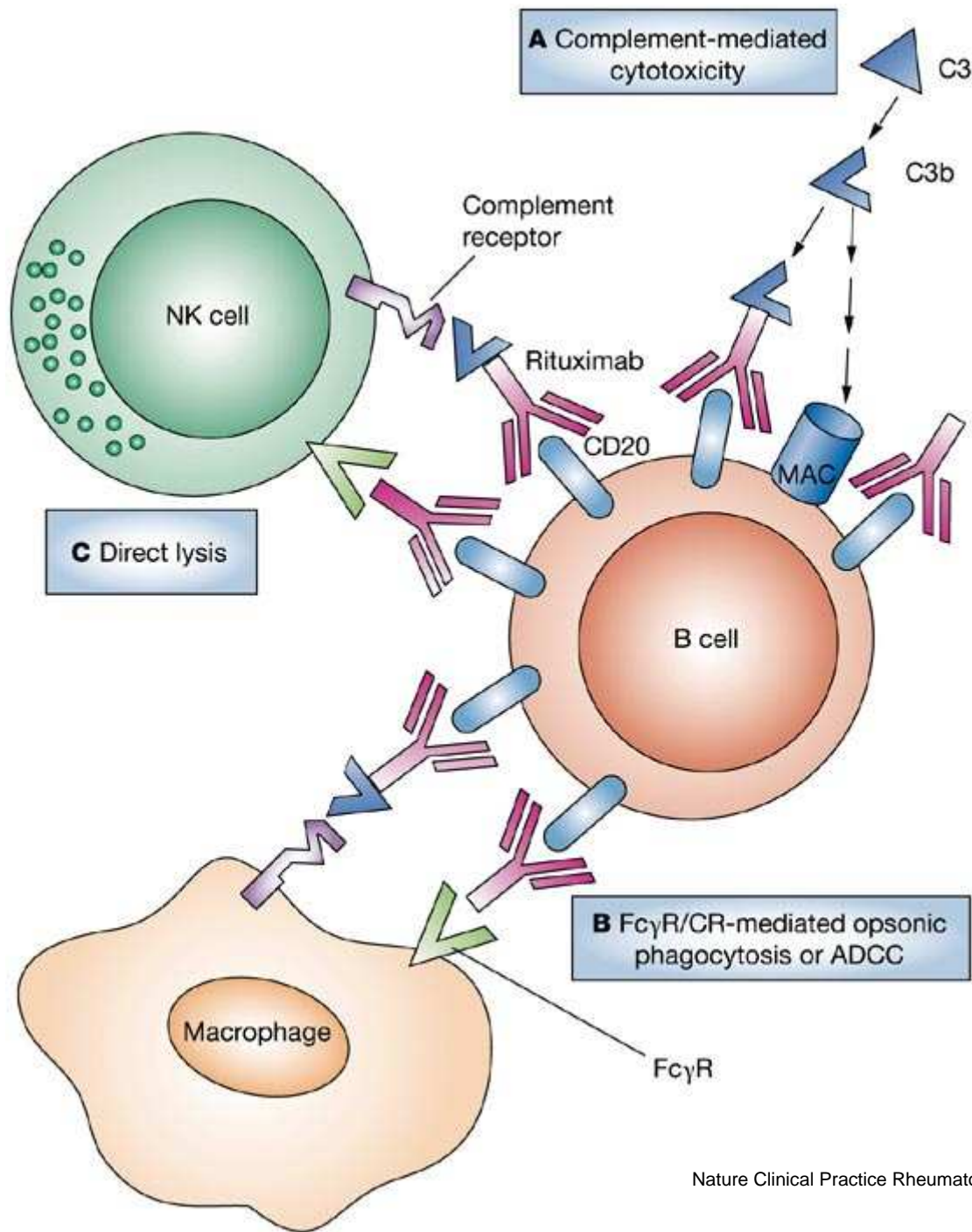
## Exámenes de laboratorio:

- Hemograma
- Prueba de Coombs directa
- Citometría de flujo para medir ciertas proteínas
- Prueba de Ham (hemolisina ácida)
- Análisis de orina
- Hemoglobina en suero y haptoglobina
- Prueba de hemólisis de la sacarosa



## Tratamiento

- Los esteroides y otras drogas que inhiben el sistema inmunitario pueden ayudar a disminuir la descomposición de los eritrocitos. Es posible que se requieran transfusiones de sangre, al igual que el suministro de suplementos de hierro y ácido fólico. Del mismo modo, los anticoagulantes pueden ser necesarios para prevenir la formación de coágulos.
- Soliris (**eculizumab**), que bloquea la descomposición de los eritrocitos, es una nueva droga utilizada para tratar esta enfermedad.
- Asimismo, el trasplante de médula ósea (progenitores hematopoyéticos) puede curar esta enfermedad.
- Todos los pacientes que padecen esta enfermedad deben recibir vacunas preventivas contra ciertos tipos de bacterias para prevenir infección.



## EL COMPLEMENTO EN LA TERAPÉUTICA....

**Linfocitos B opsonizados  
con RITUXIMAB son  
destruídos por distintas  
rutas...**



# **TERAPEÚTICA DISPONIBLE PARA DEFICIENCIAS DEL COMPLEMENTO O SUS REGULADORES**

Product (company)	Activity	Stage of development
<b>Protease inhibitors (A)</b>		
C1-INH (Cetor/Sanquin, BerinertP/CSL Behring, Lev Pharma)	Purified plasma protein; inhibition of C1r/C1s, kallikrein and other proteases	Marketed (HAE) <sup>a</sup> ; clinical phase 3 (HAE) <sup>a</sup> ; preclinical/phase 1 for other indications (AMI, CABG)
Rhucin/rhC1INH (Pharming Group N.V.)	Recombinant human C1-INH from transgenic rabbits	Clinical phase 3 (HAE)
<b>Soluble complement regulators (B)</b>		
sCR1/TP10 (Avant Immunotherapeutics)	Extracellular part of CR1; decay accelerator, factor I cofactor	Clinical phase 2 (CABG) <sup>b</sup>
CAB-2/MLN-2222 (Millenium Pharmaceuticals)	Chimera of DAF and MCP; decay accelerator, factor I cofactor	Clinical phase 1 (CABG) <sup>b</sup>
<b>Therapeutic antibodies (C)</b>		
Eculizumab/ Soliris (Alexion Pharmaceuticals)	Humanized long-acting mAb against C5	Marketed (PNH), preclinical for other indications
Pexelizumab (Alexion Pharmaceuticals)	Humanized short-acting mAb against C5	Clinical phase 3 (AMI, CABG) <sup>b</sup>
Ofatumumab (Genmab A/S)	Humanized anti-CD20 mAb	Clinical phase 2
<b>Complement component inhibitors (D)</b>		
Compstatin/POT-4 (Potentia Pharmaceuticals)	Peptidic C3 inhibitor; no generation of C3a/C3b	Clinical phase 1 (AMD)
<b>Receptor antagonists (E)</b>		
PMX-53 (Peptech Ltd.)	Peptidic C5aR antagonist	Clinical phase 2 (RA, psoriasis)
<b>Other</b>		
rhMBL (Enzon Pharmaceuticals)	Recombinant human MBL as substitution therapy	Clinical phase 1b (MBL deficiency)

<sup>a</sup>C1-INH preparation are only approved in some countries, whereas they are in clinical phase 3 in the USA.

<sup>b</sup>Clinical trials have recently been performed but no product development has been undertaken since then, or the development was officially discontinued. AMD, age-related macular degeneration; AMI, acute myocardial infarction; CABG, coronary artery bypass grafting; HAE, hereditary angioedema; PNH, paroxysmal nocturnal hemoglobinuria; RA, rheumatoid arthritis.



W.C.

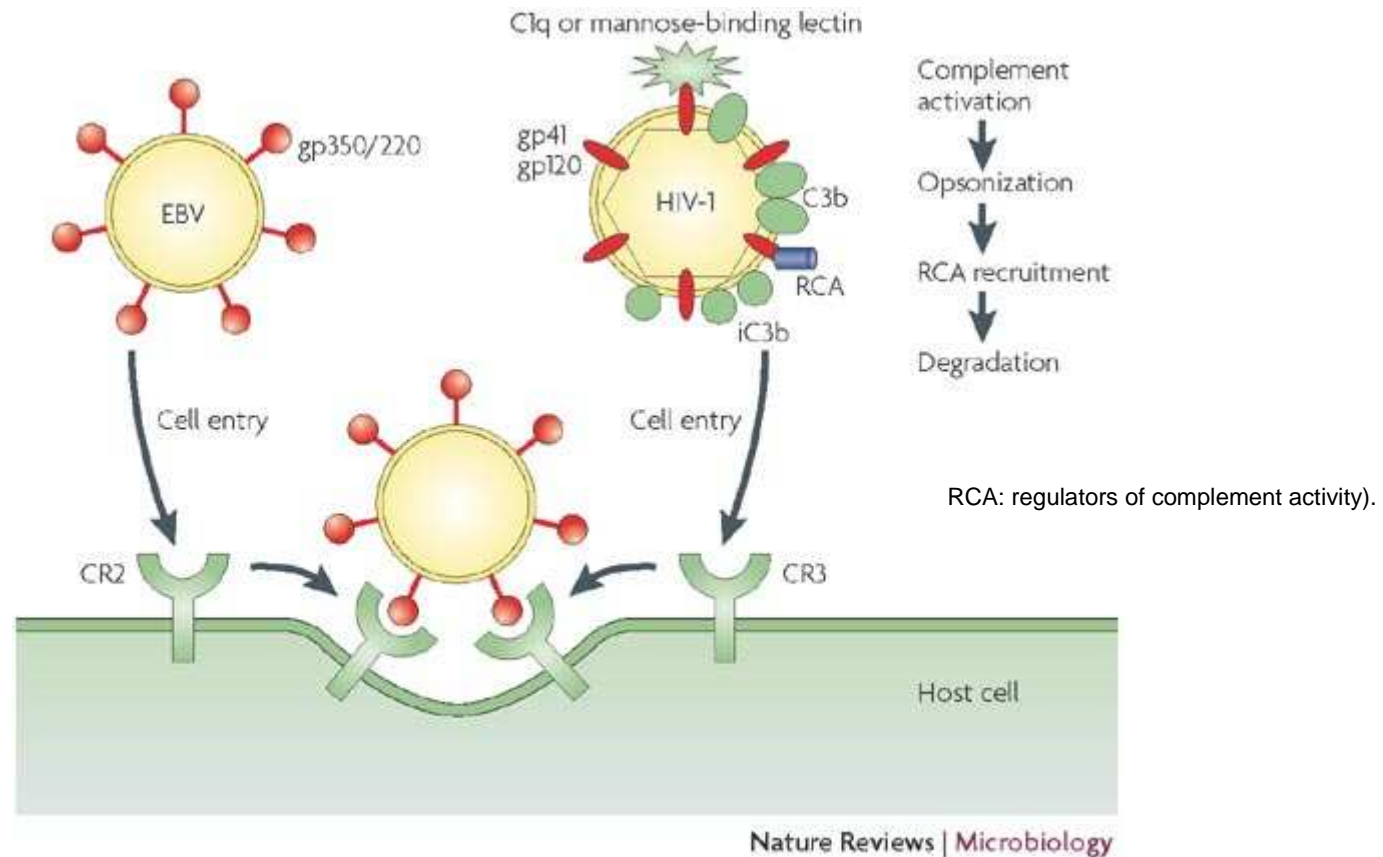
EVASIÓN.....

**TABLE 13-9 MICROBIAL EVASION OF COMPLEMENT-MEDIATED DAMAGE**

Microbial component	Mechanism of evasion	Examples
<b>Gram-negative bacteria</b>		
Long polysaccharide chains in cell-wall LPS	Side chains prevent insertion of MAC into bacterial membrane	Resistant strains of <i>E. coli</i> and <i>Salmonella</i>
Outer membrane protein	MAC interacts with membrane protein and fails to insert into bacterial membrane	Resistant strains of <i>Neisseria gonorrhoeae</i>
Elastase	Anaphylatoxins C3a and C5a are inactivated by microbial elastase	<i>Pseudomonas aeruginosa</i>
<b>Gram-positive bacteria</b>		
Peptidoglycan layer of cell wall	Insertion of MAC into bacterial membrane is prevented by thick layer of peptidoglycan	<i>Streptococcus</i>
Bacterial capsule	Capsule provides physical barrier between C3b deposited on bacterial membrane and CR1 on phagocytic cells	<i>Streptococcus pneumoniae</i>
<b>Other microbes</b>		
Proteins that mimic complement regulatory proteins	Proteins present in various bacteria, viruses, fungi, and protozoans inhibit the complement cascade	Vaccinia virus, herpes simplex, Epstein-Barr virus, <i>Trypanosoma cruzi</i> , <i>Candida albicans</i>

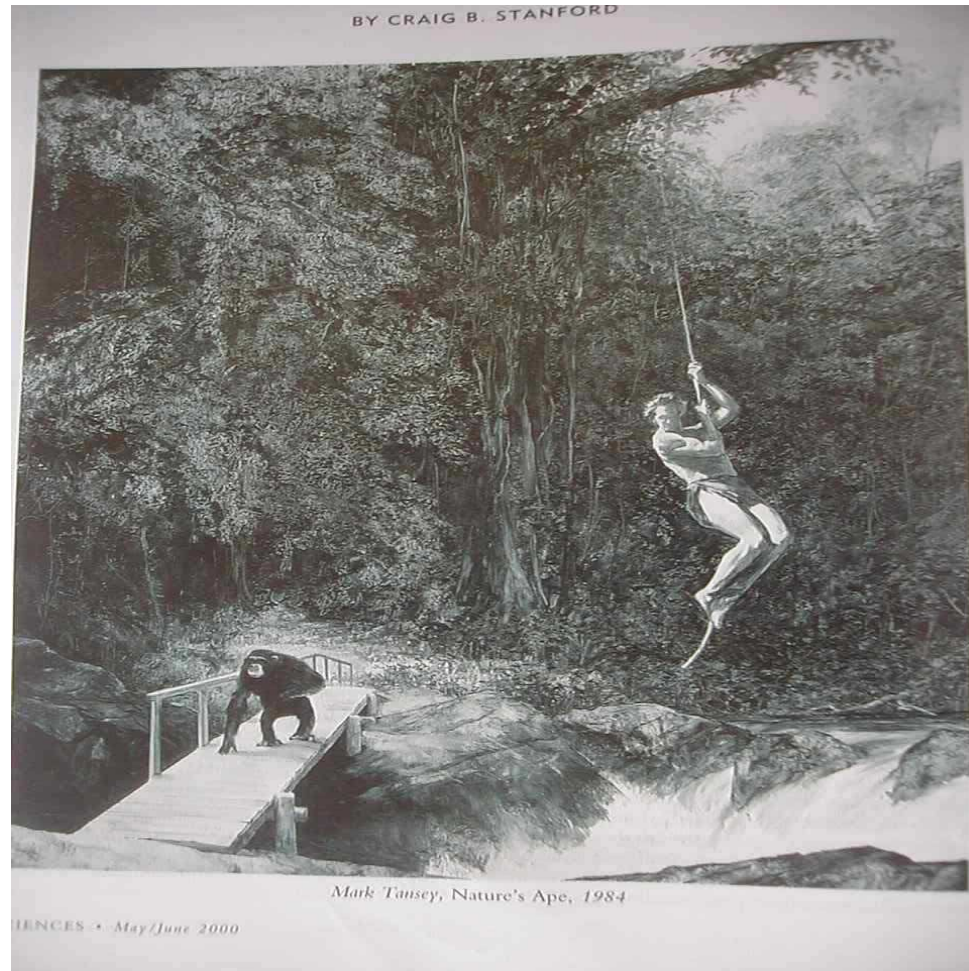
**KEY:** CR1 = type 1 complement receptor; LPS = lipopolysaccharide; MAC = membrane-attack complex (C5b-9).

# Virus y sistema del complemento



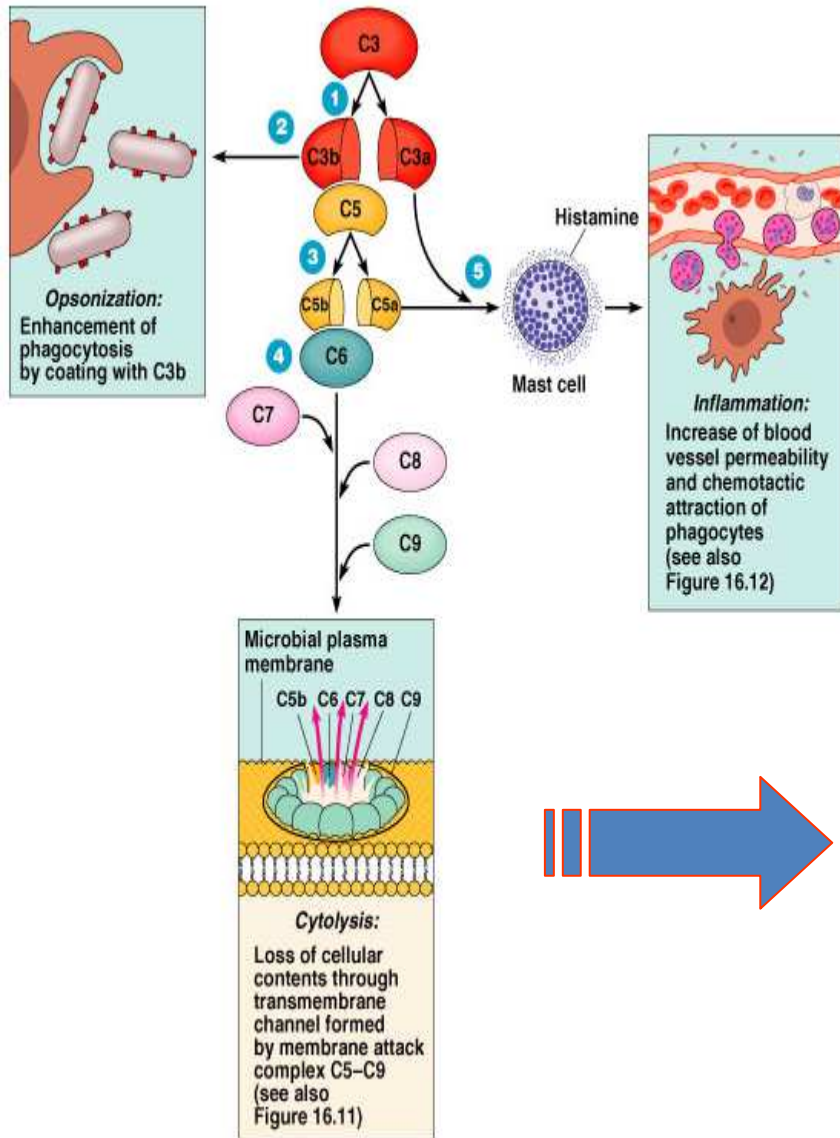
- El estrés es inherente y necesario para todos los seres vivos.
- Los niveles iniciales y moderados de estrés son beneficiosos y aún placenteros, el estrés descompensado es una enfermedad.

**El sistema inmune  
es influenciado  
por el estrés  
y ello influencia la  
génesis y la evolución  
de las enfermedades**



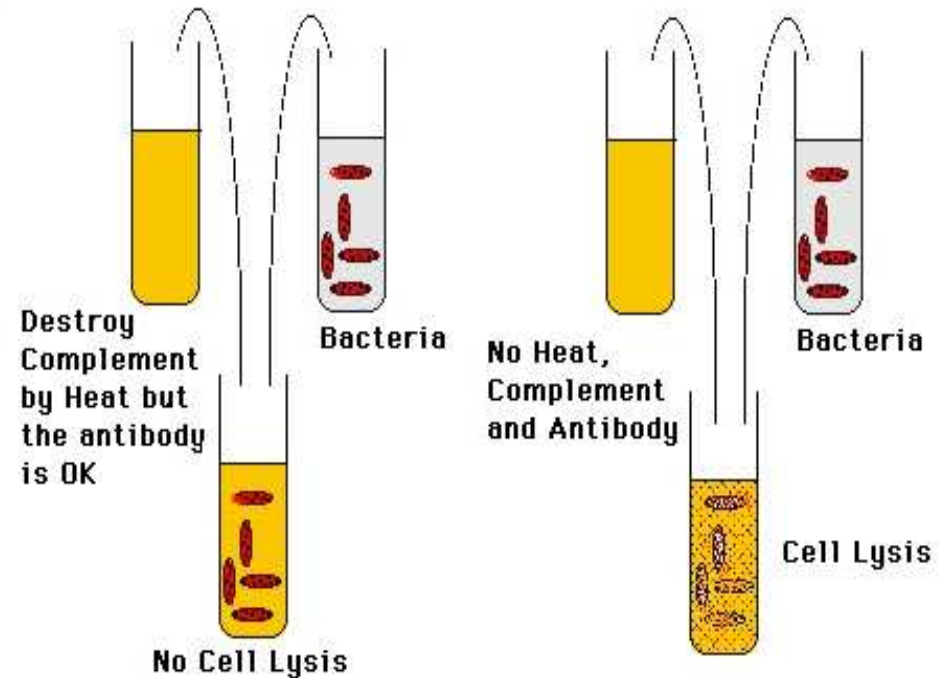


# DIAGNÓSTICO DE LABORATORIO



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### Complement (Heat-Labile Substance)



**Complement, antibodies, and cellular antigens are required for cell lysis**

La découverte du complément est due au chercheur belge Jules Bordet (1870-1961) alors qu'il menait des recherches sur le vibron cholérique (*Vibrio cholerae*) à l'Institut Pasteur de Paris. En 1896, il découvrit que le sérum d'un lapin immunisé contre le vibron cholérique (antisérum) produisait la lyse des bactéries, mais que cette action ne se produisait plus si l'antisérum avait d'abord été placé pendant quelque temps à la température de 56°C, température des étuves utilisées à l'époque pour maintenir la paraffine fondue (Jules Bordet, Prix Nobel 1919).



- La **valoración del complemento (VÍA CLÁSICA)** puede realizarse por medio de métodos que determinan la hemoglobina liberada, tras el efecto citotóxico sobre eritrocitos específicamente sensibilizados.
- Puede medirse en unidades  $CH_{100}$  o  $CH_{50}$
- La unidad de complemento hemolítica 50% ( $CH_{50}$ ) corresponde a los mililitros de suero fresco que lisan  $2,5 \times 10^8$  hematíes óptimamente sensibilizados sobre un total de  $5 \times 10^8$  eritrocitos, en presencia de cantidades óptimas de calcio y magnesio, a una fuerza iónica de 0,147, a pH 7,4 y durante una incubación de una hora a  $37^{\circ}C$  en un volumen total de 7,5 ml.
- La unidad de complemento hemolítica 100% ( $CH_{100}$ ) equivale a la cantidad de suero fresco capaz de lisis 1ml de un sistema hemolítico constituido por partes iguales de eritrocitos al 5% y hemolisina a la concentración óptima,, en presencia de cantidades óptimas de calcio y magnesio, a una fuerza iónica de 0,147, a pH 7,4 y durante una incubación de 30 minutos a  $37^{\circ}C$  y en un volumen total de 2 ml

The CH<sub>50</sub> assay (Diamedix, Miami, Fla.) utilizes sensitized sheep erythrocytes in solution and is a simplified variation of the Mayer method (6). The degree of cell lysis is proportional to the total classical complement activity present in the serum. Interpretation of CH<sub>50</sub> units is as follows: <100, low; 100 to 300, normal; and >300, high.

### 50% HEMOLYTIC COMPLEMENT ASSAY

The LIA (Waco Chemicals USA, Richmond, Va.) utilizes dinitrophenyl (DNP)-coated liposomes that contain the enzyme glucose-6-phosphate dehydrogenase. When serum is mixed with the liposomes and a substrate containing anti-DNP antibody, glucose-6-phosphate, and nicotinamide adenine dinucleotide, activated liposomes lyse, and an enzymatic colorimetric reaction occurs which is proportional to total classical complement activity. Interpretation of LIA units is as follows: <23, low; 23 to 60, normal and >60, high. LIA testing was performed with a Hitachi 717 automated analyzer per the manufacturer's protocol.

### LIPOSOME IMMUNOASSAY

### ENZYME IMMUNOASSAY

The EIA combines the principles of the hemolytic assay with the use of a monoclonal antibody specific for neoantigen (C5b-9 complex) produced as a result of complement activation (Inctar, Stillwater, Minn.). The amount of polymerized C5b-9 (final product) is proportional to the functional activity of C1 through C9. Interpretation of EIA units is as follows: <60, low; 60 to 140, normal; and >140, high.

Other than the kits to measure classical complement activity, no funds were derived from the manufacturers for these studies.

- La **valoración del complemento (VÍA ALTERNA)** es similar a la de la vía clásica.
- La diferencia fundamental radica en que **el sistema revelador está constituido por eritrocitos de conejo no sensibilizados por anticuerpos.** Estos eritrocitos por su bajo contenido en ácido siálico son capaces de activar el complemento por esta vía.
- Se utiliza:
  - ✓ suero fresco (fuente de complemento) conteniendo EGTA (ácido etilenglicol amino etil tetraacético) 10mM, sustancia quelante de  $\text{Ca}^{++}$  y no de  $\text{Mg}^{++}$  a esta concentración
  - ✓ Suspensión de eritrocitos de conejo
- **Al estar bloqueada la capacidad de activación de la vía clásica, cualquier hemoglobina liberada será un índice de la actividad funcional del complemento por la vía alterna.**
- Puede medirse en unidades  $\text{AP}_{50}$  o  $\text{AP}_{100}$
- El método de dosaje y las precauciones que deben tenerse son las mismas que para la determinación de complemento por la vía clásica.

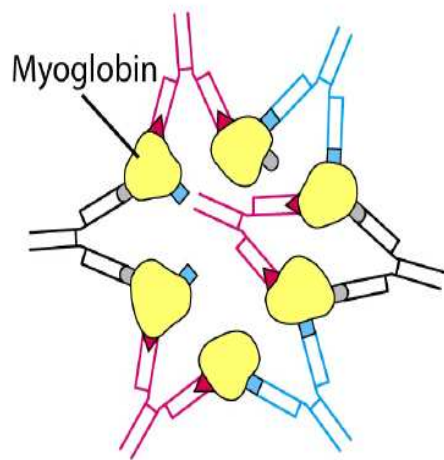
# **VALORACIÓN DE COMPONENTES DEL COMPLEMENTO**

# TÉCNICAS DEL PRECIPITACIÓN

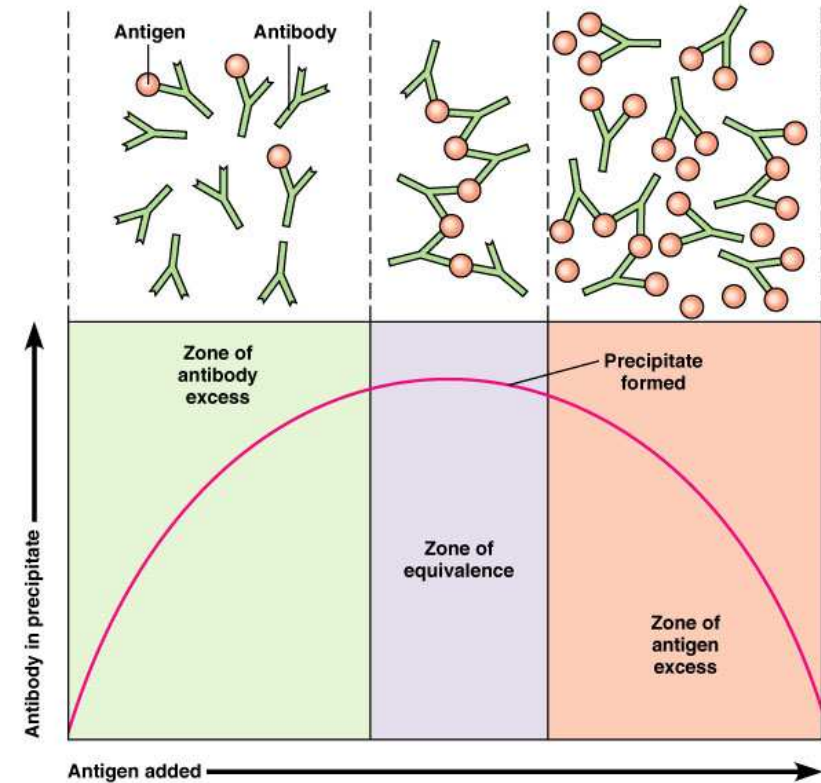
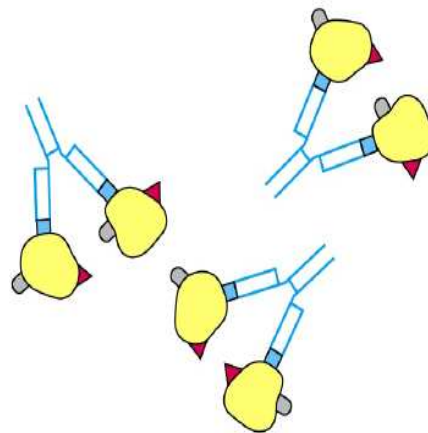
La formación del enrejado requiere:

- 1) **AntiSUELO POLICLONAL**
- 2) **Antígeno PARTICULADO bivalente o polivalente**

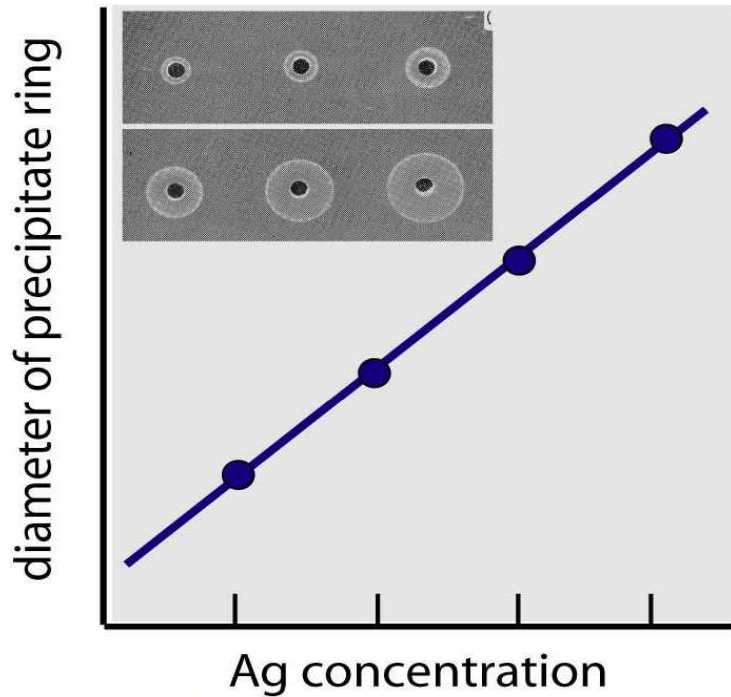
POLYCLONAL ANTISERUM



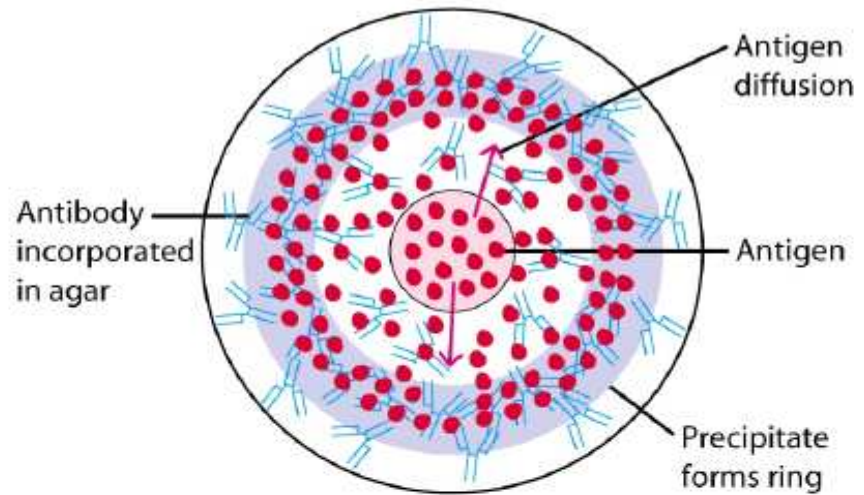
MONOCLONAL ANTIBODY



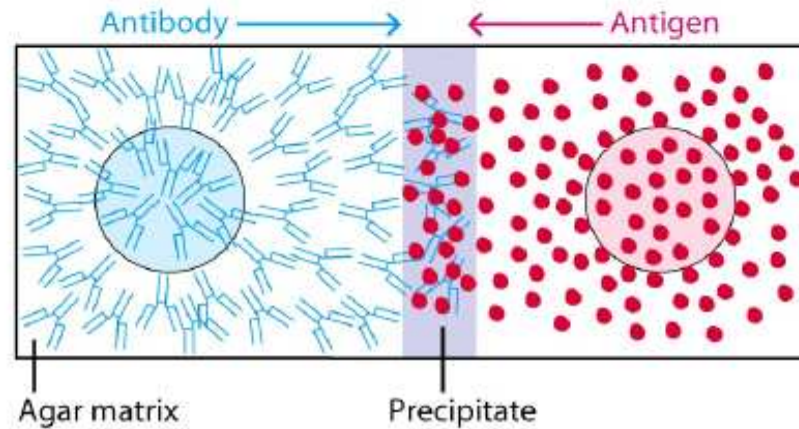
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RADIAL IMMUNODIFFUSION



DOUBLE IMMUNODIFFUSION



## TÉCNICA DE PRECIPITACIÓN: INMUNODIFUSIÓN RADIAL



## TÉCNICAS TURBIDIMÉTRICAS



## TÉCNICAS NEFELOMÉTRICAS

## INMUNOTURBIDIMETRÍA

Son métodos basados en reacciones inmunológicas, al igual que la nefelometría. Poseen una gran similitud técnica con la espectrofotometría de absorción molecular, en cuanto a integrarse dentro del mismo autoanalizador.

**Consisten, en concreto, en la valoración de la disminución de la potencia radiante, de una emisión policromática, al atravesar una solución de partículas (inmunocomplejos), medida en la misma dirección en que es emitida (es decir, el emisor y el detector están a 0°). Dicha disminución es debida a procesos de absorción, dispersión y reflexión.**

En función del momento de la reacción en que se realizan las medidas de disminución de la radiación, podemos distinguir dos tipos :

Immunoturbidimetría a punto final: se realizan únicamente dos medidas, una al principio de la reacción y otra a tiempo prefijado en la zona de equilibrio de la misma.

Immunoturbidimetría cinética: se realiza una lectura múltiple (mínimo 2 puntos) a lo largo de la reacción inmunológica, valorando la velocidad de formación de inmunocomplejos.



# NEFELOMETRÍA

**La nefelometría es un procedimiento analítico que se basa en la dispersión de la radiación que atraviesan las partículas de materia.**

Cuando la luz atraviesa un medio transparente en el que existe una suspensión de partículas sólidas, se dispersa en todas direcciones y como consecuencia se observa turbia. La dispersión no supone la pérdida neta de potencia radiante, solo es afectada la dirección de la propagación, porque la intensidad de la radiación es la misma en cualquier ángulo. La intensidad depende de: el número de partículas suspendidas, su tamaño, su forma, los índices refractivos de la partícula y del medio dispersante, y la longitud de onda de la radiación dispersada.



**La elección entre uno de ambos métodos reside en la dispersión de luz, si es extensa, es apropiado aplicar la turbidimetría, en cambio si es mínima es apropiada la nefelometría. De la nefelometría se obtienen mejores resultados, porque determina concentraciones de pocas partes por millón (ppm), con una precisión de 1 al 5%.**

**La nefelometría se basa en la medición de radiación dispersa, en cambio la turbidimetría en la medición de la intensidad de un haz disminuido.**

**El instrumento usado en la nefelometría, el nefelómetro se asemeja al fluorómetro. En cambio en turbidimetría se utiliza el turbidímetro que es un fotómetro de filtro.**

## MBL PATHWAY TESTING

---

- IBT Laboratories ([www.ibtrefflab.com](http://www.ibtrefflab.com))
  - MBL protein
    - sandwich ELISA
    - Uso de anticuerpos monoclonales contra CRD oligoméricos
    - definir normal > 100 ng/ml
  - Test funcional para la vía MBL
    - ELISA C4b deposition assay
    - Ensayo funcional de la fase inicial de la vía (MBL, MASP)
  - ProGenotyper MBL panel
    - rtPCR for MBL variant alleles
  - ProGenotyper MASP2 polymorphisms
    - DNA sequencing (D105G or entire gene?)



## PCR a Tiempo Real (RT-PCR)

Esta reciente técnica está demostrando ser una excelente herramienta **para la identificación y cuantificación de ácidos nucleicos.**

Entre las numerosas aplicaciones que se están desarrollando figuran los estudios de cuantificación absoluta o relativa de perfiles transcripcionales, medida de número de copias de un gen en DNAs genómicos o virales, estudios de discriminación alélica (SNPs) (detección de polimorfismos a nivel de nucleótidos), ensayos de detección e identificación de patógenos, etc.





## ✓ Bienvenida



✓ Introducción



✓ Inmunidad innata



Inmunidad adaptativa



Hipersensibilidad



Vacunas



Inmunodeficiencias



Autoinmunidad

  
**Gracias**

