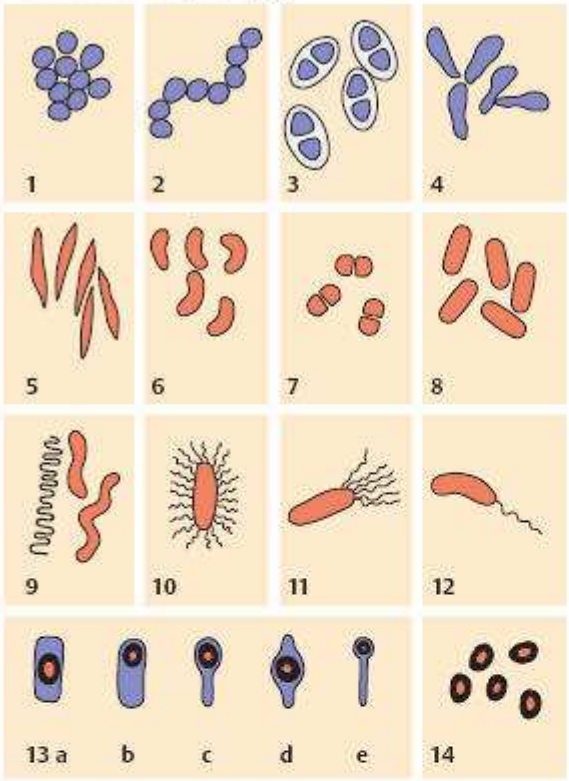
Inmunología Clínica 2009

Bioq Graciela R Svibel de Mizdraji

Respuesta inmune frente a bacterias

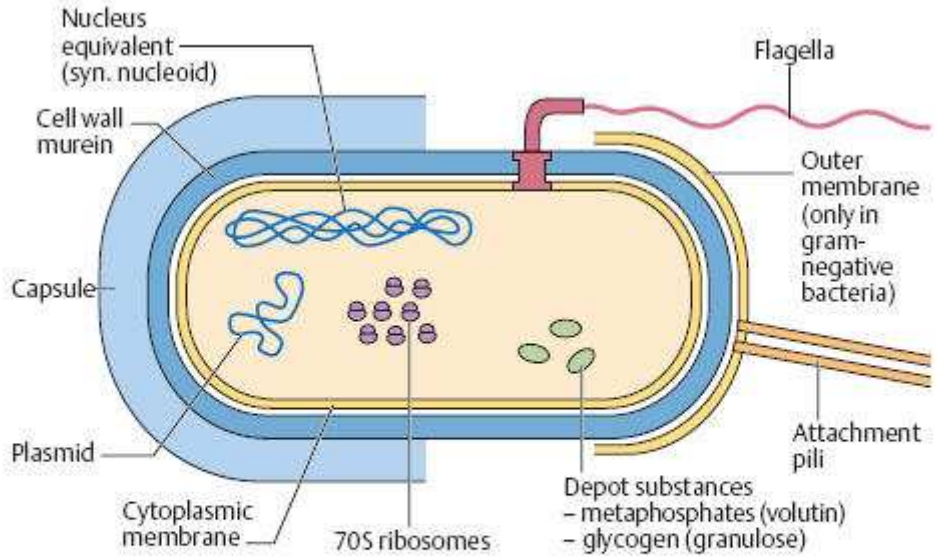
EVASIÓN DE LA RESPUESTA INMUNE

Bacterial Morphology



1. Gram-positive cocci in grapelike clusters (staphylococci)
2. Gram-positive cocci in chains (streptococci)
3. Gram-positive cocci with capsules (pneumococci)
4. Gram-positive, clubshaped, pleomorphic rods (corynebacteria)
5. Gram-negative rods with pointed ends (fusobacteria)
6. Gram-negative curved rods (here commashaped vibrios)
7. Gram-negative diplococci, adjacent sides flattened (neisseria)
8. Gram-negative straight rods with rounded ends (coli bacteria)
9. Spiral rods (spirilla) and Gram-negative curved rods (*Helicobacter*)
10. Peritrichous
11. Lophotrichou
12. Monotrichou
13. Formation of endospores (sporulation) in cells of the genera *Bacillus* and *Clostridium* (spore stain)
    a) Central spore, vegetative cell shows no swelling
    b) Terminal spore, vegetative cell shows no swelling
    c) Terminal spore ("tennis racquet")
    d) Central spore, vegetative cell shows swelling
    e) Terminal spore ("drumstick")
14. Free spores (spore stain)

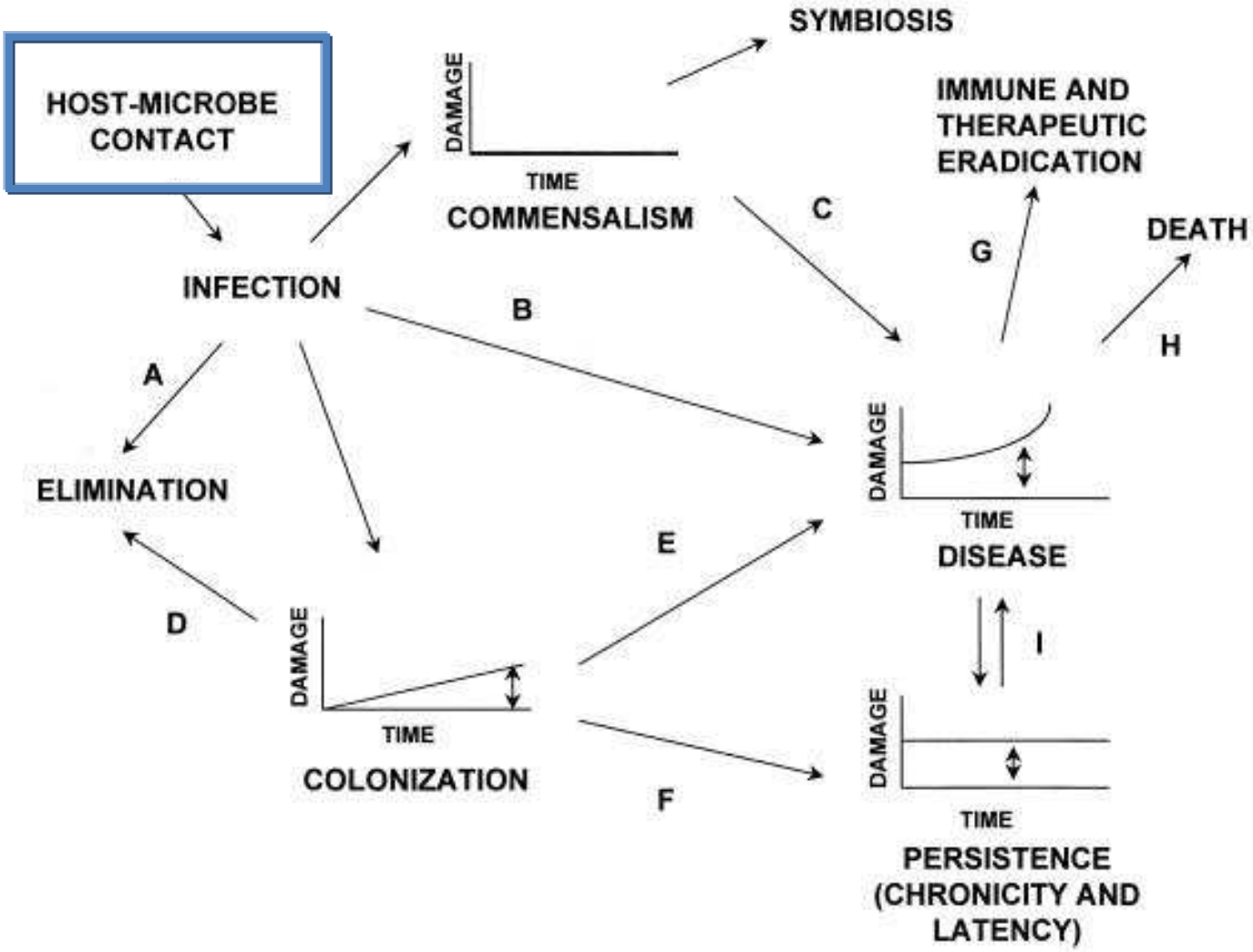
Basic Bacterial Cell Structure



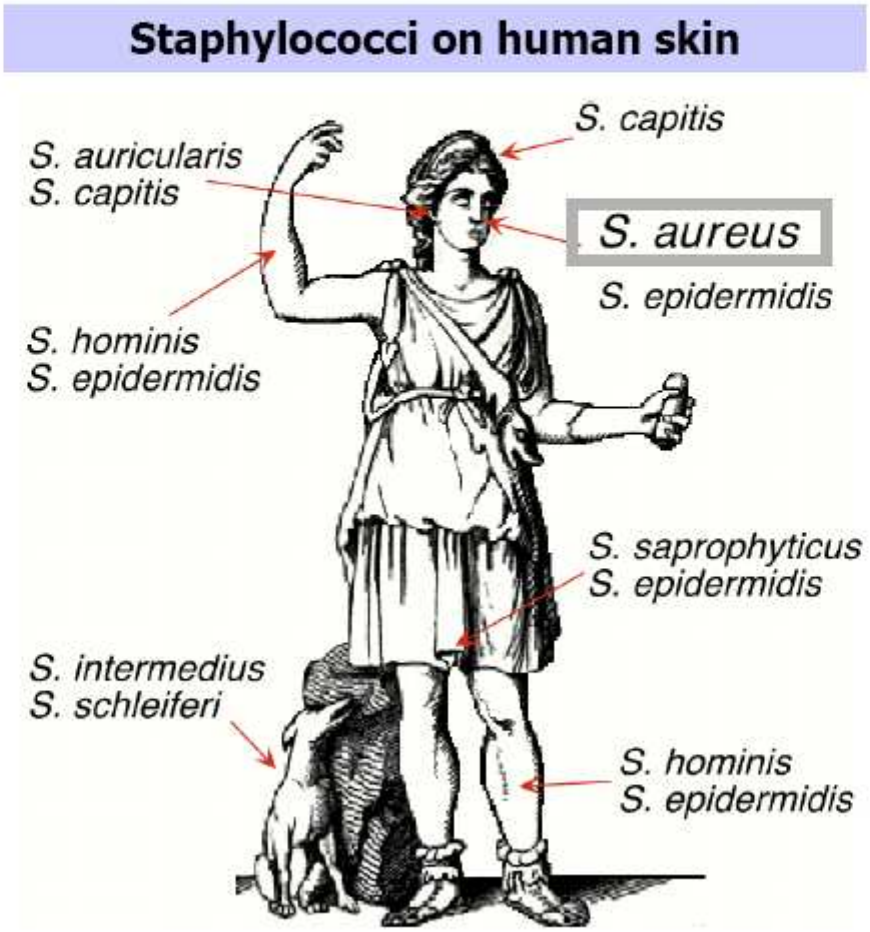
All bacteria have the same basic structure (not to scale).

Interacción Patógeno-Huésped

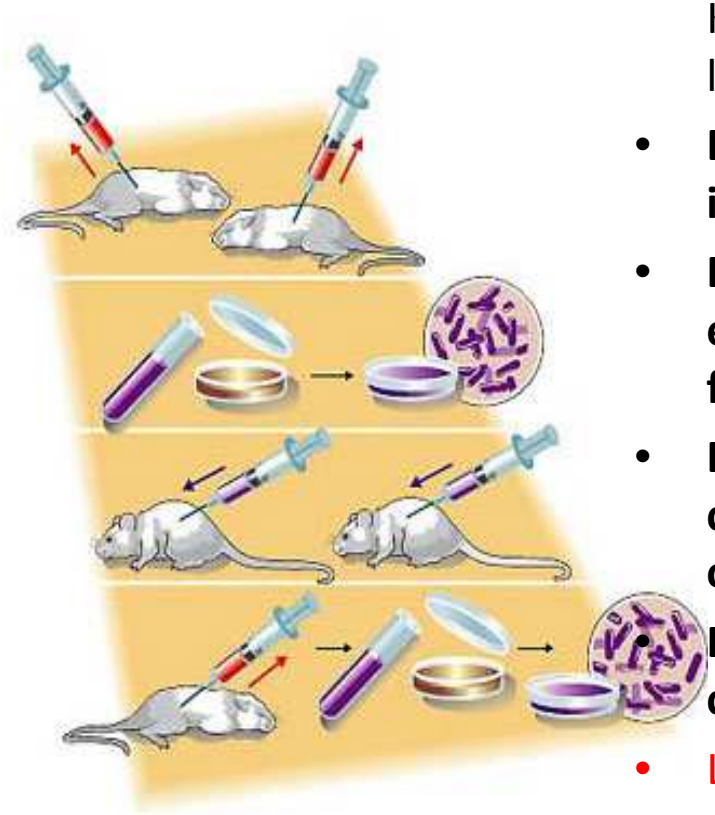
- Comensalismo
- Colonización
- Infección
- Enfermedad


HOST-MICROBE CONTACT
INFECTION
SYMBIOSIS
DAMAGE
TIME
COMMENSALISM
IMMUNE AND THERAPEUTIC ERADICATION
DEATH
A
B
C
G
H
ELIMINATION
DAMAGE
TIME
DISEASE
D
E
I
DAMAGE
TIME
COLONIZATION
F
DAMAGE
TIME
PERSISTENCE (CHRONICITY AND LATENCY)

El cuerpo humano es un ECOSISTEMA para más de 500 especies microbianas



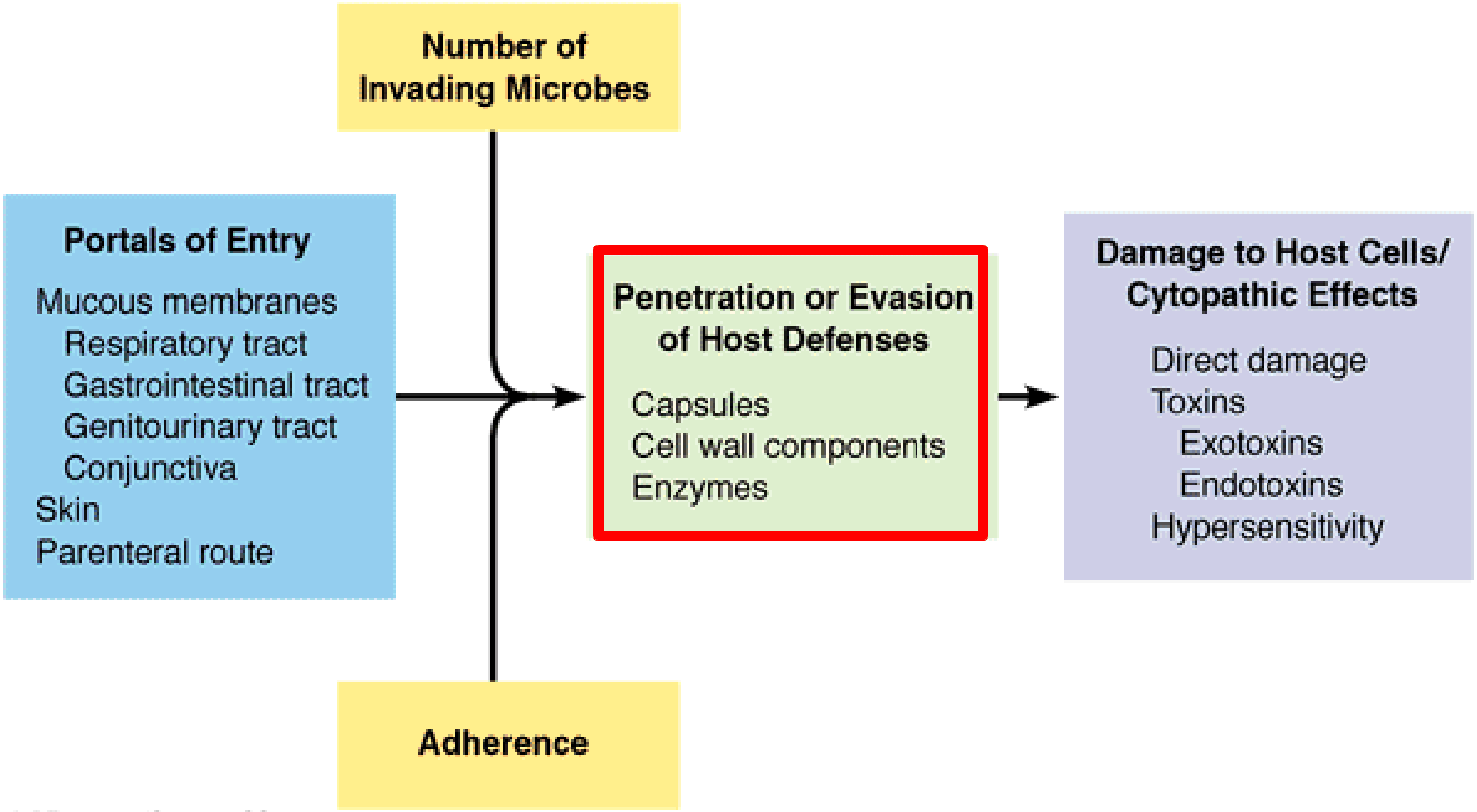
Postulados de koch: establecen la relación entre el agente infeccioso y el huésped



Los **Postulados de Koch** fueron formulados por Robert Koch y tratan de los principios que rigen la etiología de las enfermedades infecciosas.

- **El microorganismo debe estar presente en todos los individuos con la misma enfermedad.**
- **El microorganismo debe ser recuperado del individuo enfermo y poder ser aislado en medio de cultivo en forma pura (cultivo axénico).**
- **El microorganismo proveniente de ese cultivo debe causar la misma enfermedad cuando se lo inocula a otro huésped.**
- **El individuo experimentalmente infectado debe contener el micoorganismo.**
- La mayoría de las bacterias que causan enfermedad en el humano se ajustan a los postulados con excepciones, a saber: Mycobacterium Leprae no cumple con el segundo enunciado de Koch.

¿CÓMO PRODUCEN ENFERMEDAD LOS MICROORGANISMOS?



FACTORES DE VIRULENCIA

Moléculas útiles para la supervivencia y la proliferación en el huésped.

ASPECTOS GENÉTICOS DE LOS FACTORES DE VIRULENCIA

Los factores de virulencia pueden estar codificados por:

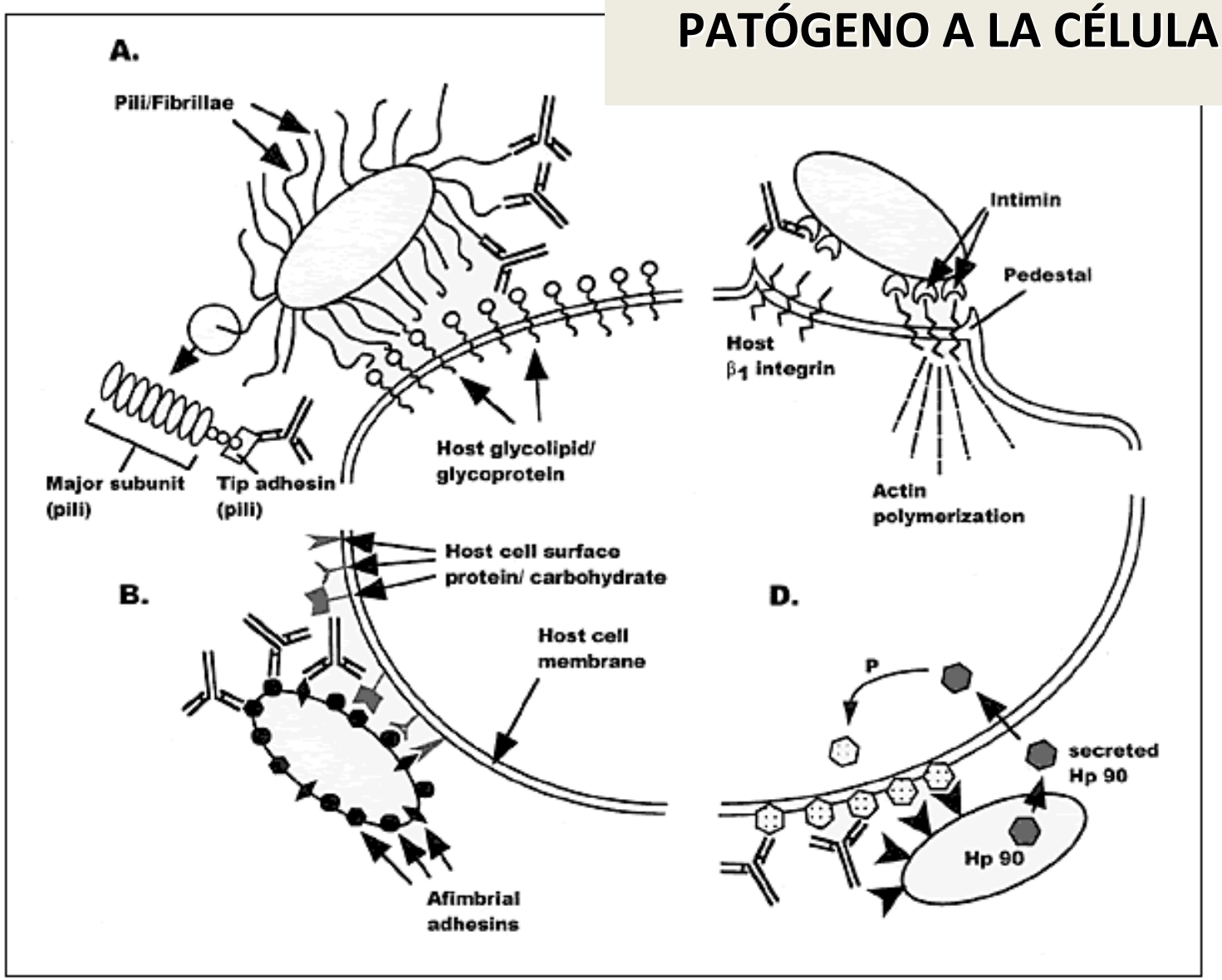
- **Plásmidos**
- **Transposones**
- **Islas de patogenicidad**

FACTORES DE VIRULENCIA

- **Promueven colonización, permanencia y funciones de la bacteria infectante…**

- **Dañan al huésped….**

MECANISMOS DE ADHERENCIA DEL PATÓGENO A LA CÉLULA HUÉSPED



- Four mechanisms of bacterial adherence where anti-adhesin vaccines could potentially block colonization and infection.

A. shows pili or fibrillae protruding from the bacterial surface. These proteinaceous appendages bind to host cell surface molecules, usually carbohydrates, by adhesin proteins located at the distal tip of the pilus/fibrillar organelle. Antibodies targeting the adhesin protein block the bacterial/host interaction.

B demonstrates a similar process of bacterial/epithelial cell interactions mediated by afimbrial adhesin proteins. In this case, antibodies directed against the bacterial surface proteins should also block attachment and colonization by impeding the ability of the bacteria to associate with mucosal tissues.

C illustrates that some bacteria establish intimate associations with eukaryotic cells by intimin proteins, resulting in cytoskeletal rearrangements, host cell signaling, possible internalization of the bacteria, and in many cases systemic disease. Blocking the intimate association/adherence may also be another strategy to prevent bacterial infections.

D shows a novel mechanism whereby bacteria secrete their own receptor protein, which is internalized by the target host cell, phosphorylated, and embedded in the eukaryotic cell as a new receptor for tight binding by the bacterium. Theoretically, blocking the secreted receptor (Hp90) before it is internalized by the host cell could provide another mechanism to block bacterial adherence and infection.

Algunos microorganismos producen infecciones persistentes…..

Table 1 | **Some persistent bacterial pathogens of humans**

Pathogen	Disease conditions	Likely sites of persistence
Mycobacterium tuberculosis	Tuberculosis	Macrophages in various sites and in granulomas
Salmonella enterica serovar Typhi	Typhoid fever	Macrophages in bone marrow, the RES and possibly the gall bladder
Chlamydia spp.	*C. pneumonia* causes respiratory and cardiovascular disease; *C. trachomatis* causes trachoma, genital-tract infections and lymphogranuloma venereum	Epithelial and endothelial cells
Helicobacter pylori	Gastritis; ulcers; gastric cancer; MALT lymphoma	Extracellular; possibly also intracellular in the stomach
Brucella spp.	Brucellosis (this can be chronic, leading to lymphadenopathy and hepatosplenomegaly)	Macrophages in the RES
Borrelia burgdorferi	Lyme disease	Disseminated in various organs
Bartonella henselae	Cat-scratch disease; bacillary angiomatosis; bacillary peliosis hepatitis	Extracellular; in erythrocytes in blood
Neisseria gonorrhoea	Genital-tract infections, which can lead to epididymitis, pelvic inflammatory disease and infertility	Extracellular; intracellular at mucosal sites
Neisseria meningitidis	Invasive infection results in meningititis	Nasopharynx; NALT?
Streptococcus pneumoniae	Acute otitis media; bacteraemia; meningitis	Nasopharynx; NALT?
Streptococcus pyogenes	Acute pharyngotonsillitis; pneumonia; endocarditis; skin, soft tissue and bone infections (necrotizing fasciitis)	Nasopharynx; NALT?
Haemophilus influenzae type B	Pneumonia; meningitis; bactereamia	Nasopharynx; NALT?

NALT, nasopharyngeal-associated lymphatic tissue; RES, reticuloendothelial system.

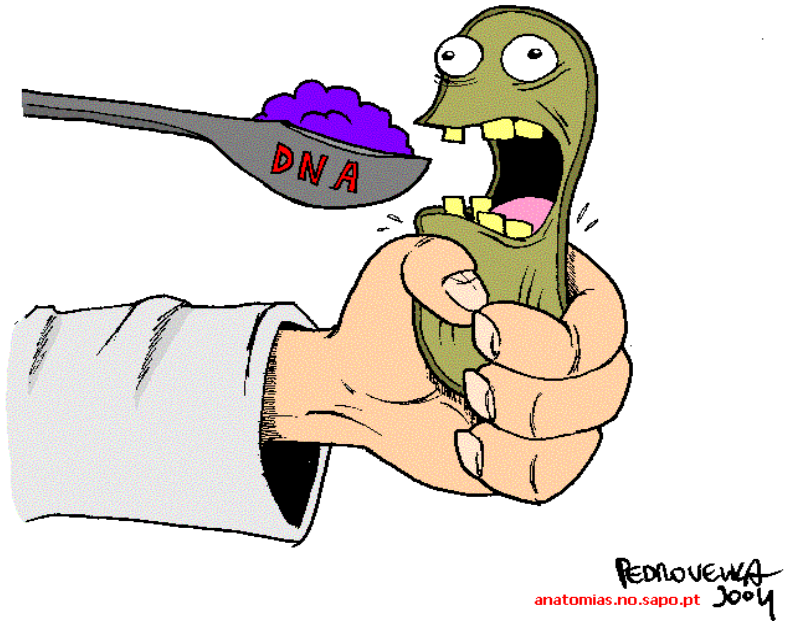
¿CÓMO PUEDEN LAS BACTERIAS EVADIR AL SISTEMA INMUNE?

Table 1. Some mechanisms of increasing persistence used by pathogenic bacteria

Mechanism	Example of pathogen	Comments
Antigenic variation	*N. meningitides* *N. gonorrhea* *B. burgdorferi* *B. fragilis*	Bacteria vary surface antigens using a repertoire of variable genes within their genome or with efficient horizontal gene transfer mechanisms[4,5]
Colonization of a particular tissue or organ	*S. enterica* Typhi	*S. enterica* Typhi can persist in the reticuloendothelial system for extended periods of time (particularly in the bone marrow)[29]; carriers are chronically infected in the gall bladder
	M. leprae	Colonization of nervous system
	H. pylori	Lives in stomach partially in layer beyond epithelial cell barrier[37]
Host mimicry	*N. gonorrhea*	Sialylation of LPS
	N. meningitidis	Mimicry of host polysaccharides
	T. pallidum	Coating with host proteins?
Resistance to immune effector mechanisms	*P. aeruginosa*	Ability to resist killing in phagocytic cells
	M. tuberculosis	Ability to persist in macrophages
Modification of the intracellular environment	*S. enterica* Typhi *Brucella* species *M. tuberculosis*	Ability to avoid fusion with the host lysosomal compartment and modify the intravacuole environment[34,52,86]
	Chlamydia species	Establishment of an obligate intracellular lifestyle
Antiphagocyte defense	*Pseudomonas aeruginosa*	Production of extracellular alginate or polysaccharide
	M. tuberculosis	Production of extracellular glycolipids[53]
Selective gene inactivation	*M. leprae*	Loss of genes and accumulation of pseudogenes compared to *M. tuberculosis*; reduction of replication rate and metabolic activity[36]
	S. enterica Typhi	Loss of attachment factors and proteins for intracellular lifestyle may promote uptake by a favored pathway promoting systemic spread and persistence[30]

Transformação de *E. coli*

"Ai não és competente?? Ora toma!!!"



BACTERIAS EXTRACELULARES

Bacterias extracelulares

- Bacterias capaces de replicarse fuera de las células, en la circulación, meninges, tejido conectivo, vías aéreas, lumen intestinal y urinario
- Cocos Gram-positivos piogénicos o productores de pus (*Staphylococcus*, *Streptococcus*), cocos Gram-negativos (meningococo y gonococo), muchos bacilos Gram-negativos (enterobacterias, *Pseudomonas*, *Haemophilus*) y Gram-positivos (*Bacillus anthracis*, *Clostridium*)
- Producen exo y endotoxinas e inducen inflamación

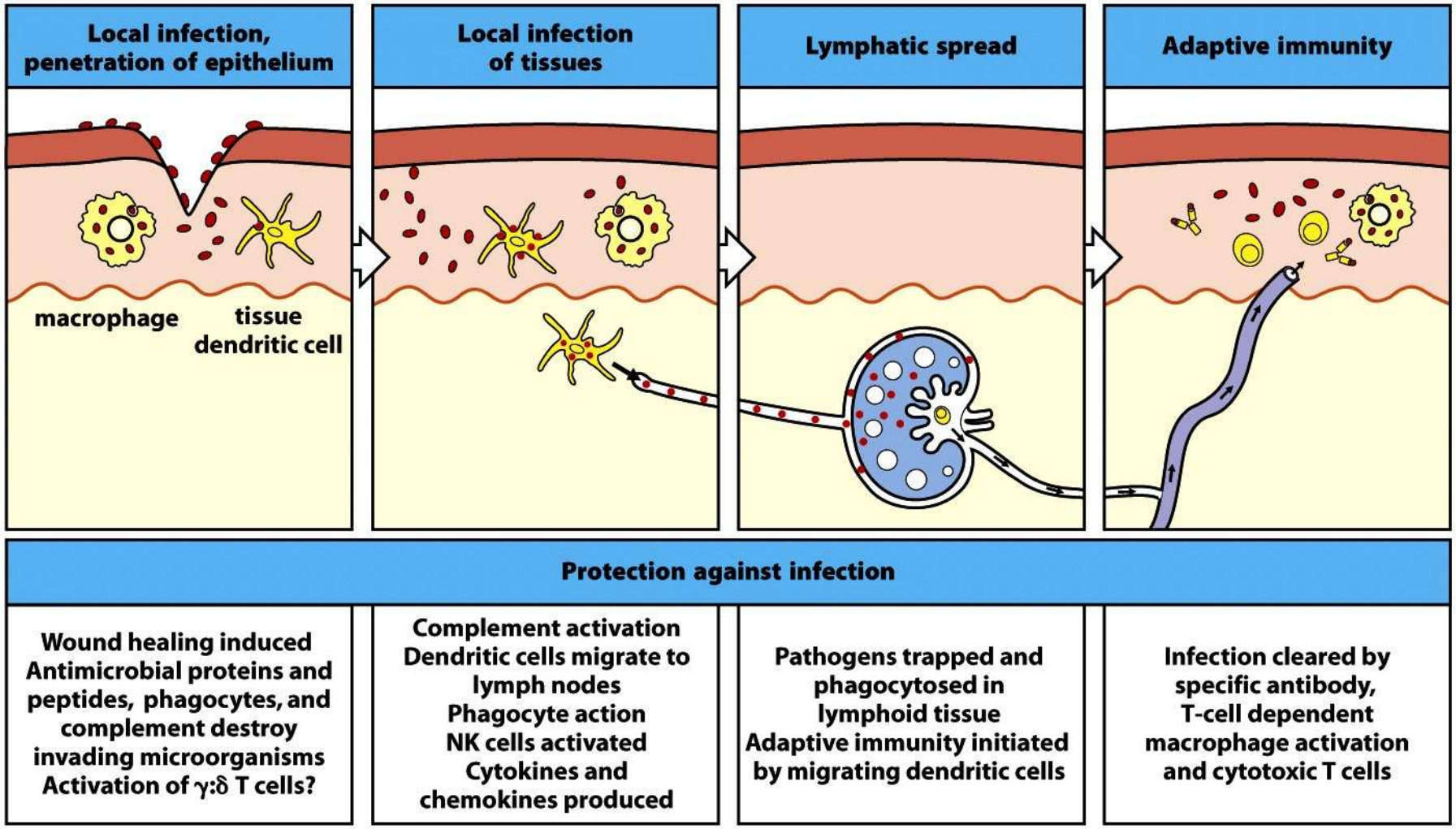


Figure 10-2 Immunobiology, 7ed. (© Garland Science 2008)

RESPUESTA INMEDIATA FRENTE A BACTERIAS EXTRACELULARES

	Immediate (0–4 hours)
	Nonspecific Innate No memory No specific T cells
Barrier functions	Skin, epithelia
Response to extracellular pathogens	Phagocytes Alternative and MBL complement pathway
Response to intracellular bacteria	Macrophages
Response to virus-infected cells	Natural killer (NK) cells

Figure 10-28 Immunobiology, 7ed. (© Garland Science 2008)

Las bacterias que expresan manosa en su superficie se unen a MBL

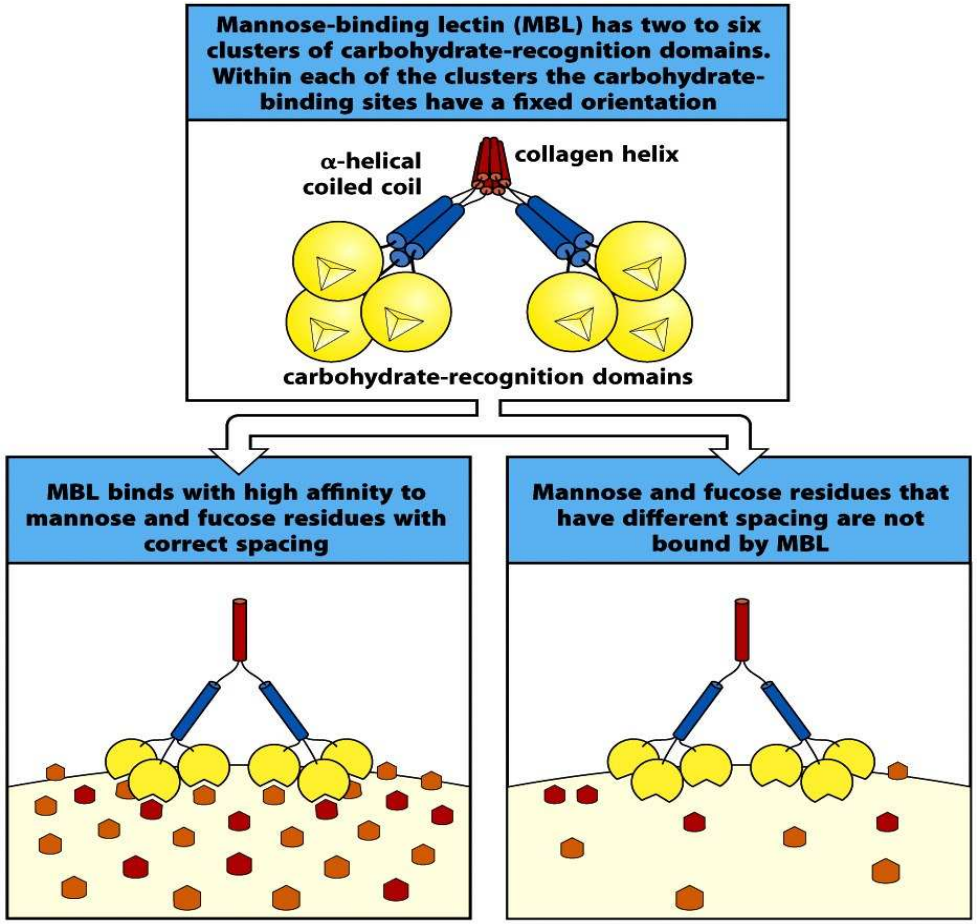


Figure 2-15 Immunobiology, 7ed. (© Garland Science 2008)

OPSONIZACIÓN Y FAGOCITOSIS

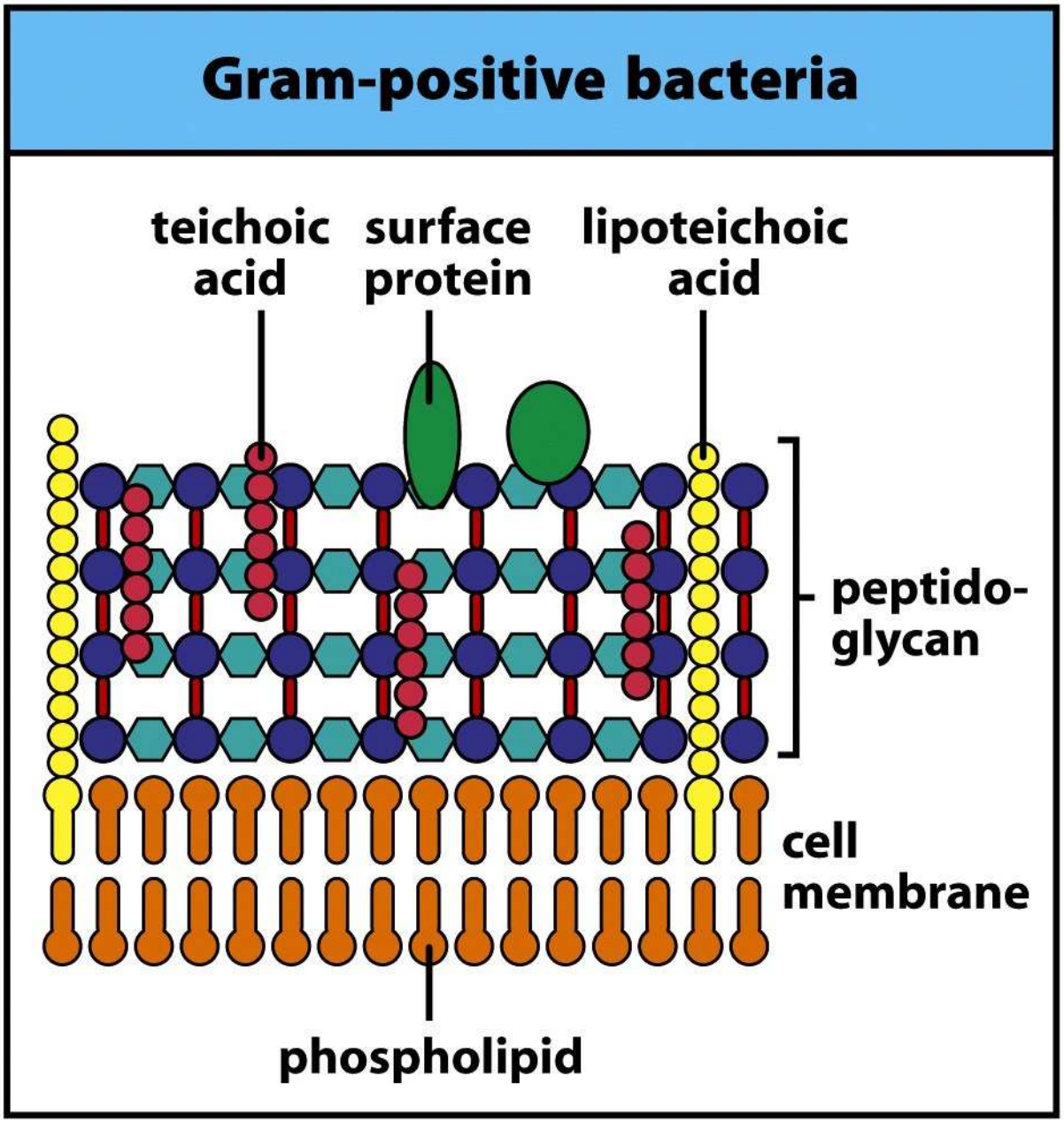


Figure 2-14 part 1 of 2 Immunobiology, 7ed. (© Garland Science 2008)

PEPTIDOGLICANO de la pared bacteriana es capaz de activar el complemento por la VÍA ALTERNATIVA

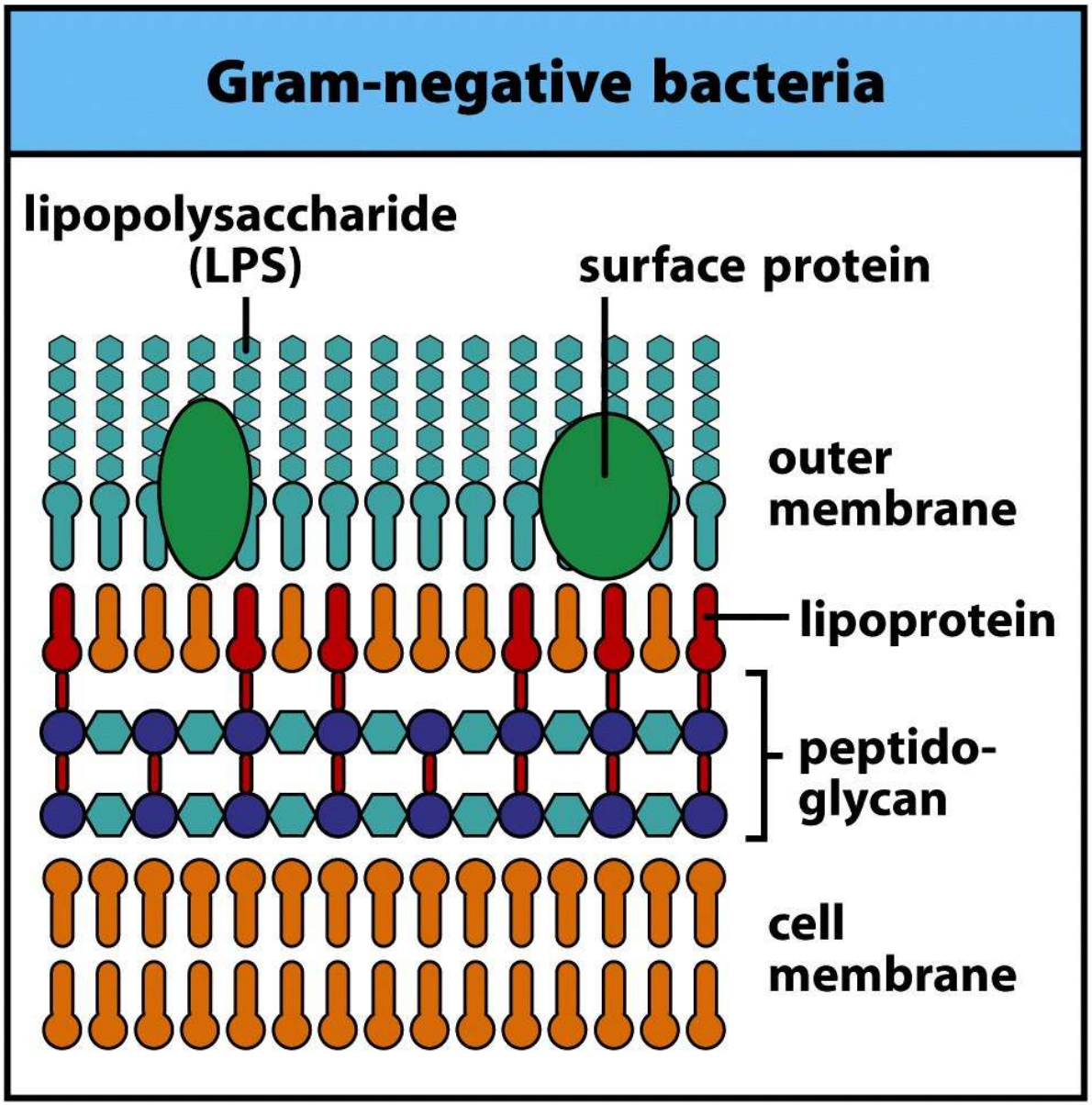


Figure 2-14 part 2 of 2 Immunobiology, 7ed. (© Garland Science 2008)

EL LPS de la pared bacteriana es capaz de activar el complemento por la VÍA ALTERNATIVA

CONSECUENCIAS DE LA ACTIVACIÓN DEL COMPLEMENTO….

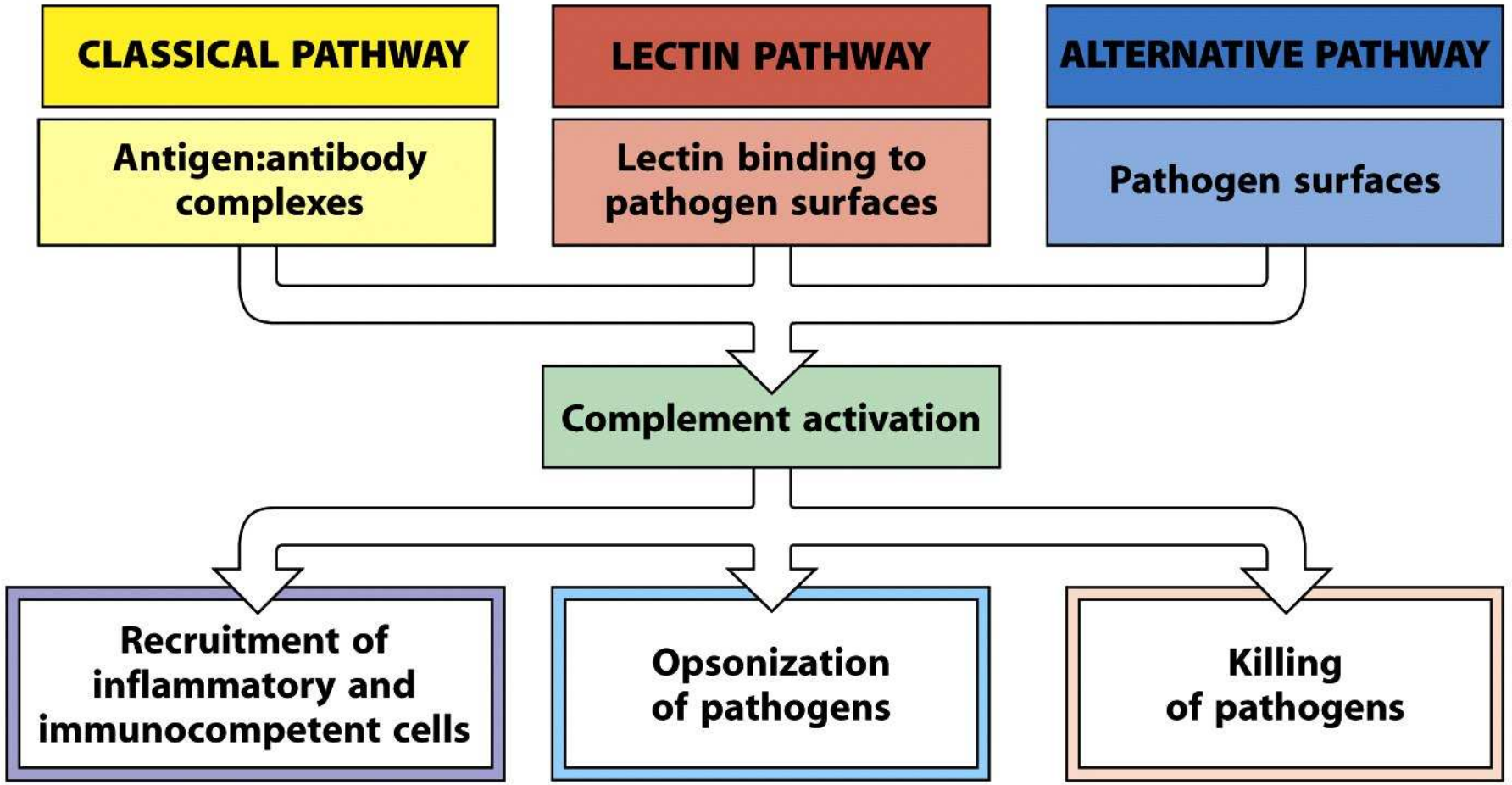
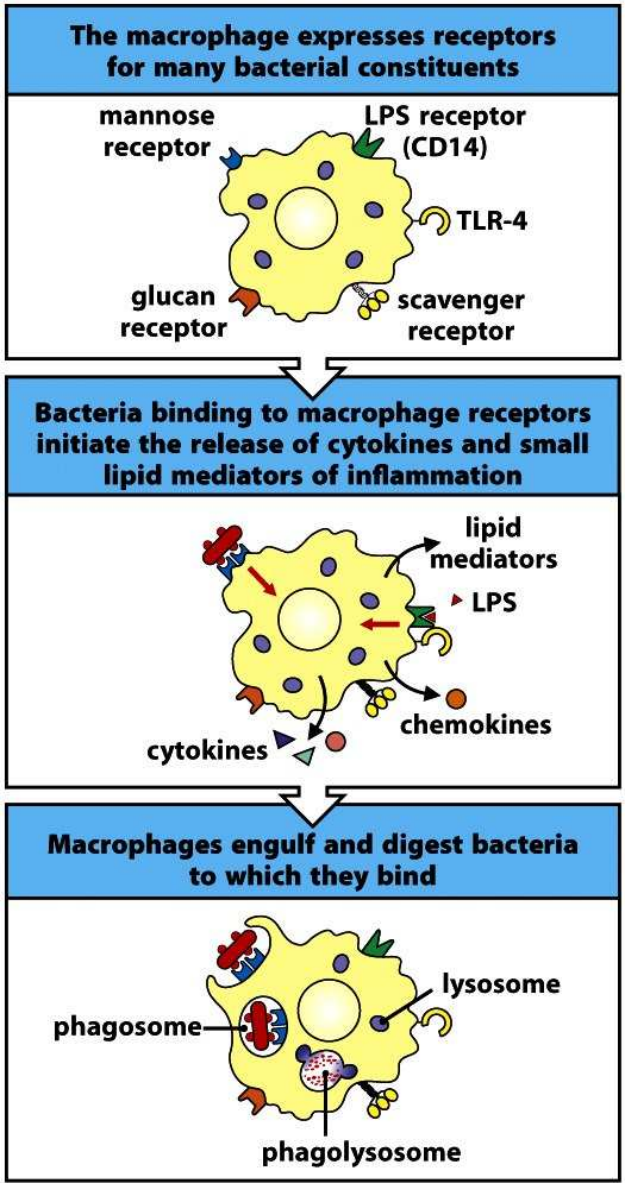


Figure 2-24 Immunobiology, 7ed. (© Garland Science 2008)



LOS MACRÓFAGOS TISULARES Y CÉLULAS DENDRÍTICAS CON LOS PRIMEROS EN ENCONTRAR A LOS PATÓGENOS EXTRACELULARES….

ACTIVACIÓN DE LOS TLR POR LAS BACTERIAS

Innate immune recognition by Toll-like receptors	
Toll-like receptor	**Ligand**
TLR-1:TLR-2 heterodimer	Peptidoglycan Lipoproteins Lipoarabinomannan (mycobacteria) GPI (*T. cruzi*) Zymosan (yeast)
TLR-2:TLR-6 heterodimer	
TLR-3	dsRNA
TLR-4 dimer (plus MD-2 and CD14)	LPS (Gram-negative bacteria) Lipoteichoic acids (Gram-positive bacteria)
TLR-5	Flagellin
TLR-7	ssRNA
TLR-8	G-rich oligonucleotides
TLR-9	Unmethylated CpG DNA

Figure 2-16 Immunobiology, 7ed. (© Garland Science 2008)

MEDIADORES INFLAMATORIOS RECLUTAN LEUCOCITOS DEL LECHO VASCULAR A LOS TEJIDOS…..

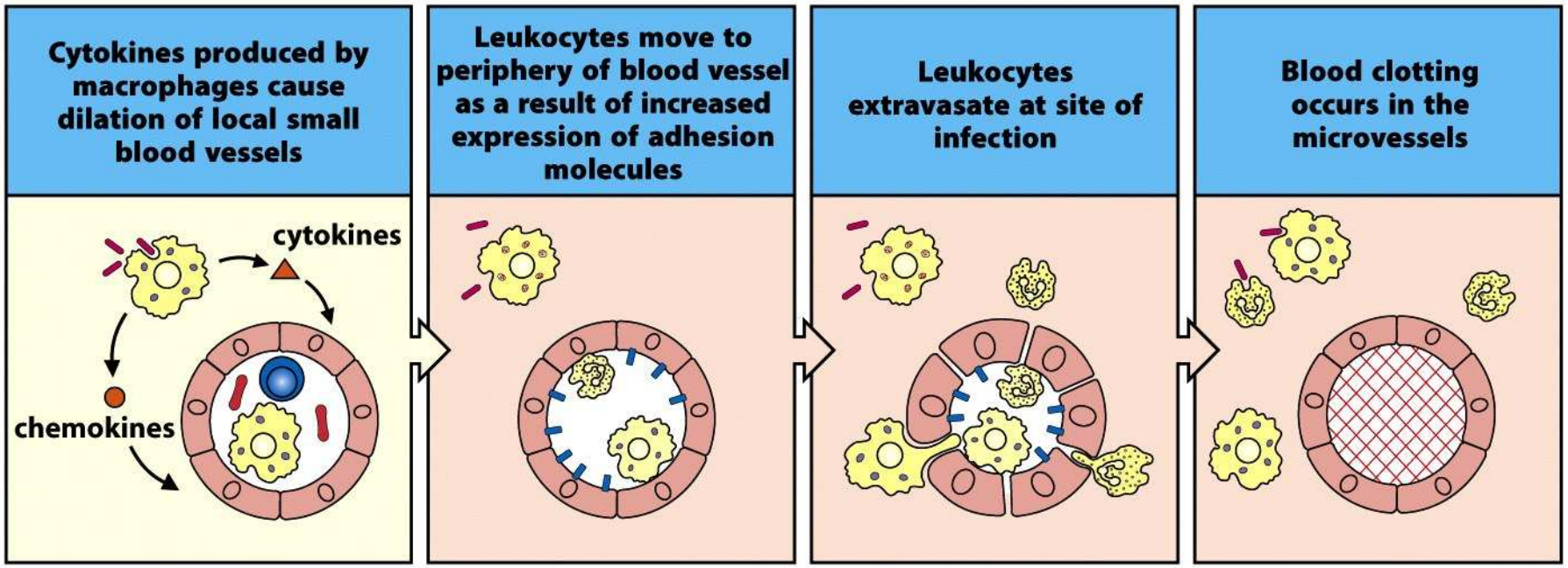


Figure 2-11 Immunobiology, 7ed. (© Garland Science 2008)

LAS BACTERIAS SON DESTRUIDAS EN EL INTERIOR DEL MACRÓFAGO

Class of mechanism	Specific products
Acidification	pH=~3.5–4.0, bacteriostatic or bactericidal
Toxic oxygen-derived products	Superoxide O_2^-, hydrogen peroxide H_2O_2, singlet oxygen $^1O_2^*$ hydroxyl radical $^*OH$, hypohalite OCl^-
Toxic nitrogen oxides	Nitric oxide NO
Antimicrobial peptides	Defensins and cationic proteins
Enzymes	Lysozyme—dissolves cell walls of some Gram-positive bacteria. Acid hydrolases—further digest bacteria
Competitors	Lactoferrin (binds Fe) and vitamin B_{12}-binding protein

Figure 2-9 Immunobiology, 7ed. (© Garland Science 2008)

	Phases of the immune response		
	Immediate (0–4 hours)	**Early (4–96 hours)**	**Late (96–100 hours)**
	Nonspecific Innate No memory No specific T cells	Nonspecific + specific Inducible No memory No specific T cells	Specific Inducible Memory Specific T cells
Barrier functions	Skin, epithelia	Local inflammation (C5a) Local TNF-α	IgA antibody in luminal spaces IgE antibody on mast cells Local inflammation
Response to extracellular pathogens	Phagocytes Alternative and MBL complement pathway	Mannan-binding lectin C-reactive protein T-independent B-cell antibody Complement	IgG antibody and Fc receptor-bearing cells IgG, IgM antibody + classical complement pathway
Response to intracellular bacteria	Macrophages	Activated NK-dependent macrophage activation IL-1, IL-6, TNF-α, IL-12	T-cell activation of macrophages by IFN-γ
Response to virus-infected cells	Natural killer (NK) cells	IFN-α and IFN-β IL-12-activated NK cells	Cytotoxic T cells IFN-γ

Figure 10-28 Immunobiology, 7ed. (© Garland Science 2008)

FUNCIONES DE LOS ANTICUERPOS

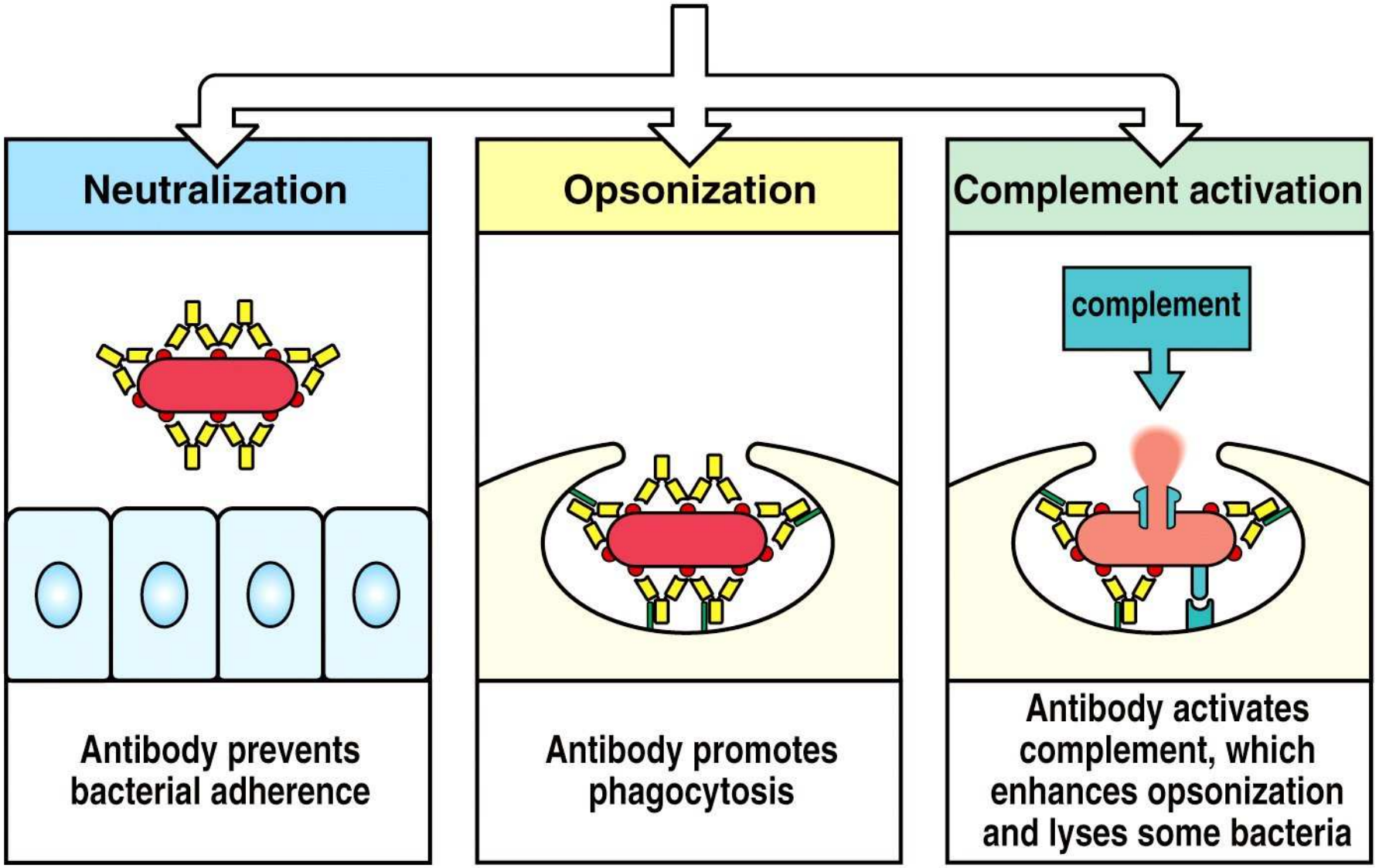
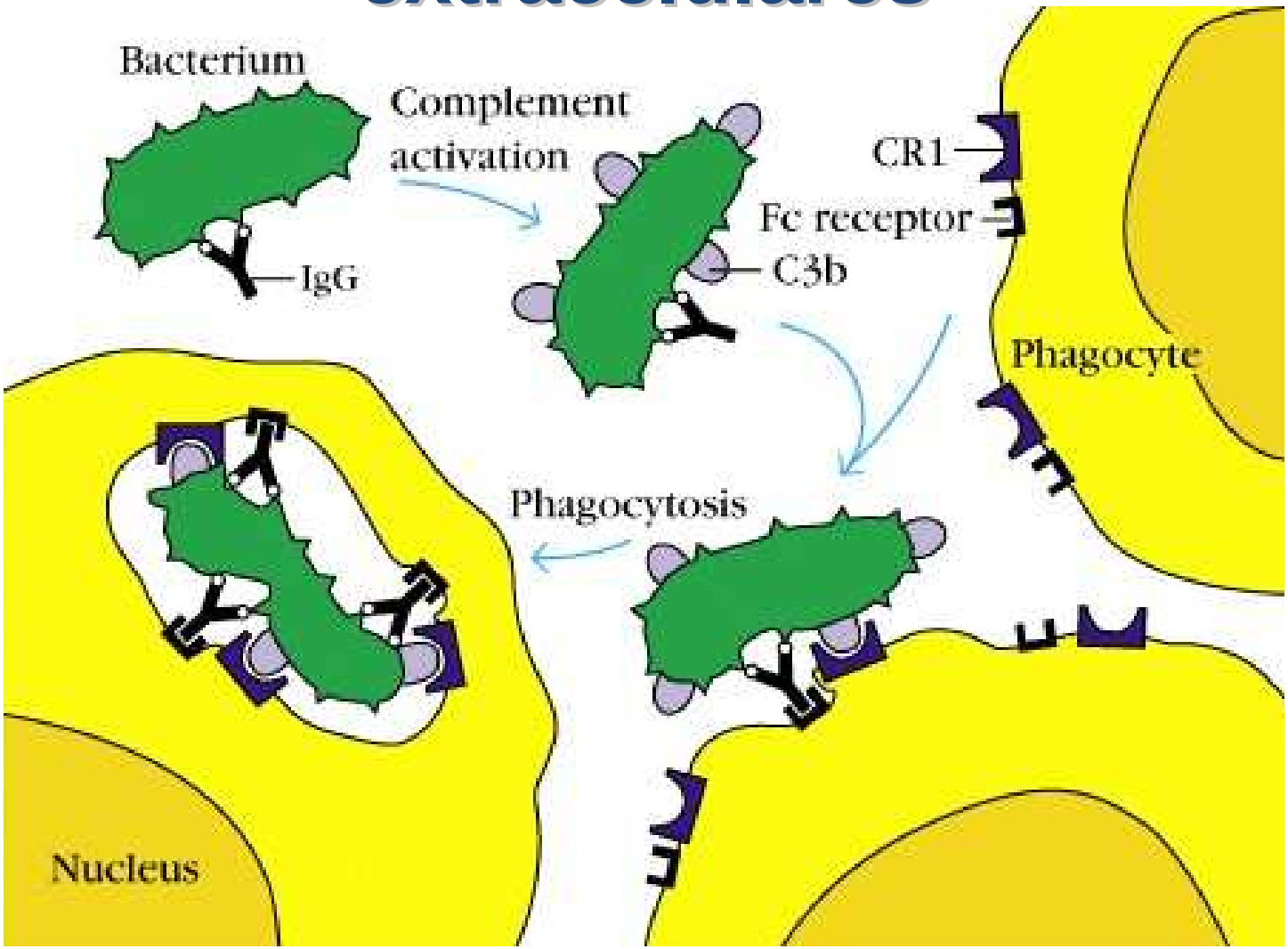


Figure 9-1 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Remoción de bacterias extracelulares



Bacterias Extracelulares

- **Se dividen fuera de las células del huésped:**

- Vías aéreas, Tracto genitourinario, Luz Intestinal, Tejido conectivo, Circulación sanguínea.

- **Estas bacterias crecen rápido y producen toxinas** por lo que es necesaria una respuesta muy rápida. Suelen provocar reacciones tisulares purulentas y formación de abcesos.

- **Cocos Gram +**
  - **Estafilococos**
  - **Estreptococos**

- **Cocos Gram -**
  - **Meningococos**
  - **Gonococos**

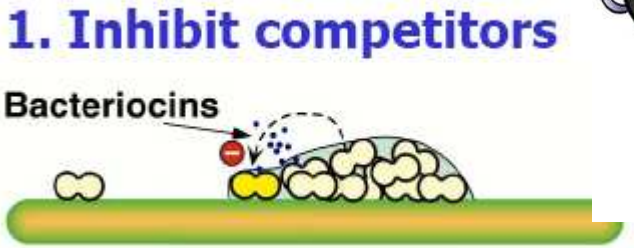
- **Bacilos Gram + (Anaerobios)**
  - ***Clostridium***

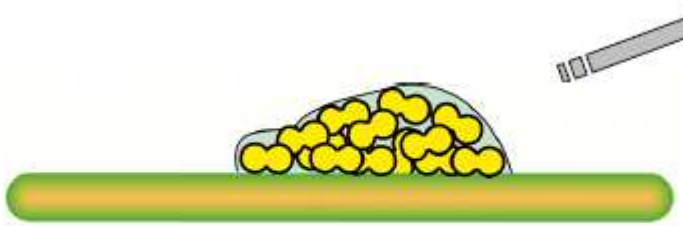
- **Bacilos Gram -**
  - ***E. coli***

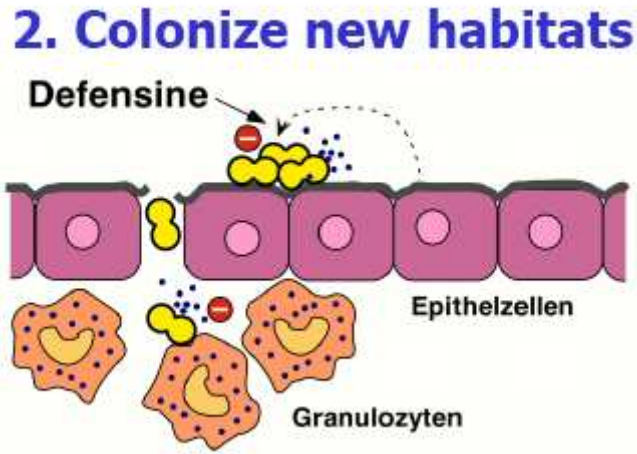
Mecanismos de enfermedad

- Inflamación y destrucción tisular
  - Formación de pus: Cocos piógenos
- Producción de toxinas
  - Exotoxinas:
    1. Citotóxicas: Shigella, B. Anthracis
    2. Interfieren con las funciones celulares
       - Cólera, difteria, tétanos
    3. Estimulan la producción de citoquinas
       - TSST-1
  - Endotoxinas:
    - Gram -. LPS

¿Por qué ciertas bacterias ocasionan ENFERMEDAD?

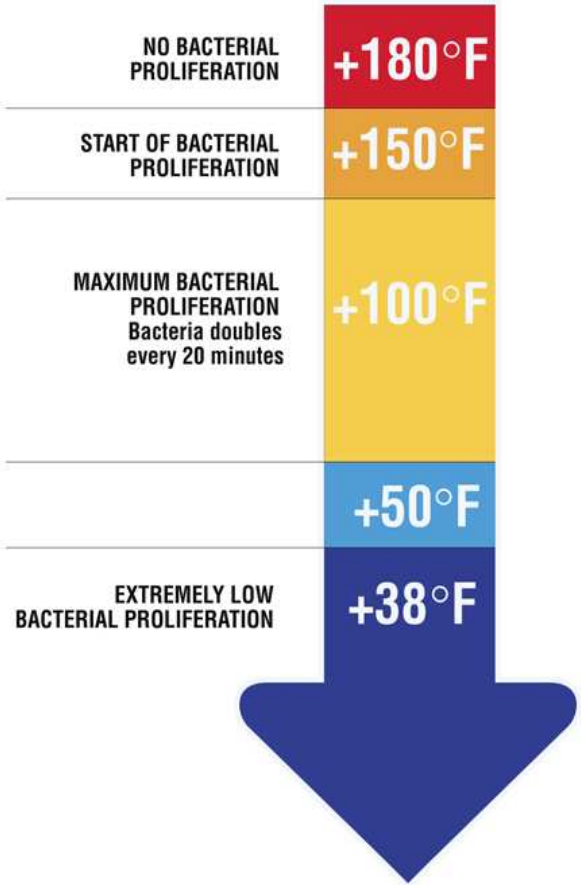






LOS FACTORES DE VIRULENCIA LES PERMITEN INVADIR OTROS TEJIDOS

LOS MICROORGANISMOS SON CAPACES DE ADAPTARSE A HABITATS DIFERENTES…



Extremely *hot*:

E.g. *Pyrolobus*: life at 113°C

Extremely *acidic*:

E.g. *Picrophilus*: life at pH 0,0

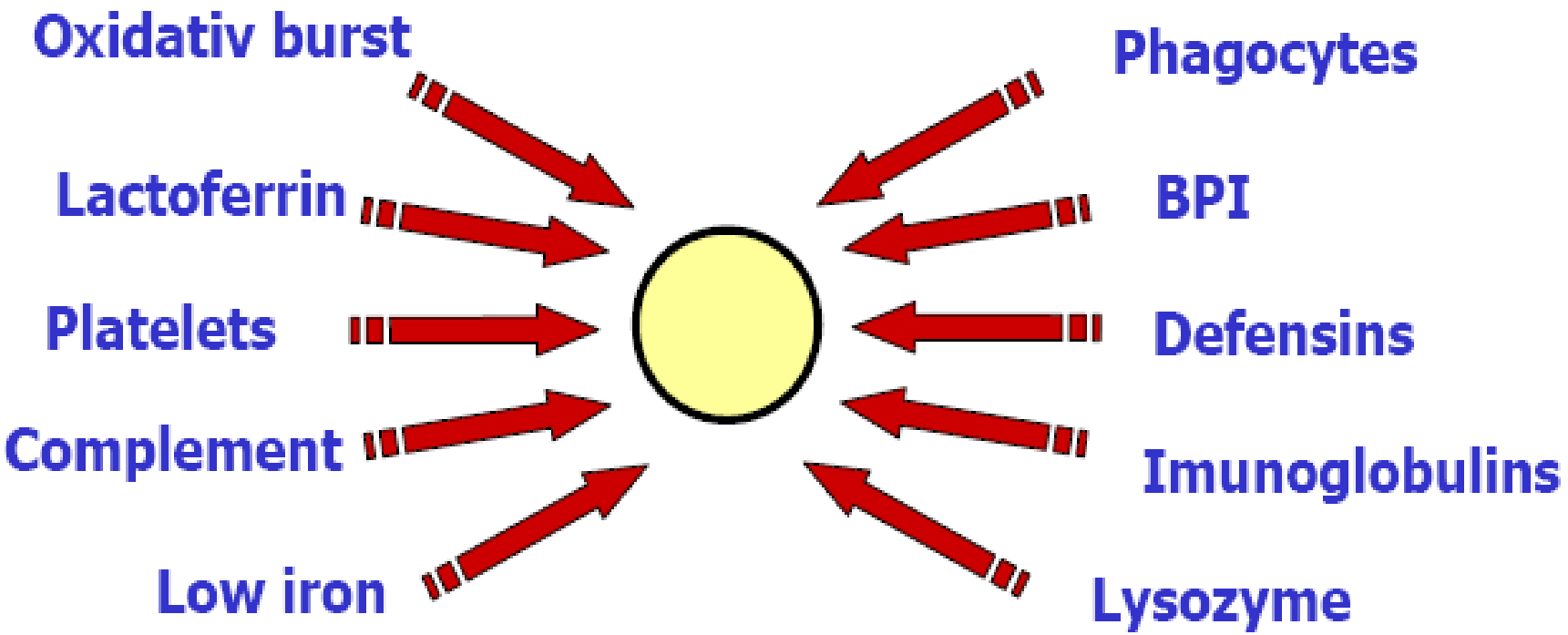
Extremely *alcaline*:

E.g. *Natronobacterium*: life at pH 12

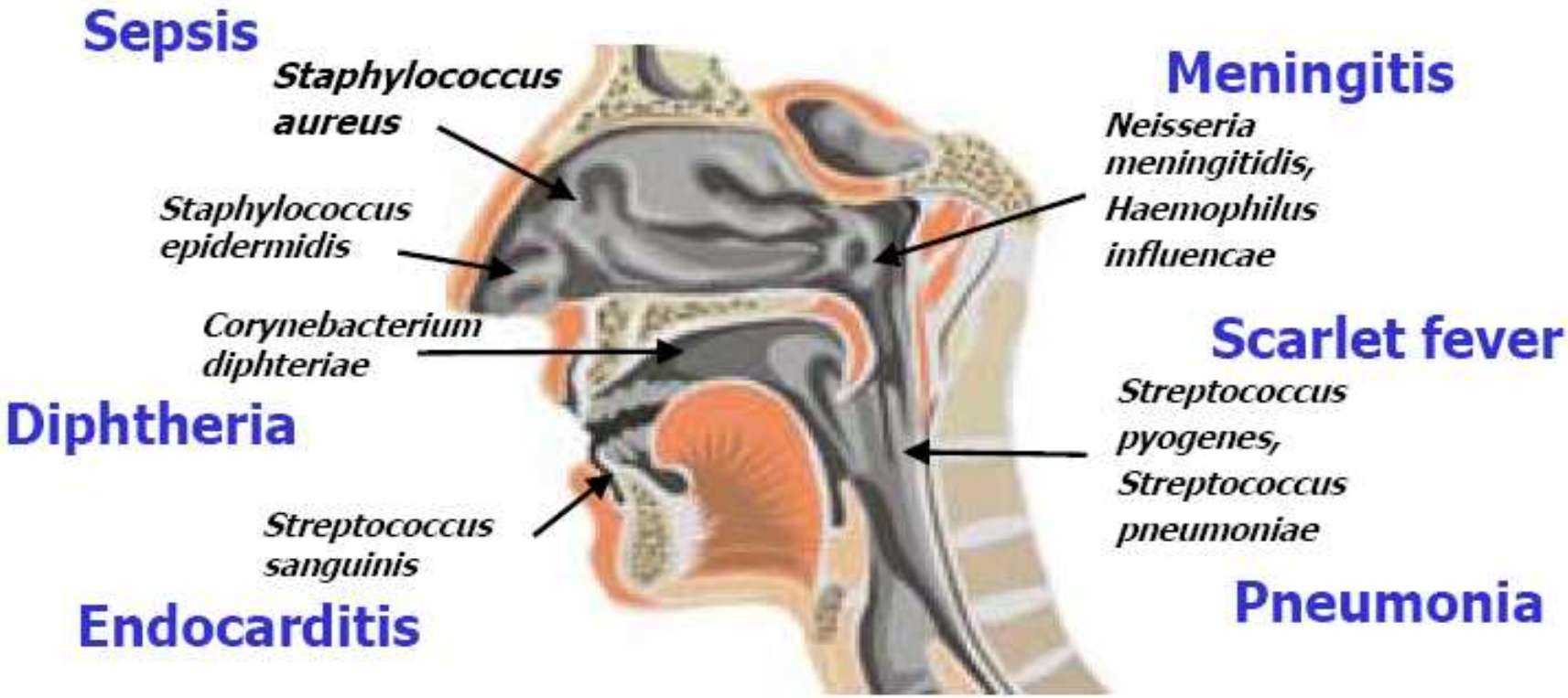
Extremely *salty*:

E.g. *Halobacterium*: life at 32% NaCl

CADA MICROORGANISMO ESTÁ EXPUESTO A CONDICIONES EXTREMAS



SIN EMBARGO……..



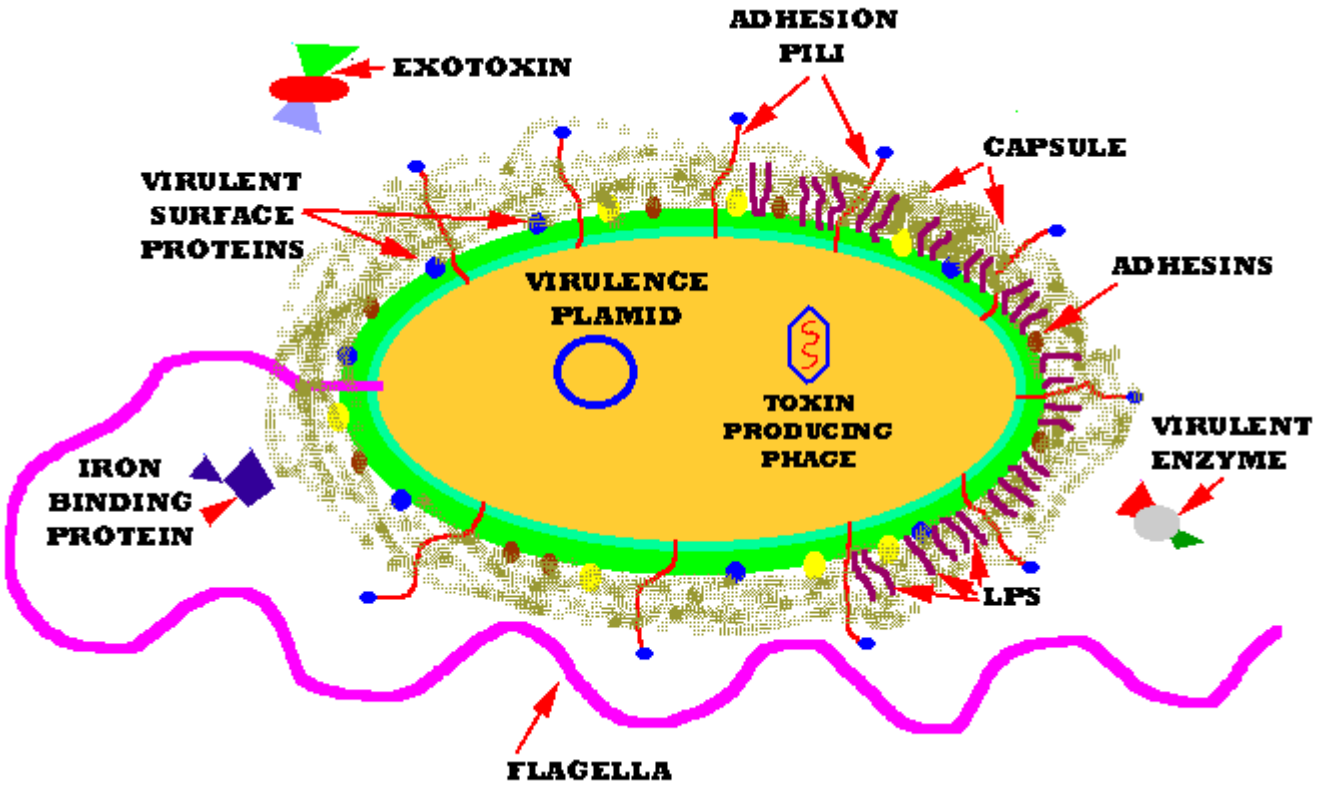
EXISTE UNA EXPOSICIÓN PERMANENTE A

FACTORES DE VIRULENCIA

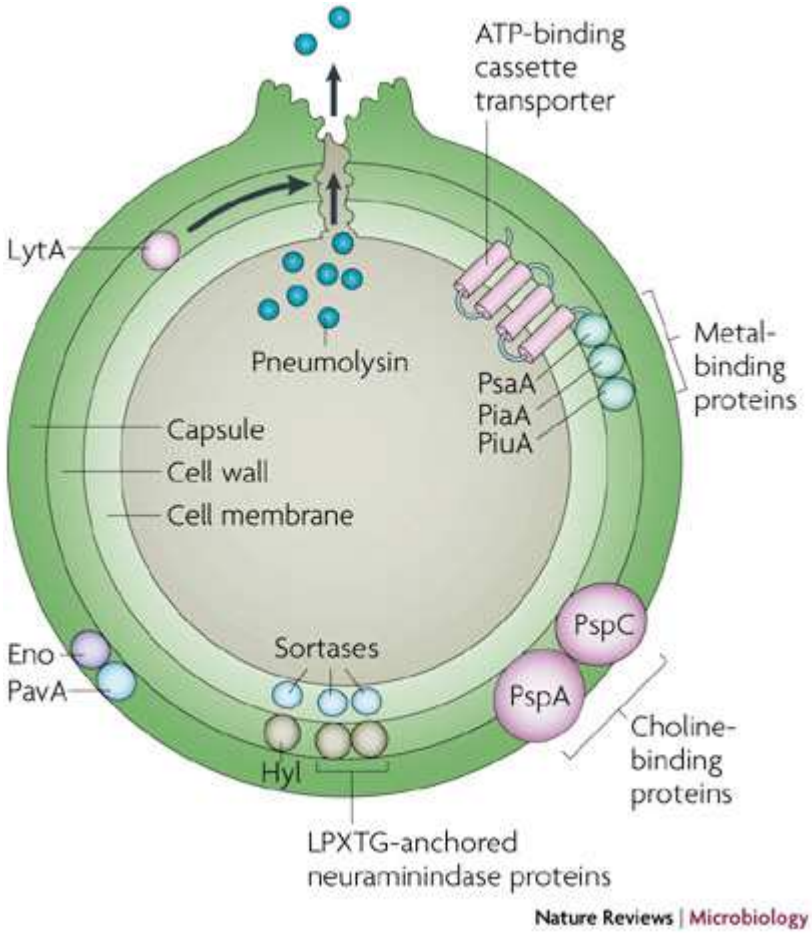
FACTORES DE VIRULENCIA

- ….CONFIEREN LA HABILIDAD PARA INVADIR Y MULTIPLICARSE EN LOS TEJIDOS DEL HUÉSPED
- ….SON MUY DIVERSOS EN SU ORIGEN Y FUNCIÓN
- …..SON FRECUENTEMENTE PRODUCIDOS POR LA FLORA MICROBIANA

Factores de virulencia



Streptococcus pneumoniae



Nature Reviews Microbiology 6, 288-301 (April 2008)

Important pneumococcal virulence factors include:

the capsule;

the cell wall;

choline-binding proteins;

pneumococcal surface proteins A and C (PspA and PspC);

the LPXTG-anchored neuraminidase proteins;

hyaluronate lyase (Hyl);

pneumococcal adhesion and virulence A (PavA);

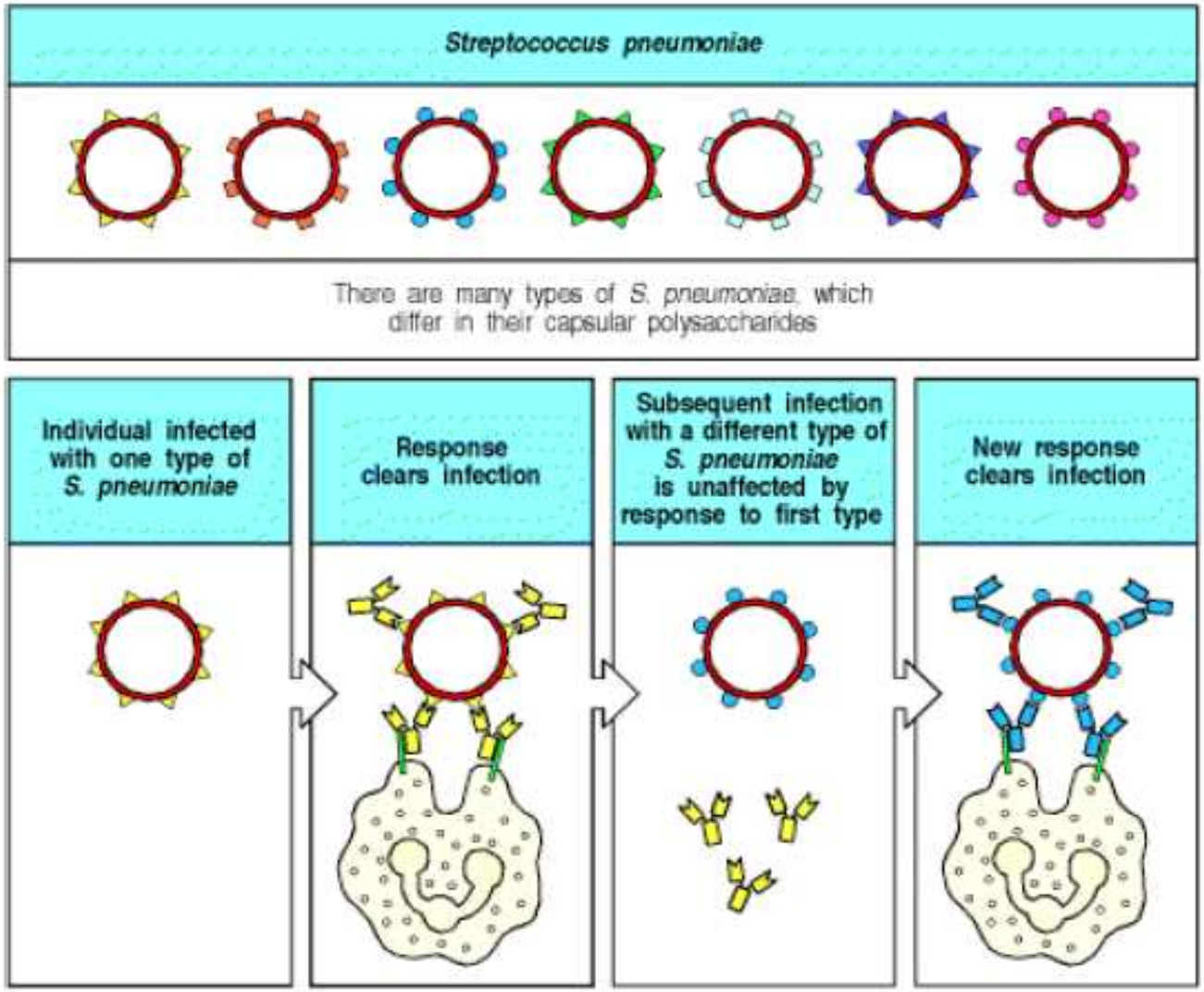
enolase (Eno);

pneumolysin;

autolysin A (LytA);

the metal-binding proteins pneumococcal surface antigen A (PsaA), pneumococcal iron acquisition A (PiaA) and pneumococcal iron uptake A (PiuA).

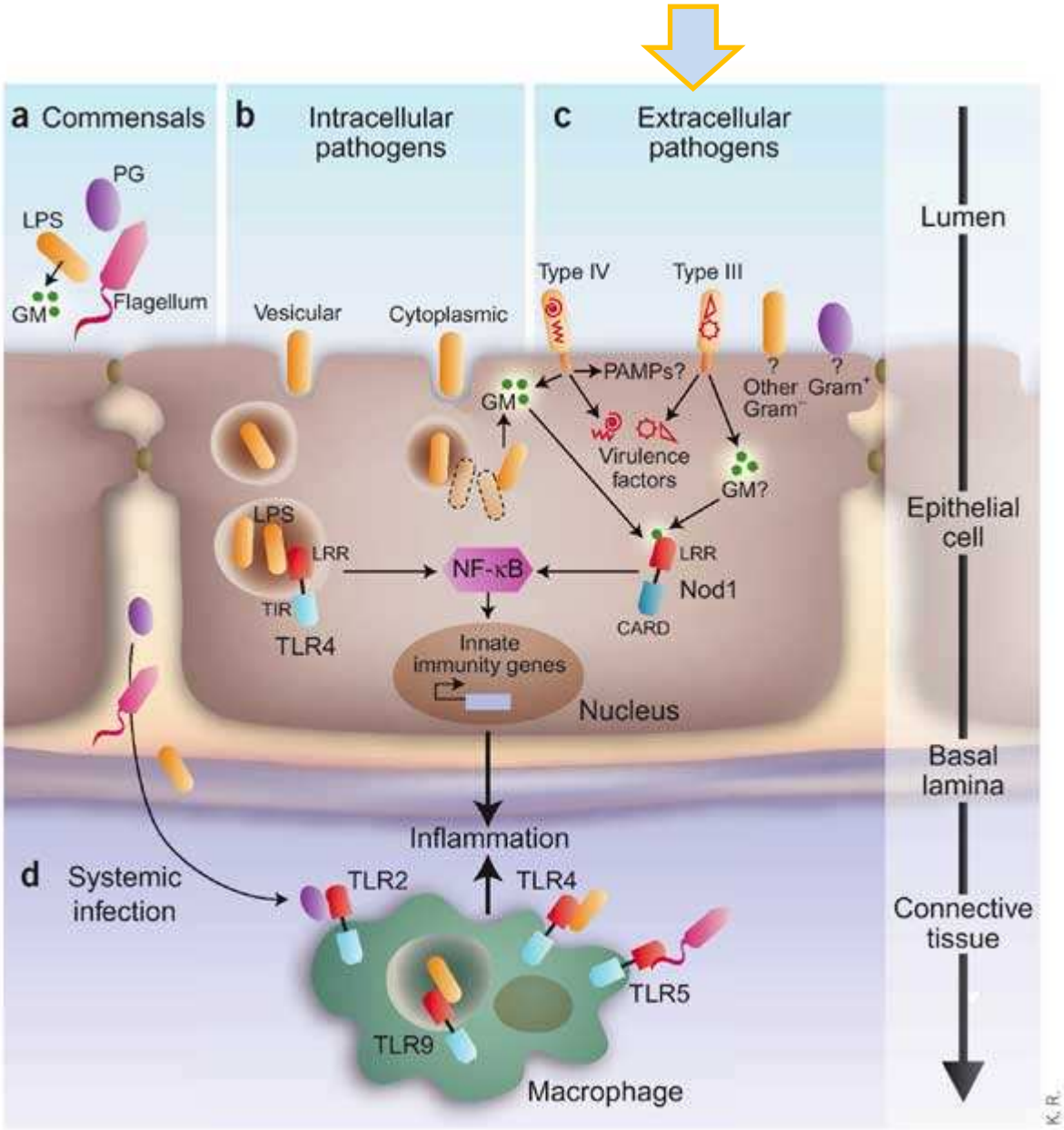
Streptococcus pneumoniae produces >100 types of capsular polysaccharides



Pneumococcal virulence factors and disease	Main role in colonization
Upper-airway colonization	
Capsule	Prevents entrapment in the nasal mucus, thereby allowing access to epithelial surfaces. Also inhibits effective opsonophagocytosis.
ChoP	Binds to rPAF on the epithelial surface of the human nasopharynx.
CbpA (also known as PspC)	Binds to human secretory component on a polymeric Ig receptor during the first stage of translocation across the epithelium.
NanA, BgaA and StrH	Act sequentially to cleave terminal sugars from human glycoconjugates, which might reveal receptors for adherence.
Hyl	Breaks down hyaluronan-containing extracellular matrix components.
PavA	Binds to fibronectin.
Eno	Binds to plasminogen.
Competition in upper airway	
Bacteriocin (pneumocin)	Small antimicrobial peptide that targets members of the same species.
Respiratory-tract infection and pneumonia	
Ply	Cytolytic toxin that also activates complement. An important determinant of virulence in *in vivo* models of disease. Wide range of effects on host immune components at sub-lytic concentrations.
PspA	Prevents binding of C3 onto pneumococcal surface. Also binds lactoferrin.
LytA	Digests the cell wall, which results in the release of Ply.
PsaA	Component of the ABC transport system, which is involved in resistance to oxidative stress.
PiaA and PiuA	Component of the ABC transport system.
NanA and NanB	Aid colonization by revealing receptors for adherence, modifying the surfaces of competing bacteria that are within the same niche and/or modifying the function of host clearance glycoproteins.
IgA	Cleaves human IgA1.

This list of virulence factors is not exhaustive and only selected examples are shown. For detailed descriptions of virulence factors see the main text. BgaA, β-galactosidase; CbpA, choline-binding protein A; ChoP, phosphorylcholine; Eno, enolase; Hyl, hyaluronate lyase; IgA, IgA1 protease; IgA1, immunoglobulin A1; LytA, autolysin A; Nan, neuraminidase; PavA, pneumococcal adhesion and virulence A; PiaA, pneumococcal iron acquisition A; PiuA, pneumococcal iron uptake A; Ply, pneumolysin; PsaA, pneumococcal surface antigen A; PspA, pneumococcal surface protein A; StrH, β-*N*-acetylglucosaminidase.

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.



Nature Immunology **5**, 1099 - 1100 (2004)

Helicobacter pylori

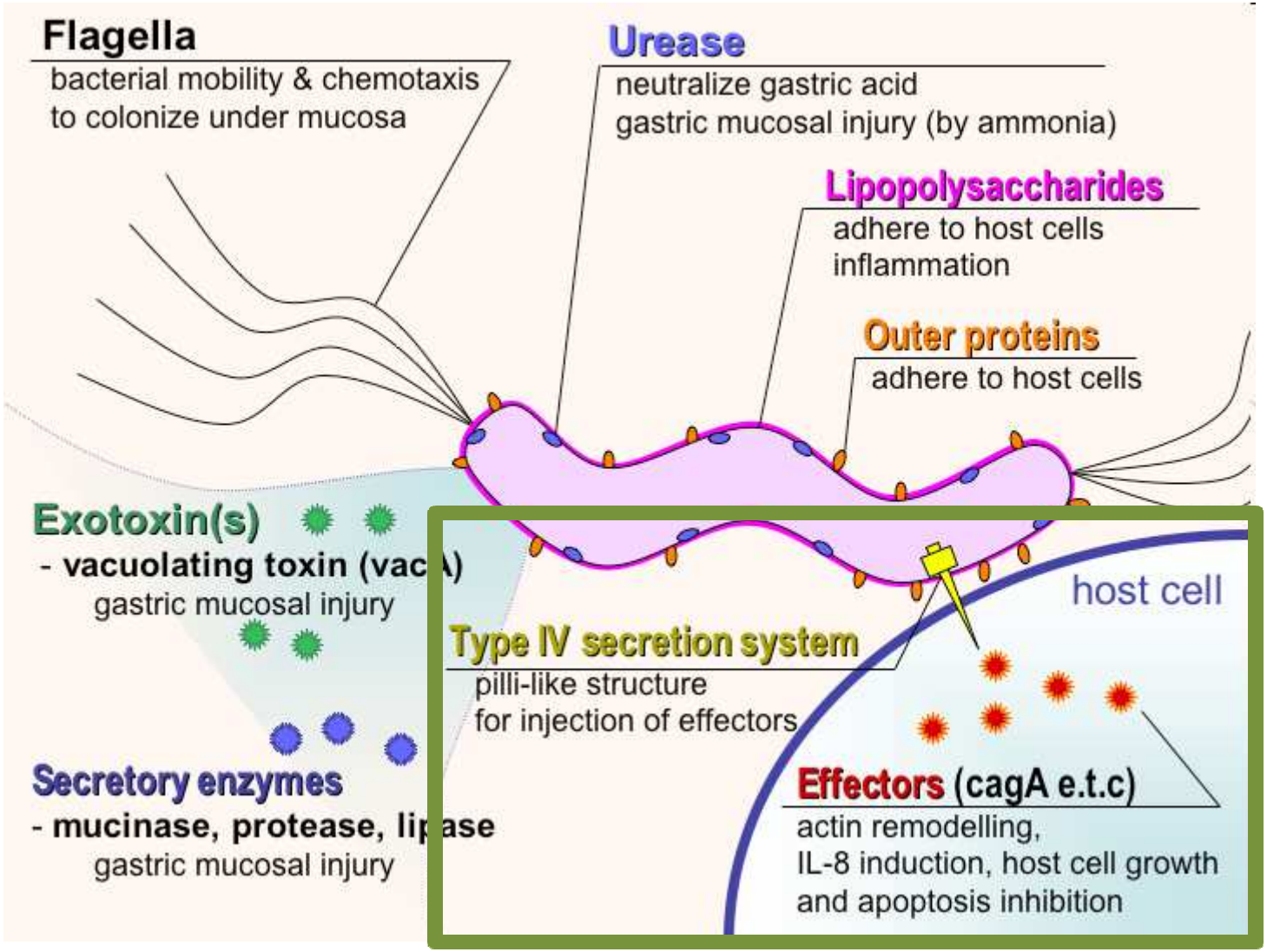
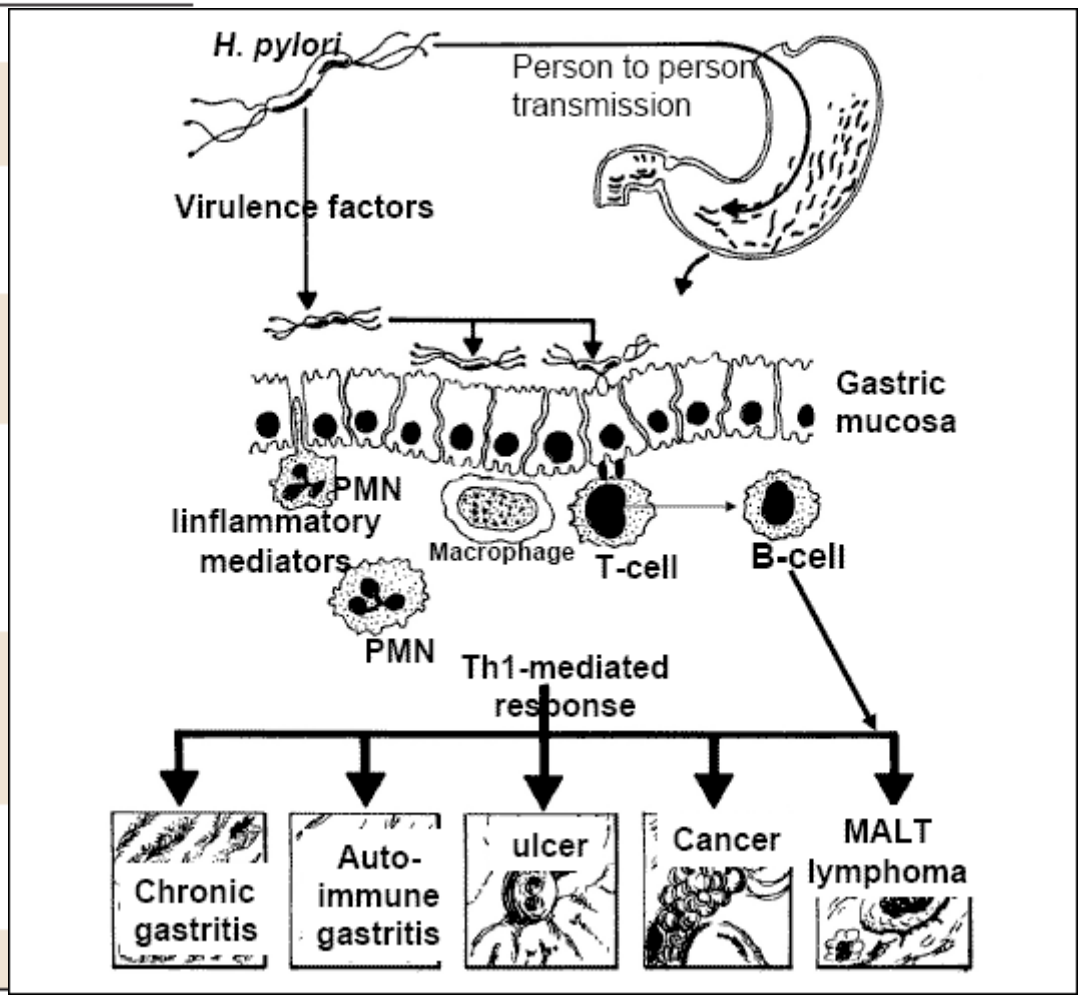
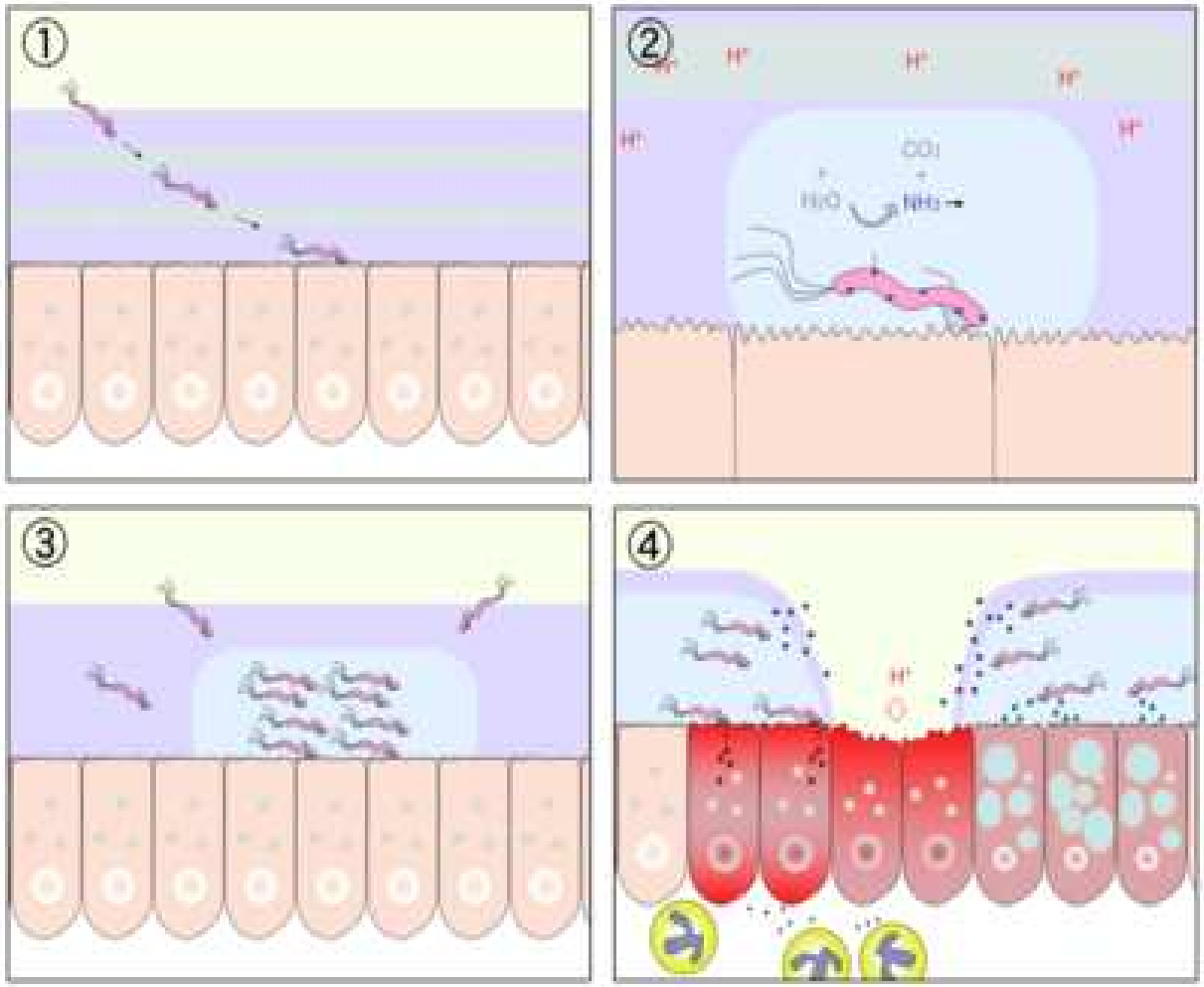


Table 3 | ***Helicobacter pylori* virulence determinants**

Virulence determinant	Description/potential role in pathogenesis
VacA	95-kDa secreted vacuolating toxin; induces apoptosis; involved in immunomodulation and colonization of mouse stomach
Cag-PAI	37-kb genomic fragment; contains 29 genes that encode a type IV secretion apparatus
CagA	120-kD protein; translocated into host cell by type IV secretion apparatus encoded on Cag-PAI; phosporylated in host cell and binds SHP-2 tyrosine phosphatase; disrupts tight junctions; epidemiologic link to cancer
BabA	78-kDa outer membrane protein; binds to fucosylated Lewis B blood group antigen; mediates adhesion to epithelial cells and possibly stomach epithelium
Urease	Resists acidic conditions in the stomach; activates innate immune responses during early steps of infection
Flagella	Involved in motility; essential for colonization

PAI, pathogenicity island.





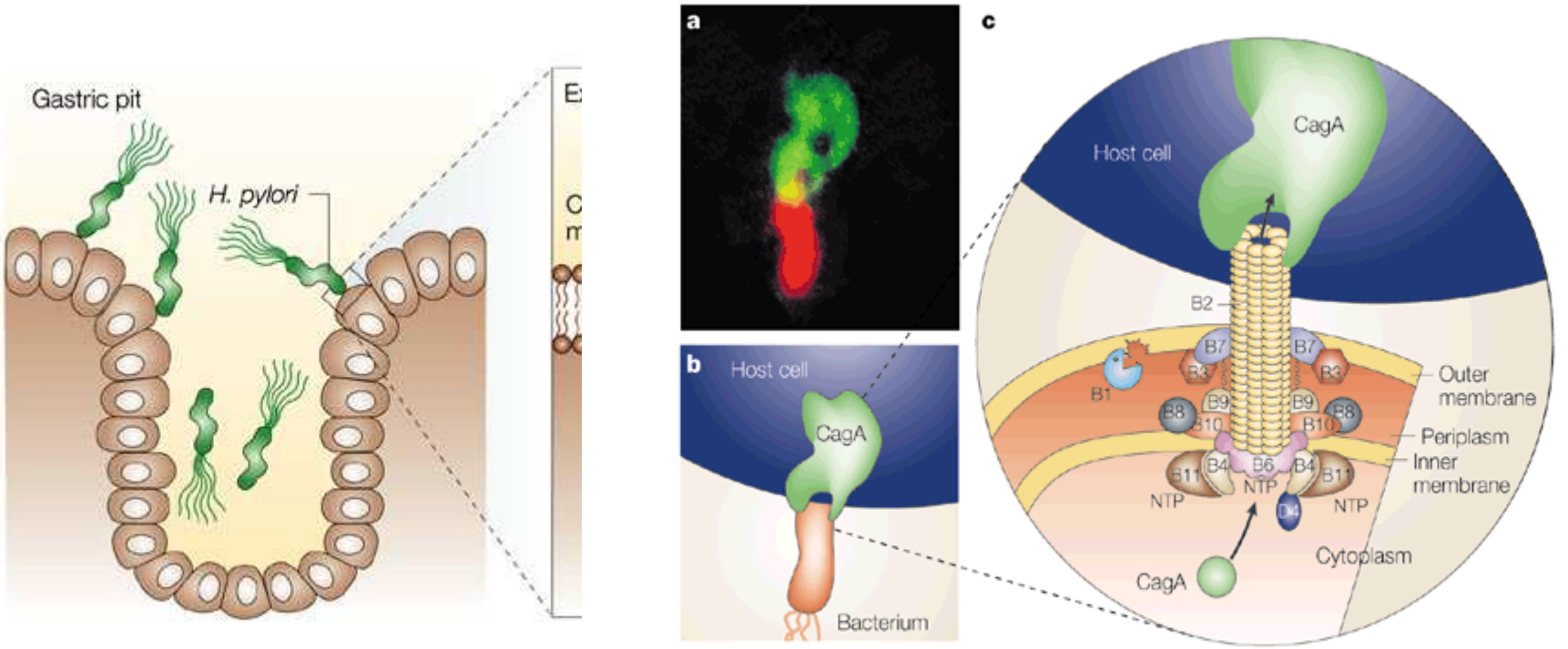
Modo de infección de *H. pylori*:

1. *H. pylori* penetra la capa mucosa del estómago y se adhiere a la superficie de la capa mucosa epitelial gástrica.

2. Produce amoníaco a partir de la urea, para neutralizar la acidez gástrica.

3. Migración y proliferación de *H. pylori* al foco de infección.

4. Se desarrolla la ulceración gástrica con destrucción de la mucosa, inflamación y muerte de las células mucosas.



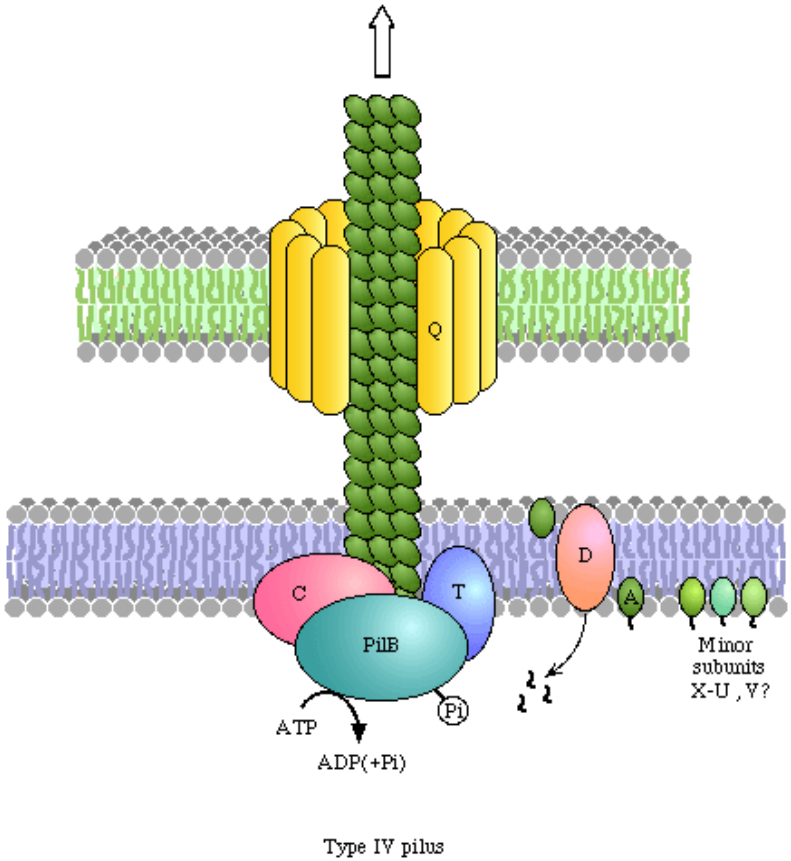
Nature Reviews Cancer 4, 688-694 (Septemb

Helicobacter pylori that is positive for the **cytotoxin-associated antigen A (cagA) gene** adheres to gastric epithelial cells of the gastric pit through an interaction between bacterial adhesin and the host-cell receptor. Following attachment of H. pylori to the cell, **CagA is translocated into the intracellular region of host cells through the type IV secretion system, which is encoded by the cag pathogenicity island.** The translocated CagA protein localizes to the plasma membrane and then undergoes tyrosine phosphorylation at EPIYA sites, mediated by the SRC-family tyrosine kinases. Phosphorylated CagA interacts with intracellular signal transducers, deregulates their activities and elicits pathobiological actions.

Cell Biology

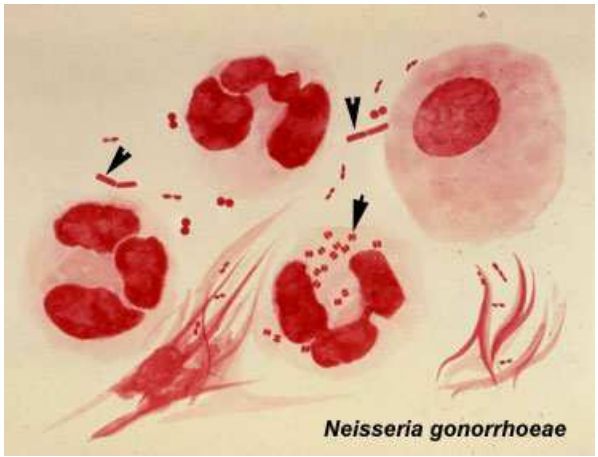
TYPE SECRETION SYSTEM

The **type IV secretion** pathway [Finlay and Falkow 1997] **comprises a group of so-called auto-transporters**, **including gonococcal immunoglobulin A and other proteases, the vacuolating cytotoxin of *Helicobacter pylori*, a family of outer membrane proteins in *B. pertussis*, and the secreted proteins SepA and EspC from *S. flexneri* and EPEC, respectively**. Apparently, these **auto-transporters form a pore in the outer membrane through which they pass, and autoproteolytic cleavage releases the proteins into the supernatant.**

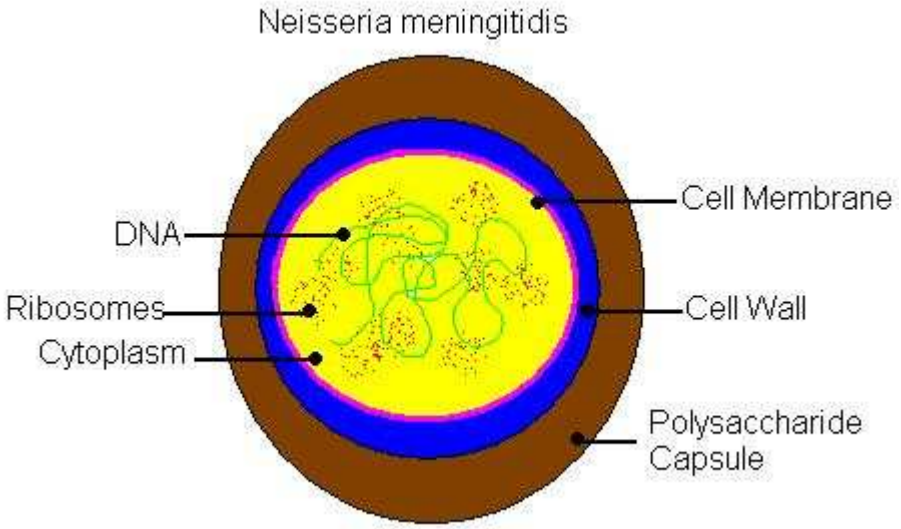


Type IV pilus

MECHANISM	EXAMPLE
Bypassing the TLR system	- Flagellin fails to activate TLR5 (Gewirtz et al., 2004) - Tetraacylated LPS poorly activates TLR4 and may actually work as an antagonist (Moran and Aspinall, 1998) - DNA is rich in A/T nucleotides and frequently methylated, making TLR9 response more unprobable (Blaser and Atherton, 2004)
Minimization of innate immunity	- Modification of LPS (long 3-hydroxy fatty acids of lipid A, unusual phosphorylation pattern…) (Moran et al., 1997, 1998)
Mimicry of host antigens	- Lewis expression (O antigen region of *H. pylori* LPS) (Wirth et al., 1997)
Antigenic variation	CagY (Aras et al., 2003)
Host gene expression modulation	- Upregulation of specific inflammatory and immune mediators including β-defensin, protease inhibitor, chemokine receptor, interleukin-1β, tumor necrosis factor-α-inducible protein… (Israel and Peek, 2006)
Downregulation of immune effectors	- Blocking the proliferation of T cell through VacA and B cell proliferation through CagA-induced supression- Interference with phagocytosis (Baldari et al., 2005)
Avoidance of attack by ROIs and RNIs	- Enzymes involved in ROI scavenging, such as catalase and superoxide dismutase - Arginase regulates NO synthesis (Baldari et al., 2005)
Ability to colonize gastric environment	- Neutralizing pH around the organism with urease enzyme - Interaction between adhesins and local cell receptors - Expressing mucolytic molecules - Relative absence of immune cells in gastric mucosa and rare competition with commensal bacteria
High mutational and recombinational frequency, high diversity	- Rapid development in the bacterial population of high-level resistance to commonly used antibiotics - High competence for uptake of DNA from other *H. pylori* strains (Sansonetti and Di Santo, 2007)

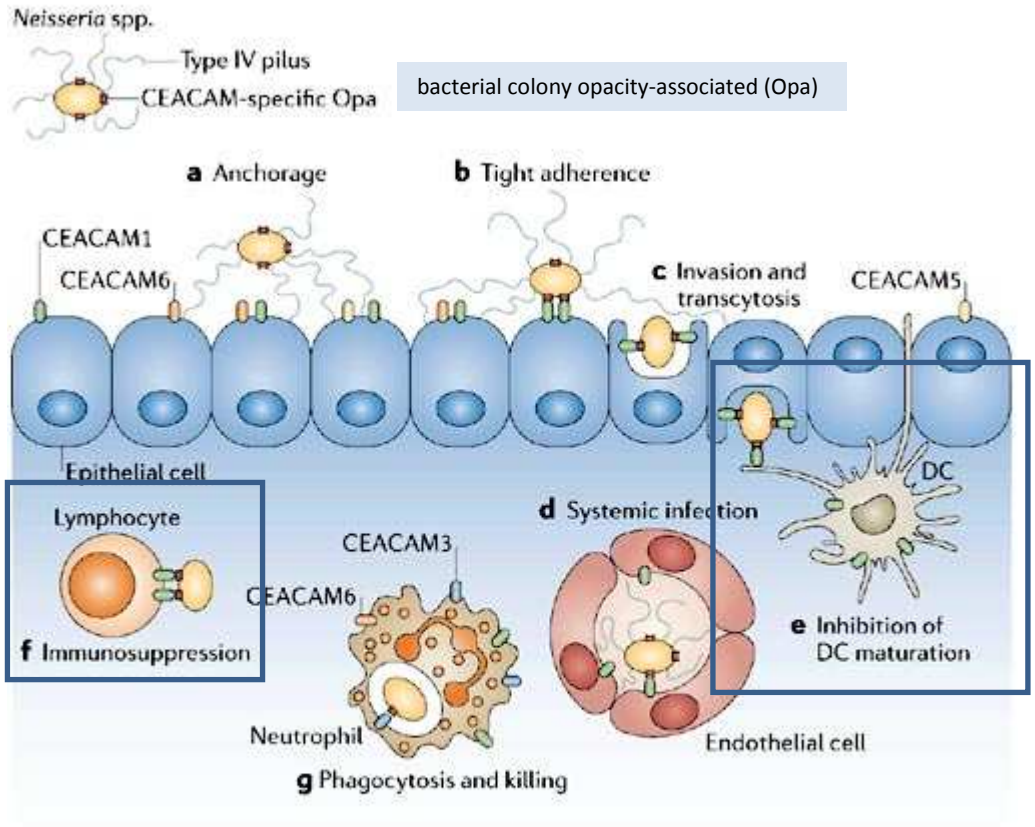


Pathogenic Neisserria	
N. gonorrhoeae	• Antigenically variable surface antigens, no capsul • Colonizes the genital tract • Does <u>not</u> form acid from maltose
N. meningitidis	• Presence of a carbohydrate capsule • Colonizes the respiratory tract • <u>Forms</u> acid from maltose
Non-Pathogenic Neisserria	
	All colonize the human respiratory tract



Es un comensal de la mucosa rinofaríngea. Su capacidad de evadir al sistema inmune le permite atravesar la BHE y causar MENINGITIS…..

N. MENINGITIDIS



Primary anchorage of Neisseria spp. to the apical surface of mucosal epithelial cells is mediated by type IV pili (a), which retract to allow a tight secondary adherence through the bacterial colony opacity-associated (Opa) proteins binding to carcinoembryonic-antigen-related cell-adhesion molecule 1 (CEACAM1), CEACAM5 and CEACAM6 (b). This interaction leads to the transcellular transcytosis of bacteria through the epithelial cells to emerge in the subepithelial spaces (c). Rarely, Neisseria spp. enter the blood and cause a systemic infection (d). Although entering the subepithelial spaces allows the Neisseria spp. access to abundant nutrients, this also exposes the bacteria to cells involved in immune surveillance.

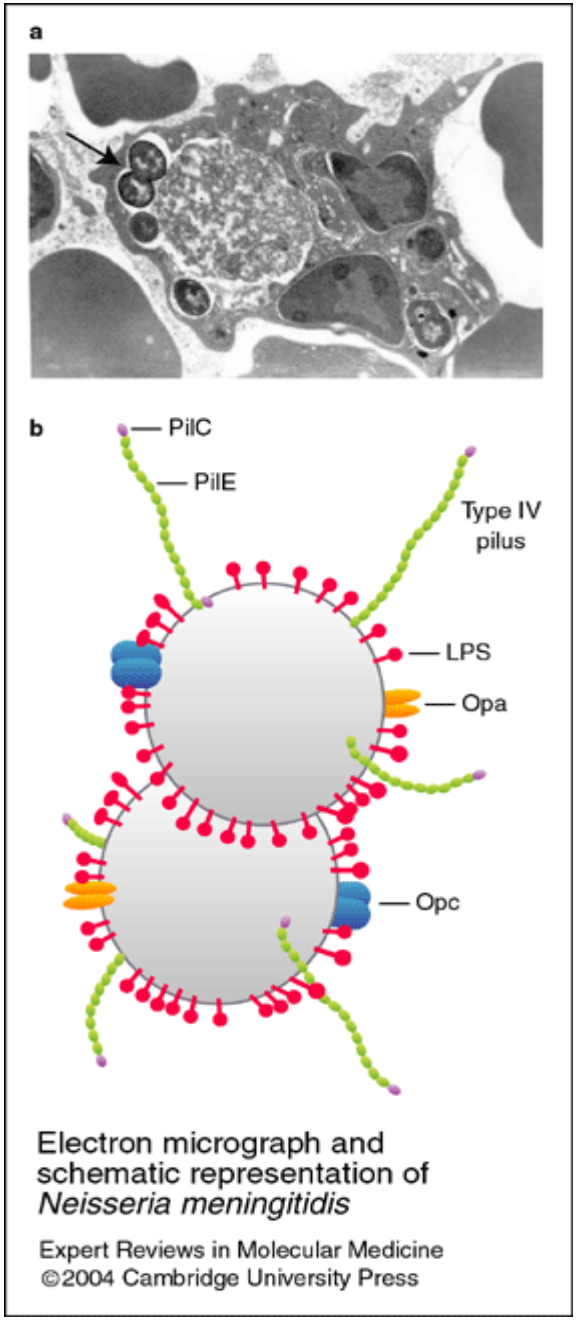
Opa binding to CEACAM1 impairs normal maturation of immature dendritic cells (e), and suppresses lymphocyte responses to activating stimuli (f).

CEACAM1 binding should also impair phagocytic engulfment of the bacteria, however neutrophil expression of the decoy receptor CEACAM3 provides a potent activation signal that allows the rapid engulfment and killing of Opa-expressing bacteria (g).

Nature Reviews Immunology 6, 433-446 (June 2006)

Proteína Opa se une a receptor inhibitorio CECAM1 de LTCD4 y DC….

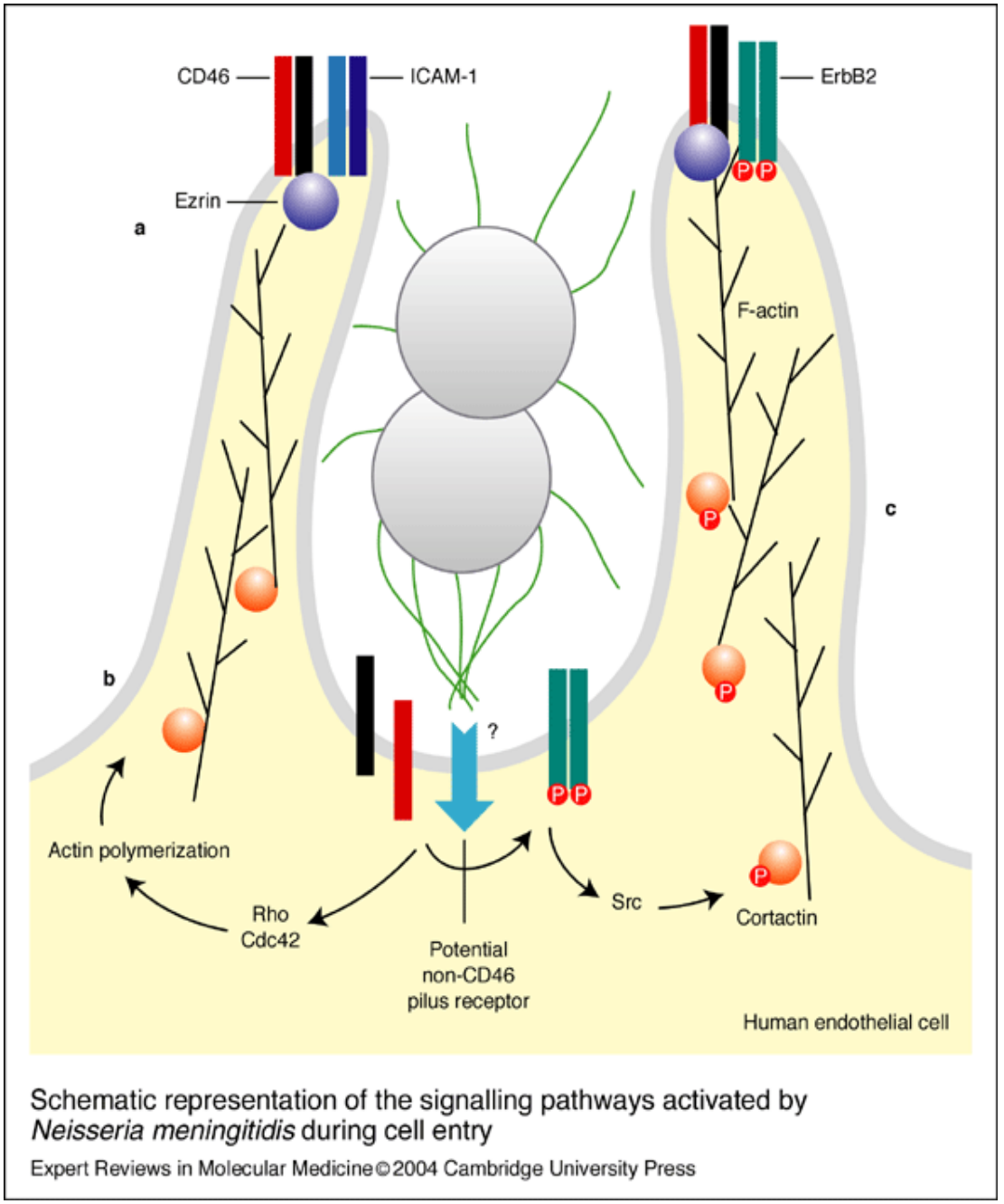
(a) Electron micrograph showing paired bacteria (arrow) within a splenic macrophage. Approximate magnification = X10 000. (b) Schematic representation showing the major adhesins. Pathogenic *N. meningitidis* possesses several surface-expressed adhesins. The type IV pilus mediates initial interactions with host cells. The opacity proteins Opa and Opc are phase-variable outer membrane proteins believed to play a role in later stages of adhesion and invasion, and enable adhesion in the absence of pili. The lipopolysaccharide (LPS) molecules display variation in side chains; the unsialylated forms of LPS might contribute to adhesion by binding to asialoglycoprotein receptors. The polysaccharide capsule is believed to inhibit adhesion to cells, possibly due to negatively charged molecules such as sialic acid that repel host cells or by masking the exposure of other surface adhesins.



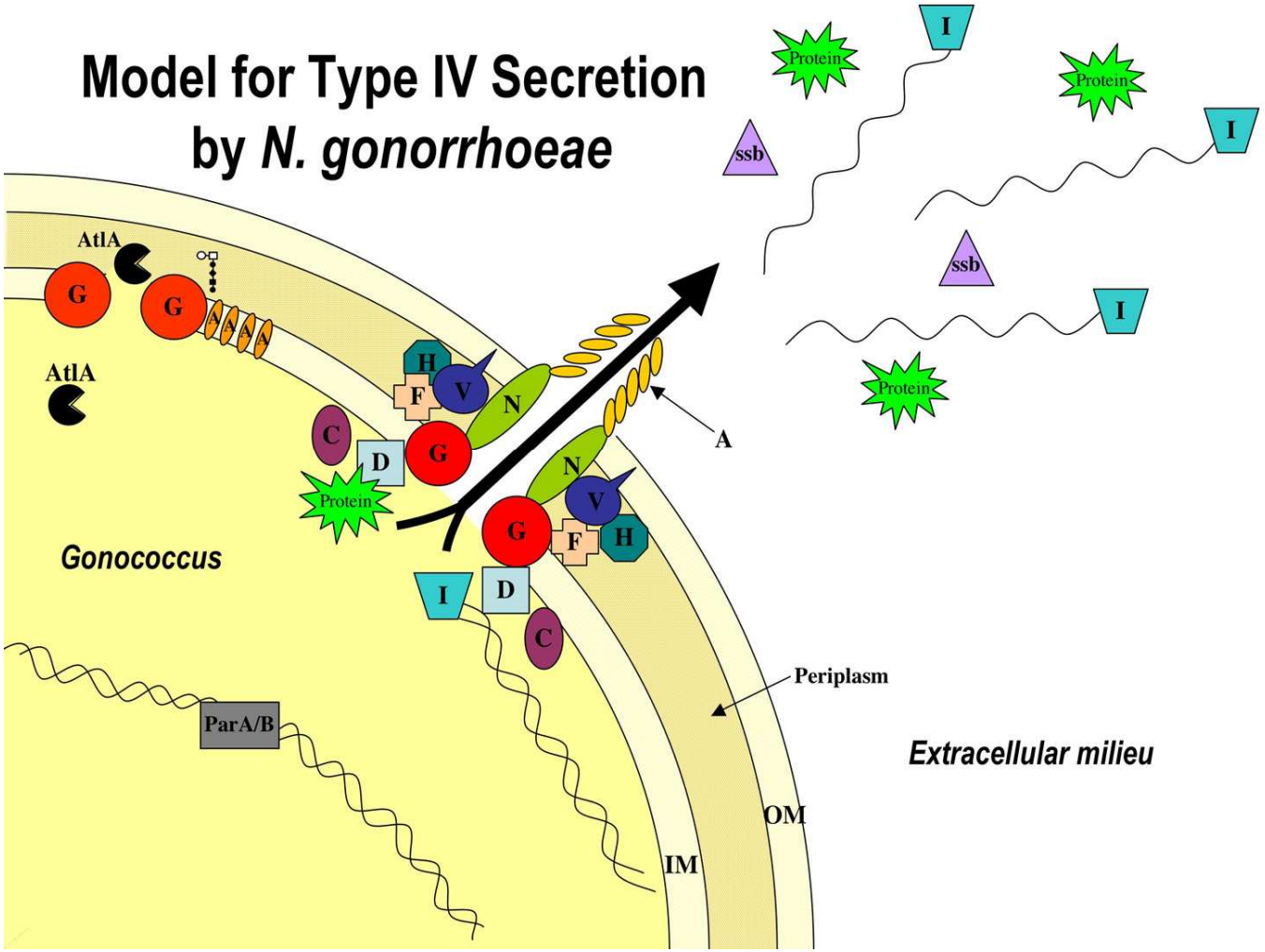
Electron micrograph and schematic representation of *Neisseria meningitidis*

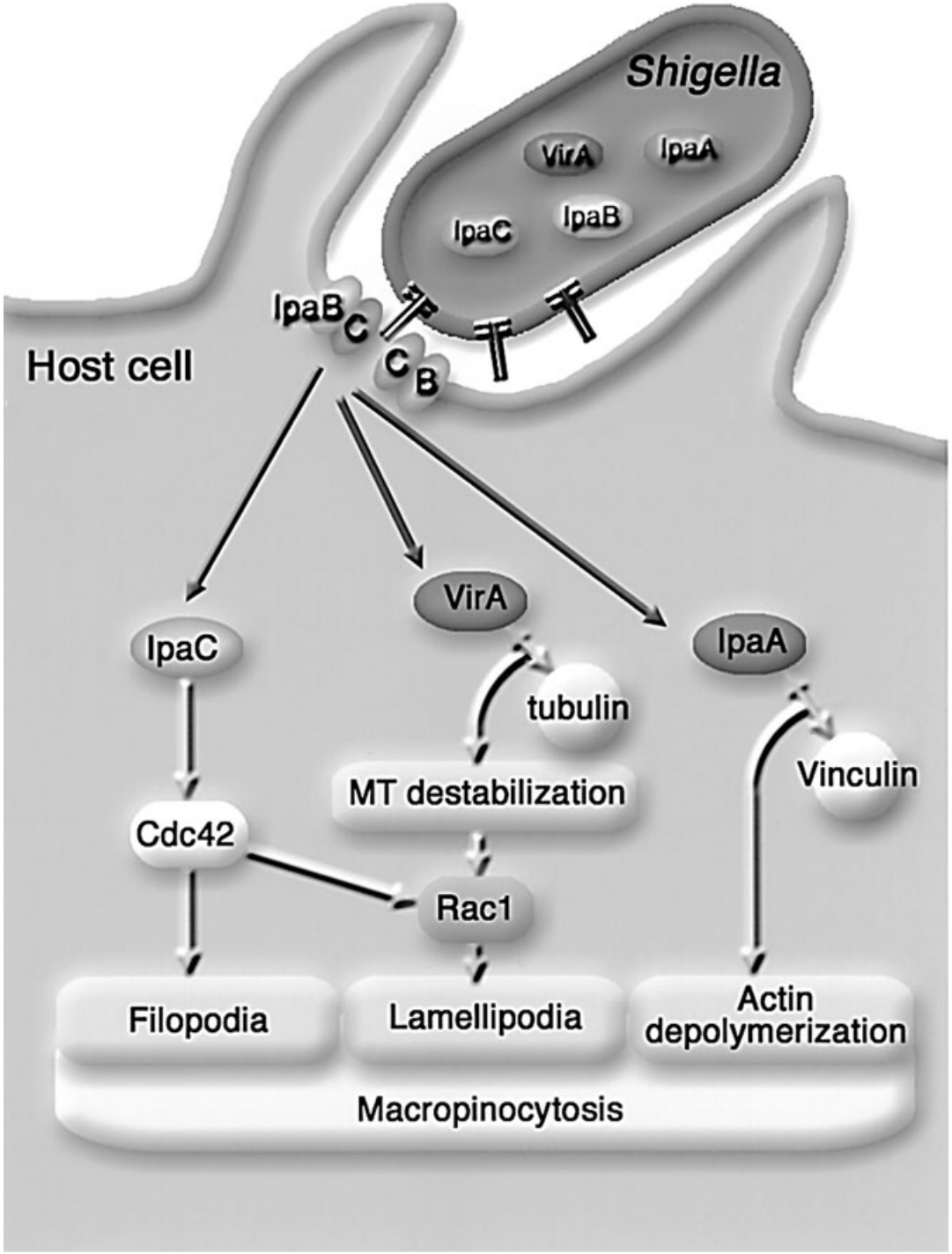
In the case of the meningococcus, the presence on its surface of a sialylated lipo-oligosaccharide and a capsule is known to play an important role against the bactericidal activity of serum (Vogel and Frosch, 1999; Wetzler et al., 1992) and to protect the bacteria against phagocytosis by macrophages and monocytes (McNeil et al., 1994; Read et al., 1996).

Adhesion of *N. meningitidis* on endothelial cells interferes with the transendothelial migration of leukocytes by preventing the formation of the endothelial docking structures required for proper leukocyte diapedesis.



Schematic representation of the signalling pathways activated by *Neisseria meningitidis* during cell entry

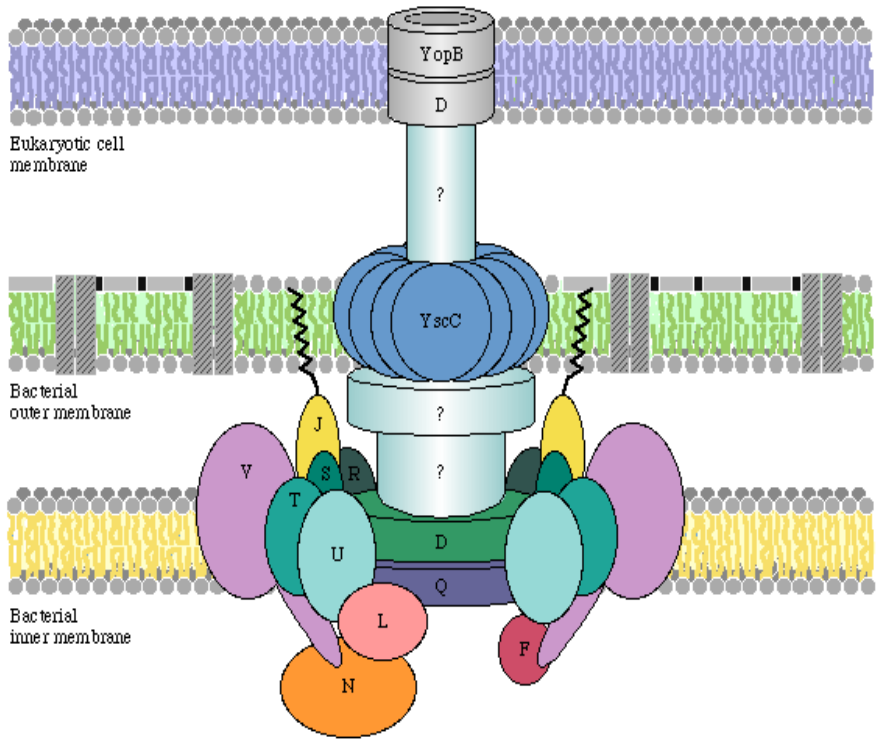

Model for Type IV Secretion
by N. gonorrhoeae
AtlA
AtlA
G
G
H
F
V
N
C
D
G
Protein
A
N
V
G
F
H
I
D
C
Gonococcus
ParA/B
Periplasm
IM
OM
Extracellular milieu
ssb
ssb
Protein
Protein
Protein
I
I
I



Cytoskeletal rearrangements induced during *Shigella* invasion of epithelial cells. *Shigella* secretes the effector proteins, such as IpaB, IpaC, IpaA and VirA, into host cells.

Sistema de secreción tipo III

SHIGELLA

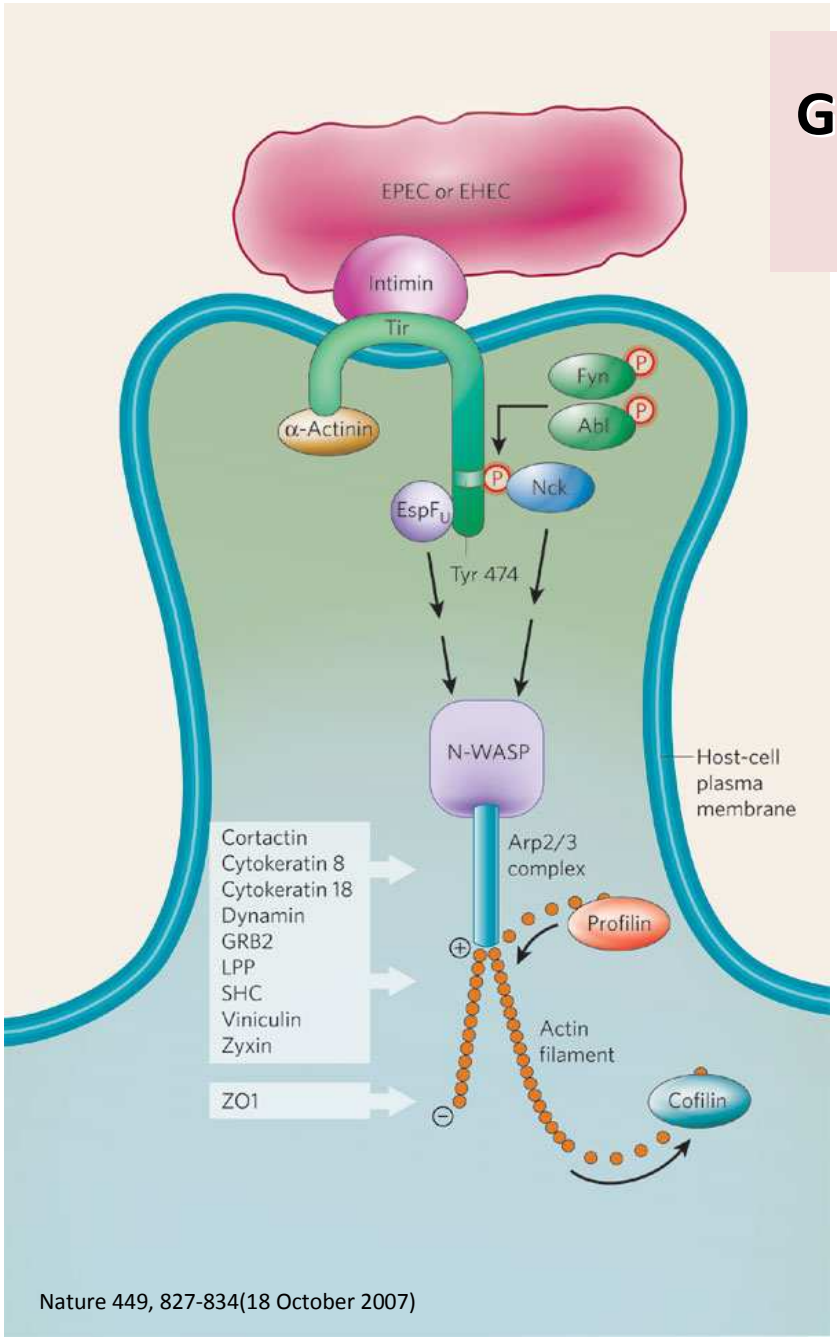


Type III secretion enables gram-negative bacteria to secrete and inject pathogenicity proteins into the cytosol of eukaryotic host cells.

Fascinatingly, while the type III secretion apparatus is conserved in pathogens as distantly related as ***Yersinia*** **and** ***Erwinia*****,** the secreted proteins differ entirely; illustrating how one bacterial pathogenicity mechanism can give rise to a multitude of diseases that range from bubonic plague in humans to fire blight in fruit trees. Secretion of bacterial pathogenicity proteins by the type III pathway and their injection into the cytosol of animal or plant cells initiates a sophisticated "biochemical cross-talk" between pathogen and host. The injected proteins often resemble eukaryotic factors with signal transduction functions and are capable of interfering with eukaryotic signalling pathways. Redirection of cellular signal transduction may result in disarmament of host immune responses or in cytoskeletal reorganization, establishing subcellular niches for bacterial colonization and facilitating a highly adapted pathogenic strategy of "stealth and interdiction" of host defense communication lines.

As in type I secretion, **the proteins secreted via the type III pathway are not subjected to amino-terminal processing during secretion. T**

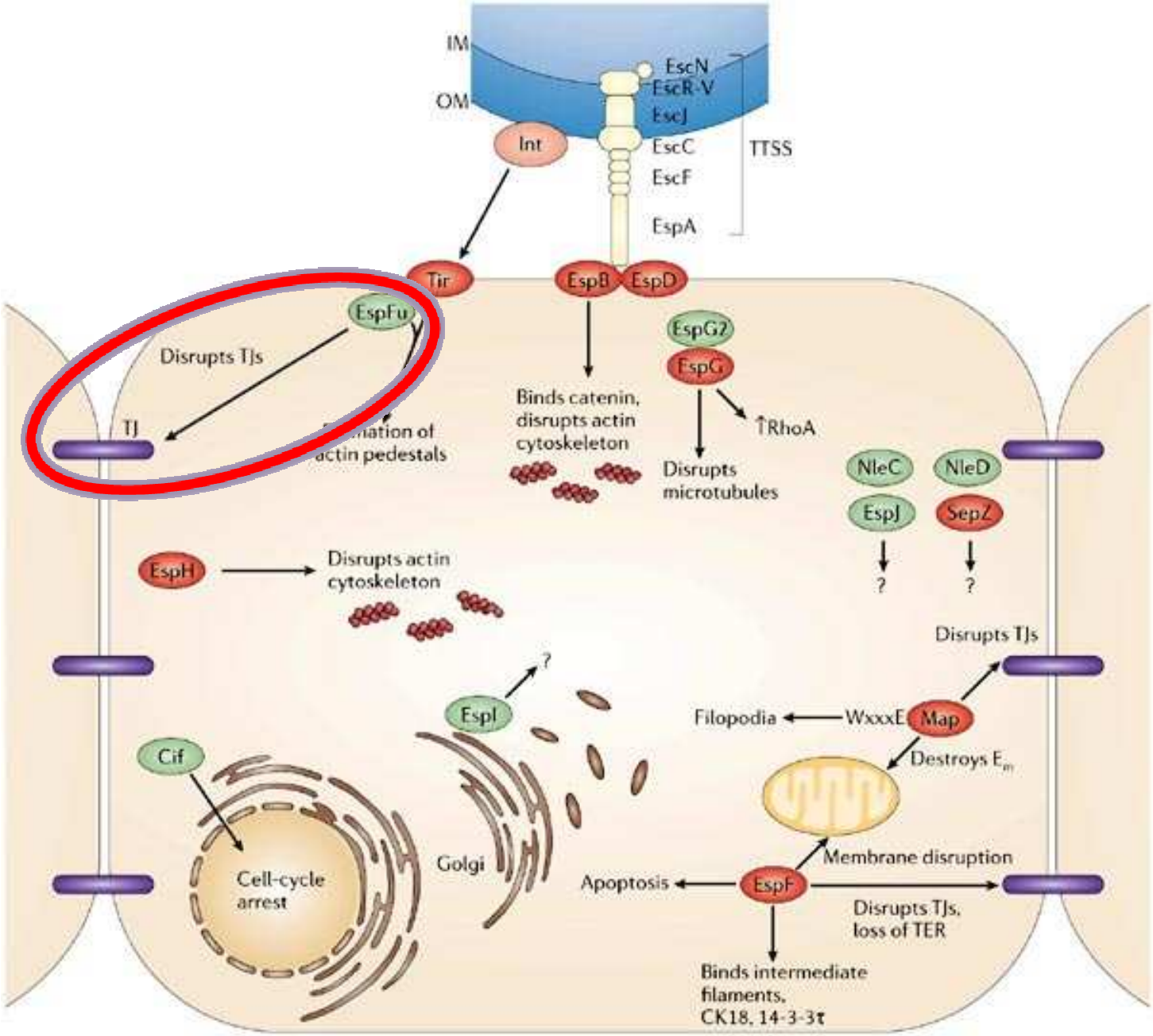
Generación de PEDESTALES por la EPEC Y EHEC (O157:H7)



Nature 449, 827-834(18 October 2007)

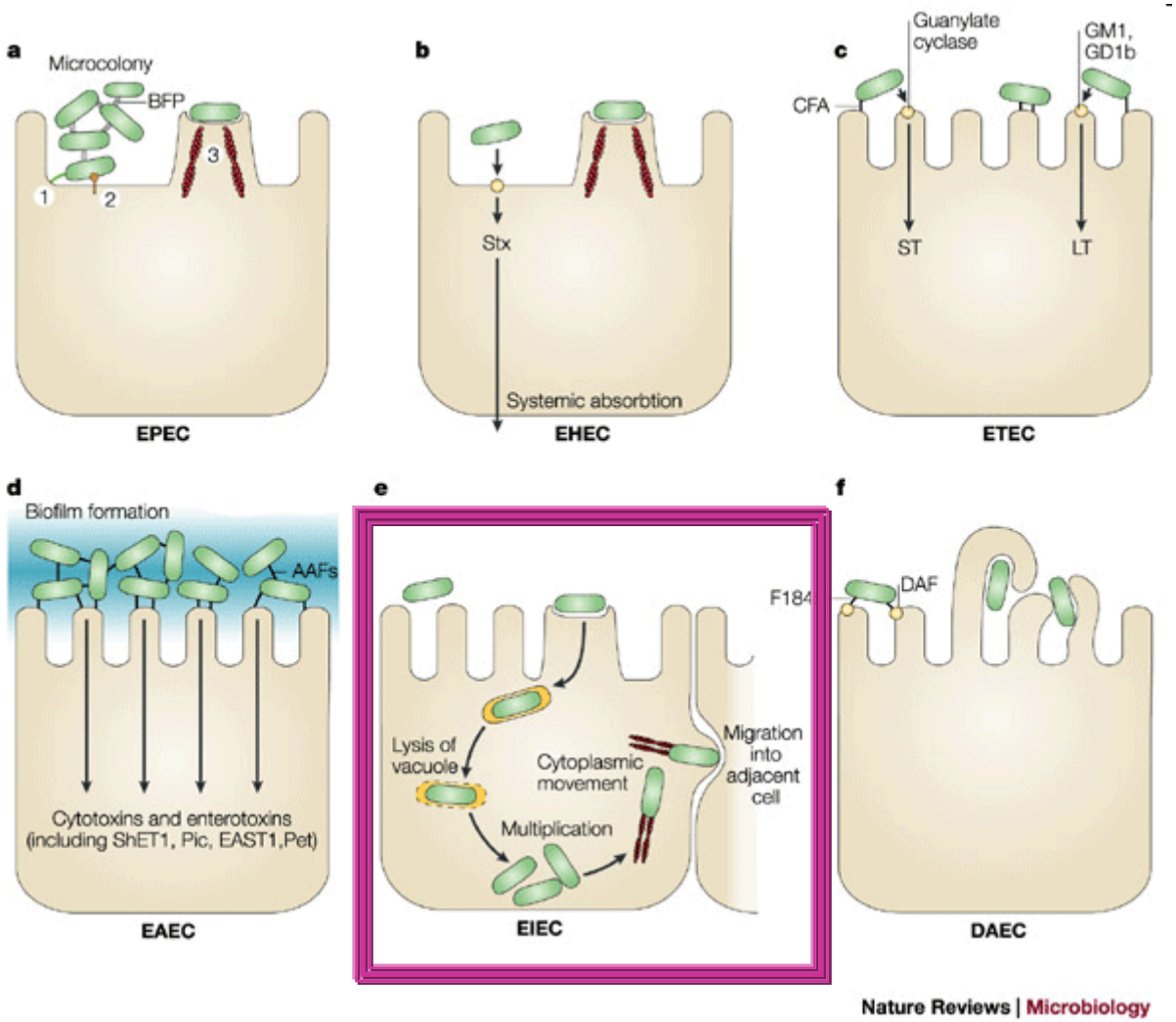
During infection with the extracellular bacterium EPEC, the intimin receptor (Tir) translocates into the host cell and inserts itself into the host-cell plasma membrane (a process mediated by the T3SS). This receptor interacts with intimin on the bacterial surface, thereby firmly anchoring the bacterium to the host cell.

- During infection with the extracellular bacterium EPEC, the intimin receptor (Tir) translocates into the host cell and inserts itself into the host-cell plasma membrane (a process mediated by the T3SS). This receptor interacts with intimin on the bacterial surface, thereby firmly anchoring the bacterium to the host cell. The carboxy terminus of EPEC Tir becomes phosphorylated on the tyrosine residue at position 474 by at least two host protein kinases, Fyn and Abl, resulting in host adaptor protein Nck being recruited and binding directly to Tir. During infection with EHEC, by contrast, the tyrosine-phosphorylation event is subverted by the EHEC effector $EspF_u$, so Nck is not required. During EPEC or EHEC infection, N-WASP and the Arp2/3 complex (which consists of seven host proteins) are recruited downstream of the Tir-interacting protein (Nck or $EspF_u$), leading to the generation of actin filaments beneath the attached bacteria and the formation of the pedestal structure. Numerous proteins are found in EPEC pedestals (some of which are listed in the shaded box); however, the precise organization of these proteins in EPEC- and EHEC-induced pedestal generation has not been clearly shown. It has been demonstrated that the tight-junction-associated protein ZO1 localizes to the distal portion of the actin filaments of the EPEC pedestal. In addition, the actin-disassembly protein cofilin has been shown to localize to pedestals and presumably, together with the actin-assembly protein profilin, regulates the actin-filament dynamics in pedestals. Also, the amino terminus of Tir has been shown to bind directly to -actinin, but the effect of this interaction is unknown.

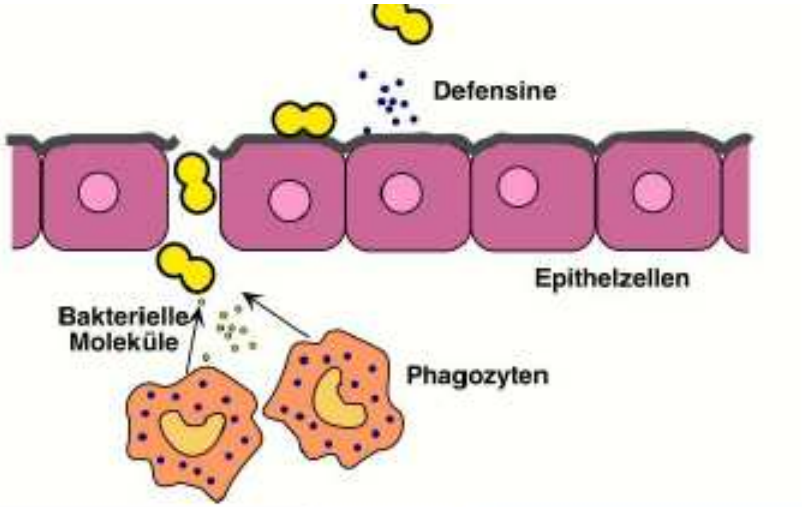


Nature Reviews Microbiology 4, 358-370 (May 2006)

EIEC: LISA EL FAGOSOMA



LAS BACTERIAS EVADEN AL SISTEMA INMUNE INNATO



Defense mechanisms:

Antimicrobial peptides

Phagocytes

Recognition by preformed receptors

Virulence factors:

Peptide resistance

Evasion of phagocytosis

Disguise mechanisms, receptor antagonists

Host defenses factors are *'positive by nature'* - Bacteria are *'negative by nature'*

Antimicrobial host factors are

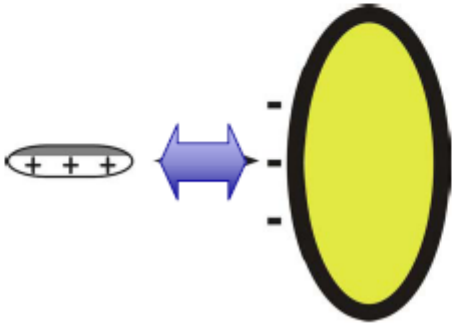
Positively charged:

- Antimicrobial peptides
- Class IIA phospholipase A2
- Lactoferrin
- Myeloperoxidase
- Lysozyme, ...

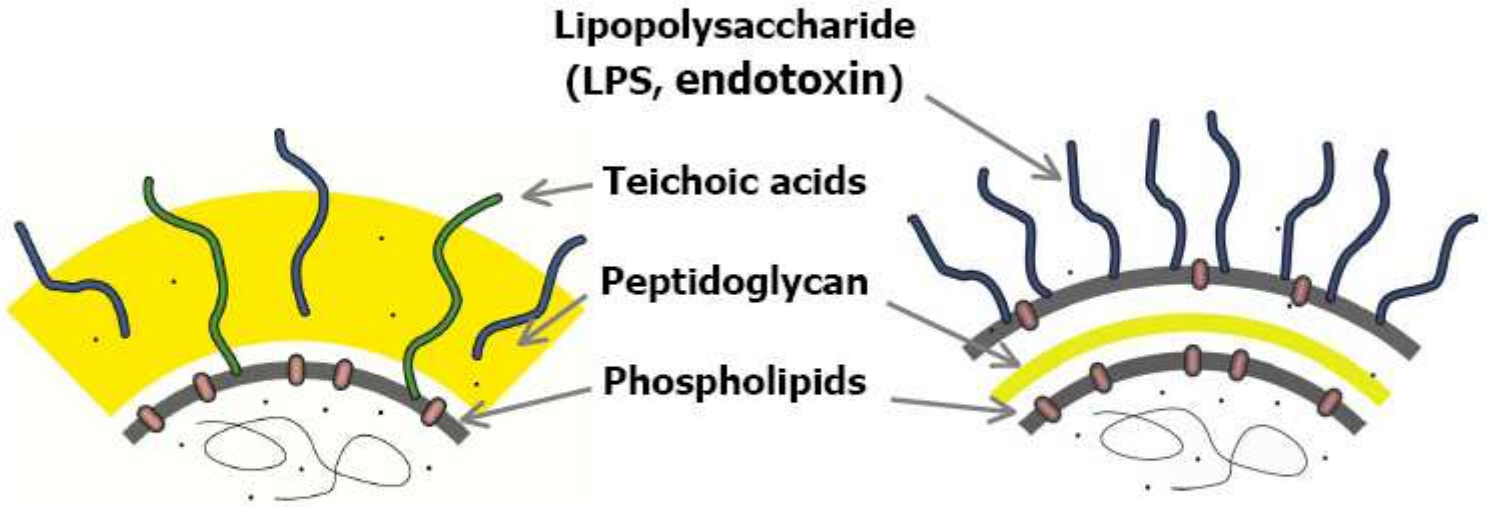
Bacterial cell envelope components are

Negatively charged:

- Peptidoglycan
- Teichoic acids
- Teichuronic acids
- Phospholipids (most)
- Lipid A, LPS,...



The negatively charged bacterial cell envelope:



Gram-*positive* bacteria
(*Staphylococcus aureus*)

Gram-*negative* bacteria
(*Shigella flexneri*)

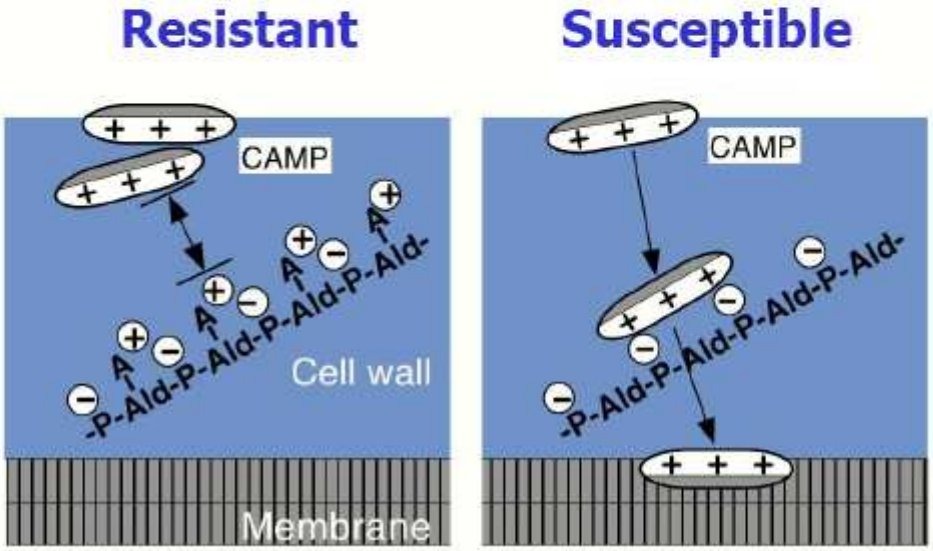
Staph. aureus is resistant to defensins

Minimal inhibitory concentration of defensin hNP1-3:

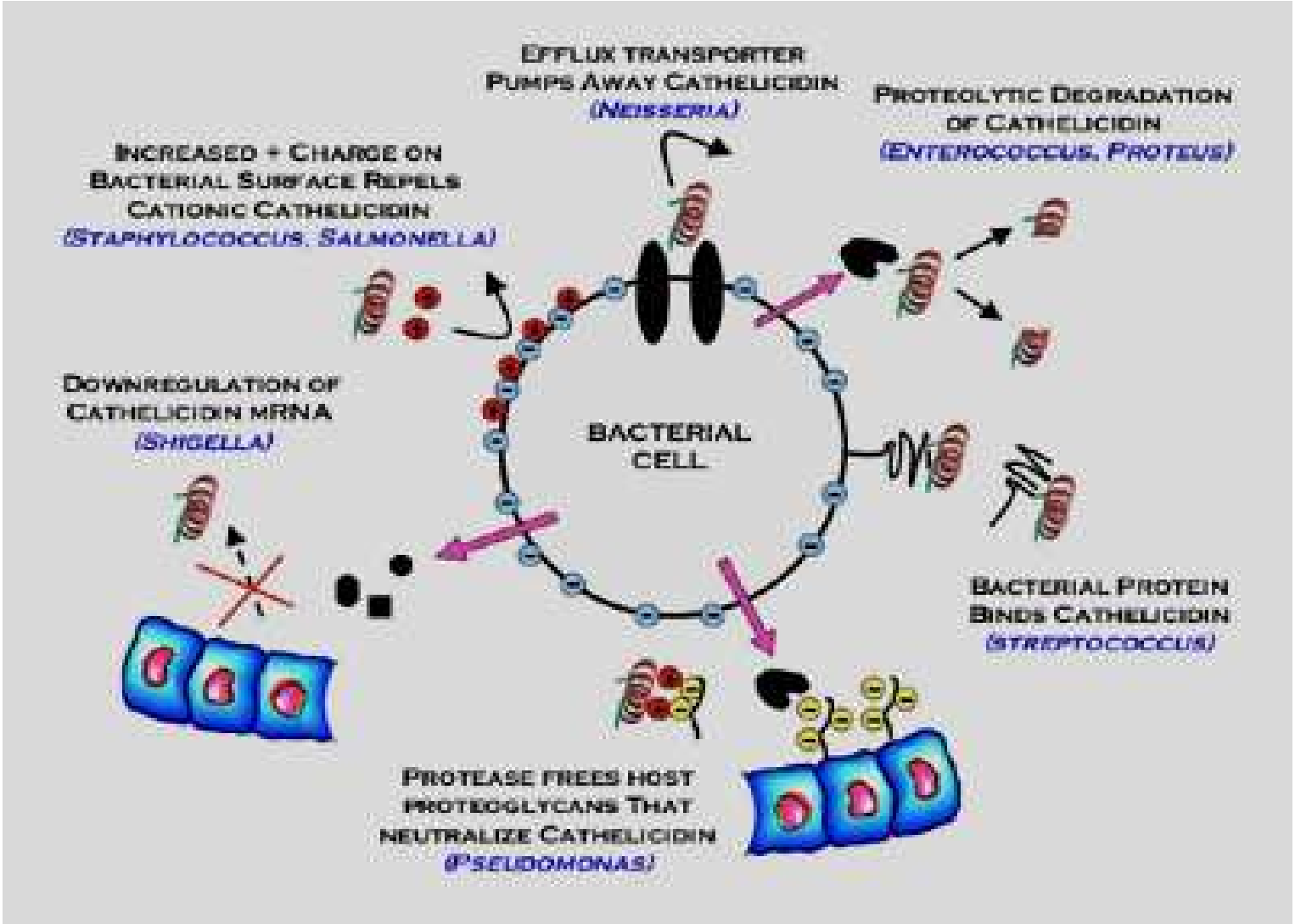
S. aureus **wild-type**: **>60** µM

mutant Δ*dltA*: **2.9** µM

Resistance mechanism:
Introduction of positive charges into the cell wall

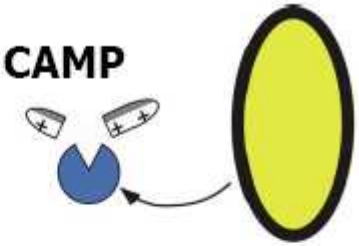


LAS BACTERIAS EVADEN A LOS AMP



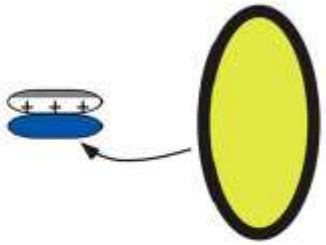
Bacterial CAMP resistance mechanisms

1. Cleavage of CAMP



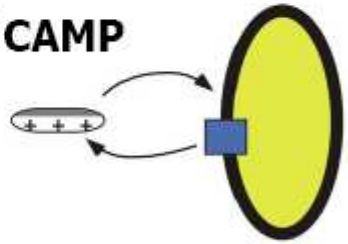
PgtE protease:
Salmonella, Escherichia, ...

2. Anti-CAMP



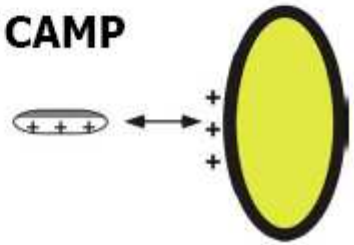
Staphylokinase:
Staphylococcus, ...

3. Extrusion of CAMP



MtrCDE efflux pump:
Neisseria,...

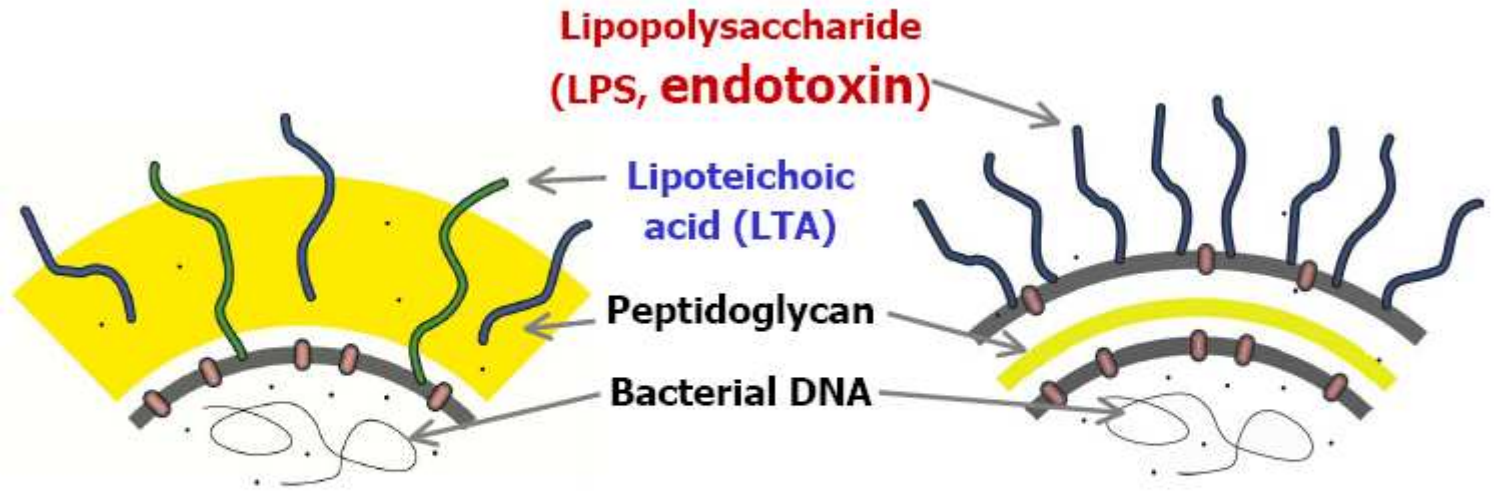
4. Repulsion of CAMP



Modification of teich. acids and lipids:
Staphylococcus, Listeria, Streptococcus, ...

Modification of lipid A:
Salmonella, Pseudomonas, Legionella,...

CIERTAS MOLÉCULAS BACTERIANAS, ACTIVAN AL SISTEMA INMUNE INNATO Y CAUSAN INFLAMACIÓN…..



Gram-*positive* bacteria
(*Staphylococcus aureus*)

Gram-*negative* bacteria
(*Shigella flexneri*)

LA RESPUESTA INFLAMATORIA INLCUYE ACTIVACIÓN DE DIFERENTES TIPOS CELULARES……

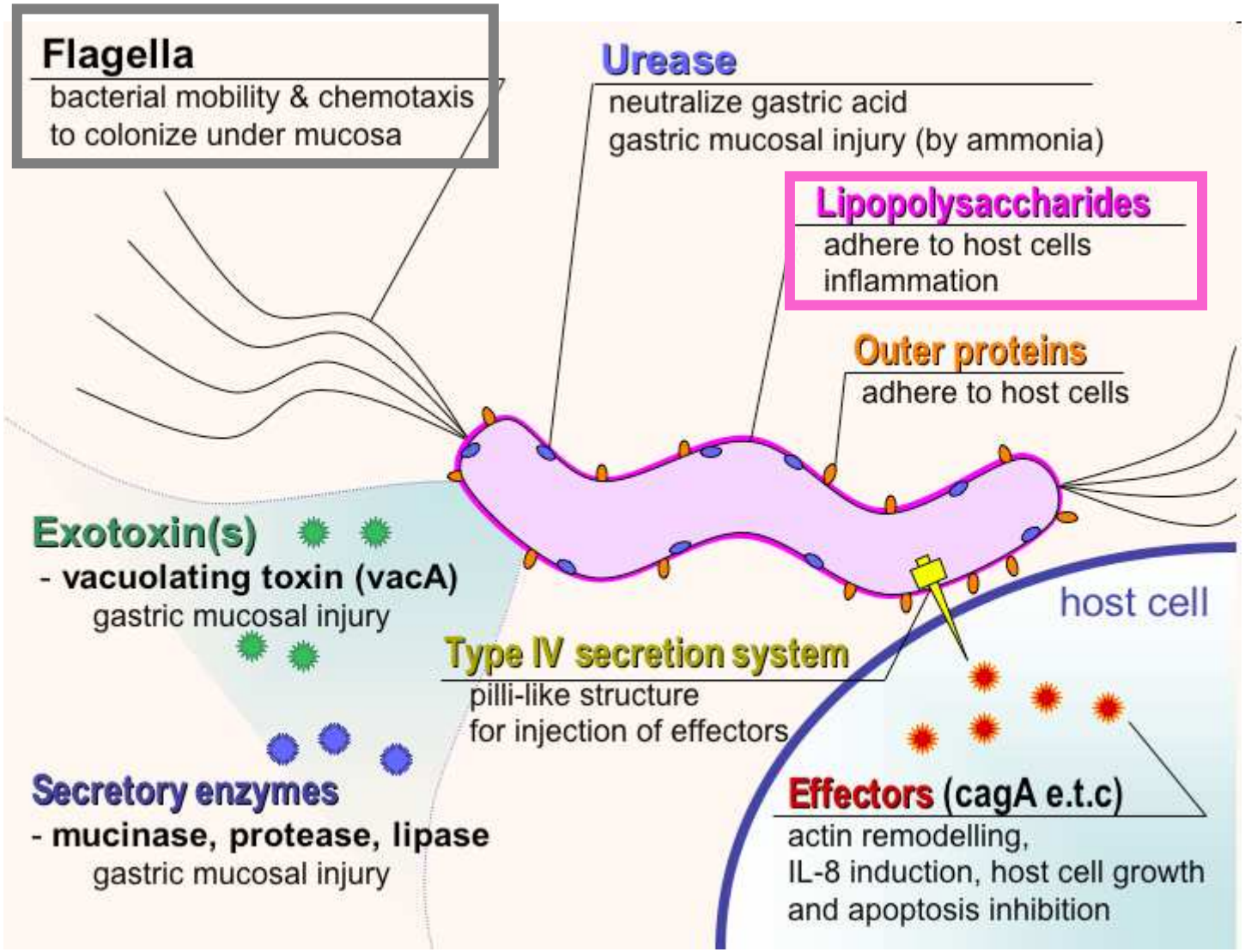
TLRs → NF-κB
(transcription factor)

Epithelial cells:
- → **Defensin** production
- → **IL-8** produktion

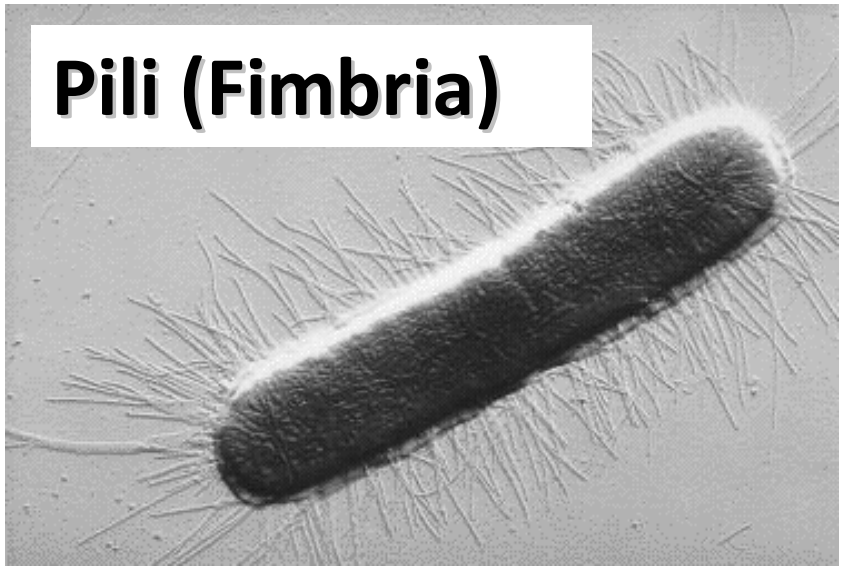
Endothelial cells:
- → Adhesive for **leukocytes**
- → **Permeabilisation**

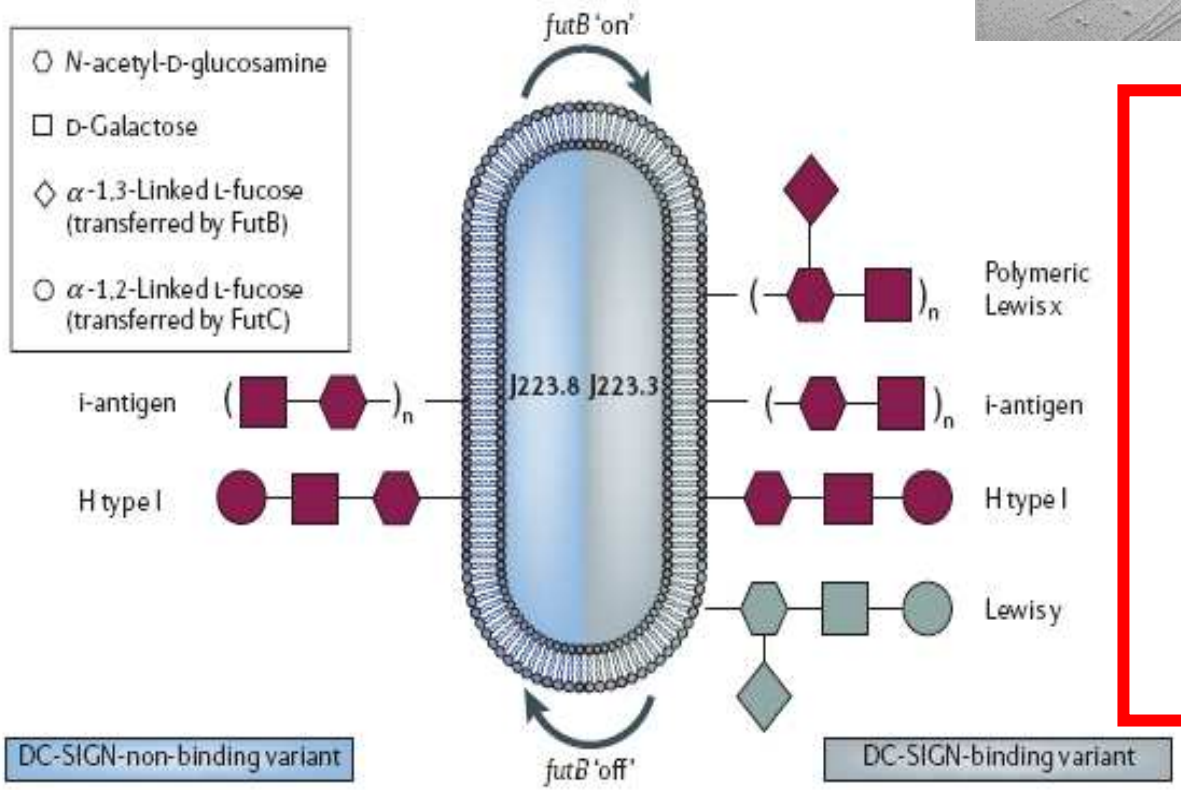
Phagocytes:
- → **Cytokine** production
- → increased **killing**

Helicobacter pylori



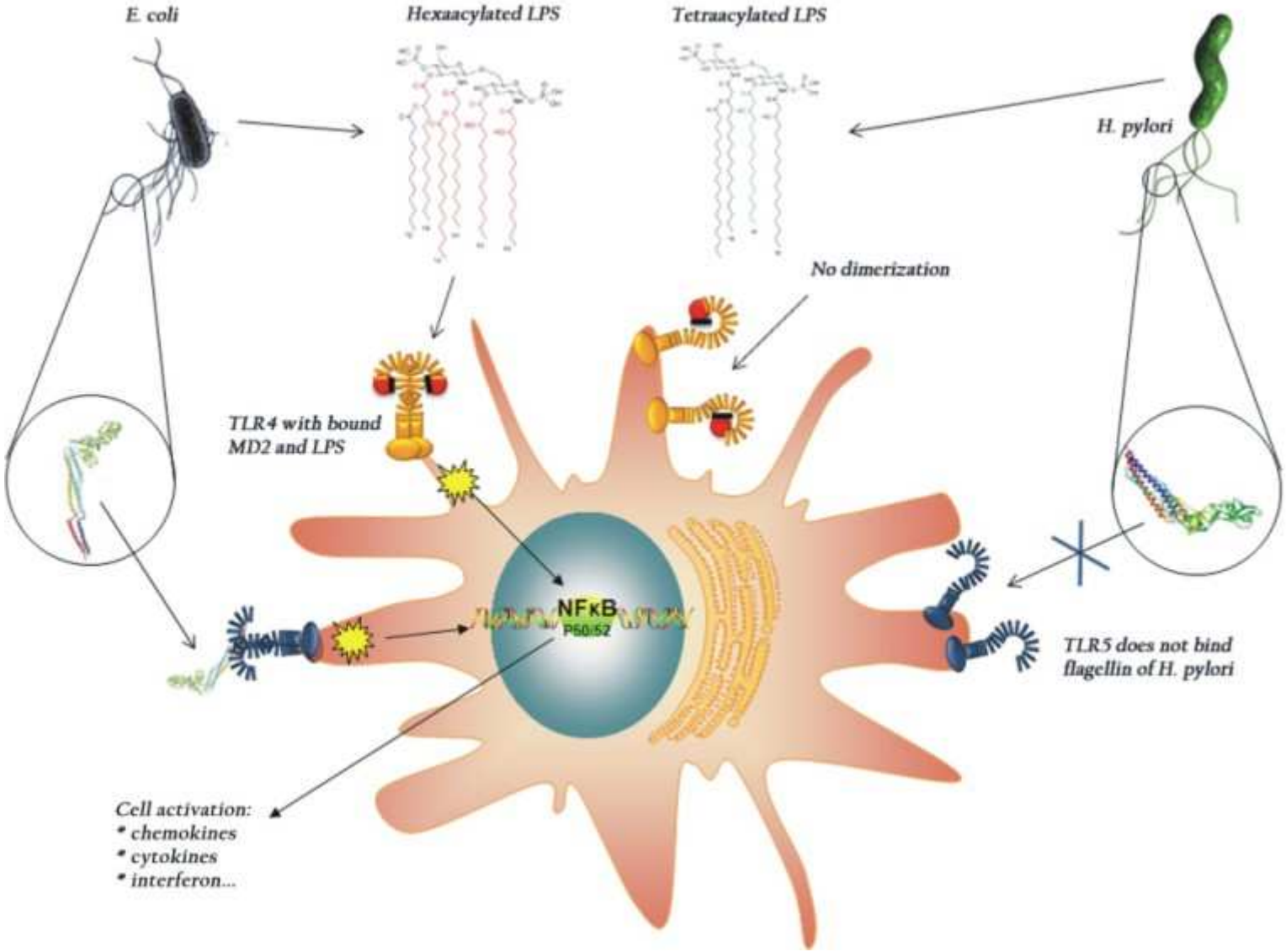
HELICOBACTER PYLORI

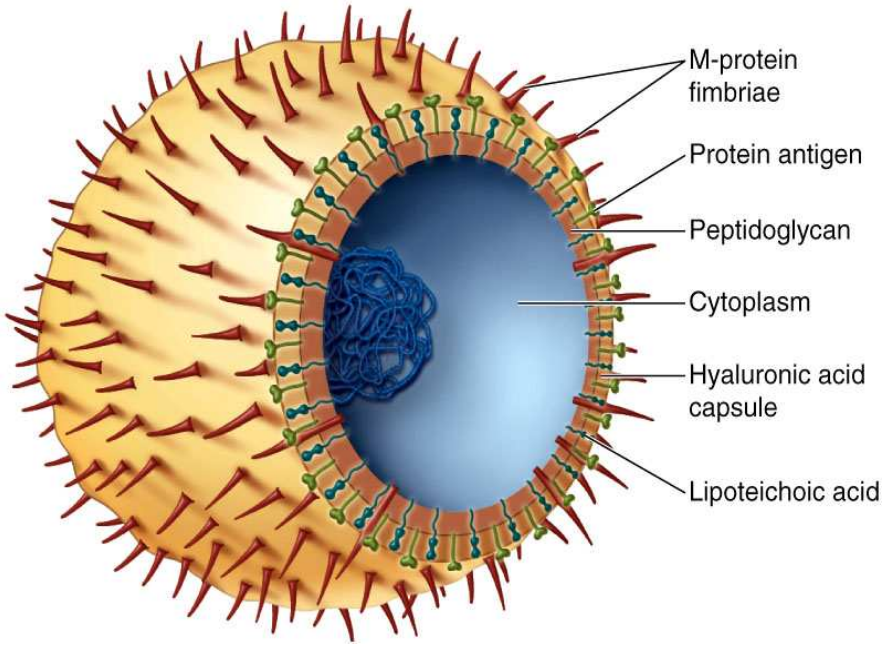


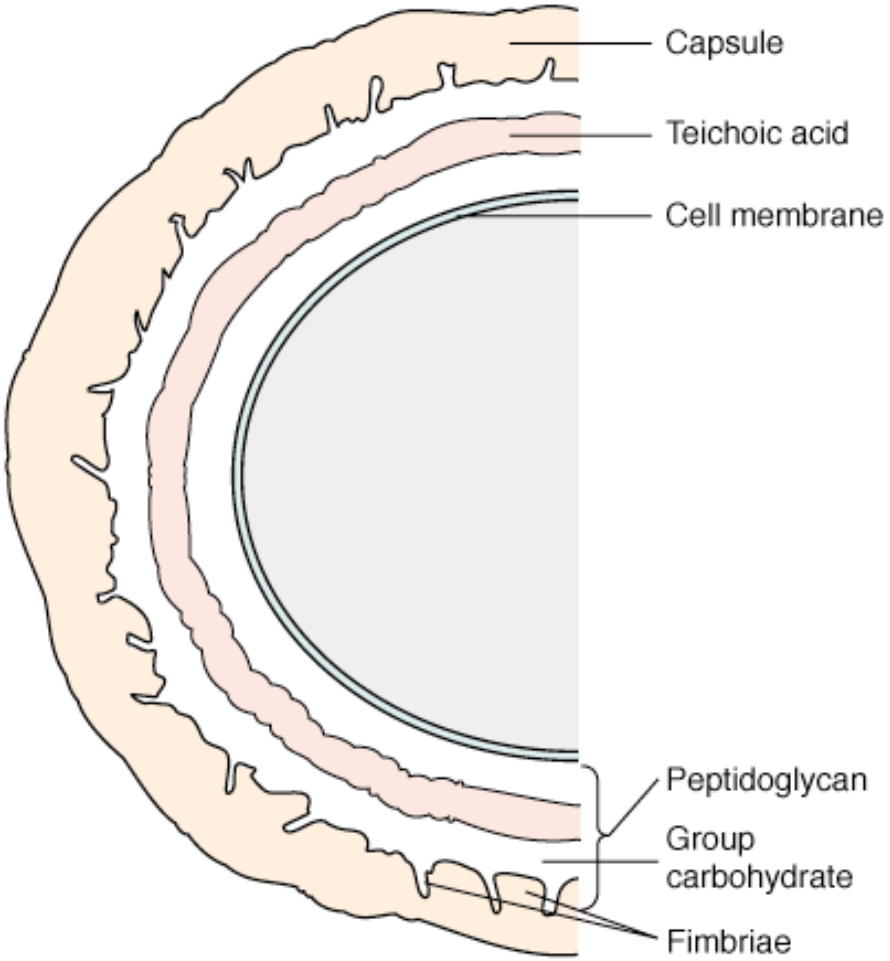


Helicobacter Pilori

Incorpora HC a sus LPS y le confieren propiedades similares a los antígenos Lewis de los grupos sanguíneos humanos


E. coli
Hexaacylated LPS
Tetraacylated LPS
H. pylori
No dimerization
TLR4 with bound
MD2 and LPS
NFκB
P50/52
TLR5 does not bind
flagellin of H. pylori
Cell activation:
* chemokines
* cytokines
* interferon...





ESTREPTOCOCOS

Streptococcus: estructura antigénica

- **Antígenos de la pared específico del grupo**: Se trata de un carbohidrato que ha permitido clasificarlos serológicamente en los grupos de Lancefield. El grupo viene determinado por el aminoazúcar y los grupos son A, B, C, D y F
- **Proteína M: Pertenece a la pared celular y forman los llamados pilis (aparece como pelos). Constituye el principal factor de virulencia para el S. pyogenes y resisten la acción fagocítica y está implicada en los procesos de adherencia a células epiteliales.**

Streptococcus classification

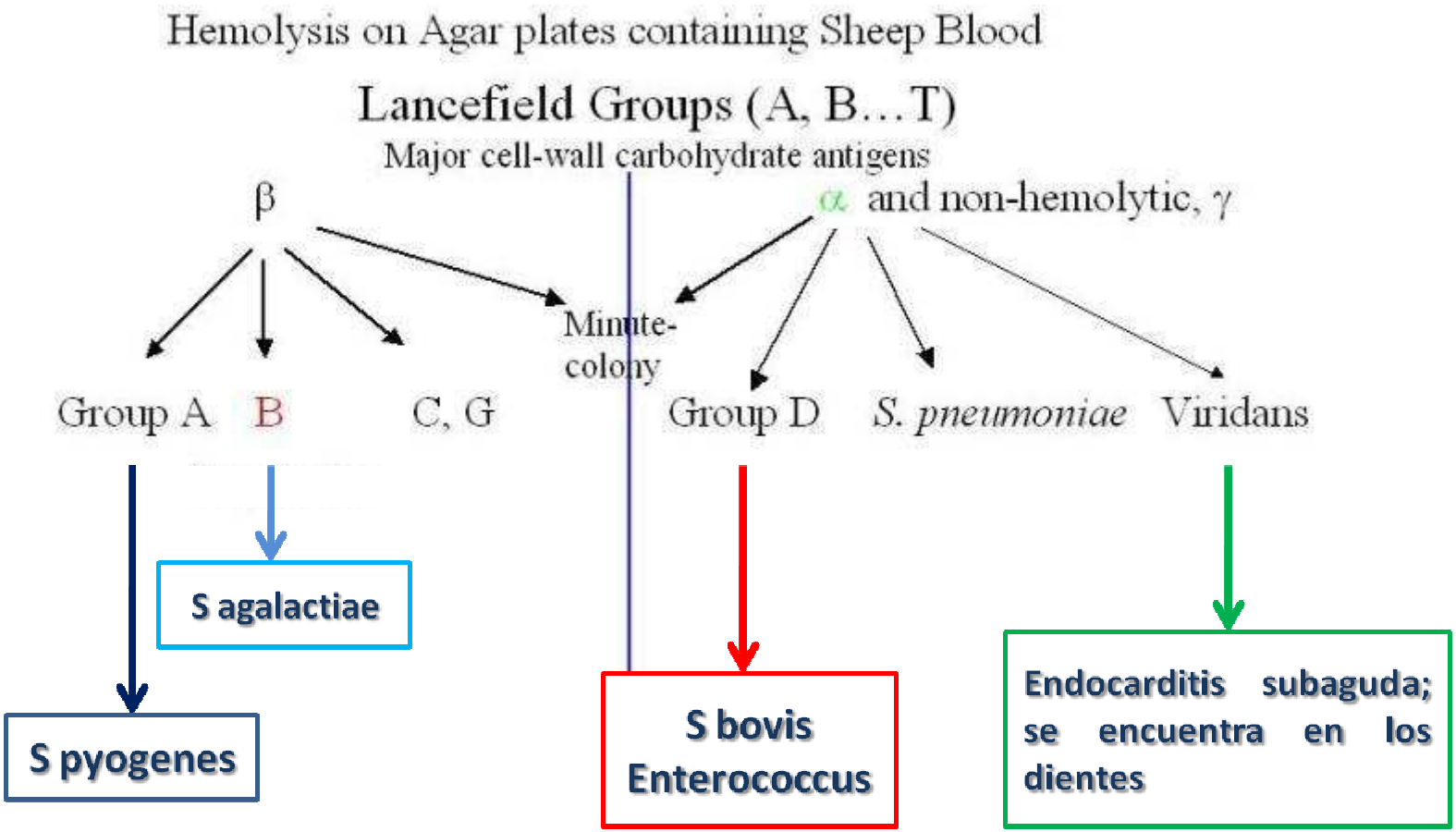


Table 1 | **Streptococcal diseases and virulence factors**

Organism	Diseases	Virulence factors
Streptococcus mutans	Dental caries, endocarditis	Adhesins: antigen I/II (SpaP), glucosyltransferases and glucan-binding proteins A and C, WapA, SloC, PavA-like protein Acid production
Streptococcus agalactiae	Neonatal sepsis and meningitis; systemic infection in immuno-compromised individuals	Capsule Laminin-binding protein α- and β-proteins Fibronectin-binding proteins: FbsA, PavA-like protein C5a-peptidase Other LPXTG-anchored proteins Haemolysin (CylE)
Streptococcus pyogenes	Pharyngitis; cellulitis; scarlet fever; streptococcal toxic-shock syndrome; necrotizing fasciitis; rheumatic fever as sequela; glomerulonephritis as sequela	Capsule Fibronectin-binding proteins: SfbI, SfbII, FBP54 (PavA-like), F2, PFBP C5a-peptidase Mac SIC Haemolysins: SLO, SLS Pyogenic exotoxins: SpeA, SpeC, SpeG, SpeH, SpeJ, SpeK, SpeL, SSA, SMEZ, SMEZ2 GRAB DNAses A, B, C and D Streptokinase Hyaluronidase SpeB (cysteine protease)
Streptococcus pneumoniae	Otitis media; bacteraemia; pneumonia; meningitis	Capsule Cell wall Pneumolysin Surface proteins: LPXTG-anchored, choline anchored (PspA, PspC, autolysin), PavA, PsaA Hydrogen peroxide NADH oxidase Superoxide dismutase

The list of virulence factors is not exhaustive and represents selected examples. FbsA, fibrinogen-binding protein from *S. agalactiae*; FBP54, fibronectin-binding protein 54; GRAB, G-related α_2-macroglobulin-binding protein; Mac, Mac1-like protein; PavA, pneumococcal adhesion and virulence A; PFBP, *pyogenes* fibronectin-binding protein; Psa, pneumococcal surface antigen; Psp, pneumococcal surface protein; Sfb, streptococcal fibronectin-binding protein; SIC, streptococcal inhibitor of complement; SLO, streptolysin O; SloC, *S. mutans* LraI operon C; SLS, streptolysin S; SMEZ, streptococcal mitogenic exotoxin Z; SpaP, streptococcal protein antigen P; Spe, streptococcal pyogenic exotoxin; SSA, streptococcal superantigen A; WapA, wall-associated protein A.

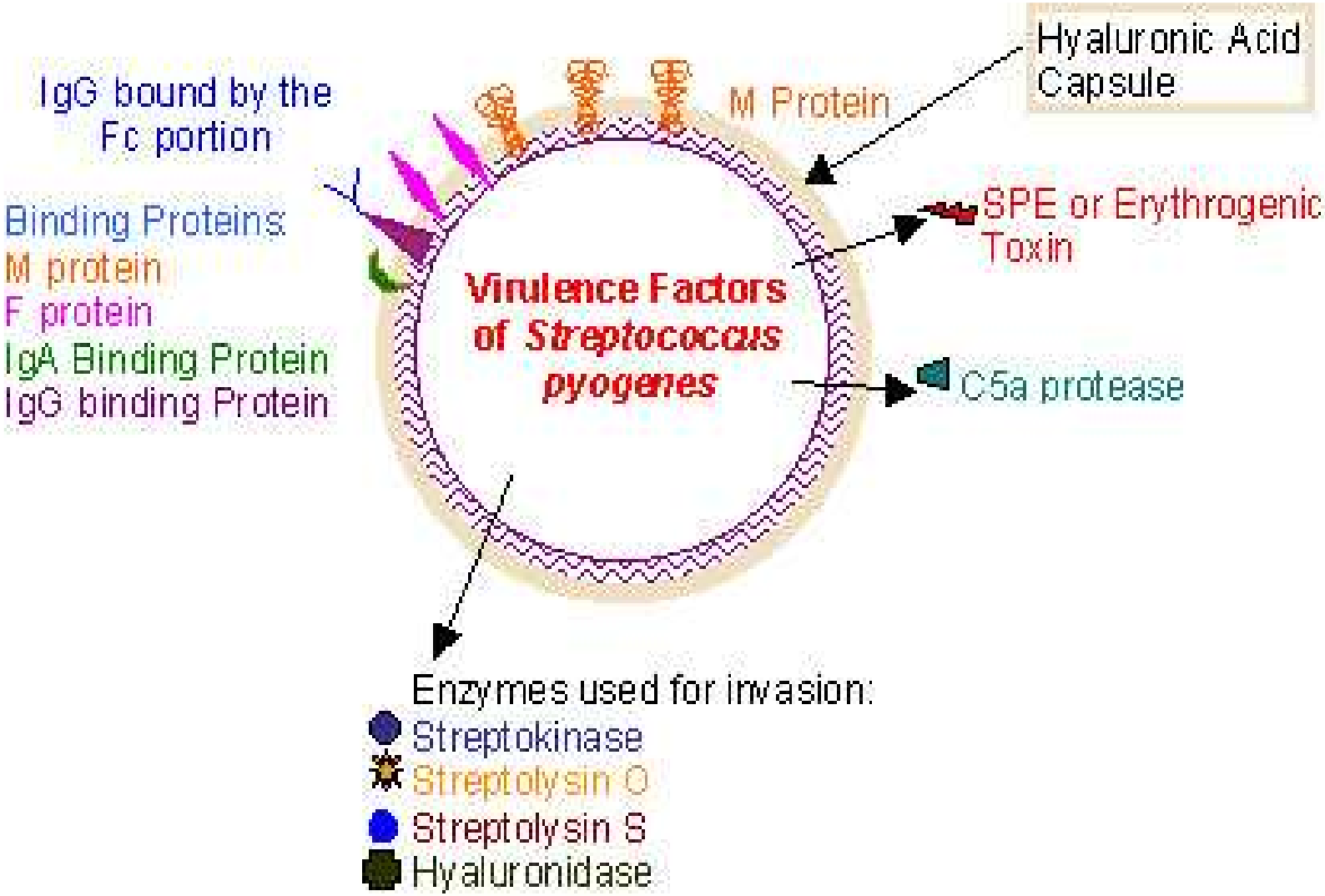
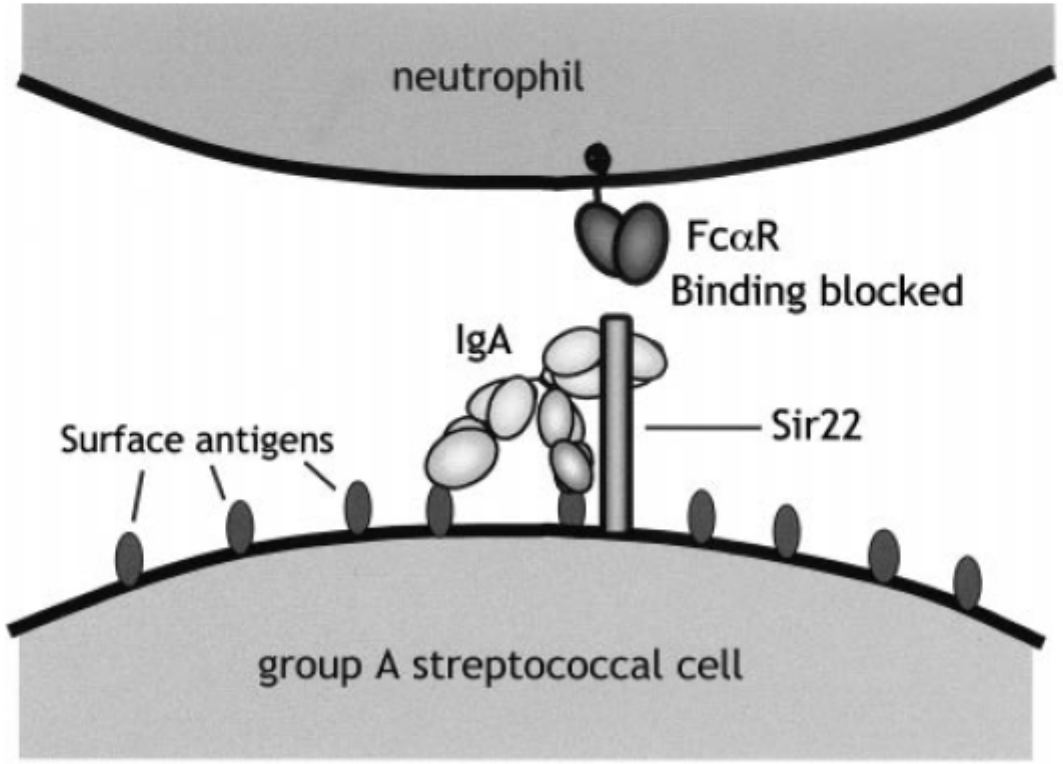
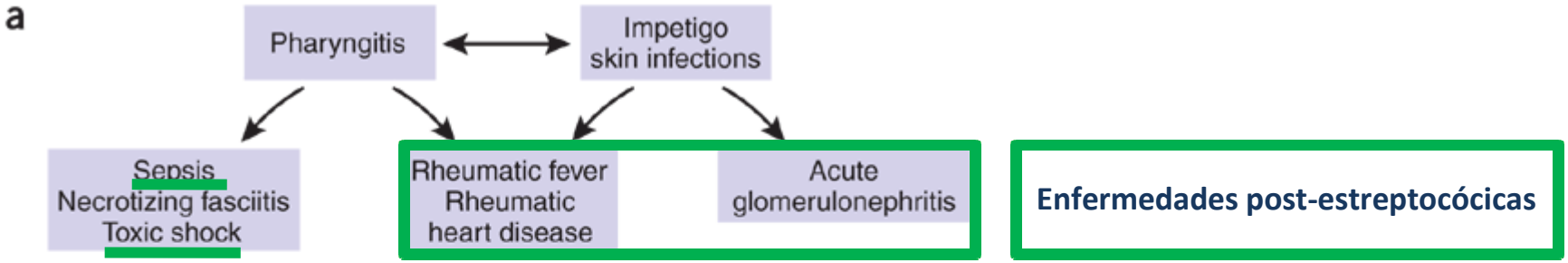

IgG bound by the Fc portion
Binding Proteins:
M protein
F protein
IgA Binding Protein
IgG binding Protein
M Protein
Hyaluronic Acid Capsule
SPE or Erythrogenic Toxin
Virulence Factors of Streptococcus pyogenes
C5a protease
Enzymes used for invasion:
Streptokinase
Streptolysin O
Streptolysin S
Hyaluronidase

Figure 2

A simplified view of a possible mechanism by which Sir22 IgA-BP might interfere with IgA-mediated clearance of group A streptococci

An IgA molecule specific for surface antigens on a group A streptococcal cell is shown binding to two antigen molecules through its Fab arms. The IgA-binding region of the Sir22 protein is simultaneously interacting with the interdomain region in the IgA Fc, thereby blocking access to the same region by the FcαR on an adjacent neutrophil.

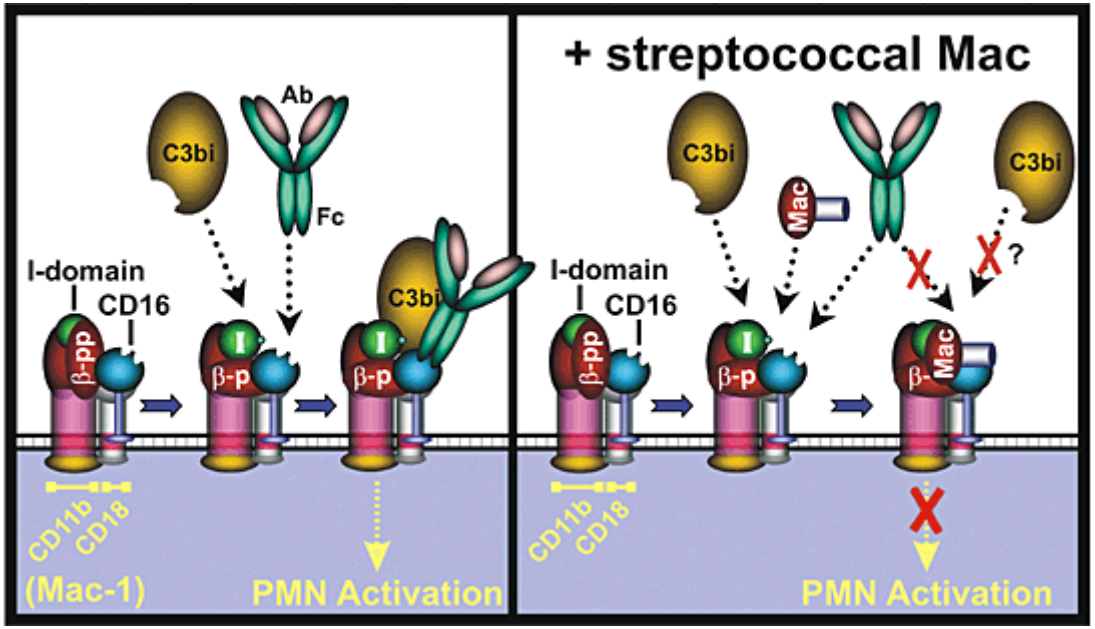




- **Group A *Streptococcus* (GAS) is a gram-positive bacterial pathogen that is responsible for human morbidity and mortality globally.**
- **The organism causes infections such as pharyngitis, cellulitis, bacteremia, necrotizing fasciitis and post-infectious sequelae such as acute rheumatic fever (ARF) and acute glomerulonephritis.**
- **The success of GAS as a pathogen is dependent on its ability to avoid phagocytosis and killing by PMNs, and complement-mediated effects.**

Streptococcus grupo A (GAS)

Streptococcal Mac: inhibe opsonofagocitosis



Based on recent observations the β-propeller domain (red oval labeled -pp) may serve to modulate the binding of C3bi with the I-domain of CD11b. Therefore, in our model, the C3bi binding site on the I-domain is sequestered by the -propeller domain until a conformation shift allows binding (left panel). On human PMNs, CD16 is associated with CD11b/CD18 and upon antibody binding transduces host-response signals through CD11b/CD18 (left panel). It is possible that a conformational shift in the β-propeller domain provides an additional link between CD16 and human Mac-1, thus permitting 'outside-in' signal transduction (left panel). Streptococcal Mac binds to CD16, sequestering its Fc-binding region and blocking its ability to interact with antibody (right panel). Mac may also block binding of C3bi with the I-domain by associating with the I-domain of CD11b and mimicking (through its homology) the function of the β -propeller domain (right panel).

ESTAFILOCOCOS

Staphylococcus : estructura antigénica

- **Peptidoglicano:** Polímero polisacárido de N-acetilmurámico y N-acetilglucosamina del que cuelgan cadenas de pentapéptido. Estas cadenas cortas están a su vez unidas entre sí por puentes que se establecen entre el residuo L-lisina de una cadena y el de D-alanina de la siguiente. El peptidoglicano induce la producción de IL-1 y anticuerpos opsonizantes, atrae leucocitos polimorfonucleares, activa el complemento y posee actividad endotóxica.
- **Ácidos teicoicos**: Polímeros de fosfato glicerol y ribitol. En algunos procesos infecciosos se desarrollan anticuerpos frente a estos ácidos.
- **Factor de aglutinación**: También conocido como "coagulasa unida" (en la pared externa) en los S. aureus y fija el fibrinógeno.
- **Polisacárido:**

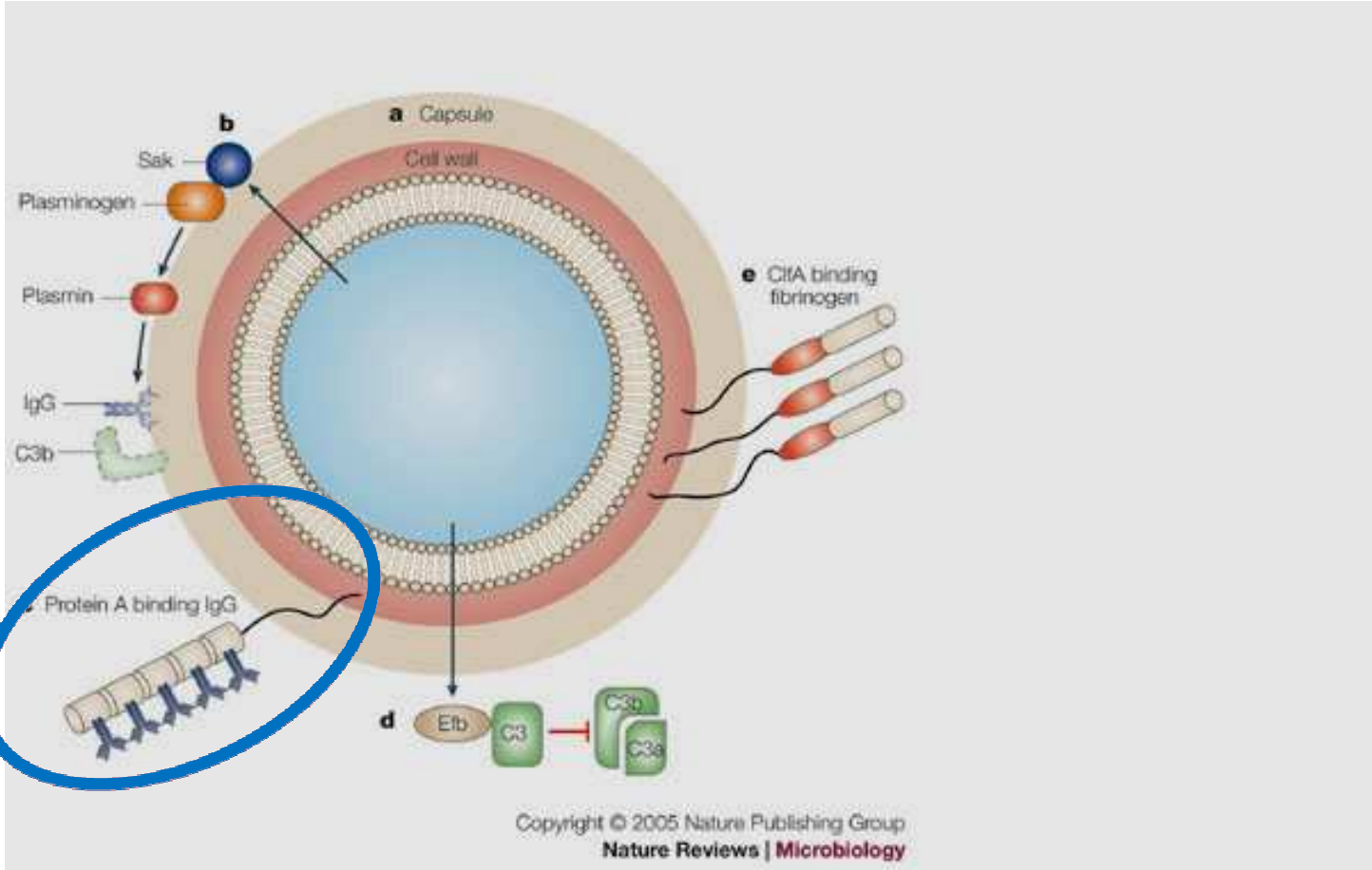
S. aureus: poseen una cápsula polisacarídica capaz de inhibir la fagocitosis

S. epidermidis: produce un exopolisacárido llamado Slime responsable de la adherencia a las superficies, de la resistencia a la fagocitosis y del fracaso de la terapéutica antimicrobiana.

TABLE 18.1 Major Virulence Factors of *Staphylococcus aureus*

Name	Enzyme/Toxin	Effect
Coagulase	Enzyme	Coagulates blood plasma
Hyaluronidase	Enzyme	Digests connective tissue of the host
Staphylokinase	Enzyme	Digests blood clots
Lipase	Enzyme	Digests oils, allowing bacteria to more easily colonize the skin
Penicillinase	Enzyme	Inactivates penicillin, rendering the bacterium resistant
Hemolysins (α, β, γ, δ)	Toxin	Lyse red blood cells
Leukocidin	Toxin	Lyses neutrophils and macrophages
Enterotoxins	Toxin	Induce nausea, vomiting, and diarrhea
Exfoliative toxins (A, B)	Toxin	Cause desquamation of the skin
Toxic shock syndrome toxin	Toxin	Induces fever, vomiting, rash, organ damage

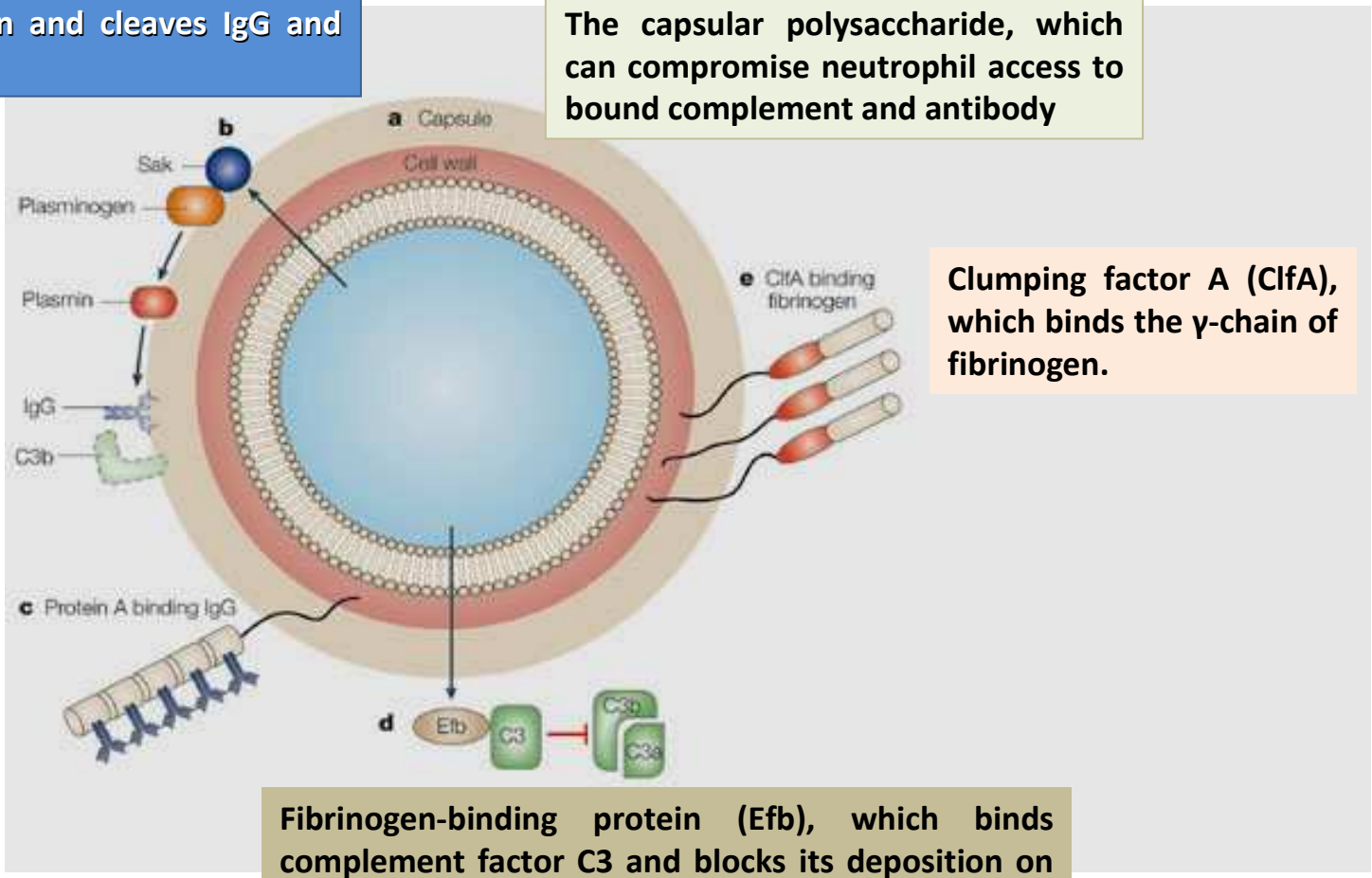
STAPHYLOCOCCUS AUREUS: Proteína A previene la correcta opsonización por IgG



Nature Reviews Microbiology 3, 948-958 (December 2005)

The extracellular staphylokinase (Sak), which activates cell-bound plasminogen and cleaves IgG and C3b

The capsular polysaccharide, which can compromise neutrophil access to bound complement and antibody

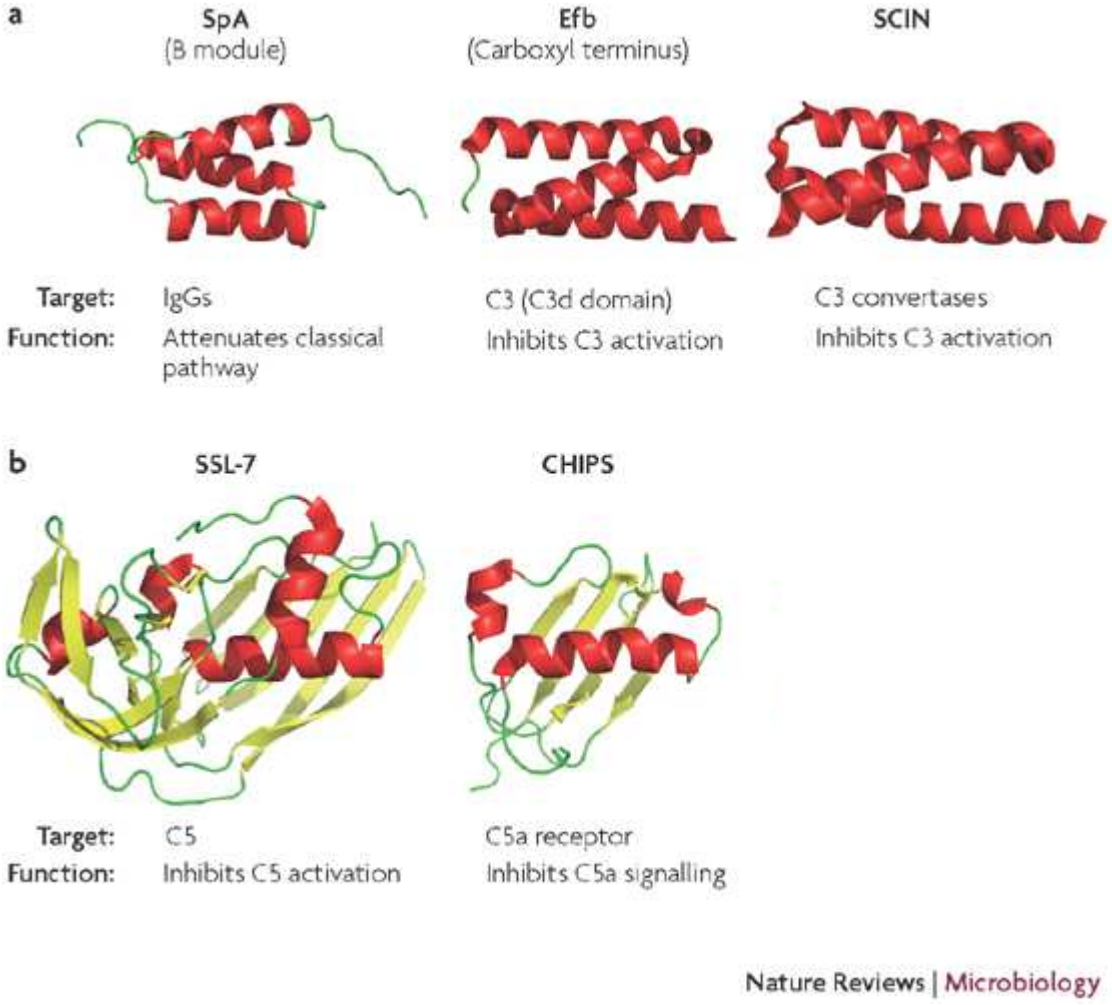


Clumping factor A (ClfA), which binds the γ-chain of fibrinogen.

Fibrinogen-binding protein (Efb), which binds complement factor C3 and blocks its deposition on the bacterial cell surface. Complement activation beyond C3b attachment is prevented, thereby inhibiting opsonization.

Nature Reviews Microbiology 3, 948-958 (December 2005)

S aureus y sistema del complemento



- **A range of S. aureus proteins that interact with targets from the complement system have been structurally analysed.**
- **a | The immunoglobulin G (IgG)-binding module of staphylococcal protein A (SpA), the C3-binding domain of the extracellular fibrinogen-binding protein (Efb) and staphylococcal complement inhibitor (SCIN) share similar structural motifs, but show differential functionality.**

b | Both staphylococcal superantigen-like protein-7 (SSL-7) and the chemotaxis inhibitory protein of S. aureus (CHIPS) attenuate complement on the level of C5, but bind to unrelated targets (C5 protein and C5a receptor, respectively). All protein-structure representations are coloured according to their secondary structure (α-helices in red and β-sheets in yellow).

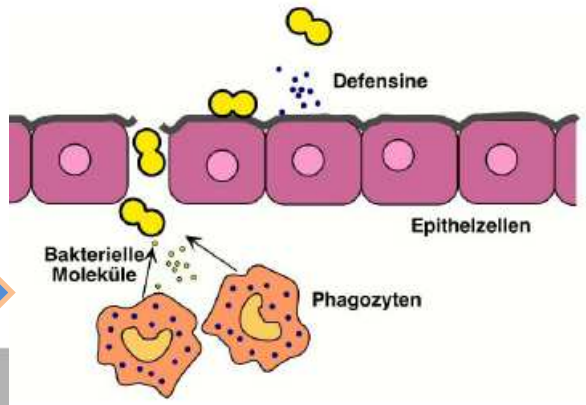
Nature Reviews Microbiology 6, 132-142 (February 2008)

Complement evasion protein	Host target
Antibody depletion	
Staphylococcal protein A (SpA)	IgG
Complement inhibition	
Extracellular fibrinogen-binding protein (Efb)	C3 and C3b-containing convertases
Staphylococcal superantigen-like protein-7 (SSL-7)	C5
Staphylococcus complement inhibitor (SCIN)	C3 convertases
Complement C2 receptor trispanning protein (CRIT)	C2
Chemotaxis inhibitory protein of *Staphylococcus aureus* (CHIPS)	C5a receptor (C5aR)
Regulators of complement activation (RCA) recruitment	
Complement-regulator-acquiring protein (CRASP)	Factor H, factor H-like protein-1 (FHL-1) and C4-binding protein (C4BP)
M protein family	Factor H, FHL-1 and C4BP
RCA mimicry	
Variola virus complement-control protein (VCP)	C3b and C3 convertases
Smallpox protein of complement enzymes (SPICE)	C3b and C3 convertases
Proteolytic degradation	
Staphylokinase	C3b and IgG (by activation of plasmin)
Pseudomonas elastase (PaE)	C3
56 kDa protease	C5a

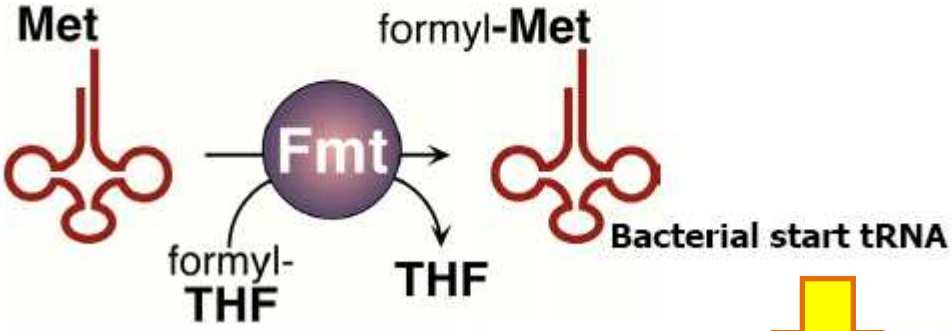
***See** Supplementary information S1 **(table) for an extended list of microbial evasion proteins.**

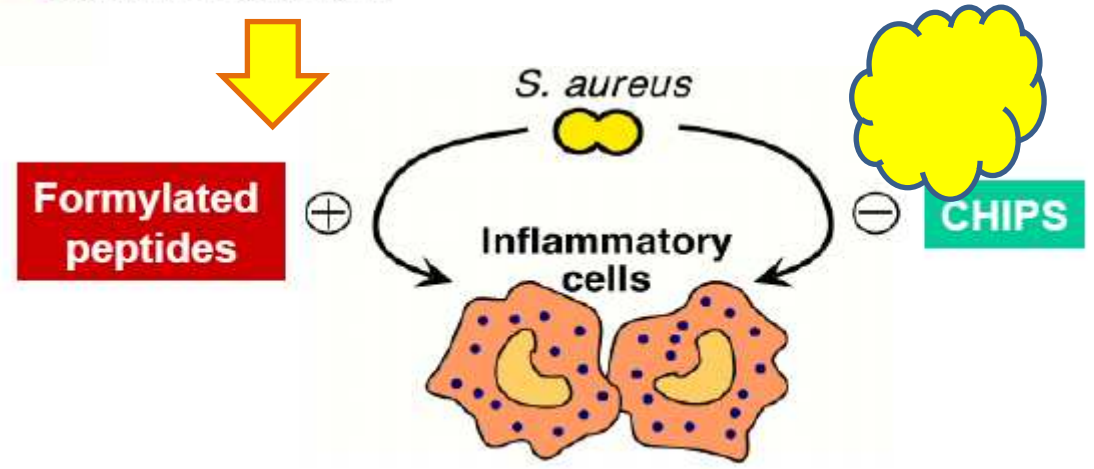
Nature Reviews Microbiology 6, 132-142 (February 2008)

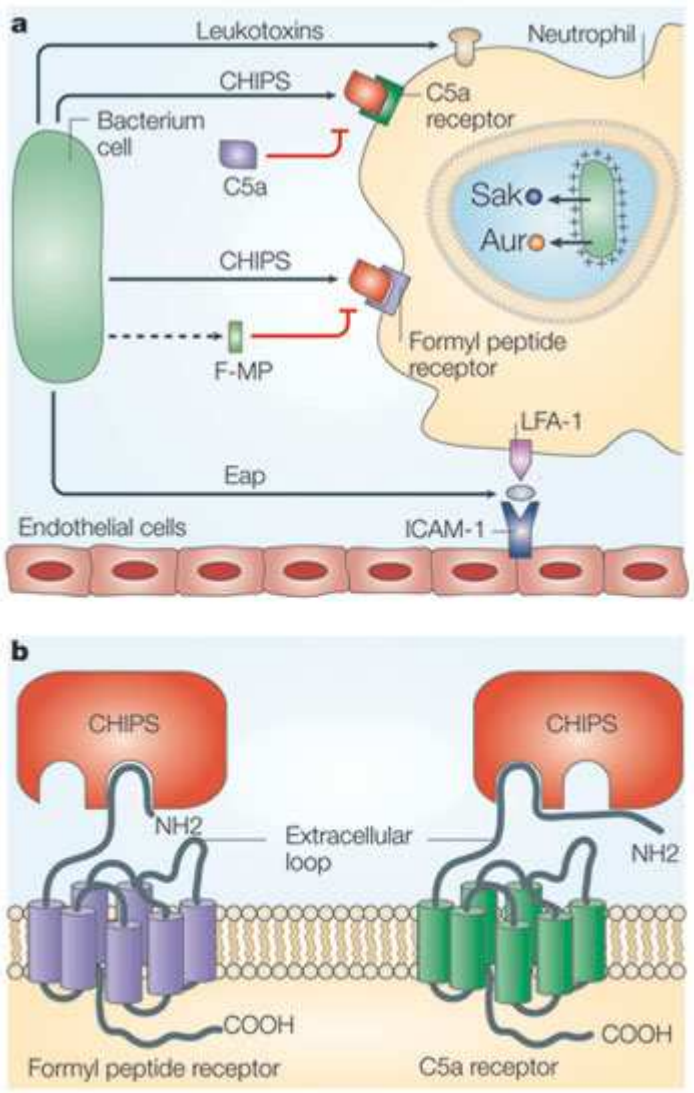
LOS PÉPTIDOS FORMILADOS INDUCEN QUIMIOTAXIS DE LOS PMNs….



Bacterial protein synthesis starts with fMet-tRNA



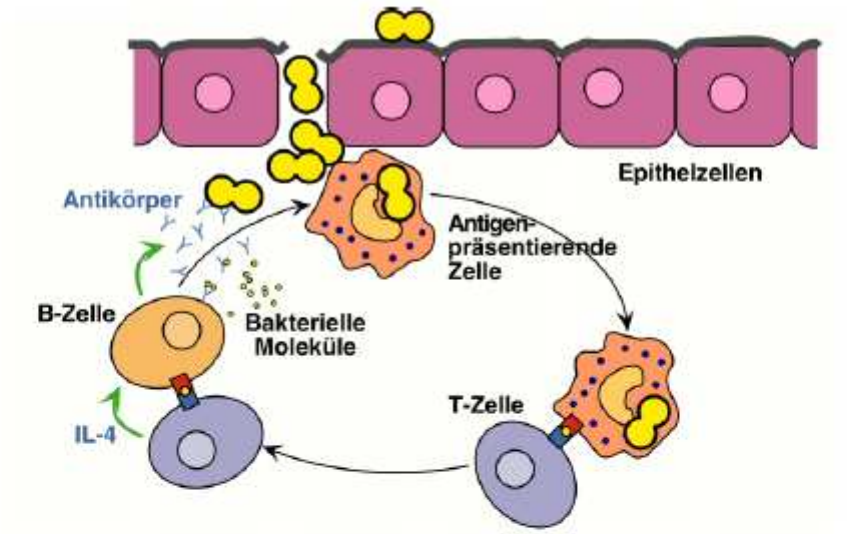




a | The chemotaxis inhibitory protein of staphylococci (CHIPS) and the extracellular adherence protein (Eap) interfere with neutrophil chemotaxis and extravasation.
Resistance to killing by antimicrobial peptides in the neutrophil phagosome is promoted by D-alanine and L-lysine modifications to cell-wall components (indicated by +), by secretion of staphylokinase (Sak) and aureolysin (Aur), and by the creation of 'spacious' phagosomes in which bacteria can survive. The pore-forming leukotoxins are shown by the mushroom-shaped insertion in the neutrophil membrane.

b | Model for interactions between CHIPS and the formyl peptide receptor (FPR) and C5a receptor. Two distinct but closely linked binding domains in CHIPS are indicated, one for the extreme N terminus of FPR involving residues F1 and F3, the second for a domain located between residues 10–20 of the C5a receptor. F-MP, N-formyl-methionyl peptide; ICAM-1, intercellular adhesion molecule-1; LFA-1, lymphocyte-function-associated antigen.

LAS BACTERIAS EVADEN AL SISTEMA INMUNE ADAPTATIVO



Defense mechanisms:

Antigen-presenting cells

Immunoglobulins

T cells

Virulence factors:

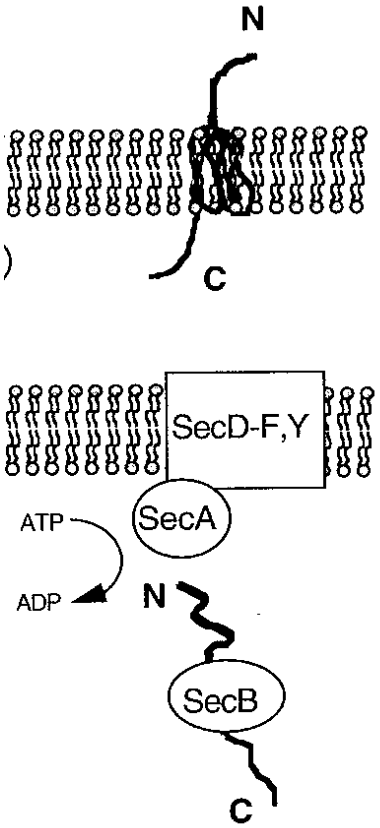
Leukocidins

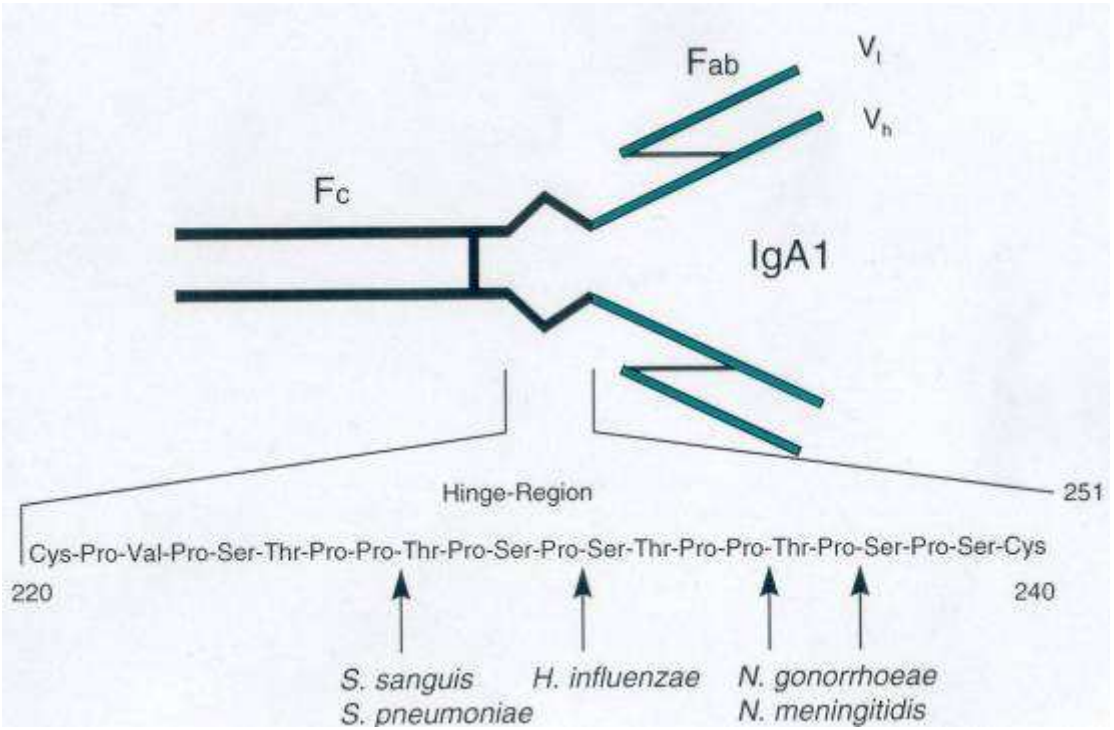
Ig proteases, capsules

Antigen variation

LAS BACTERIAS PRODUCEN IGA PROTEASAS…..

Neisseria meningitidis **serin proteasa**
Neisseria gonorrhoeae **serin proteasa**
Haemophilus spp. **serin proteasa**
Streptococcus spp. **Zinc-proteasa**





Evasión inmune por bacterias extracelulares

- Resistencia a fagocitosis:
  - Cápsulas: antiopsonizantes, voluminosas
    - Acido siálico (*Neisseria meningitidis*), ácido hialurónico (*Streptococcus pyogenes*)
  - Recubrimiento con proteínas del hospedero:
    - Fibronectina (*Staphylococcus aureus*), lactoferrina, transferrina (*Neisseria*)
  - Citotoxinas que matan leucocitos
    - Yops (*Yersinia*), leucocidinas (*S. aureus, S. pyogenes*)

Resistencia al complemento:

- Cápsulas que previenen la deposición de C3b, lo alejan del receptor celular, o estimulan su degradación
- Proteínas bacterianas que unen factores H
  - Proteína M (*S. pyogenes*), proteína II (*N. gonorrhoeae*)
- Degradación de factores
  - C5a proteasa (*S. pyogenes*)
  - Elastasa (*Pseudomonas*)
- Complejos C5b-9 no líticos
  - LPS de enterobacterias
  - Omps de *Neisseria*

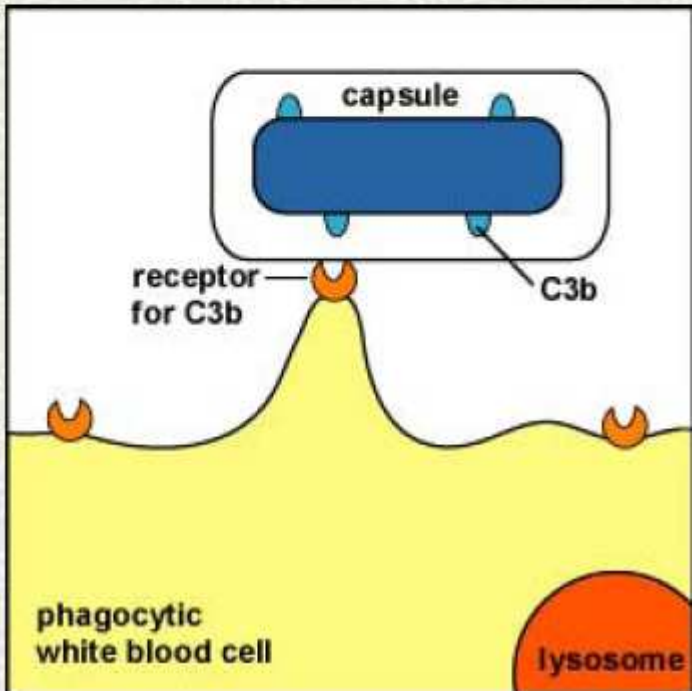


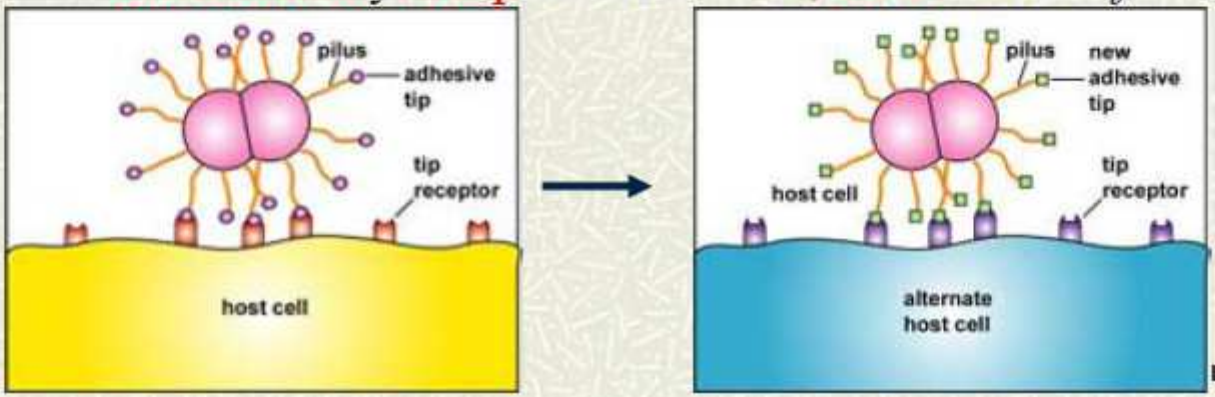
TABLE 13-9 MICROBIAL EVASION OF COMPLEMENT-MEDIATED DAMAGE

Microbial component	Mechanism of evasion	Examples
	Gram-negative bacteria	
Long polysaccharide chains in cell-wall LPS	Side chains prevent insertion of MAC into bacterial membrane	Resistant strains of *E. coli* and *Salmonella*
Outer membrane protein	MAC interacts with membrane protein and fails to insert into bacterial membrane	Resistant strains of *Neisseria gonorrhoeae*
Elastase	Anaphylatoxins C3a and C5a are inactivated by microbial elastase	*Pseudomonas aeruginosa*
	Gram-positive bacteria	
Peptidoglycan layer of cell wall	Insertion of MAC into bacterial membrane is prevented by thick layer of peptidoglycan	*Streptococcus*
Bacterial capsule	Capsule provides physical barrier between C3b deposited on bacterial membrane and CR1 on phagocytic cells	*Streptococcus pneumoniae*
	Other microbes	
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, *Trypanosoma cruzi*, *Candida albicans*

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

Resistencia a anticuerpos:

- Cápsulas antiopsonizantes
- Recubrimiento proteico
  - Proteína A (*S. aureus*), proteína G (*S. pyogenes*)
- Degradación
  - IgA proteasa (*Haemophilus, Neisseria*)
- Variación antigénica
  - Fimbrias y Omp de *Neisseria*, LPS de *H. influenzae*



BACTERIAS INTRACELULARES

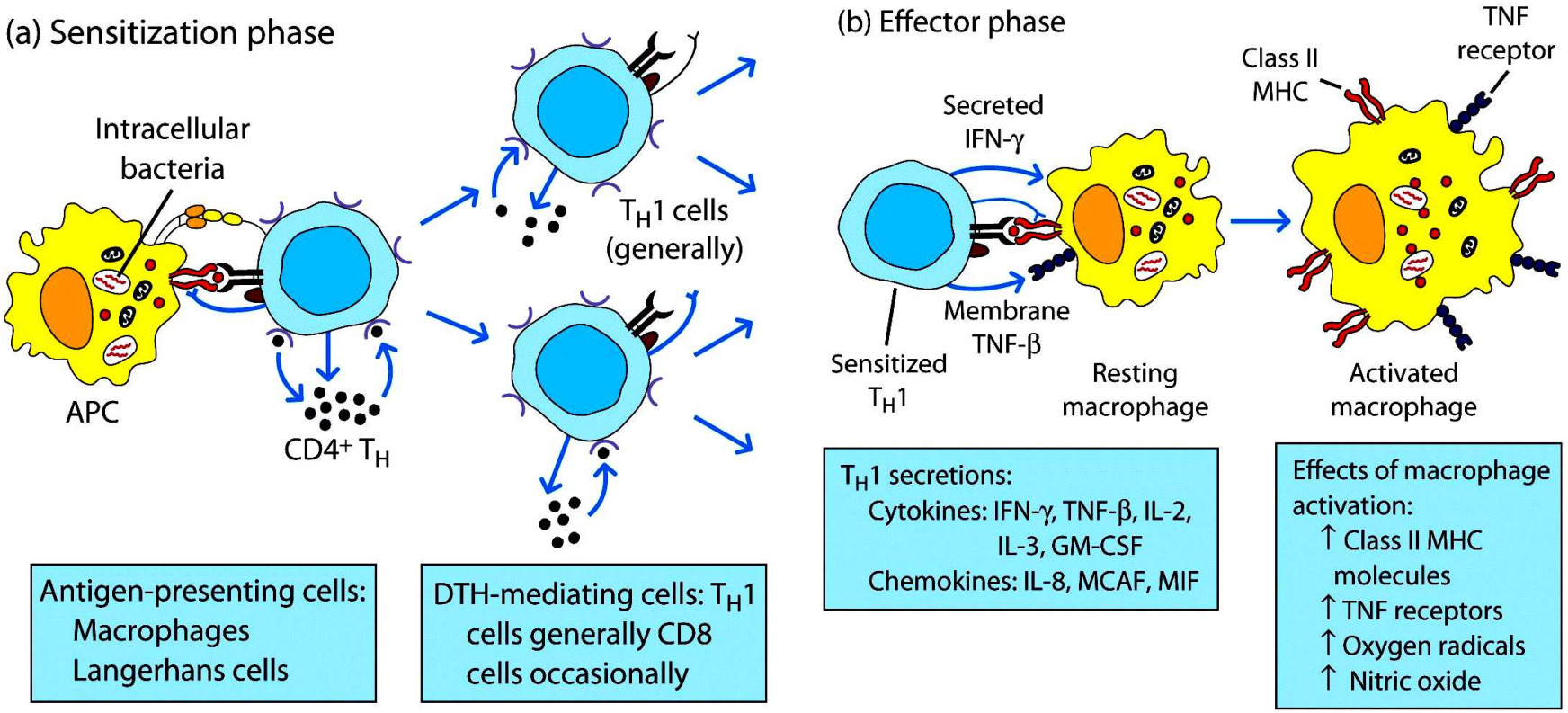
Inmunidad contra bacterias intracelulares

- Resistentes a degradación en macrófagos, sobreviven y se multiplican en nichos intracelulares
- Inmunidad natural
  - Células NK: producen IL-2, IL-12 e IFN-γ. Lisis directa y activación de macrófagos
  - Linfocitos Tγδ: producen TNF-α, IFN-γ y tienen actividad citolítica

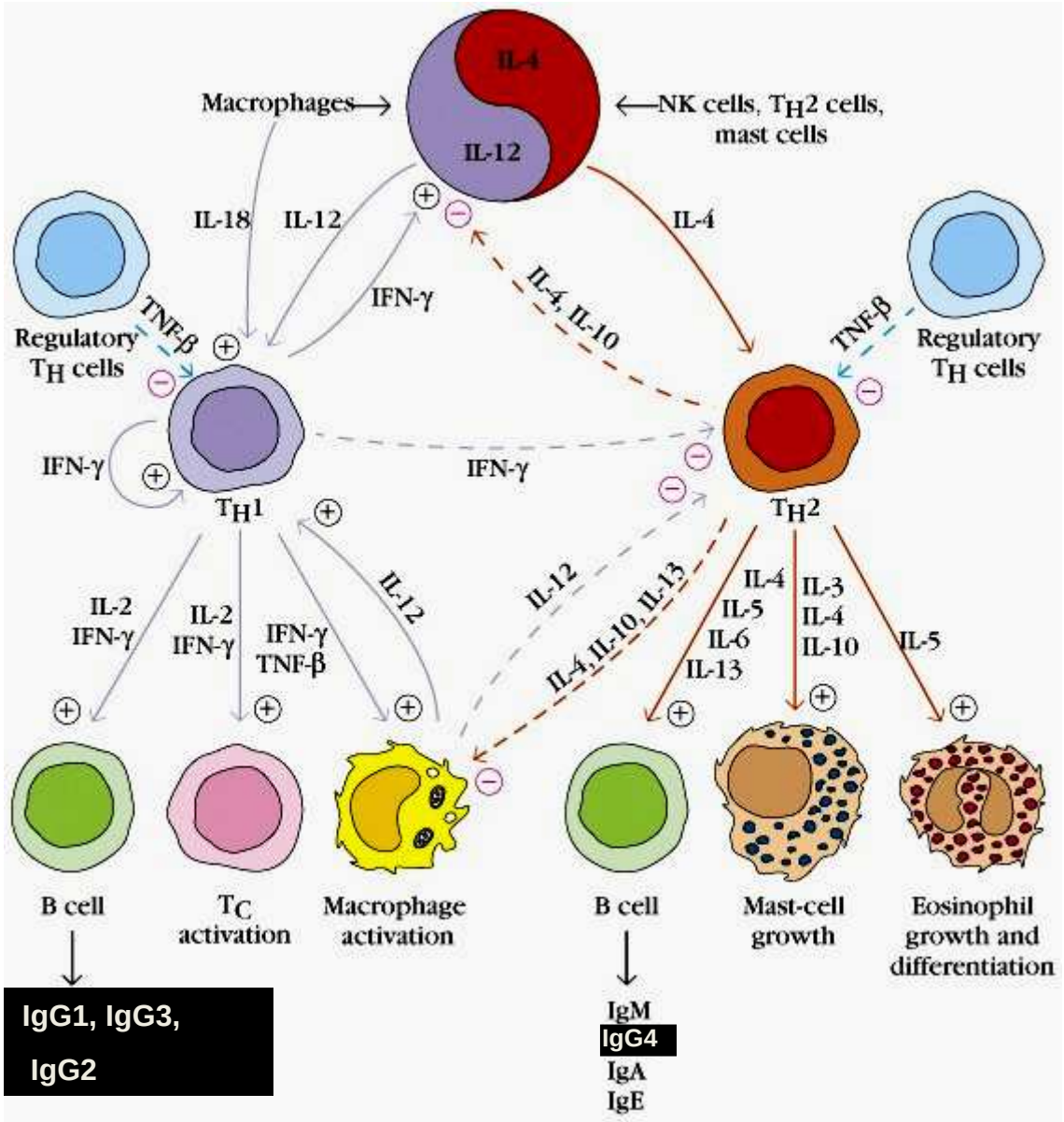
Inmunidad celular

- Linfocitos Th1: hipersensibilidad retardada
- Activación de linfocitos T citotóxicos ($CD8^+$, TcR $\alpha\beta^+$) antígeno-específicos: perforinas y granulisinas
- Linfocitos T restringidos a CD1b ($CD8^+$ y DN): antígenos no proteicos (glicolípidos)
- Producción de IFN-γ, TNF-β (linfotoxina)
- Activación de células NK y macrófagos (granulomas)

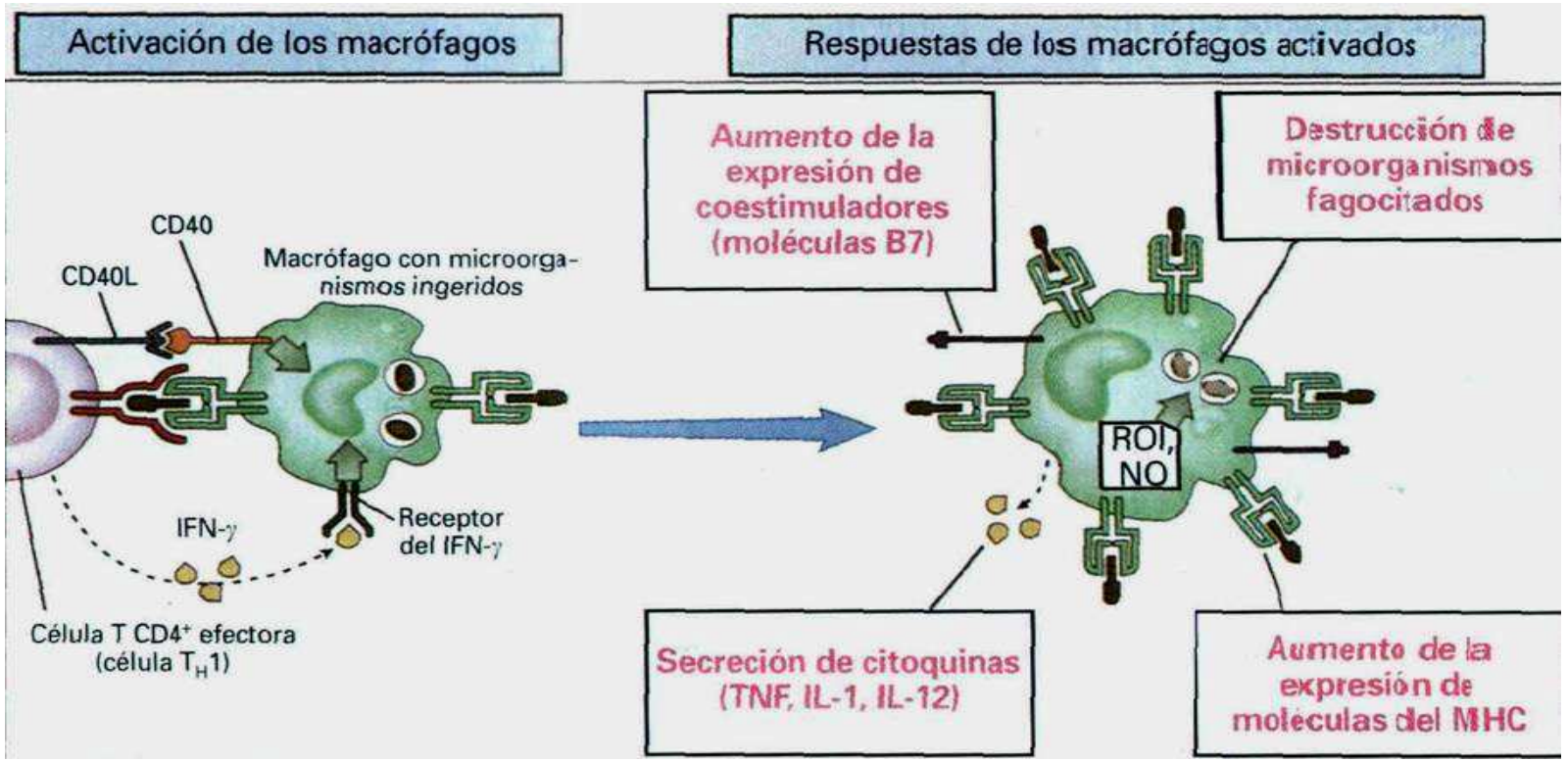
Respuesta a infecciones por bacterias intracelulares



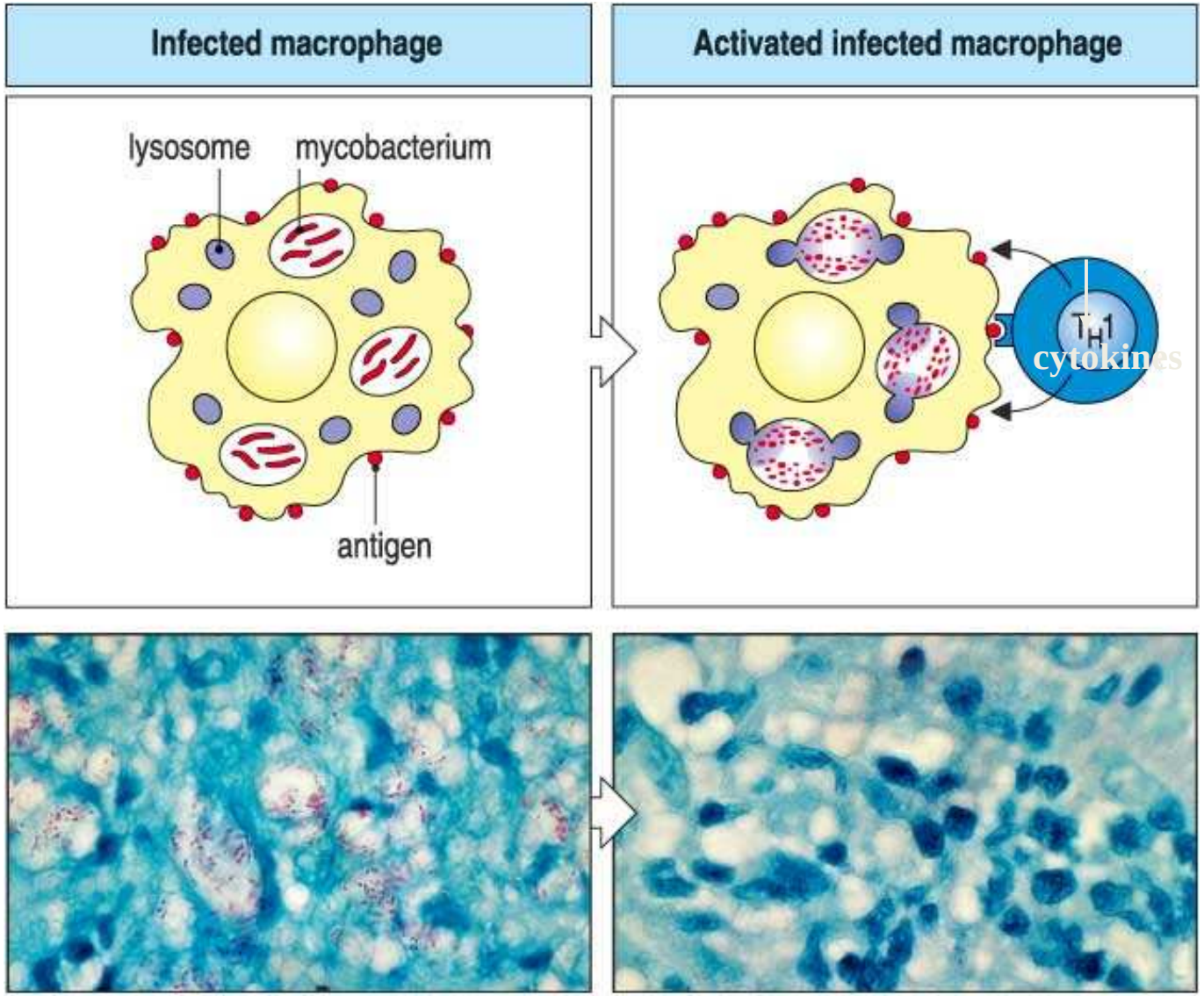
Regulación de balance T_{H1}/T_{H2}



Activación de macrófagos en la respuesta T_{H1}



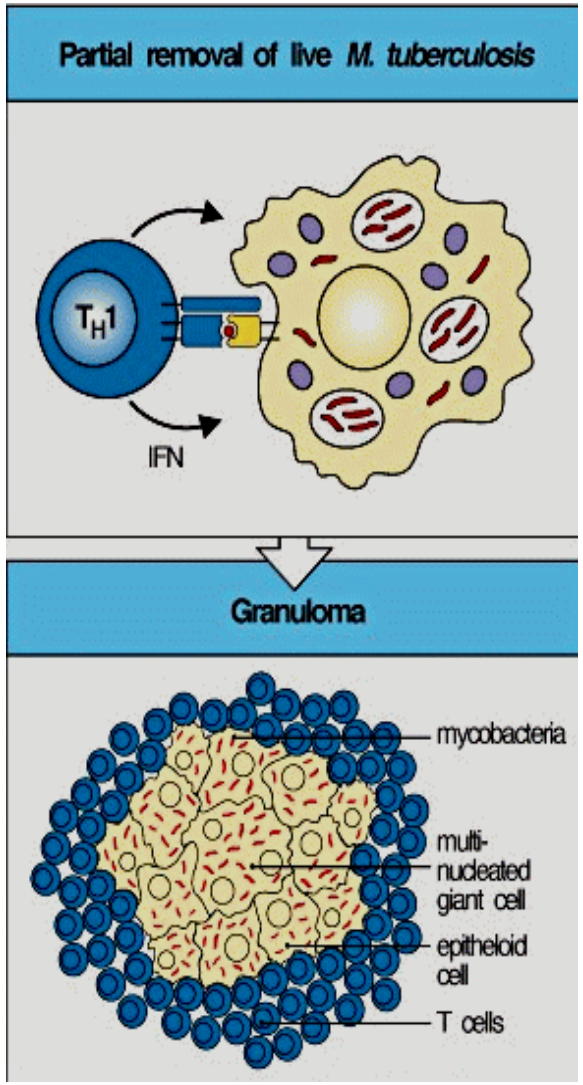
Activación de macrófagos en la respuesta T_{H1}

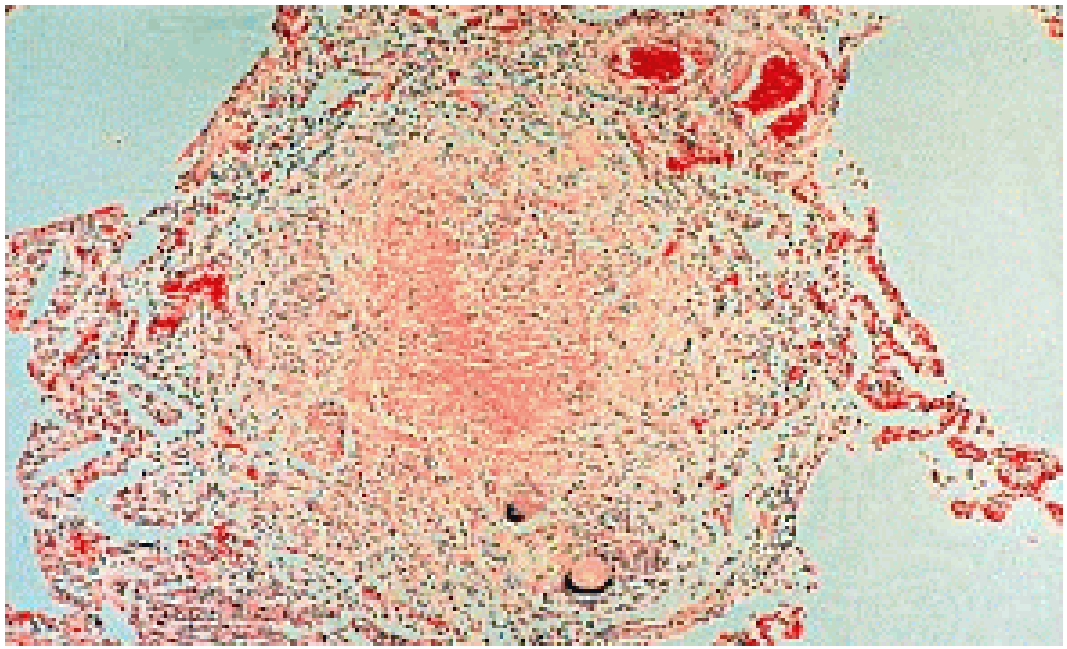


Las células T_H1 se denominan también células T inflamatorias

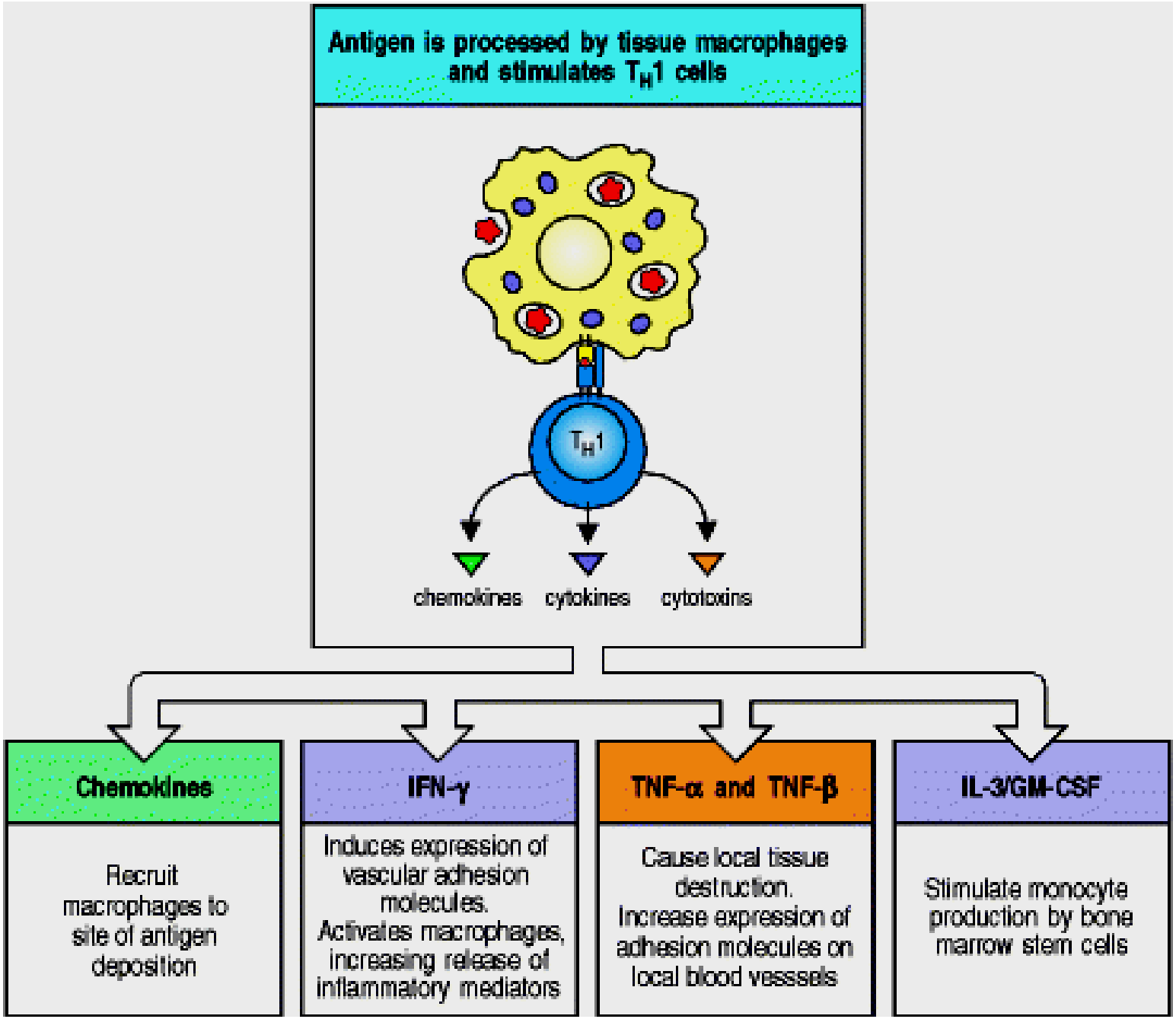
La activación de los macrófagos permite eliminar los patógenos intracelulares.

Desarrollo de granulomas

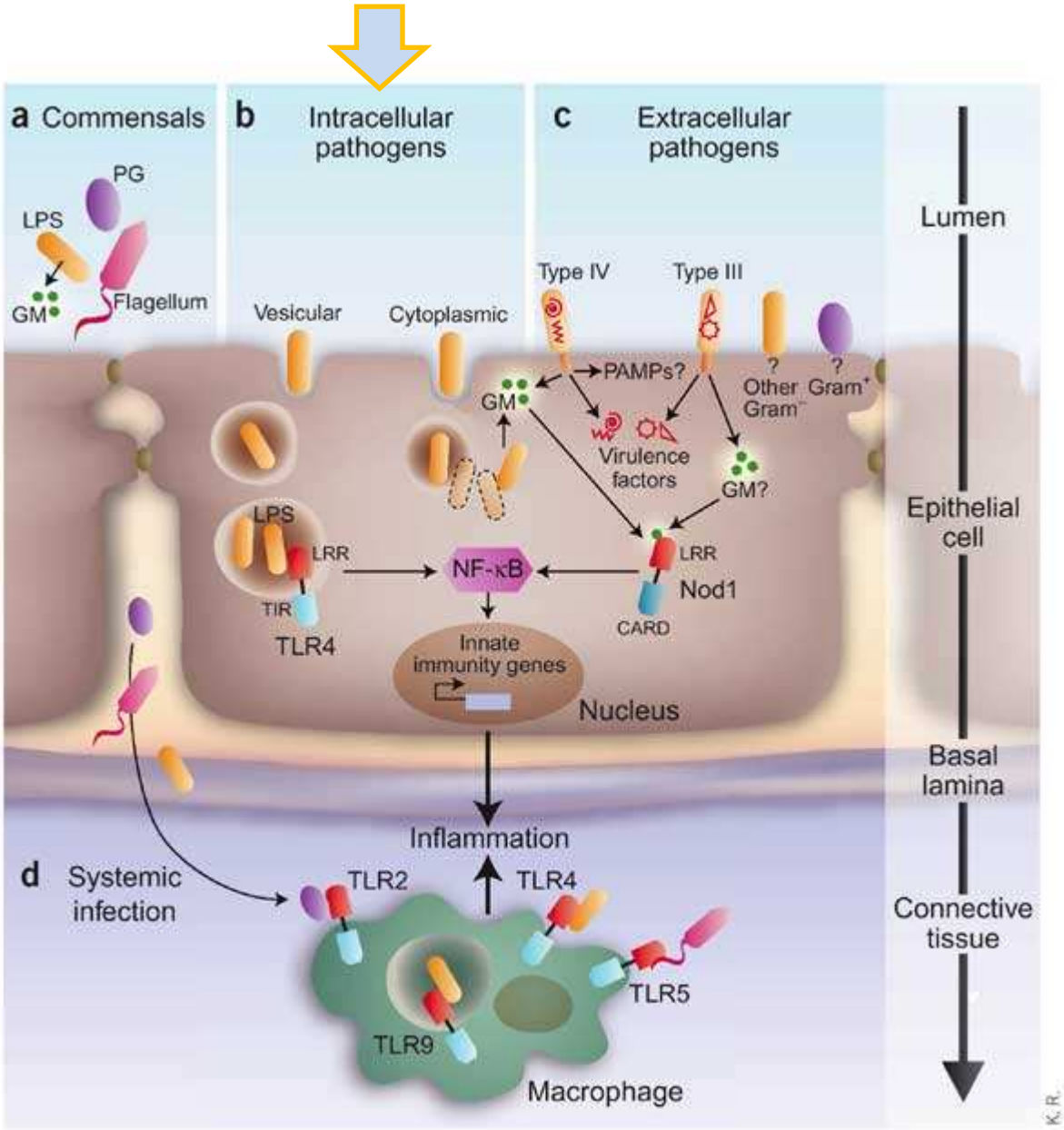




Intracellular bacteria
- *Mycobacterium tuberculosis*
- *Mycobacterium leprae*
- *Listeria monocytogenes*
- *Brucella abortus*


Antigen is processed by tissue macrophages and stimulates T_H1 cells
T_H1
chemokines
cytokines
cytotoxins
Chemokines
Recruit macrophages to site of antigen deposition
IFN-γ
Induces expression of vascular adhesion molecules. Activates macrophages, increasing release of inflammatory mediators
TNF-α and TNF-β
Cause local tissue destruction. Increase expression of adhesion molecules on local blood vesssels
IL-3/GM-CSF
Stimulate monocyte production by bone marrow stem cells

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.



Nature Immunology **5**, 1099 - 1100 (2004)

- (**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-B 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.

LA RESPUESTA INFLAMATORIA INLCUYE ACTIVACIÓN DE DIFERENTES TIPOS CELULARES……

TLRs → NF-κB
(transcription factor)

Epithelial cells:
- → **Defensin** production
- → **IL-8** produktion

Endothelial cells:
- → Adhesive for **leukocytes**
- → **Permeabilisation**

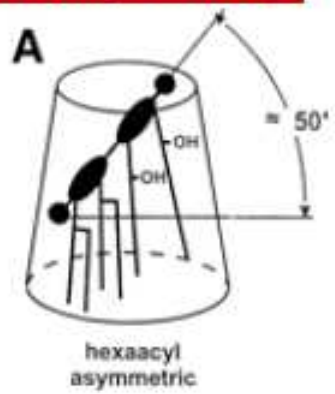
Phagocytes:
- → **Cytokine** production
- → increased **killing**

LA CHLAMYDIA PRODUCE LPS DE MUY BAJA ACTIVIDAD INFLAMATORIA…

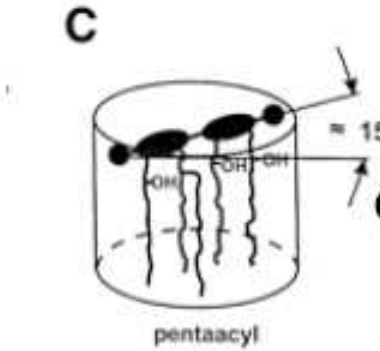
Chlamydia pneumoniae

- **Obligate intracellular pathogens**
- **Cause persistant infections**

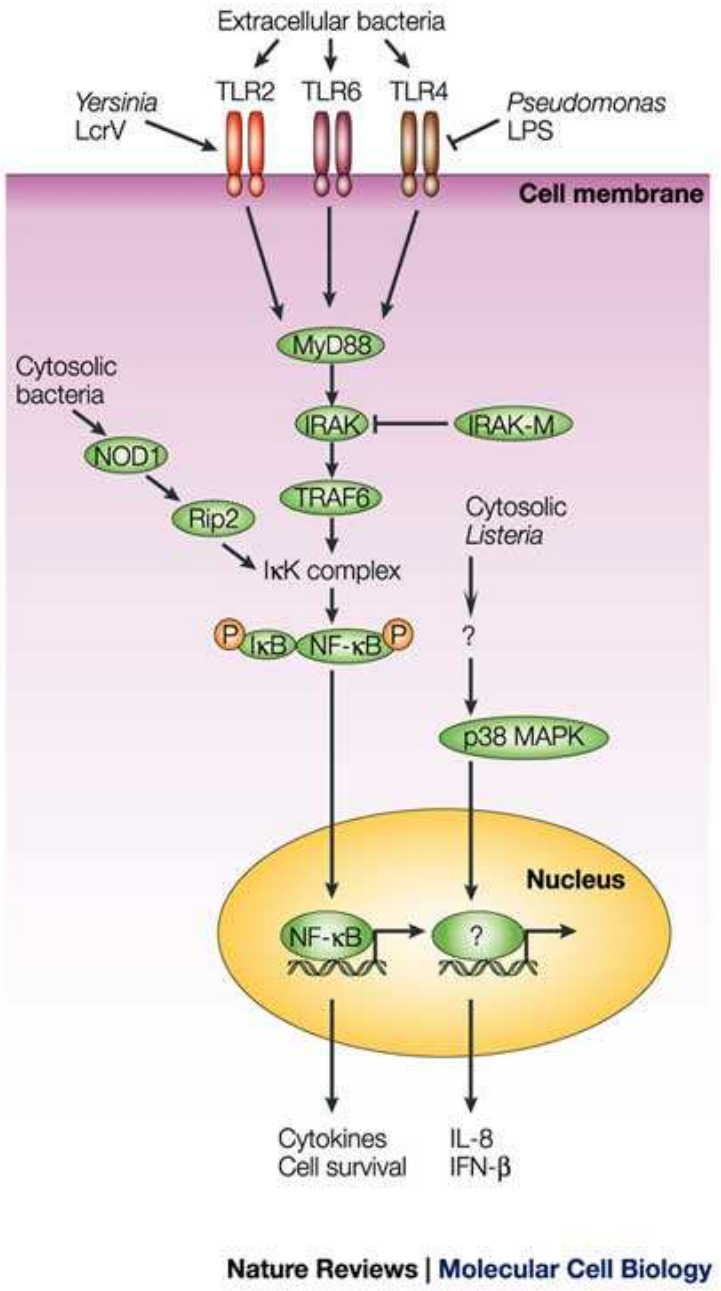
Active LPS:
(6 fatty acids)



Inactive LPS:
(4-5 fatty acids)

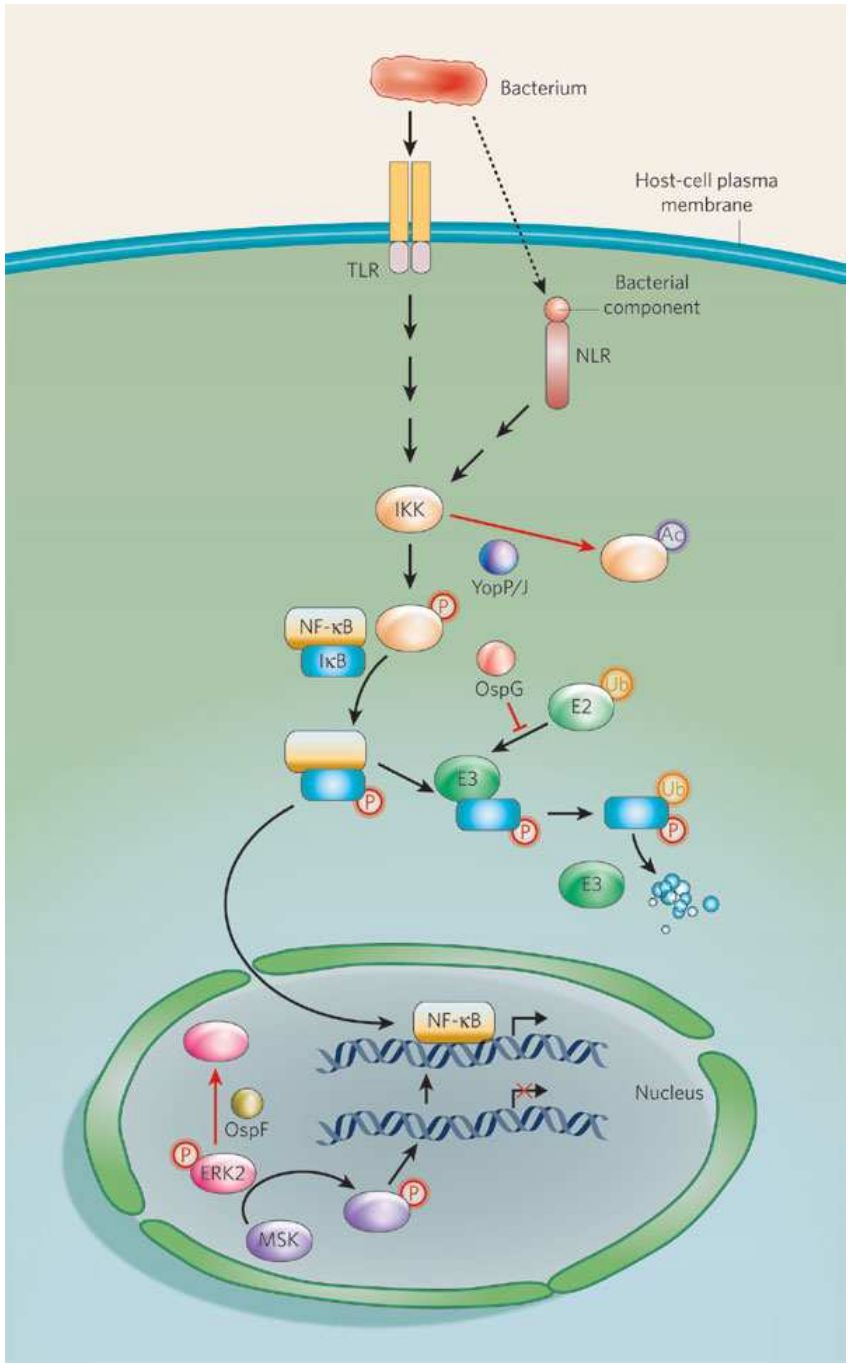


Chlamydial LPS



Pseudomonas aeruginosa decrease TLR4-mediated signalling

Yersinia pestis produces the virulence protein LcrV that increases TLR2-mediated signalling and secretion of the anti-inflammatory cytokine IL-10.



INTERFERENCIA CON EL FACTOR NF-κB

***Yersinia* spp effector YopP/J**

***S. flexneri* effector OspG**

ACTIVIDAD ENZIMÁTICA DE PROTEÍNAS BACTERIANAS: INTERFERENCIA EN LA FUNCIÓN DEL MACRÓFAGO

Table 1 | **The enzymatic activities of bacterial proteins that interfere with macrophage signalling**

Strategy	Activity	Gene product	Pathogen	Target
Avoidance of phagocytosis	Tyr phosphatase	YopH	*Yersinia*	FAK, Paxillin, p130cas
	Ser/Thr kinase	YpkA (YopO)	*Yersinia*	Actin, RhoA, Rac
	Cysteine protease	YopT	*Yersinia*	RhoA, Rac1, Cdc42
	GTPase activating	YopE	*Yersinia*	Rho, Rac, Cdc42
	GTPase activating	ExoT, ExoS	*Pseudomonas*	Rho, Rac, Cdc42
	ADP-ribosyltransferase	ExoS	*Pseudomonas*	Ras family
Disruption of trafficking	GEF	RalF	*Legionella*	ARF1
Promotion of inflammation	Protease activation	IpaB	*Shigella*	Caspase-1
	Protease activation	SipB	*Salmonella*	Caspase-1
Dampening of inflammation	Protease	Lethal factor	*Bacillus*	MEK kinase
	Ubiquitin-like protease	YopJ (YopP)	*Yersinia*	MAPKK, IKKβ
	Protease	?	*Pseudomonas*	IFN-γ, TNF-α
Cytotoxicity	Adenylate cyclase	CyaA	*Bordetella*	↑ cyclic AMP
	ADP-ribosyltransferase	SpvB	*Salmonella*	?
Alteration of signalling	Adenylate cyclase	EF	*Bacillus*	↑ cAMP

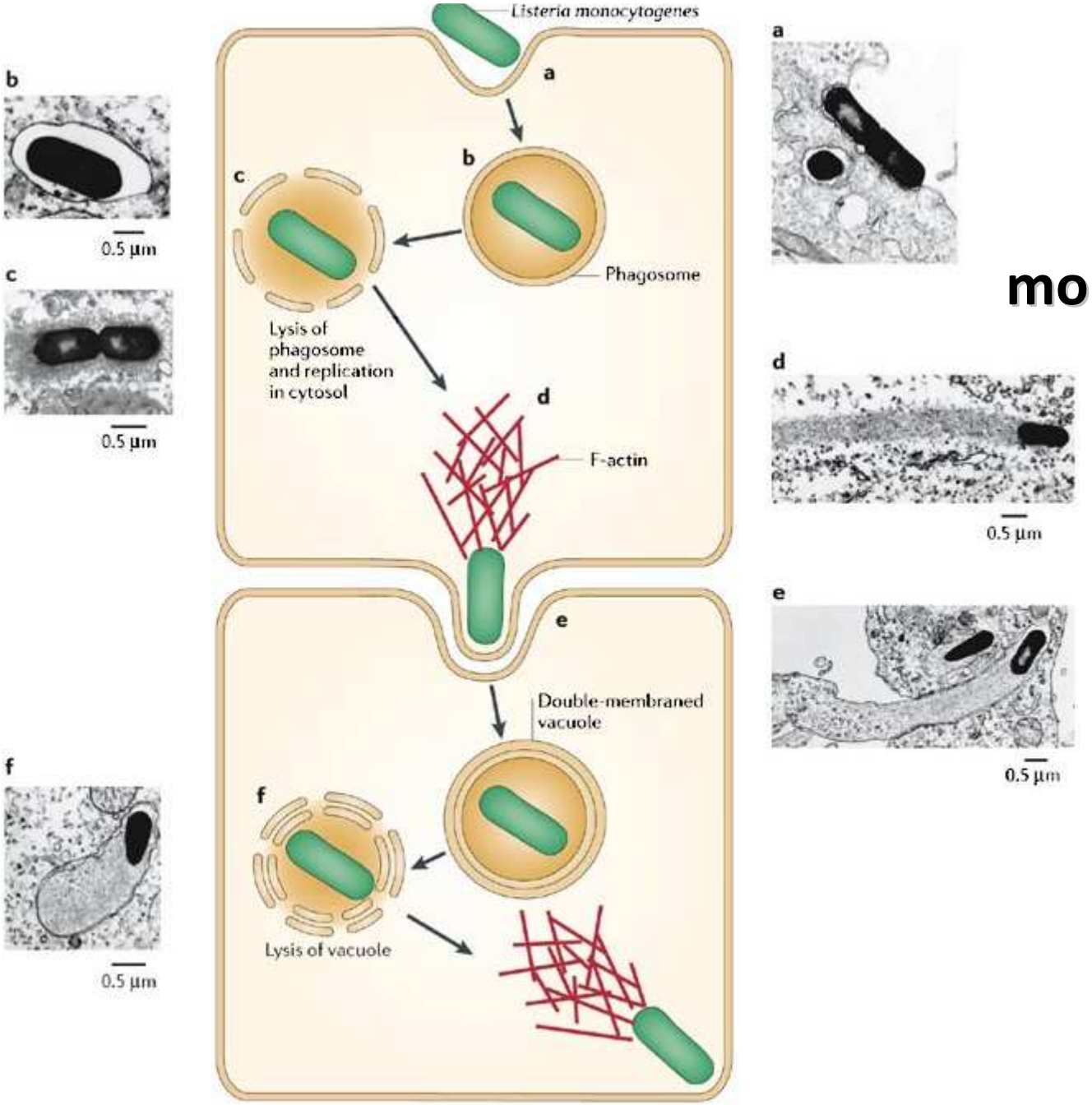
The above bacterial virulence proteins are expressed during infection — their cellular targets and biological effects are given where known. The enzymatic activity of these bacterial effectors has often been determined in non-macrophage cell lines or *in vitro* and has not always been confirmed in macrophages. These bacterial components can have several effects. For example, the virulence proteins IpaB and SipB target caspase-1 to cause macrophage apoptosis as well as release of the proinflammatory cytokine interleukin-1β. ARF1, ADP-ribosylating factor 1; CyaA, adenylate cyclase; EF, oedema factor; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GEF, guanine nucleotide exchange factor; IFN-γ, interferon-γ; IKKβ, inhibitor of NF-κB (IκB) kinase β; MAPK, mitogen-activated protein kinase; MEK, MAPK and ERK kinase; Ser, serine; Thr, threonine; TNF-α, tumour necrosis factor-α; Tyr, tyrosine.

Table 1 | **Some persistent bacterial pathogens of humans**

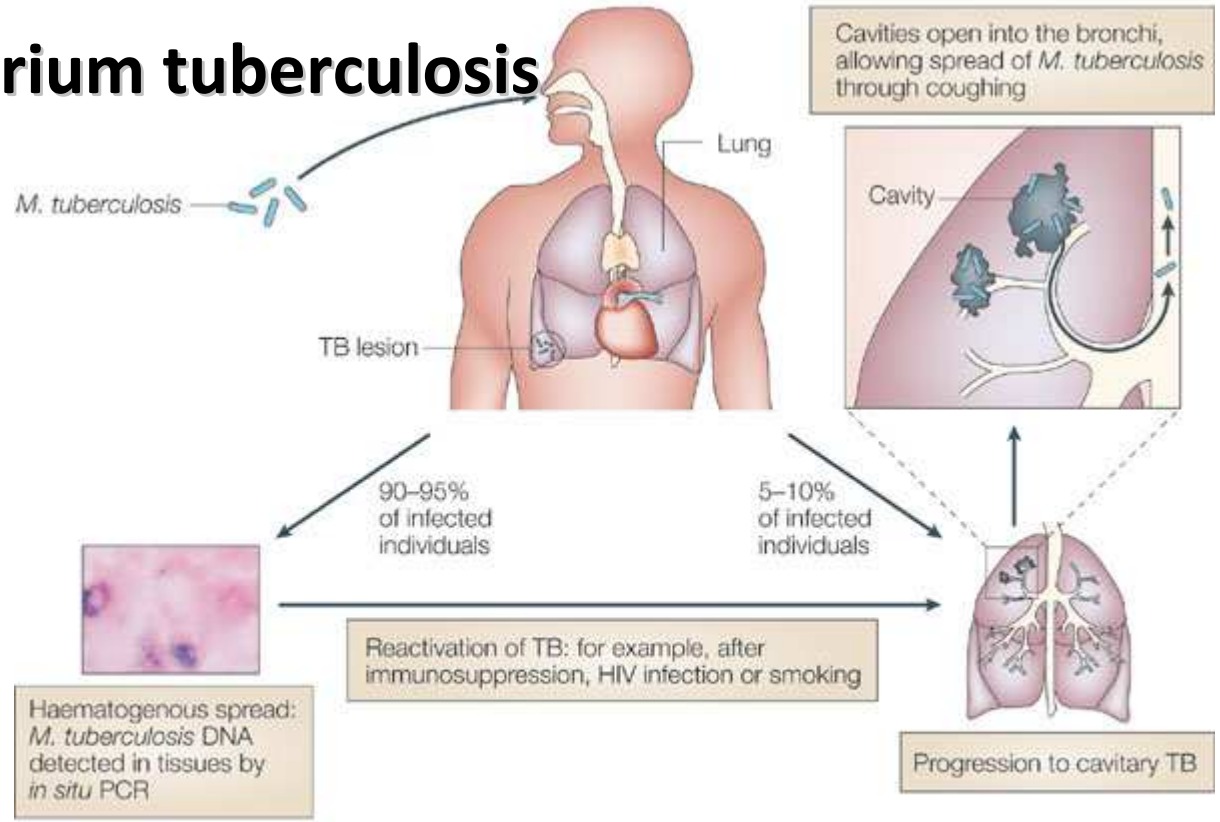
Pathogen	Disease conditions	Likely sites of persistence
Mycobacterium tuberculosis	Tuberculosis	Macrophages in various sites and in granulomas
Salmonella enterica serovar Typhi	Typhoid fever	Macrophages in bone marrow, the RES and possibly the gall bladder
Chlamydia spp.	*C. pneumonia* causes respiratory and cardiovascular disease; *C. trachomatis* causes trachoma, genital-tract infections and lymphogranuloma venereum	Epithelial and endothelial cells
Helicobacter pylori	Gastritis; ulcers; gastric cancer; MALT lymphoma	Extracellular; possibly also intracellular in the stomach
Brucella spp.	Brucellosis (this can be chronic, leading to lymphadenopathy and hepatosplenomegaly)	Macrophages in the RES
Borrelia burgdorferi	Lyme disease	Disseminated in various organs
Bartonella henselae	Cat-scratch disease; bacillary angiomatosis; bacillary peliosis hepatitis	Extracellular; in erythrocytes in blood
Neisseria gonorrhoea	Genital-tract infections, which can lead to epididymitis, pelvic inflammatory disease and infertility	Extracellular; intracellular at mucosal sites
Neisseria meningitidis	Invasive infection results in meningititis	Nasopharynx; NALT?
Streptococcus pneumoniae	Acute otitis media; bacteraemia; meningitis	Nasopharynx; NALT?
Streptococcus pyogenes	Acute pharyngotonsillitis; pneumonia; endocarditis; skin, soft tissue and bone infections (necrotizing fasciitis)	Nasopharynx; NALT?
Haemophilus influenzae type B	Pneumonia; meningitis; bactereamia	Nasopharynx; NALT?

NALT, nasopharyngeal-associated lymphatic tissue; RES, reticuloendothelial system.

Listeria monocytogenes

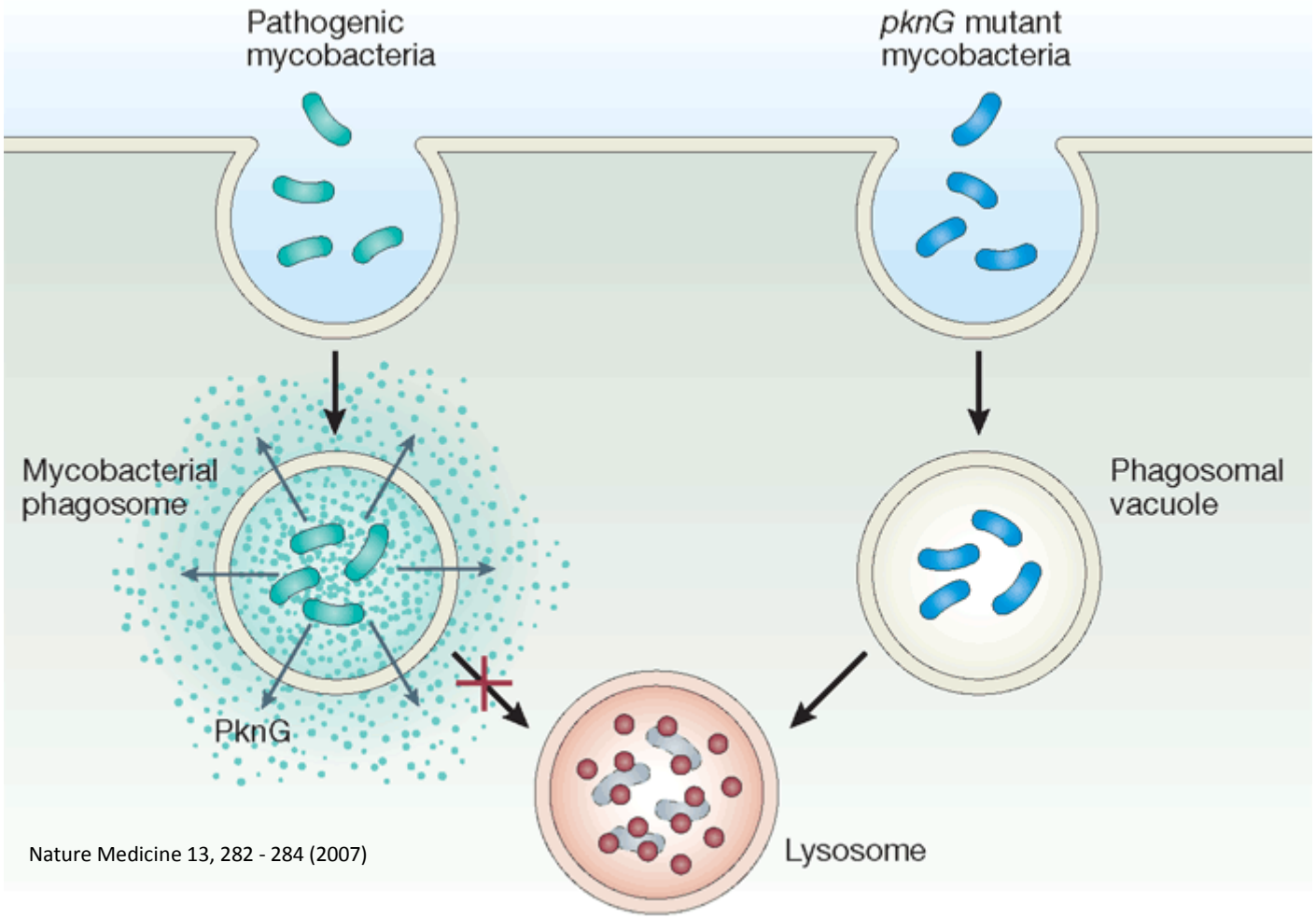


Micobacterium tuberculosis



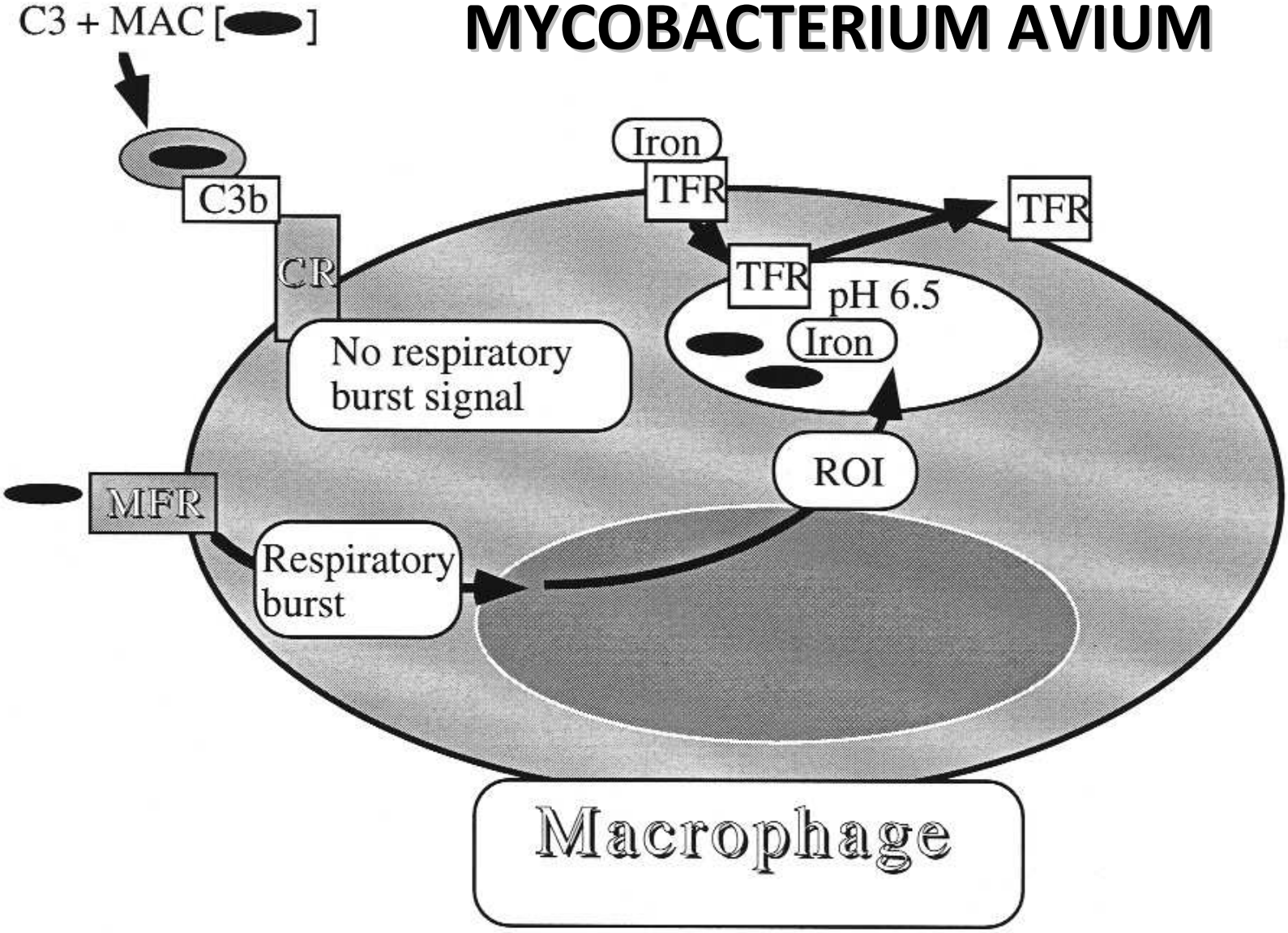
In 90–95% of individuals, the infection remains latent, which is probably reflected by the Mycobacterium tuberculosis DNA that can be detected using in situ PCR (shown as blue intracytoplasmic material) in tissue with little cellular infiltrate.

In a minority of individuals, there is progressive disease development, most often in the lung apex, where the ratio of ventilation to blood perfusion is highest. However, in most infected people, the disease remains latent for decades, although it can be reactivated when an individual is immunosuppressed, particularly through infection with HIV. Progressive disease is characterized by weight loss, toxicity of tumour-necrosis factor, cavitation and fibrosis, even though interferon-γ produced by T_H1 cells can decrease the amount of fibrosis. The cavities eventually open into the bronchi, which allows the spread of TB by aerosols during coughing.

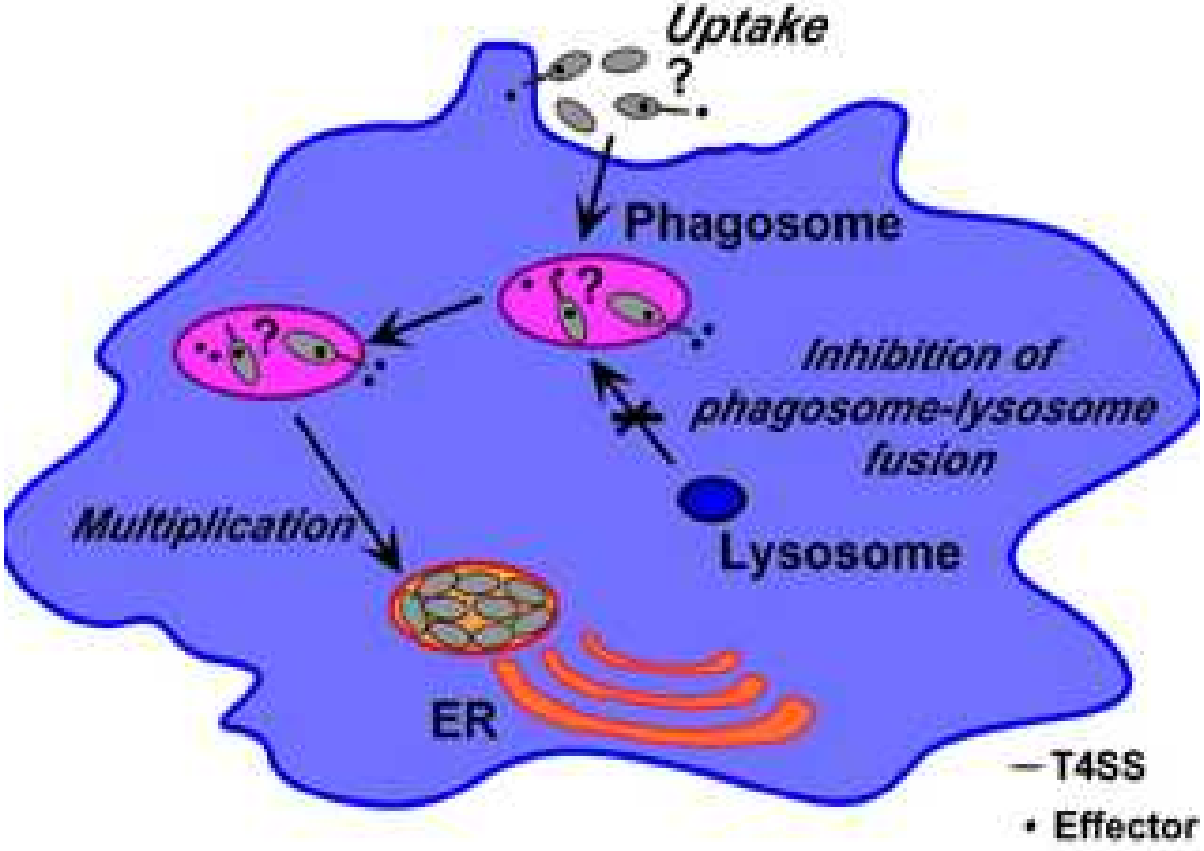


Most microbes and nonpathogenic mycobacteria quickly find themselves in lysosomes, where they are killed. By contrast, *M. tuberculosis* stays within phagosomes; the bacterium releases PknG to block phagosome-lysosome fusion. Bacteria lacking *pknG* are rapidly transferred to lysosomes and eliminated.

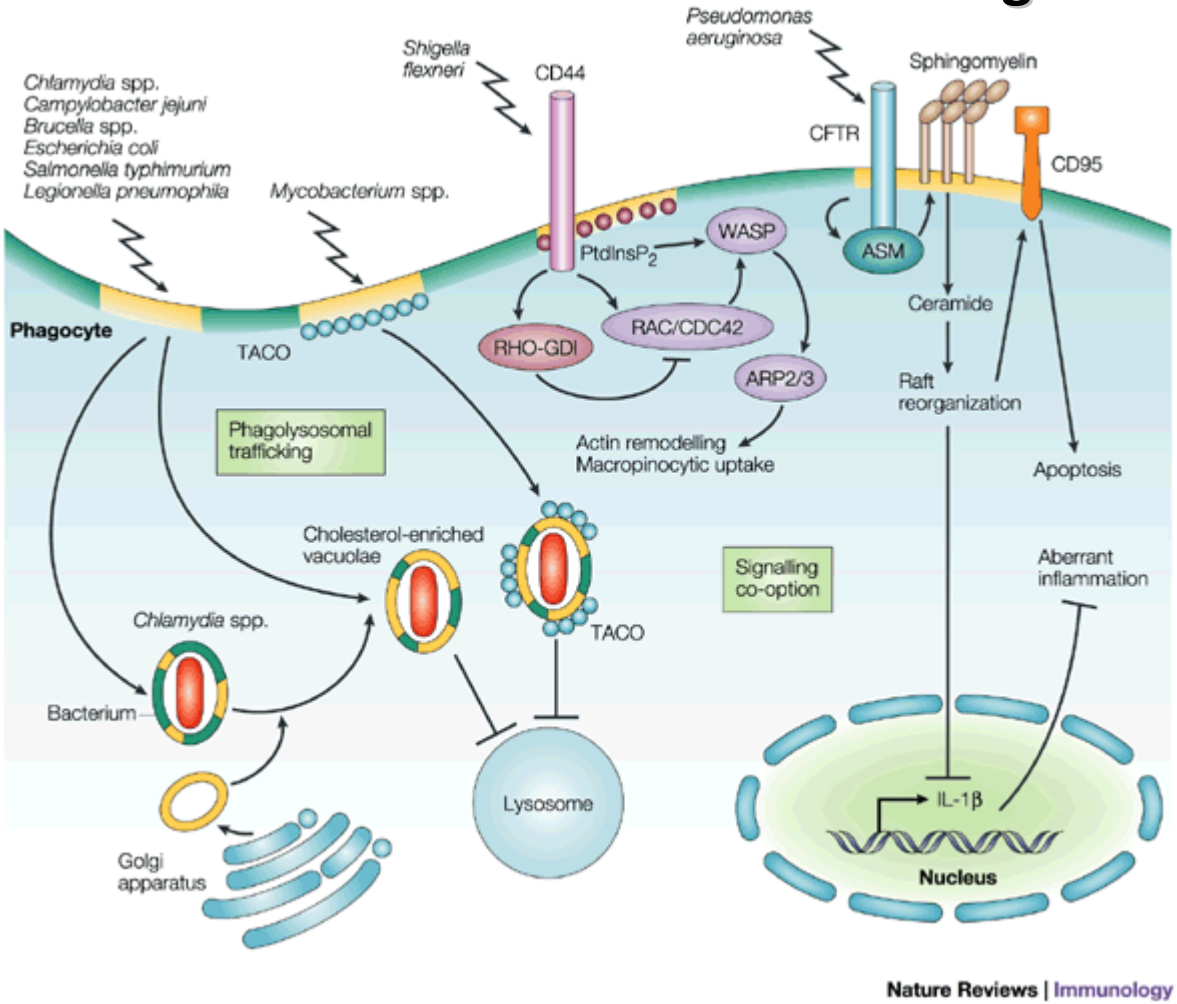
MYCOBACTERIUM AVIUM



Brucella suis redirects intracellular trafficking and multiplies in a specialized organelle



RAFTS LIPÍDICOS E INFECCIONES: evasión de la fusión fago-lisosomal



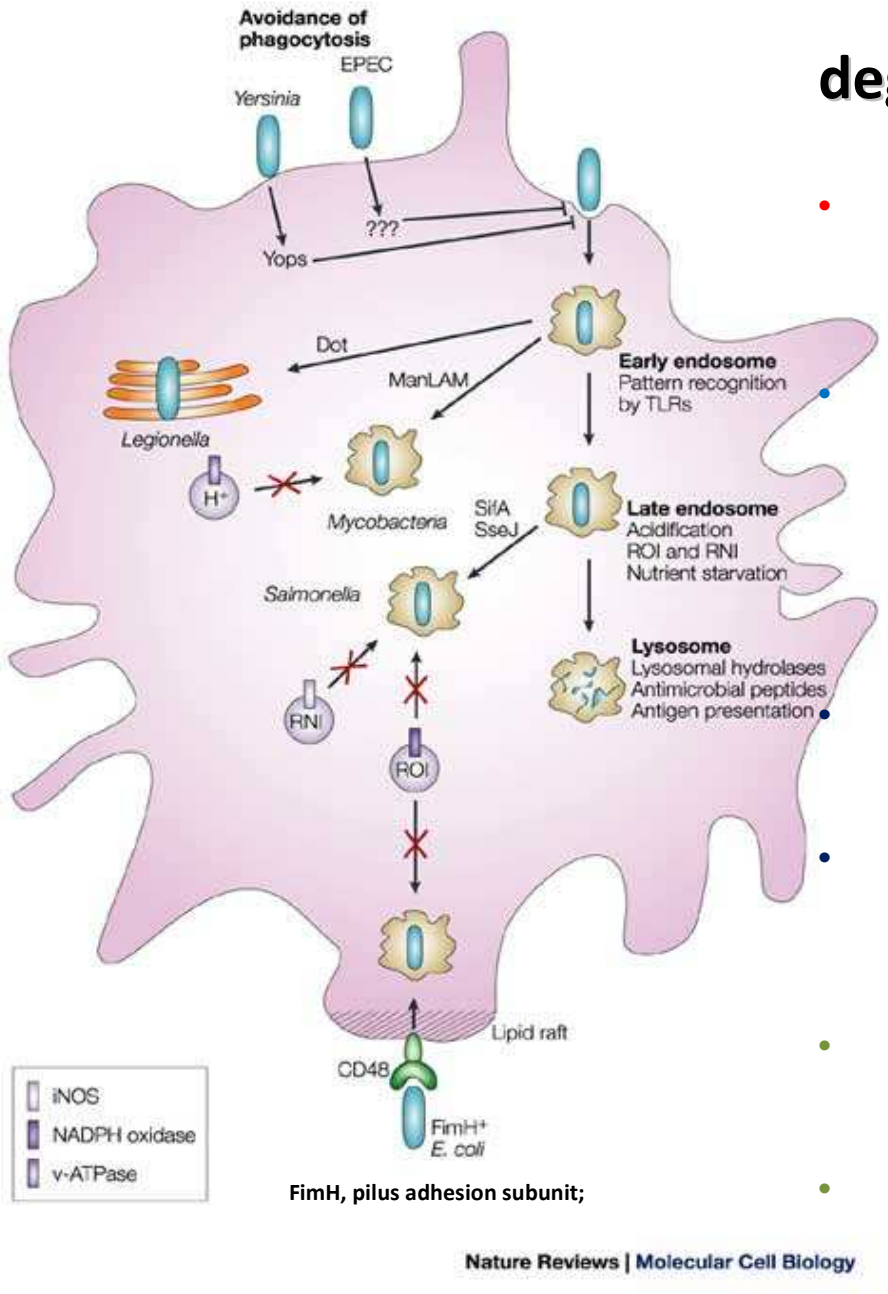
Nature Reviews Immunology 3, 557-568 (July 2003)

Many bacteria use host raft components to avoid phagosome fusion with the degradative compartments of the host cell.

Some of these bacteria use raft-associated receptors to generate vacuoles that are enriched in raft components and have a defined intracellular trafficking pathway.

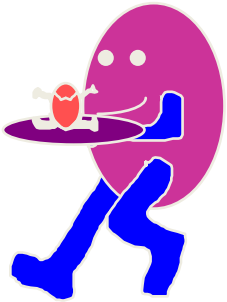
TACO (tryptophan-aspartate-containing coat protein)

Interferencia con la degradación fagolisosomal

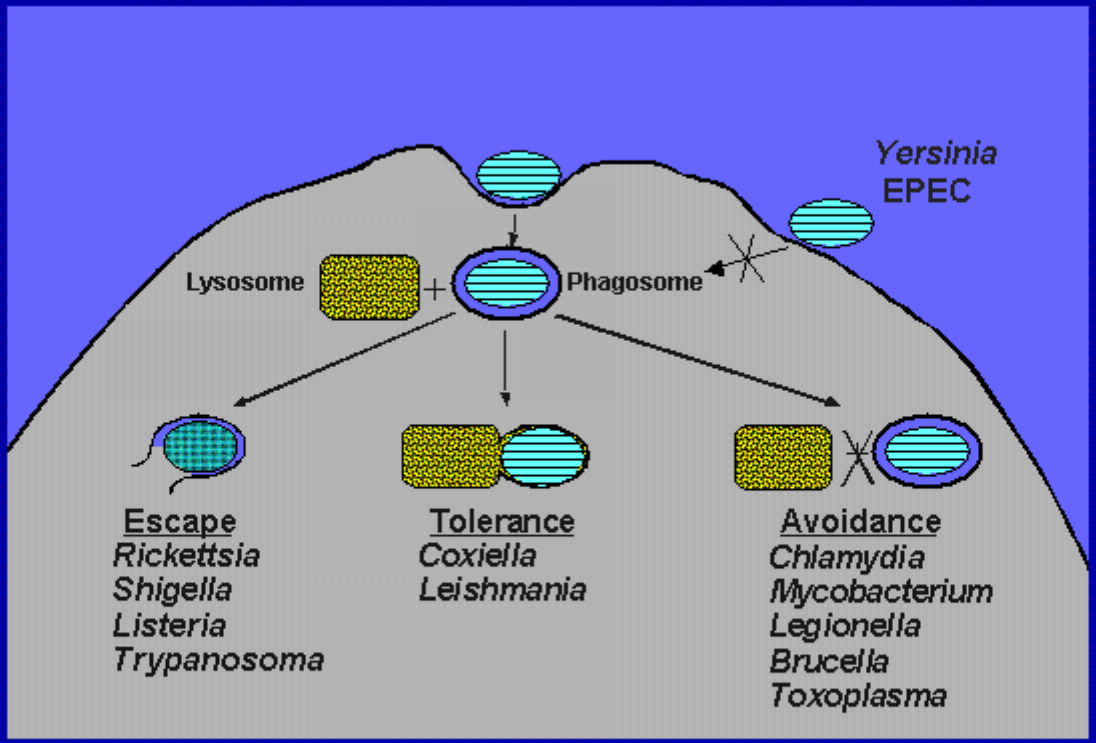


- Mycobacterium tuberculosis blocks acidification and uses mannose-lipoarabinomannan (ManLAM) to prevent interactions with other endosomal compartments.
- **Salmonella resides in an acidified compartment that resembles late endosomes but blocks the acquisition of NADPH oxidase, inducible nitric oxide synthase (iNOS) and degradative lysosomal enzymes, and uses the bacterial type III effector proteins SifA and SseJ to modify the vacuolar membrane composition.**
- **FimH⁺Escherichia coli engages alternative receptors to enter macrophages using lipid rafts and avoids the oxidative burst.**
- **Legionella pneumophila secretes proteins through the Dot secretion system to establish a replicative organelle resembling rough endoplasmic reticulum.**
- **Yersinia and enteropathogenic E. coli (EPEC) express virulence proteins (such as Yops) to inhibit phagocytosis altogether.**
- CD48, human leukocyte antigen/macrophage receptor CD48; FimH, pilus adhesion subunit; TLR, Toll-like receptor.

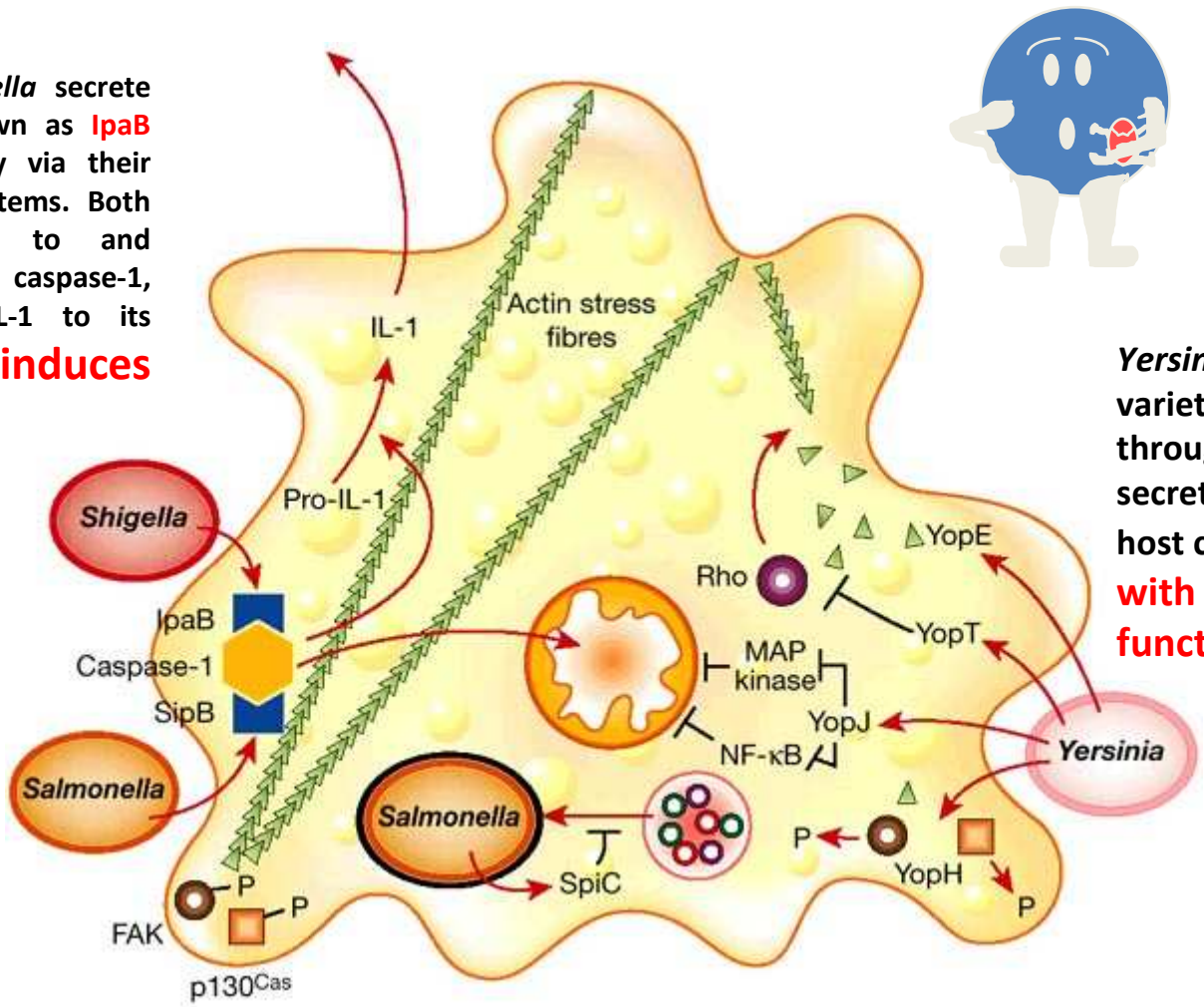
FimH, pilus adhesion subunit;



Microbial interactions with host cells



Shigella **and** ***Salmonella*** **secrete related proteins known as IpaB and SipB respectively via their type III secretion systems. Both proteins can bind to and presumably activate caspase-1, which cleaves pro-IL-1 to its mature form and induces apoptosis.**



Yersinia **delivers a variety of Yops through its type III secretion system into host cells to interfere with macrophage function.**

Salmonella **also secretes a protein known as SpiC through its SPI2 secretion system; this protein blocks vesicle trafficking (depicted as a failure of fusion between lysosome and endosome).**

- ***Yersinia*** delivers a variety of Yops through its type III secretion system into host cells to interfere with macrophage function. Macrophages infected with *Yersinia* produce smaller quantities of tumour necrosis factor-α, and stimulation of anti-apoptotic pathways through the activation of NF-B and MAP kinase is inhibited.
- **The effector molecule known as YopP in *Y. enterocolitica* and YopJ in *Y. pseudotuberculosis* is targeted to the host cell cytoplasm and is required both for the inhibition of tumour necrosis factor- α production and for apoptosis.**
- The **YopH protein** is a highly active tyrosine phosphatase that is directed to and disrupts focal adhesions, sites where actin stress fibres (light green) contact the cell membrane. Among its targets are the proteins focal adhesion kinase (FAK) and $p130^{Cas}$ (brown and orange). **The result is an inhibition of bacterial uptake by epithelial cells and macrophages.**
- **YopT,** another protein targeted by the type III system to the host cell's cytoplasm, participates in inducing a loss of actin stress fibres in the cells. It was recently realized that the electrophoretic mobility of Rho (purple), one of the small GTPases that regulates the actin cytoskeleton, is altered in cells infected with *Y. enterocolitica* and that this altered mobility is dependent on YopT. Presumably this alteration of Rho affects its function, leading to the observed cytoskeletal changes.
- The **YopE** protein is also targeted to the cytoplasm of host cells and, like YopT, disrupts the ac tin cytoskeleton. However, the mechanism of action of YopE is unknown.

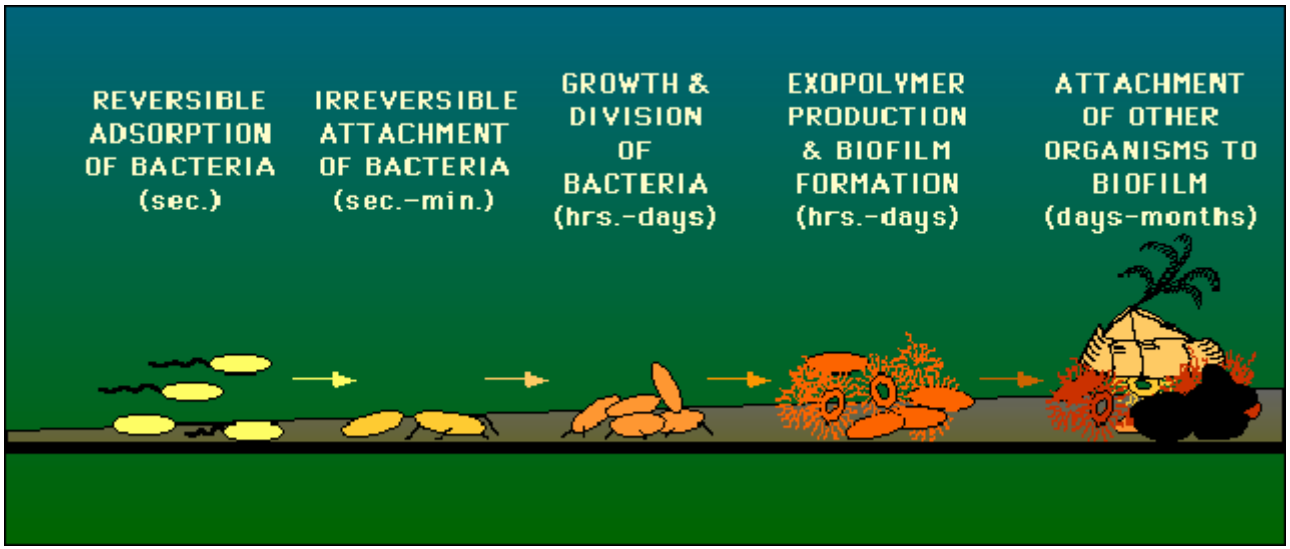
Mecanismos de evasión inmune	Ejemplos
Bacterias intracelulares	
Inhibición de formación de fagolisosomas	M tuberculosis, L penumophila, Chlamydia
Eliminación de IROs **Eliminación de peroxinitrilos**	M leprae (glucolípido fenólico) M tuberculosis
Pérdida de membrana del fagosoma	L monocytogenes (LLO)
TLR como mecanismo de escape bacteriano: **La Lp 19kD de M tuberculosis reconocida por TLR2 inhibe la expresión de MHC-II y de RFcγI.** **El microorganismo utiliza el TLR2 para inducir la liberación de IL-10.**	M tuberculosis Y entercolítica
Manipulación de los ligandos de TLR: **produce un lípido A del LPS que induce una reducida respuesta inflamatoria**	Chl trachomatis

Mecanismos de evasión inmune	Ejemplos
Bacterias intracelulares	
Evasión de la fagocitosis: se observa mayor supervivencia luego de su unión a CR1 en comparación con lo observado después de su unión a CR3 o CR4	M tuberculosis
Modulación bacteriana del procesamiento y presentación mediante CD1	M tuberculosis
Modulación del procesamiento y presentación antigénica	M tuberculosis, Chlamydia, Yersinia
Efectos inhibitorios sobre LT: Inducen la producción de TGF-β e IL-10 Interfieren en la señalización de la vía NF-κB	M tuberculosis Yersinia

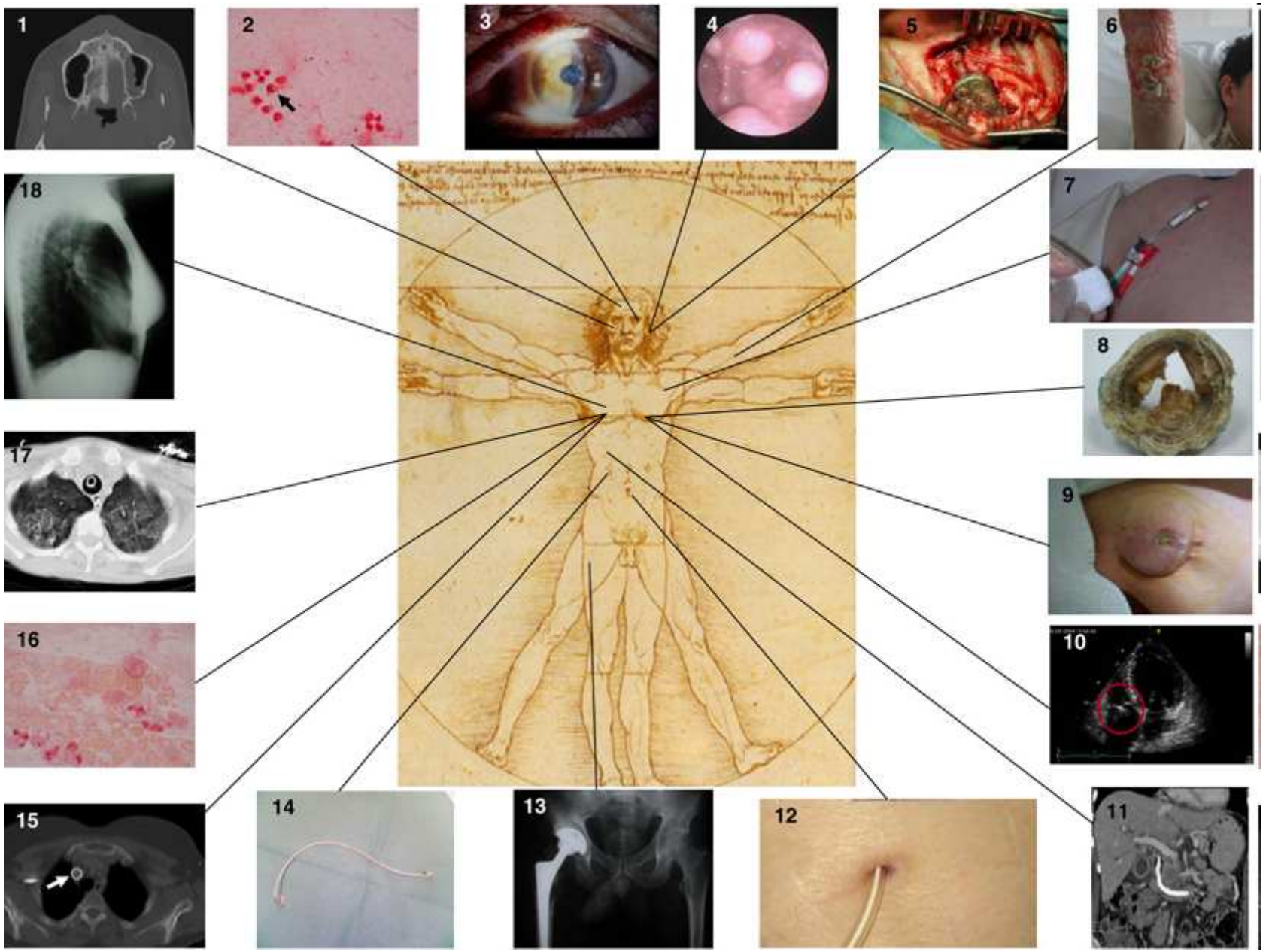
Biofilm

…….otro mecanismo de evadir al sistema inmune y a los antibióticos

Un BIOFILM es una comunidad microbiana de células adheridas a una superficie en la que las células se mantienen unidas gracias a una matriz extracelular. Pueden ser una simple monocapa de bacterias sobre una superficie a un tapete microbiano tan complejo que podríamos considerarlos auténticas "ciudades microbianas".



Nature Reviews Drug Discovery 2, 114-122 (February 2003)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- Some proposed-biofilm associated resistance mechanisms: (1) Antimicrobial agents may fail to penetrate beyond the surface layers of the biofilm. Outer layers of biofilm cells absorb damage. Antimicrobial agents action may be impaired in areas of waste accumulation or altered environment (pH, pCO2, pO2, etc). (2) Antimicrobial agents may be trapped and destroyed by enzymes in the biofilm matrix. (3) Altered growth rate inside the biofilm. Antimicrobial agents may not be active against nongrowing microorganisms (persister cells). (4) Expression of biofilm-specific resistance genes (e.g., efflux pumps). (5) Stress response to hostile environmental conditions (e.g., leading to an overexpression of antimicrobial agent-destroying enzymes).

Finalmente sacando la bacteria se termina con la patología… ¿Seguro?