Molecular and cell mechanism of mammalian immunity against infection

the main tasks are:

recognition

and

removal

of the infectious agent

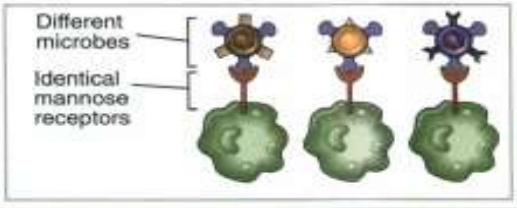
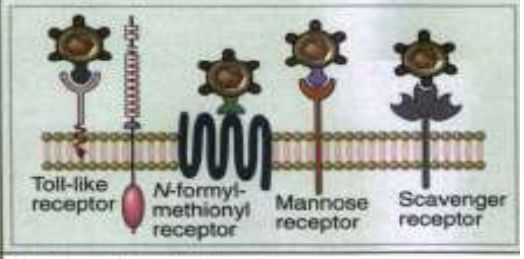
I am not exactly certain what it is yet until the tests come back but it looks like its some sort of fungal ifnection

thanks to Martina Kosova

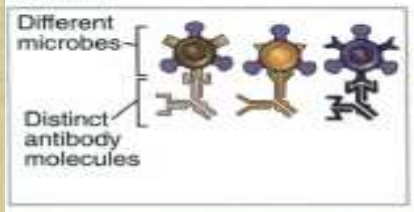
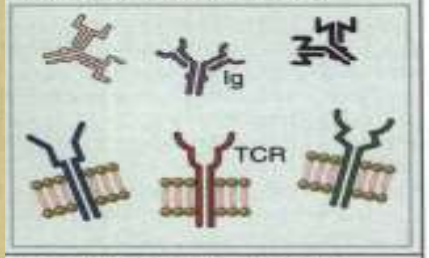
Defense	Site of action	Function
Transferrin	Blood/tissue	Limits iron availability
PMNs	Blood/tissue (attracted to infection site)	Ingest and kill bacteria
Monocytes	Blood (attracted to infection site)	Weakly phagocytic, produce cytokines
Macrophages	Tissue (especially lymph nodes, spleen, liver, lungs)	Actively phagocytic; produce cytokines; present antigens to T cells on MHC I (intracellular pathogens) or MHC II (extracellular pathogens)
Complement	Blood/tissue (inactive unless activated by foreign invader)	C3a activated phagocytes; C5a attracts phagocytes; C3b opsonizes bacteria; C5b-C9 form MAC (kills G- bact.)
Mannose binding protein	Produced by liver	Binds to bacterial surface and activated complement
T cells	Blood (attracted to infection site)	T helper cells (CD4+) stimulate B cells to produce antibodies (Th2) or produce IFN-gamma to activate macrophages (Th1); Cytotoxic T cells (CD8+) kill infected host cells; cytokine production
B cells	Blood (attracted to infection site)	Produce antibodies
Antigens (IgG, IgM)	Blood	Opsonize bacteria (IgG); activate complement (IgM >IgG); neutralize toxins

innate

- physical barriers, skin dryness, pH, mucus layer
- limitation of iron availabilty
- production of bactericidal molecules
- professional phagocytes (neutrophiles, macrophages)
- complement cascade
- mechanical clearence of mucosal surfaces

	Innate immunity
Specificity	For structures shared by classes of microbes ("pathogen-associated molecular patterns") 
Receptors	Encoded in germline; limited diversity ("pattern recognition receptors") 
Distribution of receptors	Nonclonal: identical receptors on all cells of the same lineage
Discrimination between self and nonself	Yes; host cells are not recognized or they may express molecules that prevent innate immune reactions

Immunity

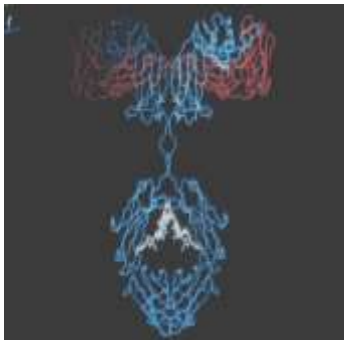
	Adaptive immunity
Specificity	For structural detail of microbial molecules (antigens); may recognize nonmicrobial antigens 
Receptors	Encoded by genes produced by somatic recombination of gene segments; greater diversity 
Distribution of receptors	Clonal: clones of lymphocytes with distinct specificities express different receptors
Discrimination between self and nonself	Yes; based on selection against self-reactive lymphocytes; may be imperfect (giving rise to autoimmunity)

- humoral - antibodies
- cellular - lymphocytes
- presentation of antigens leads to specificity
- recognition of self x nonself
- immunological memory

adaptive

Cílem očkování je manipulace adaptivního imunitního systému

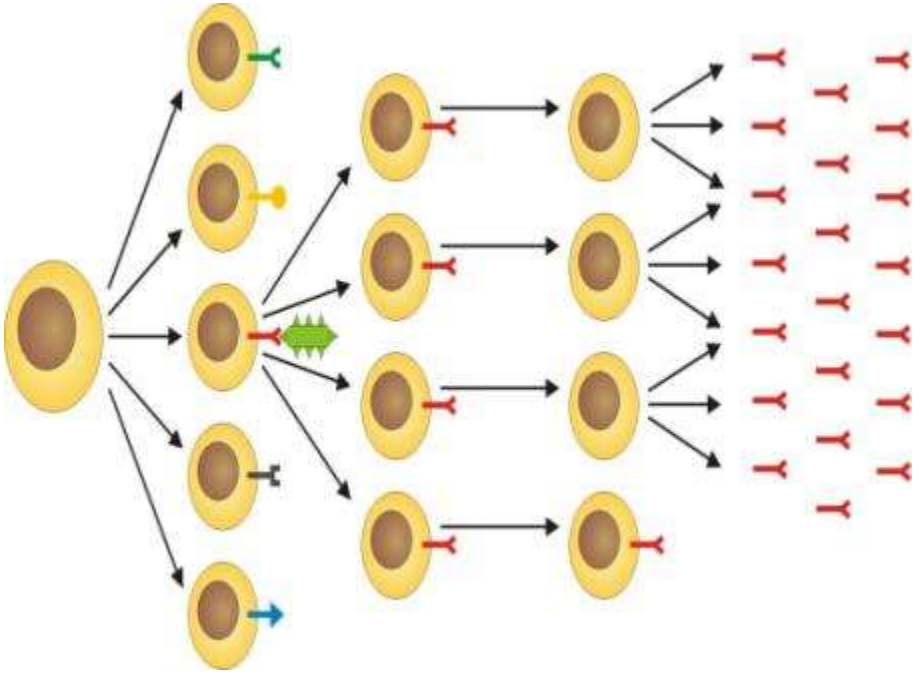
• založený na obrovském repertoáru individuálních klonů B a T lymfocytů, vzniklých reorganizací genů a somatickými mutacemi, z nichž každý receptor nese unikátní, specifický receptor (BCR or TCR)



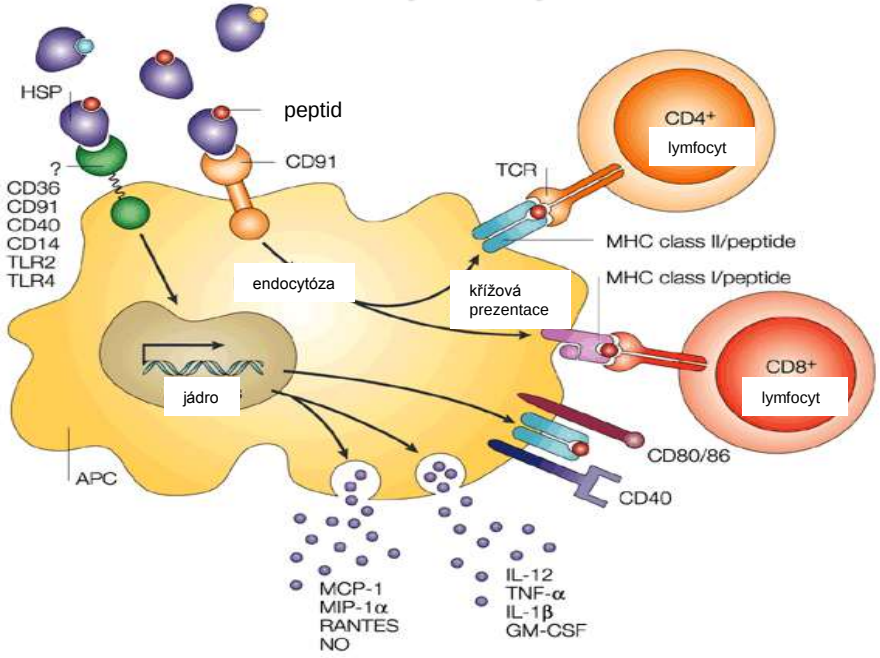
• Rozpustnými receptory adaptivního systému jsou **protilátky**

• Adaptivní systém je **“předvídavý”, klonální, “předimenzovaný”**

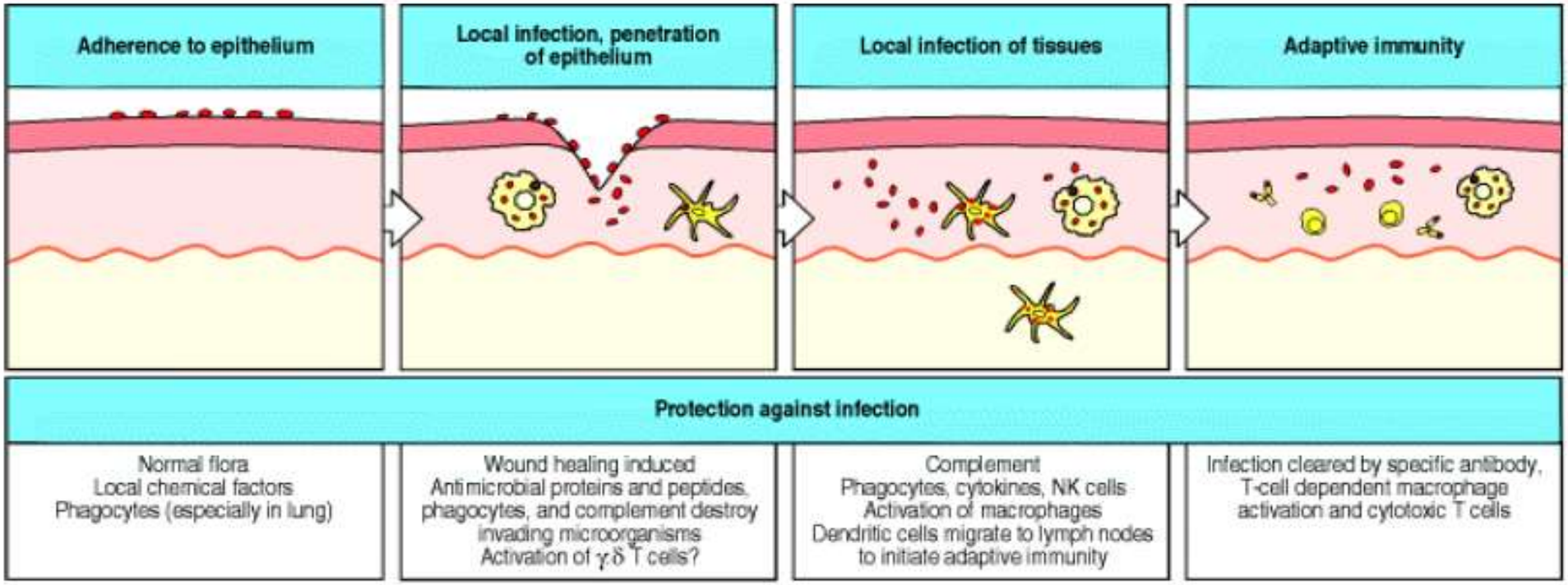
klonální expanze B lymfocytů



Aktivace T lymfocytů

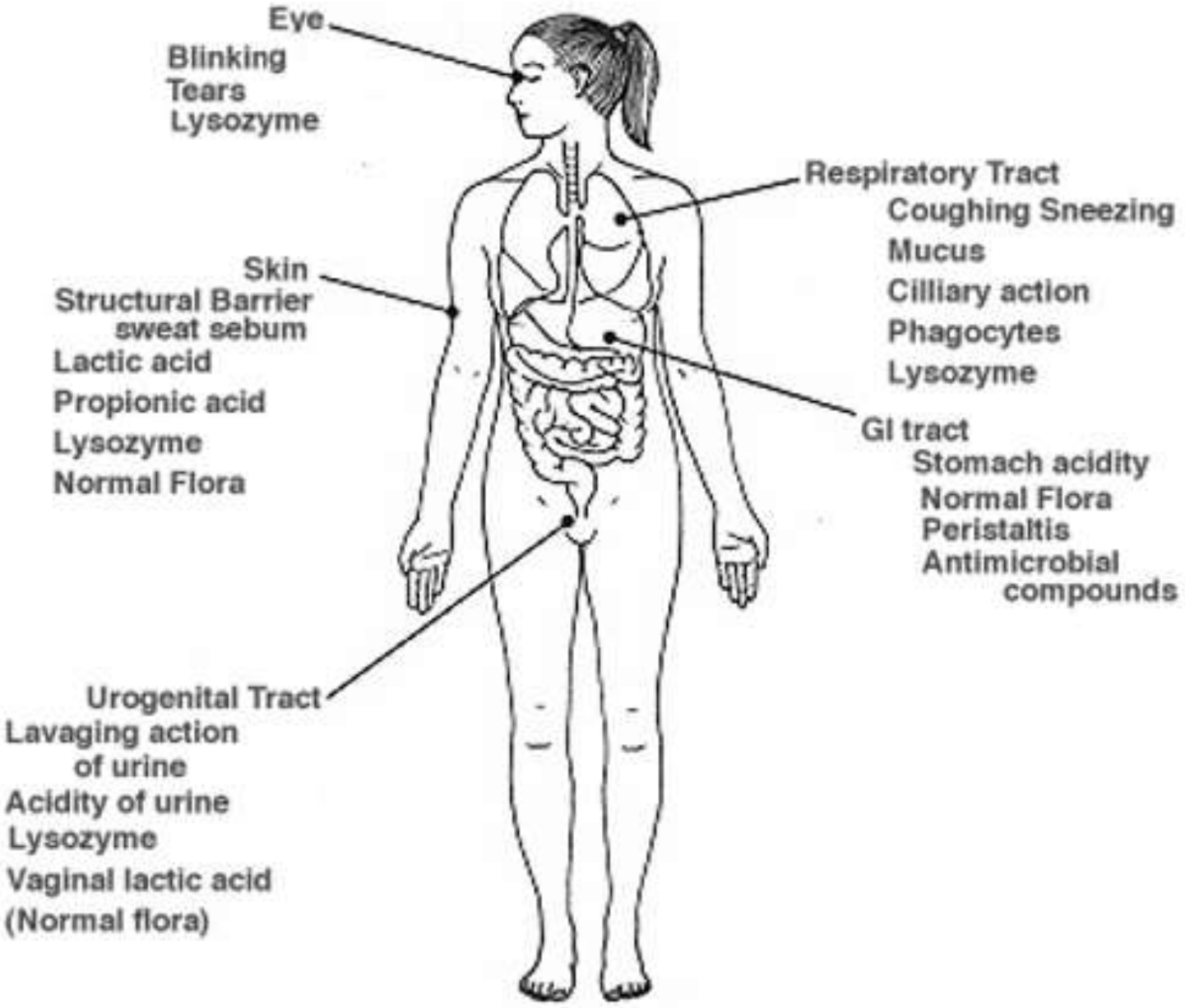


Phases of infection



(Janeway, 2001)

Innate protection mechanisms

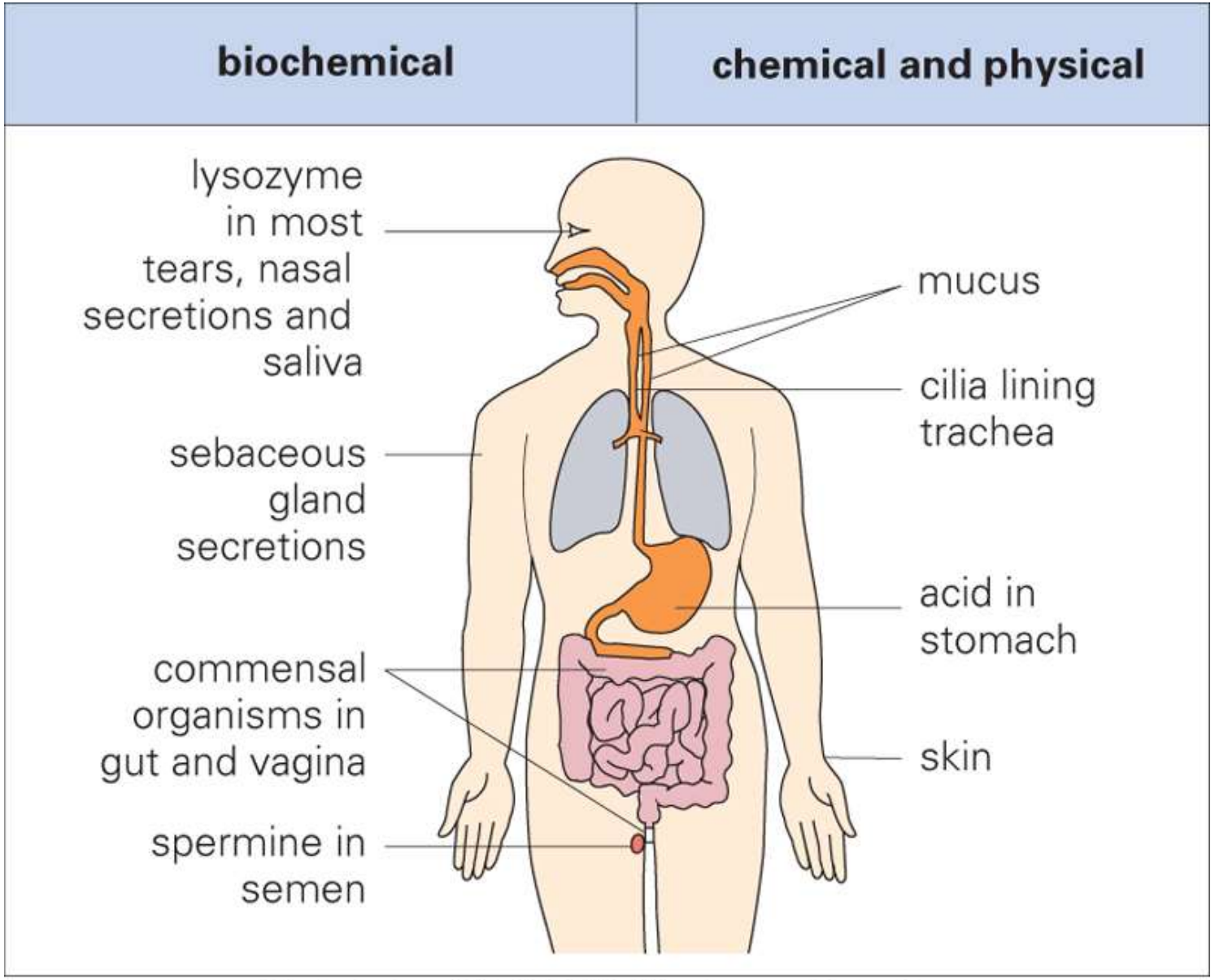


Innate immunity - antimicrobial compounds on mucosal surfaces

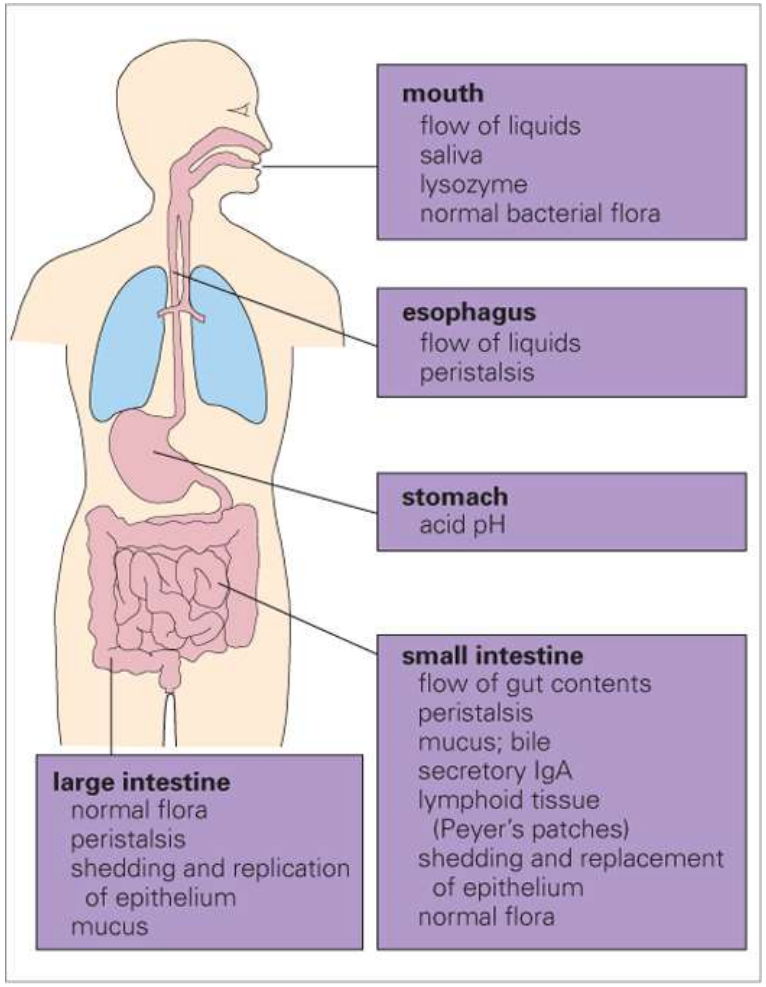
Inorganic:	hydrogen peroxide
	nitric oxide
Peptides:	defensins
	catelicidins
Proteins:	lysozyme
	lactoferrin
	cathepsin G
	phospholipase A2
	lactoperoxidase
Mucins	

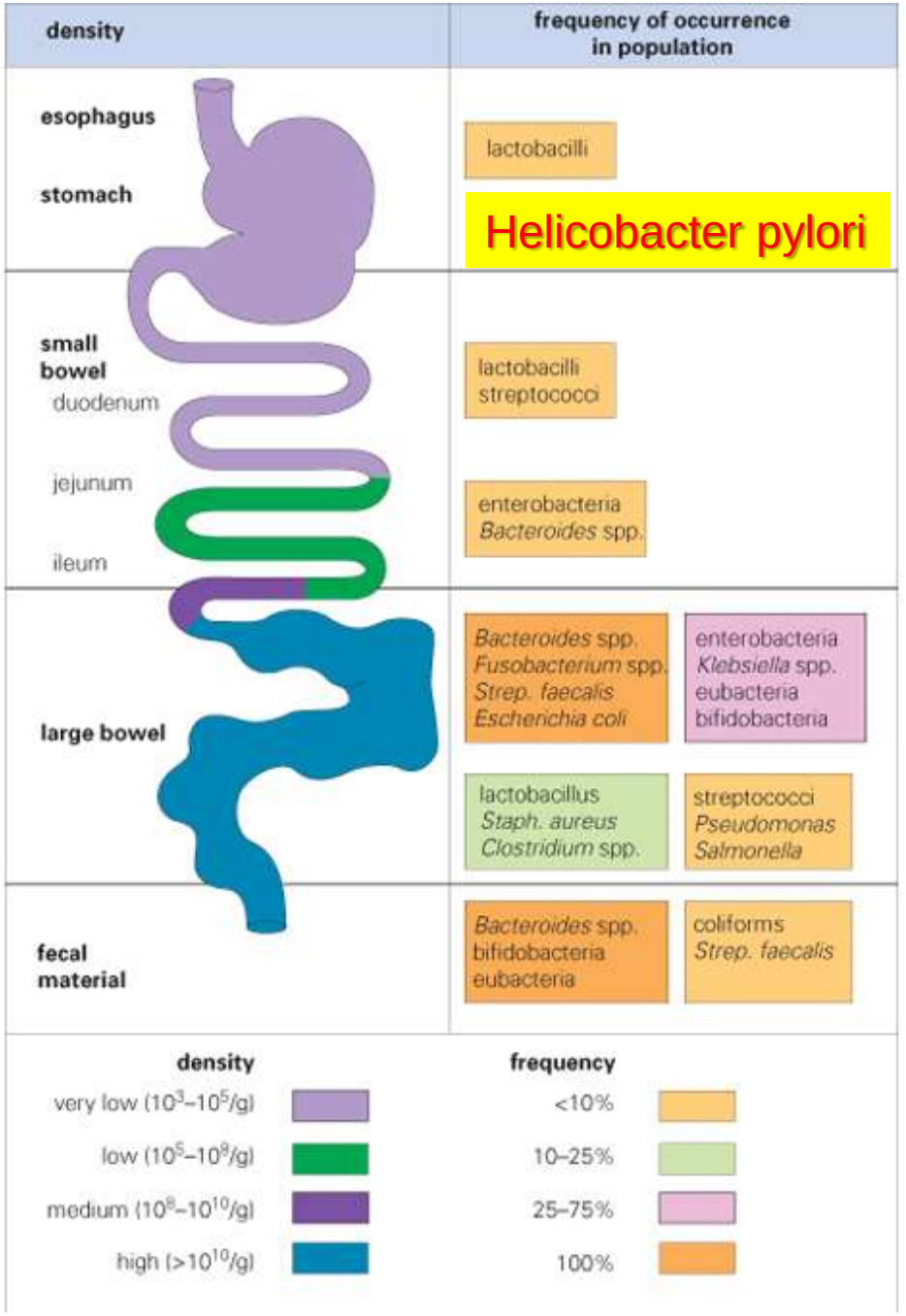
Secretory Ig	

Important innate barriers to infection



Innate immunity is extremely Important at mucosal surfaces heavily colonized by bacteria





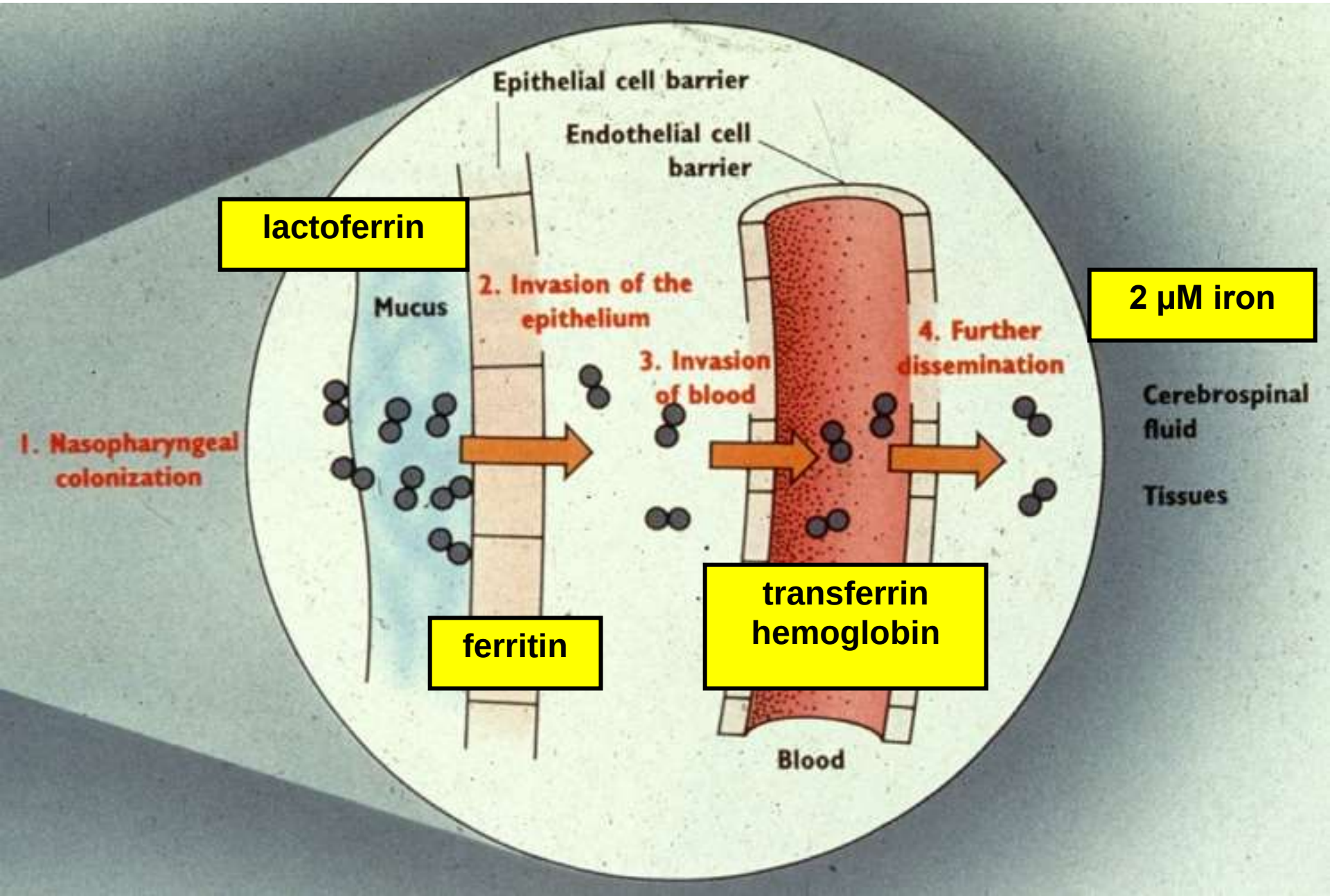
IRON AVAILABILITY IN THE HUMAN HOST:

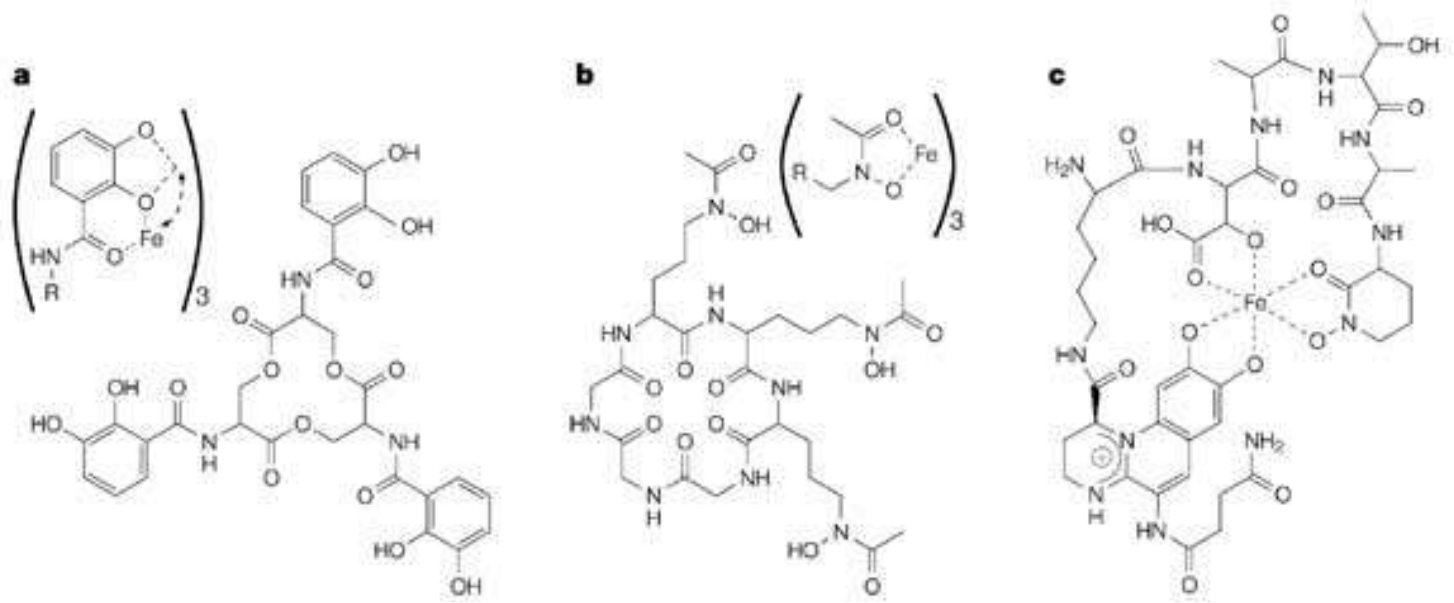
- free iron concentrations are low in aqeous environment, in aerobic conditions, iron exists in the oxidized, ferric form (Fe^{III}), which at pH 7, soluble to $1.4x10^{-9}$ M
- erroneously it used to be given as 10^{-18} M, however, at pH 7 the principal ionic species is polymeric $Fe(OH)^{+}_{2}$ not $Fe(OH)_3$
- For invasion and proliferation bacteria need to induce specific pathways capable of scavenging iron from the host to about 10^5-10^6 iron atoms per bacterium or 10^{14} per 1 L culture
- free iron concenration in host is about 10 x lower that in media
- **In human body fluids more than 99,9% of iron is bound to transport (transferrin, lactoferrin) and storage proteins (ferritin, heme-containing compounds) with extremely high binding constants of 10^{36}, 30-40% saturated** :
  - transferrin in plasma and lymph
  - lactoferrin in milk and secretions
  - Another source of iron is hemoglobin

    (i.e. meningococci are capable of using haptoglobin-hemoglobin and free heme as sources of iron)

- **iron concentration tells the pathogen it is inside the host**
- **Two general mechanisms of iron acquisition in bacteria have been described:**
  - **siderophore-mediated iron acquisition by cognate receptors**
  - **receptor-mediated iron acquisition from host iron-binding proteins**

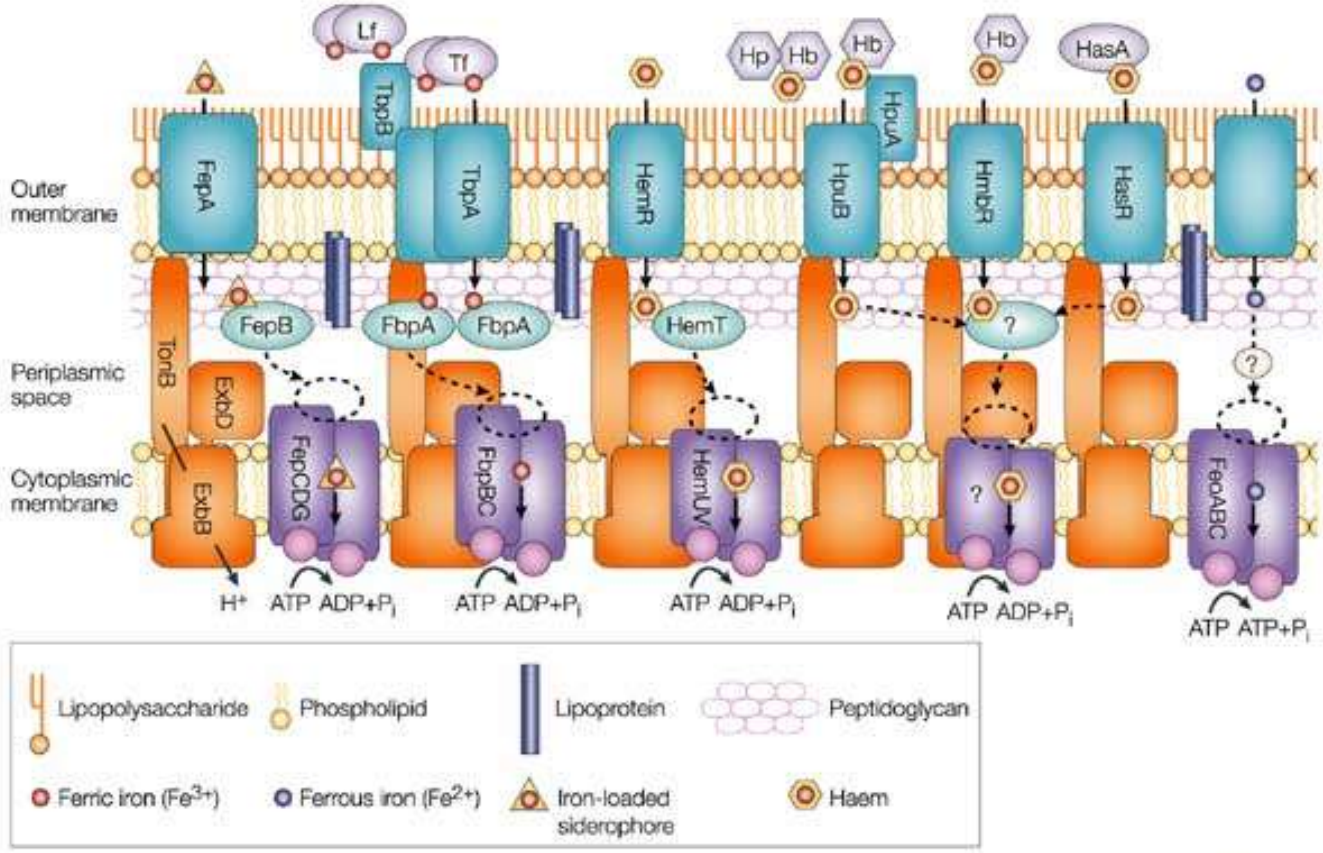
Iron availability in the human host





Siderophores are low-molecular-weight molecules that are synthesized by bacteria and fungi and are excreted into the medium to sequester and make soluble ferric iron (Fe3+).

- capture iron from proteins such as haemoglobin or transferrin.
- iron-loaded siderophores are transported into the bacterial cell, where iron is released
-siderophores chelate ferric iron with high affinity
- siderophores can be divided into hydroxamates and catecholates
- Enterobactin (a) is a 669-Da catecholate (*Escherichia coli* and enterobacteria)
- Ferrichrome (b) is a 740-Da hydroxamate-type siderophore of fungi scavenged by bacteria, -
- Mycobactins, which are synthesized by mycobacteria, are distinct from other siderophores
- Pyoverdins, such as pseudobactin B10 (c), are the most prevalent siderophores of *Pseudomonas*.
- Siderophore–antibiotic conjugates can be used to deliver antimicrobial agents into the cell, which reduces the minimal inhibitory concentration several-hundredfold.



Nature Reviews | Molecular Cell Biology

Under aerobic conditions, bacteria rely on high-affinity surface receptor proteins (blue) that bind Fe3+-containing proteins (light purple), such as iron-loaded siderophores or haem, and that subsequently facilitate their translocation into the periplasmic space. This process is activated by the Ton complex (TonB–ExbBD). Periplasmic-binding proteins (light blue) and ATP-driven transporters (purple) that are in the cytoplasmic membrane are used to ensure further transport into the cell (the same is true for Gram-positive species, except that in these bacteria the binding proteins are membrane-anchored).

Antimicrobial peptides

- **more than 500 antimicrobial peptides already** found in various species
- all share suprisingly similar characteristic:
  - constitutive or inducible gene expression
  - 12-50 AAs, cationic charge (Arg, Lys), 50% of hydrophobic residues
  - membrane active agents
  - broad spectrum of antimicrobial actvities (against G^- and G^+ bacteria, viruses, fungi)
  - non toxic to the host cells
- direct antimicrobial activity
- product of immune and epithelial cells
- additional immunological fuction:
  - **mediators of inflammation – effect on epithelial + immune cells**
  - chemotactic activity for monocytes, DC, T cells
  - influence proliferation, immune induction, cytokine release, wound healing, protease-anti-protease balance, redox homeostasis
- could be... the next generation of antibiotics ??? – almost certainly not!!!

Antimicrobial peptides - classification

GROUP I: LINEAR, α-HELICAL PEPTIDES WITHOUT CYSTEINES

LL-37/hCAP-18 LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES

← Classification of antimicrobial peptides – based on motifs in the amino acids sequence.

GROUP II: PEPTIDES WITH CYSTEINES LINKED BY DISULFIDE BRIDGES

Defensins

α-defensins

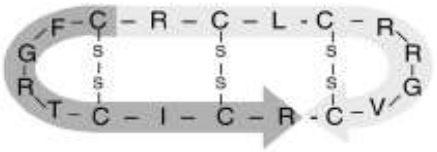
HNP-1 ACYCRIPACIAGERRYGTCIYQGRLWAFCC
HNP-4 VCSCRLVFCRRTELRVGNCLIGGVSFTYCCTRVD
HD-6 GSTRAFTCHCRR-SCYSTEYSYGTCTVMGINHRFCCL

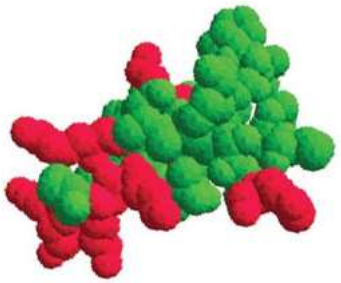
β-defensin

hBD-1 GLGHRSDHYNCVSSGGQCLYSACPIFTKIQGTCYRGKAKCCKZ
hBD-2 GIGDPVTCLKSGAICHPVFCPRRYKQIGTCGLPGTKCCKKP
hBD-3 GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK

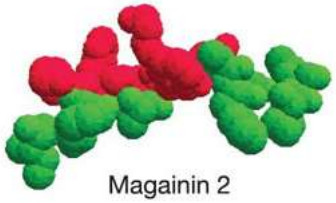
θ-defensin

rTD-1

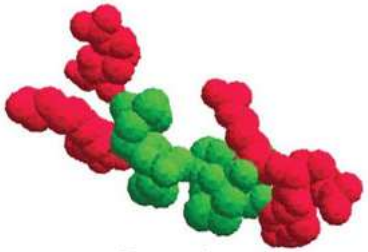




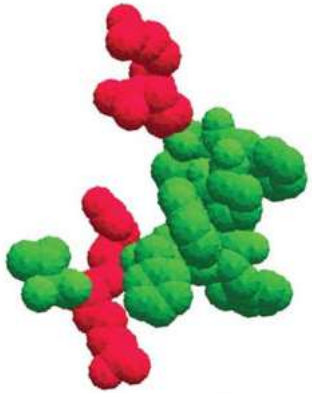
Human α-defensin 3



Magainin 2



Protegrin



Indolicidin

GROUP III: UNUSUAL HIGH PROPORTION OF SPECIFIC AMINO ACIDS

PR-39 RRRPRPPYLPRPRPPPFFPPRLPPRIPPGFPPRFPPRFPGKR

Histatins

His-1 DSHEKRHHGYRRKFHEKHHSHREFPFYGDYGSNYLYDN
His-3 DSHAKRHHGYKRKFHEKHHSHRGYRSNYLYDN

(Bals, 2000)

Clustering of cationic and hydrophobic amino acids into distinct domains. Red, basic (positively charged) amino acids; green, hydrophobic ('oily') amino acids. Other amino acids are not shown.

(Zasloff, 2002)

Antimicrobial peptides - activities

➢electrostatic interaction between the negatively charged bacterial wall and the positively charged peptides

- insertion as pores, forming carpet-like structures, ..

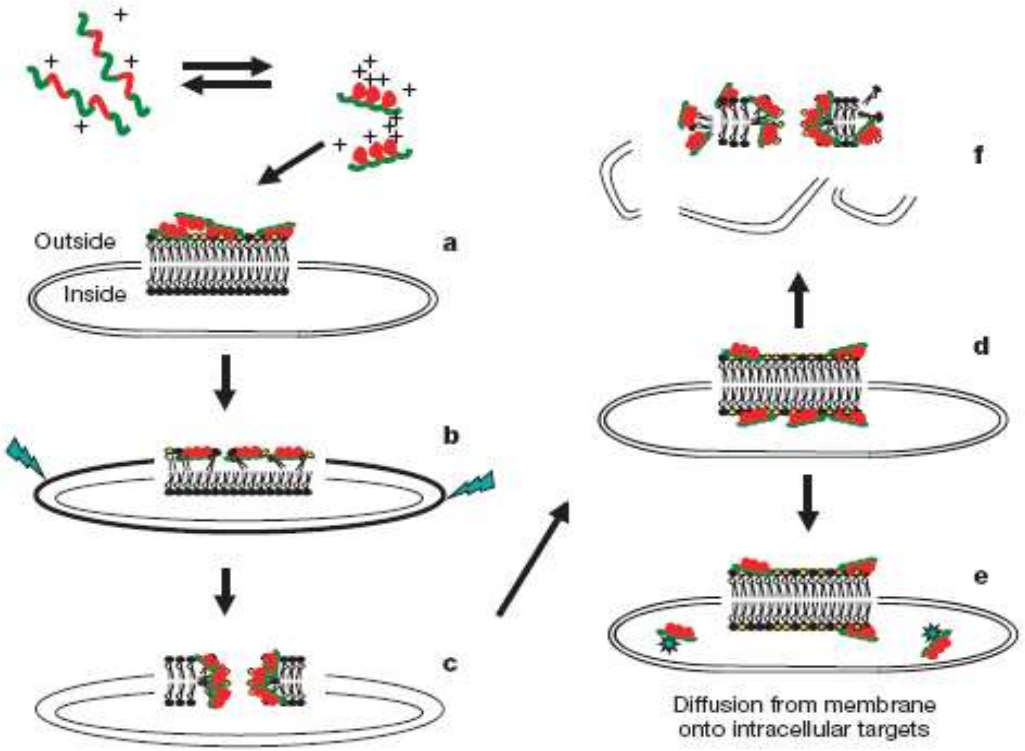


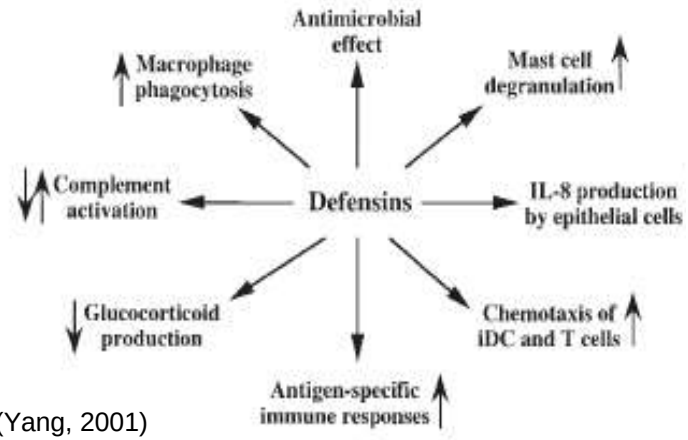
Figure 3 The Shai–Matsuzaki–Huang model of the mechanism of action of an antimicrobial peptide. An α-helical peptide is depicted. **a**, Carpeting of the outer leaflet with peptides. **b**, Integration of the peptide into the membrane and thinning of the outer leaflet. The surface area of the outer leaflet expands relative to the inner leaflet, resulting in strain within the bilayer (jagged arrows). **c**, Phase transition and 'wormhole' formation. Transient pores form at this stage. **d**, Transport of lipids and peptides into the inner leaflet. **e**, Diffusion of peptides onto intracellular targets (in some cases). **f**, Collapse of the membrane into fragments and physical disruption of the target cell's membrane. Lipids with yellow headgroups are acidic, or negatively charged. Lipids with black headgroups have no net charge.

(Zasloff, 2002)

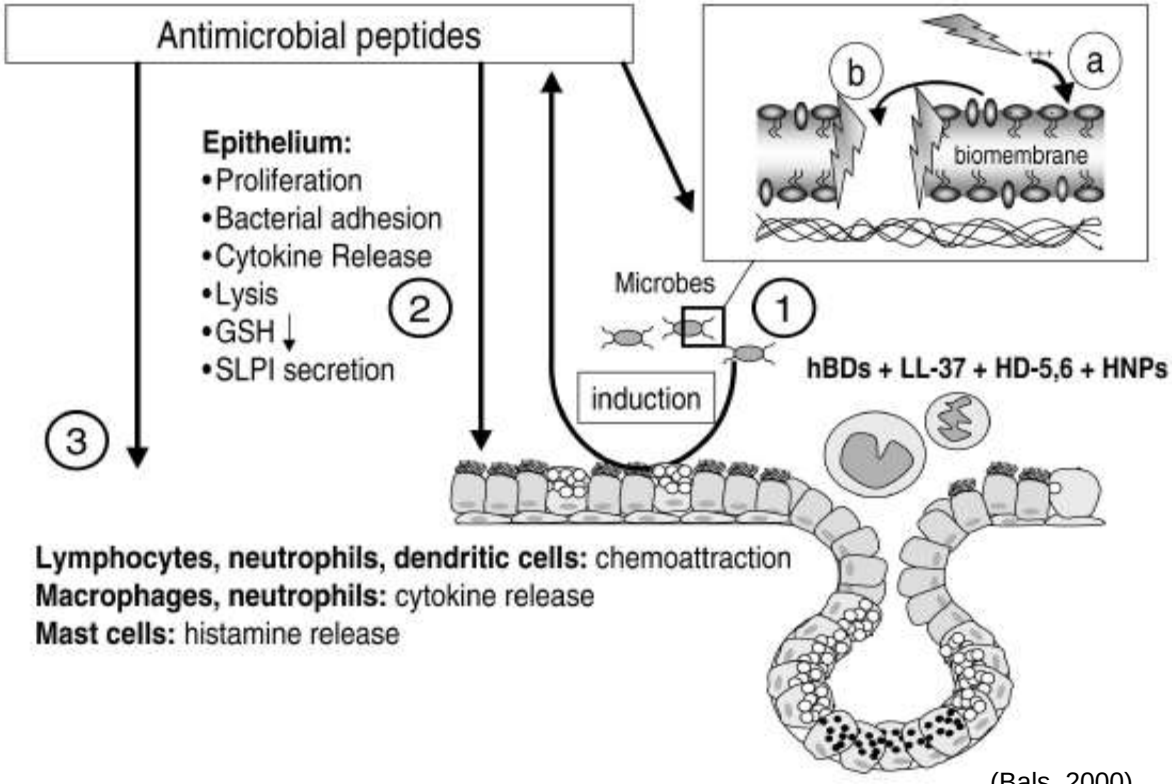
Antimicrobial peptides - activities

- the release of various mediators influencing immunological balance

Innate defence regulators – IDRs as novel anti-infective agents are already in clinical trials



(Yang, 2001)



(Bals, 2000)

Innate immune system

- present in all organism (x adaptive immunity only in vertebrates)
- **ancient recognition system** – due to long evolution effective discrimination between self x nonself
- forms the earliest barrier to infection (0-96 hours)
- depends upon germline-encoded receptors to recognize common pathogen features – *pathogen-associated molecular patterns or PAMPs*
- does not generate long-term protection (immunological memory)
- necessary prerequisition for induction of adaptive immune response
- Relies on:
  - complement
  - Phagocytes
  - polysecific antibodies

BASIC TASKS:

- PROTECTION FROM PATHOGENS

- REMOVAL OF ABNORMAL SELF CELLS

Innate immune mechanism - pathogen removal

- killing by production of bactericidal molecules (act as chemical weapons):
  - antimicrobial peptides
  - reactive oxygen species (ROS)
  - reactive nitrogen species (RNS)

- phagocytosis
- inflammation (chemokines, cytokines)
- direct killing of infected cells

RECOGNITION OF PATHOGENS AND ABNORMAL SELF CELLS BY MEANS OF:

- SURFACE RECEPTORS

- “SOLUBLE RECEPTORS”

EFFECTOR MECHANISMS OF PATHOGEN REMOVAL

(FOLLOWING RECOGNITION BY EITHER INNATE OR ADAPTIVE RECEPTORS):

KILLING BY MIROBICIDAL PEPTIDES, REACTIVE OXYGEN SPECIES, OR OTHER "CHEMICAL WEAPONS"

PHAGOCYTOSIS

INFLAMMATION (BASED ON CYTOKINES, CHEMOKINES)

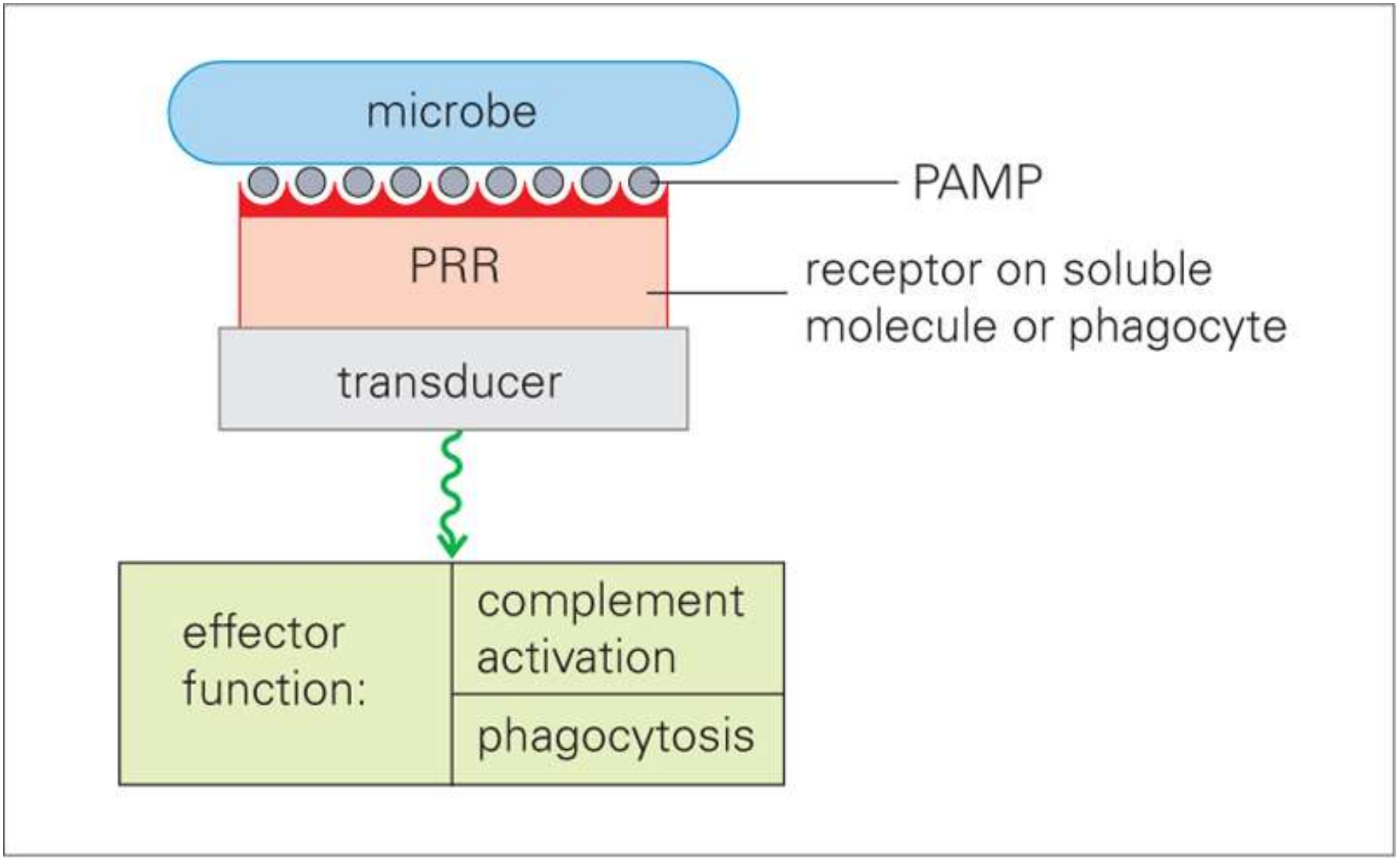
KILLING (NOT CURING!!) OF INFECTED CELLS

SOLUBLE AND MEMBRANE RECEPTORS OF THE INNATE SYSTEM (MAINLY ON VARIOUS TYPES OF PHAGOCYTES) RECOGNIZE:

PATHOGEN-ASSOCIATED MOLECULAR PATTERNS (PAMPs)

The number of the innate receptors is limited, shared structural features are recognized

PAMpology…



Recognition by complement

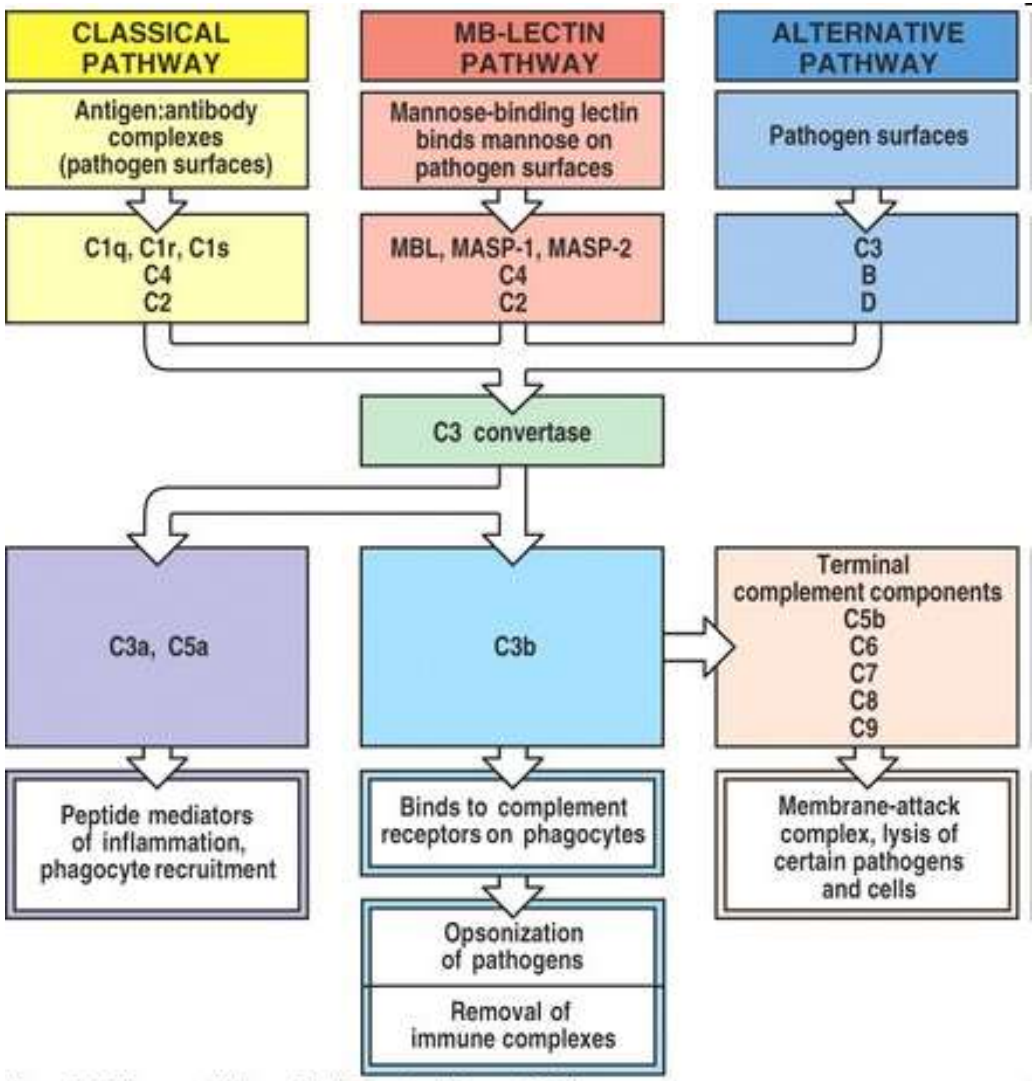
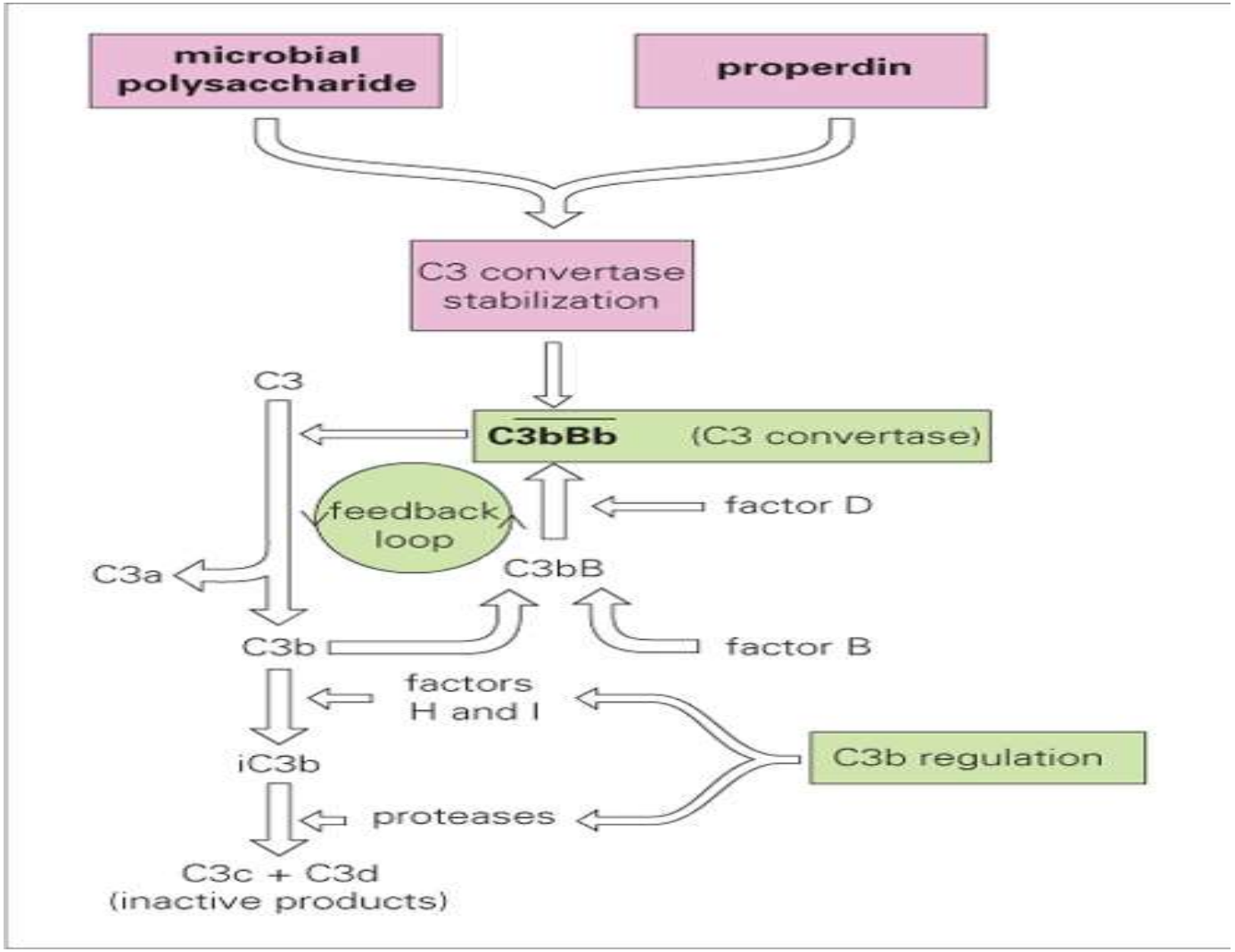


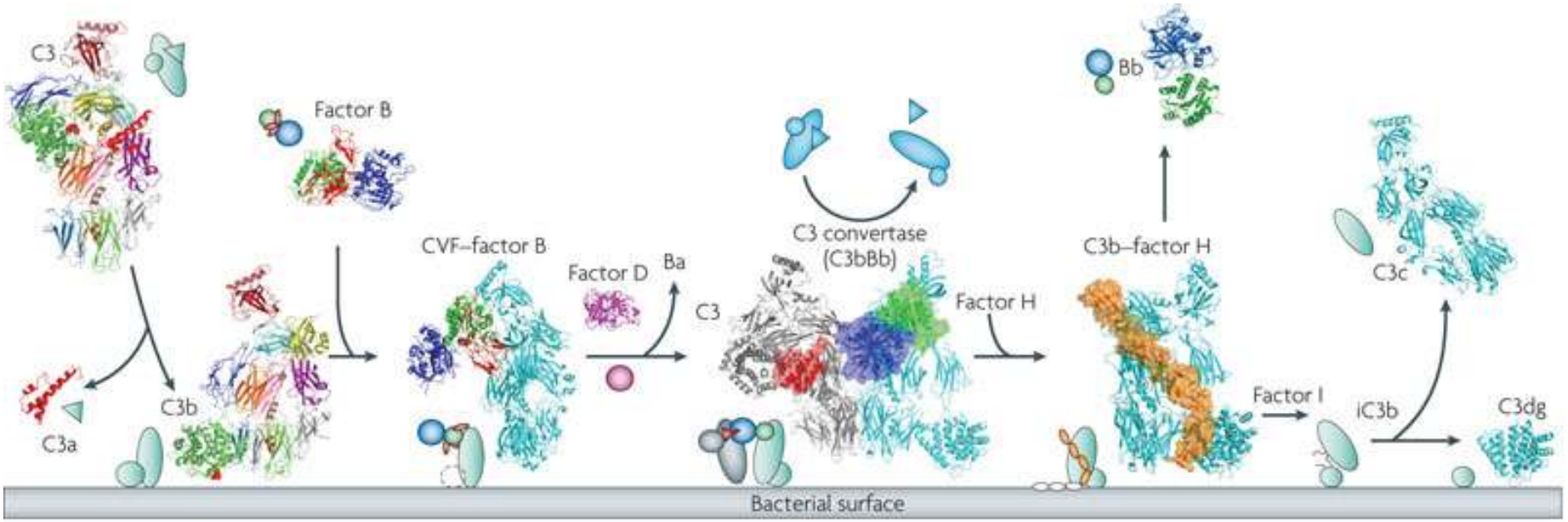
Figure 2-19 Immunobiology, 6/e. (© Garland Science 2005)

Activation of complement by microorganisms.

C3b is formed by the spontaneous breakdown of C3 complexes with factor B to form C3bB which is split by factor D to produce a C3 convertase , capable of further cleaving C3. The convertase is heavily regulated by factors H and I but can be stabilized on the surface of microbes and properdin. The horizontal bar indicates an enzymically active complex. iC3b, inactive C3b.



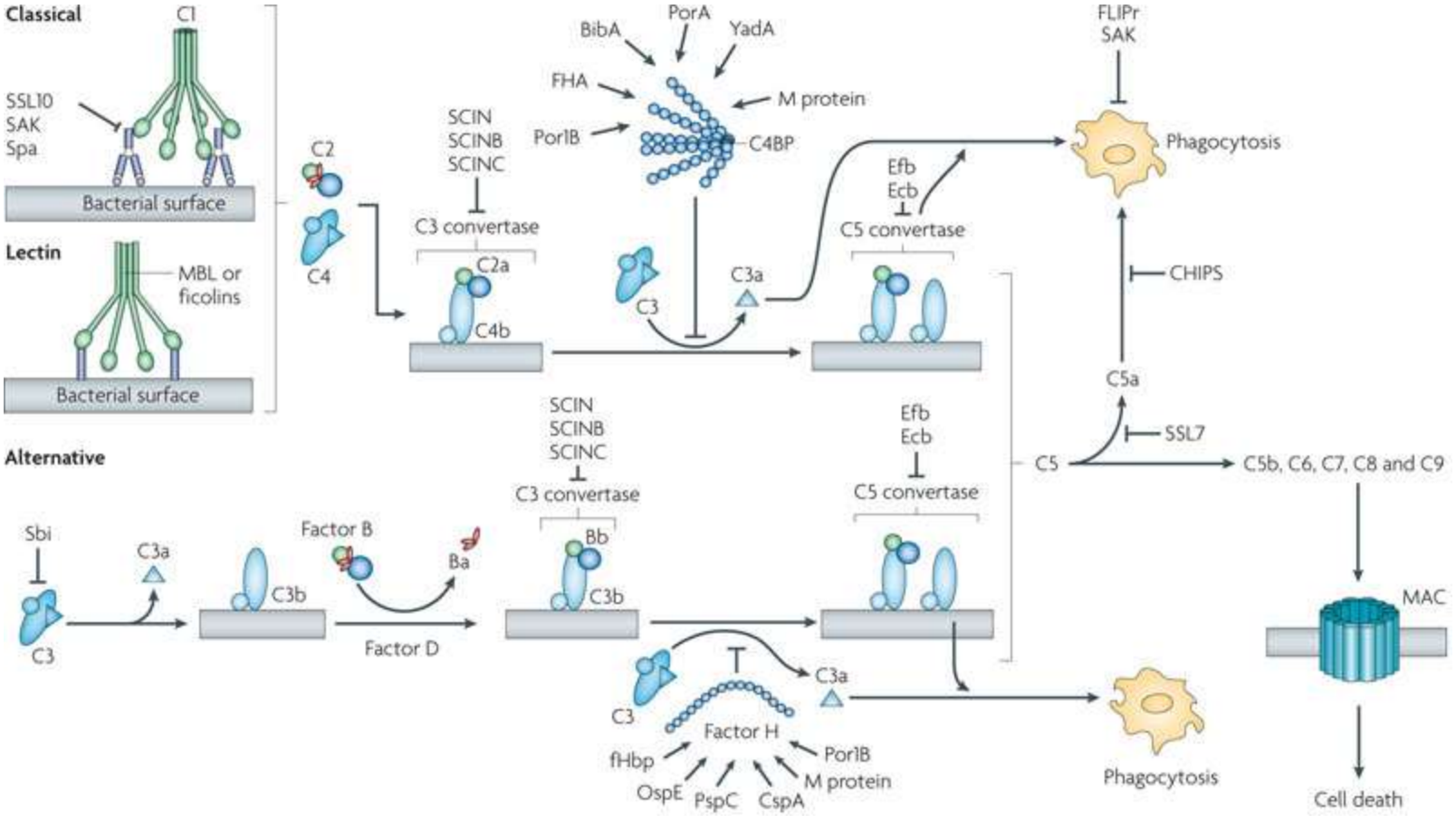
Avoiding complement killing - II



Nature Reviews | Microbiology

The central molecule C3 consists of 13 domains, shown by the different colours in the left-most structure. C3 is cleaved by the C3 convertase (generated either through the classical and lectin pathways or through the alternative pathway). Cleavage results in release of the small anaphylatoxin C3a and the large opsonin C3b. The activated C3b exposes its thioester and covalently binds to hydroxyls on adjacent surfaces. C3b binds pro-enzyme factor B, which consists of five domains: the Von Willebrand factor A-type domain (green), the serine protease domain (dark blue) and the domains comprising fragment Ba (red). Binding of factor B to C3b is indicated by the complex formed by factor B and the C3b homologue cobra venom factor (CVF). Next, factor B is cleaved by factor D, yielding C3bBb, which is the active C3 convertase of the alternative pathway. In the scheme, the putative binding of C3 to C3bBb is indicated (here, C3 is in grey, with the C3a domain in red; C3b is shown in light blue); the factor B protease fragment Bb (dark blue and green) is associated with the carboxyl terminus of the C3 substrate. The C3 convertase cleaves additional C3 molecules into C3a and C3b, thereby amplifying complement activation. **Host cells are protected by complement regulators such as factor H.** Factor H dissociates the C3bBb complex by binding to C3b (shown is the complex of C3b and factor H domains CCP1–CCP4). **Subsequent binding of protease factor I cleaves C3b into inactive C3b (iC3b) and the further degradation products C3dg and C3c**.

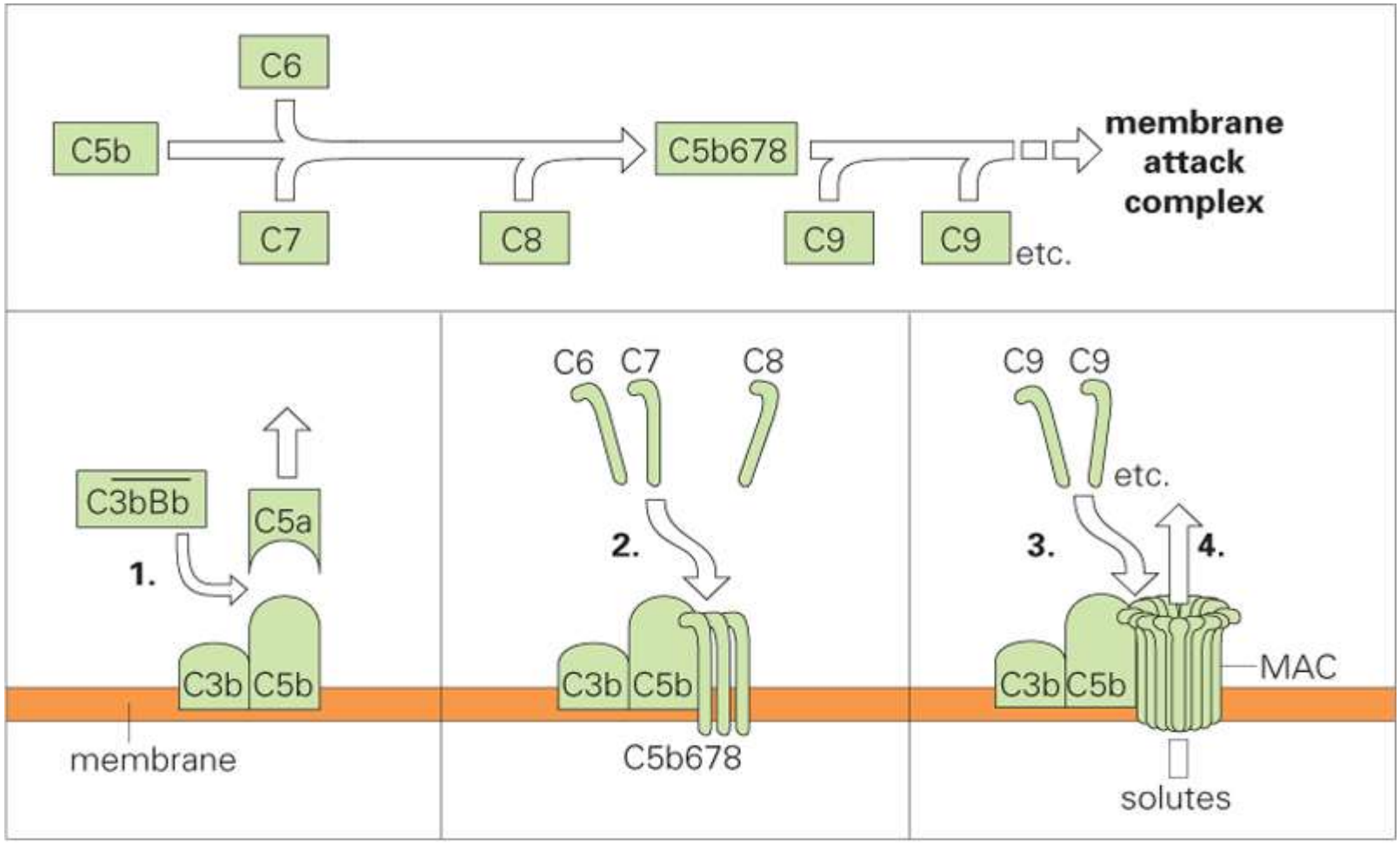
Complement and how to escape its killing action



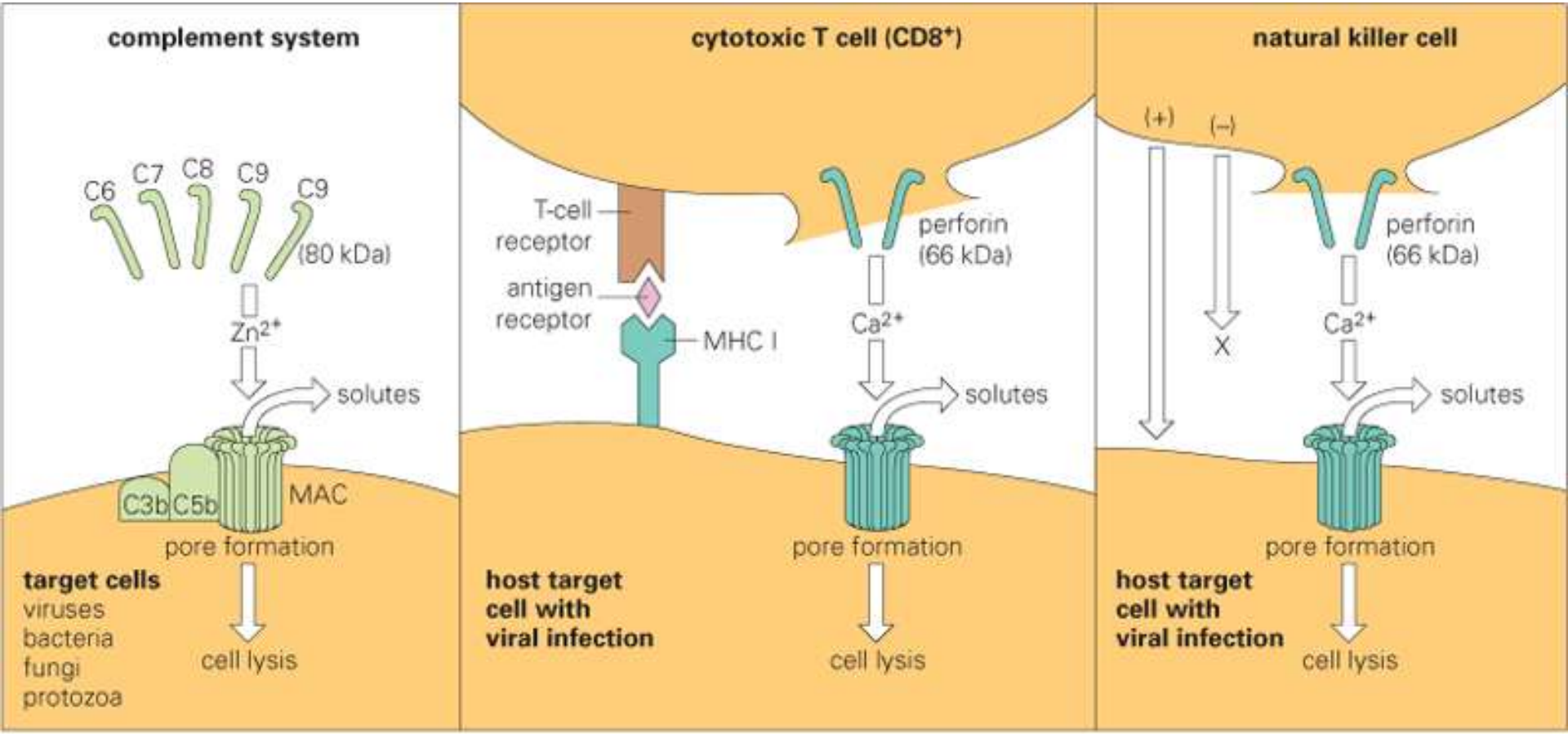
The three pathways of the human complement system and the bacterial factors that target the complement regulators factor H and C4-binding protein (C4BP). Ba, fragment Ba of factor B; Bb, fragment Bb of factor B; CHIPS, chemotaxis inhibitory protein of staphylococci; Ecb, extracellular complement-binding protein; Efb, extracellular fibrinogen-binding protein; FHA, filamentous haemagglutinin; fHbp, factor H-binding protein; FLIPr, formyl peptide receptor-like 1 inhibitory protein; MAC, membrane attack complex; MBL, mannose-binding lectin; OspE, outer surface protein E; PspC, pneumococcal surface protein C; SAK, staphylokinase; Sbi, staphylococcal binder of immunoglobulin; SCIN, staphylococcal complement inhibitor; Spa, staphylococcal protein A; SSL, staphylococcal superantigen-like; YadA, Yersinia adhesin A. **Nature Reviews Microbiology 8, 393-399 (June 2010) | doi:10.1038/nrmicro2366**

Assembly of the C5b-9 membrane attack complex (MAC).

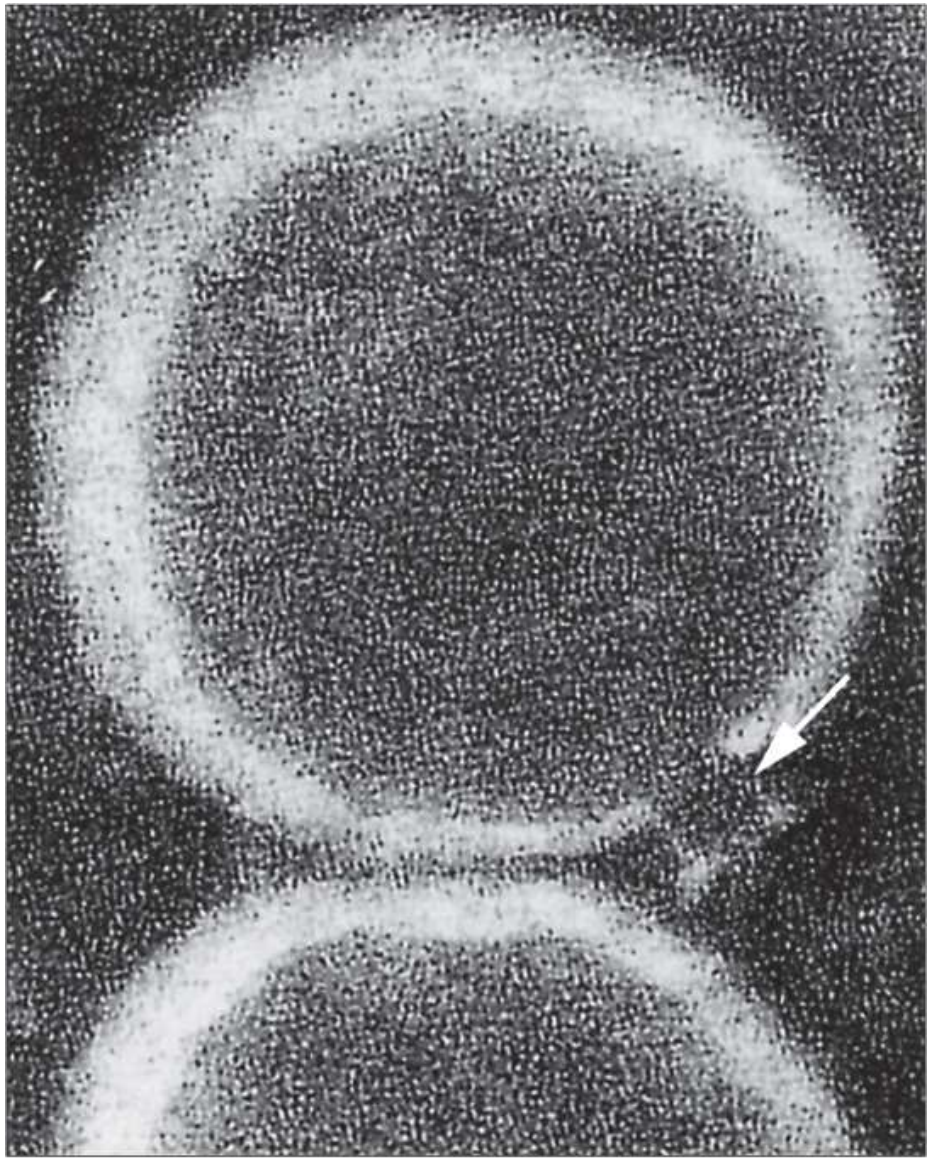
(1) Recruitment of a further C3b into the C3bBb enzymic complex generates a C5 convertase which cleaves C5a from C5 and leaves the remaining C5b attached to the membrane. (2) Once C5b is membrane bound, C6 and C7 attach themselves to form the stable complex C5b67, which interacts with C8 to yield C5b678. (3) This unit has some effect in disrupting the membrane, but primarily causes the polymerization of C9 to form tubules traversing the membrane. The resulting tubule is referred to as a MAC. (4) Disruption of the membrane by this structure permits the free exchange of solutes, which are primarily responsible for cell lysis.



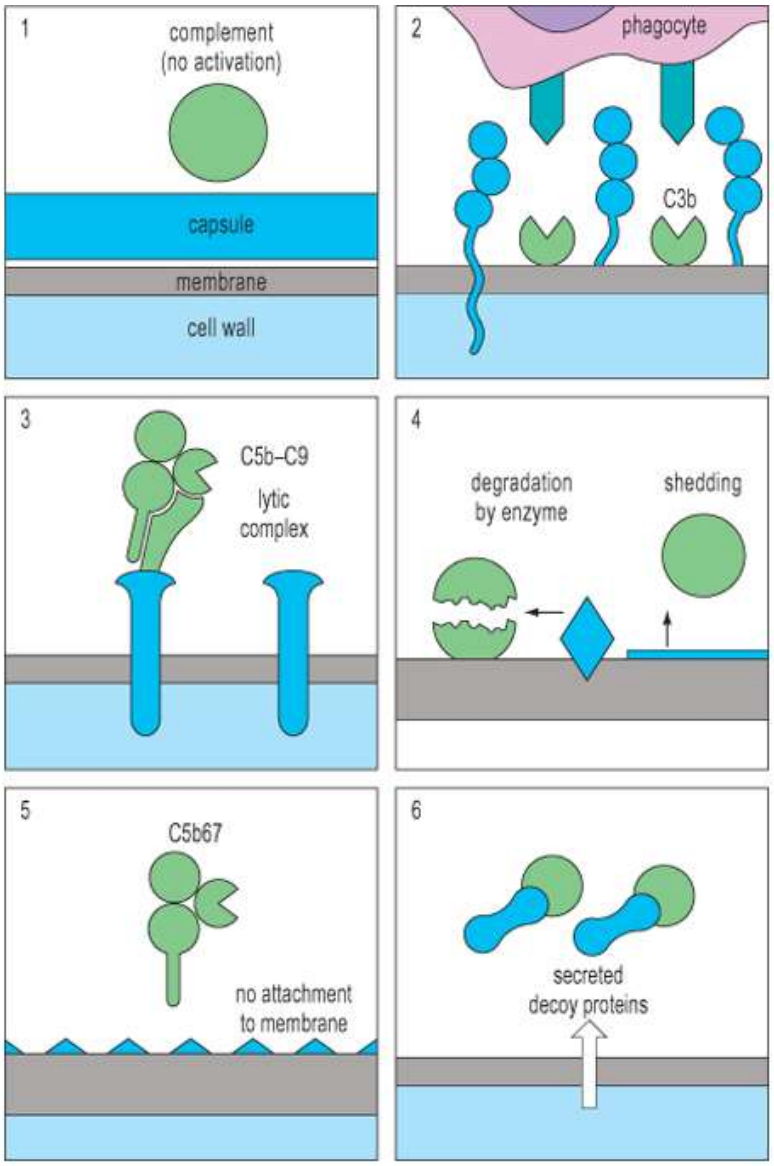
Common features of complement and perforin pores



The complemement membrane attack pore



Bacteria avoid complement-mediated damage by a variety of strategies.



(1) An outer capsule or coat prevents complement activation – „invisible capsule, such as that of *N. meningitidis* serogroup B. or *H. pylori*

(2) An outer surface can be configured so that complement receptors on phagocytes cannot obtain access to fixed C3b.,

(3) Surface structures can be expressed that divert attachment of the lytic complex (MAC) from the cell membrane.

(4) Membrane-bound enzyme can degrade fixed complement or cause it to be shed.

(5) The outer membrane can resist the insertion of the lytic complex.

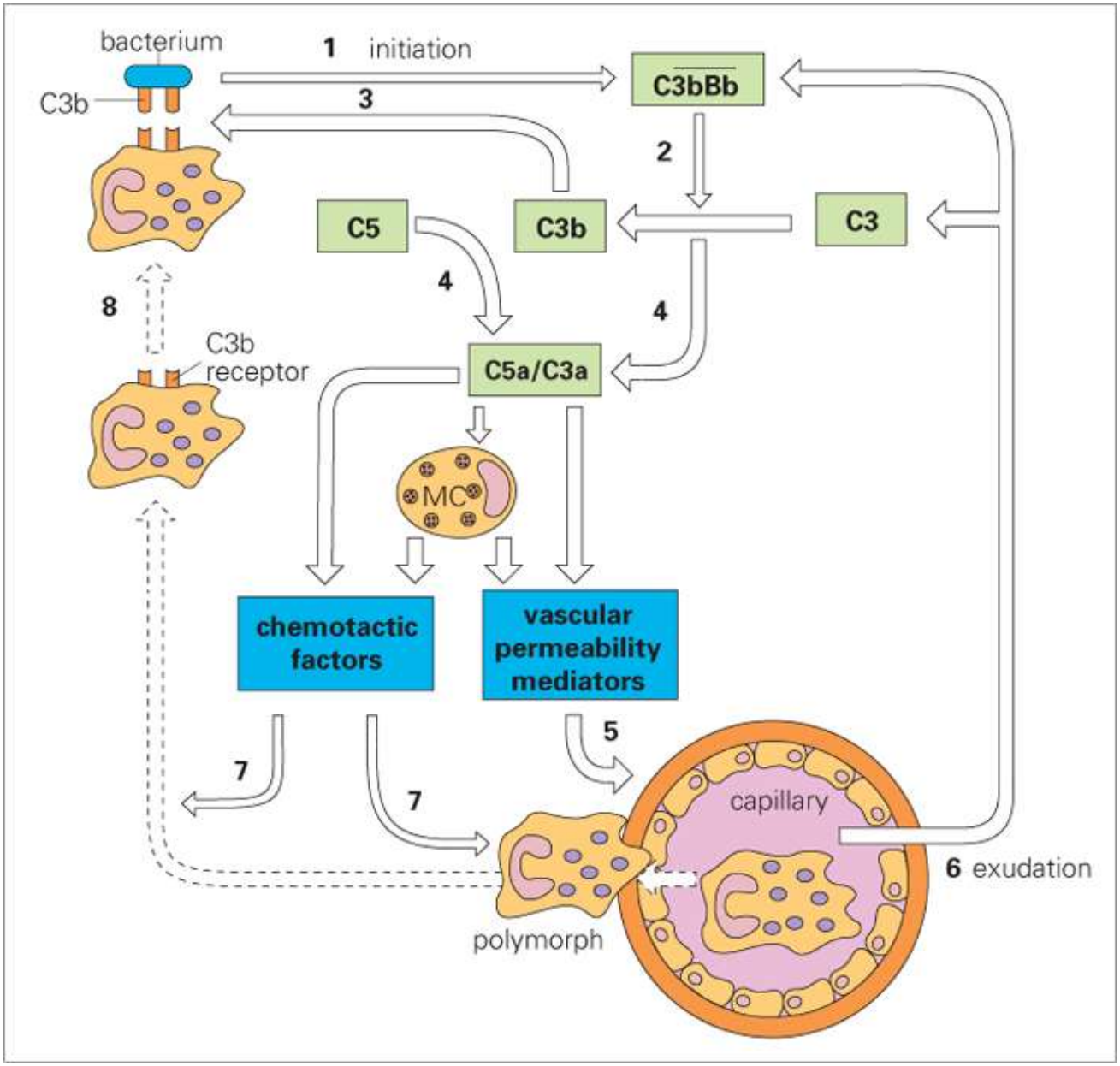
(6) Secreted decoy proteins can cause complement to be deposited on them and not on the bacterium itself.

The insertion of the C567 complex is prevented by the long side chains of the cell wall polysaccharides of smooth strains of *Salmonellae* and by the capsules of staphylococci, which, unlike the cell wall, do not activate complement.

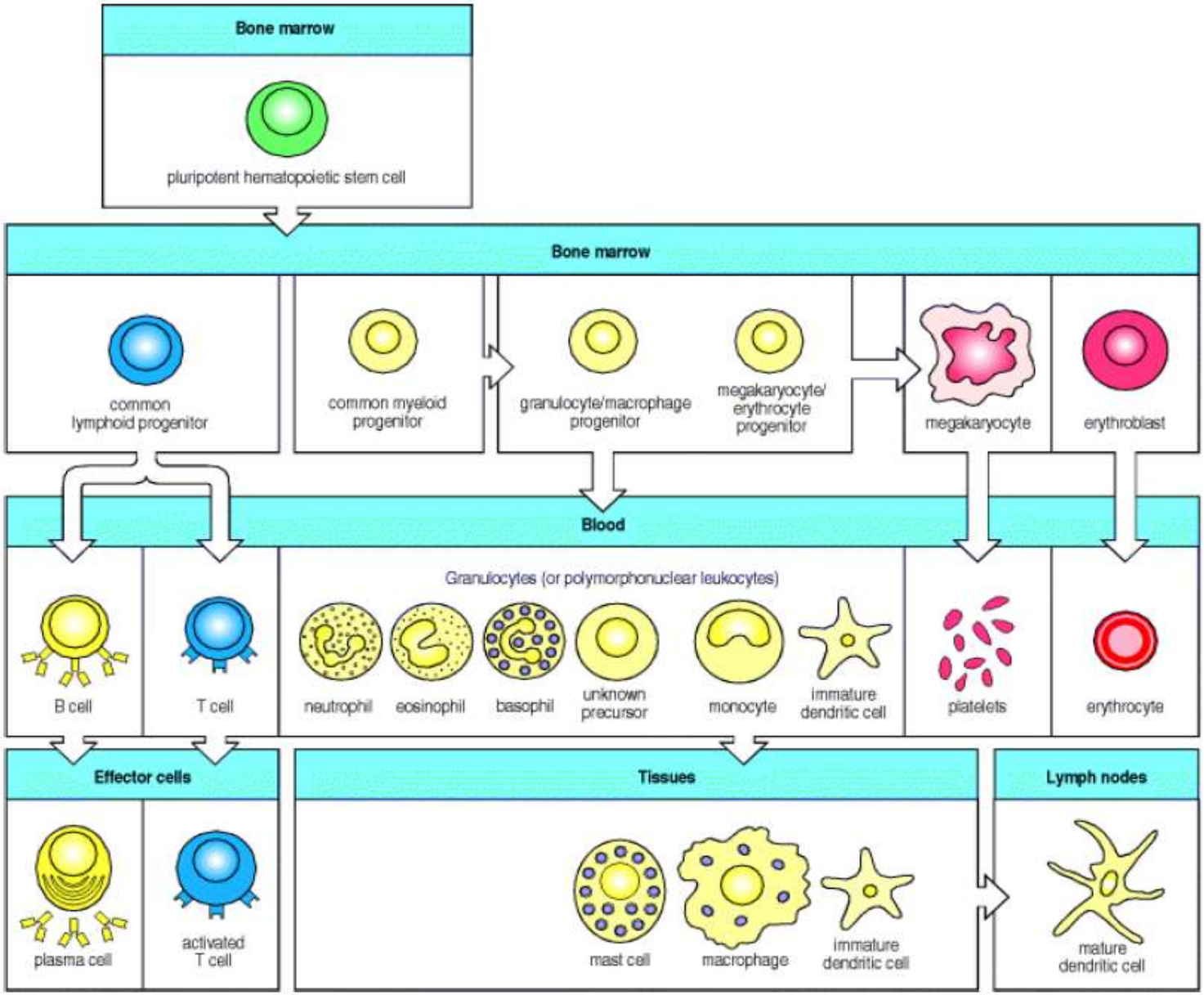
Protein A and G bing Ig through Fc fragments, preventing complement activation.

surface proteins bind finbrin, or factor H, making the bacteria invisible to complement, „stealth technology…Certain bacteria, for example streptococci and Campylobacter, actively inhibit complement activation, while a covering of non-complement fixing antibody, for example IgA, is yet another way of avoiding lysis.

Activation of complement attracts phagocytes



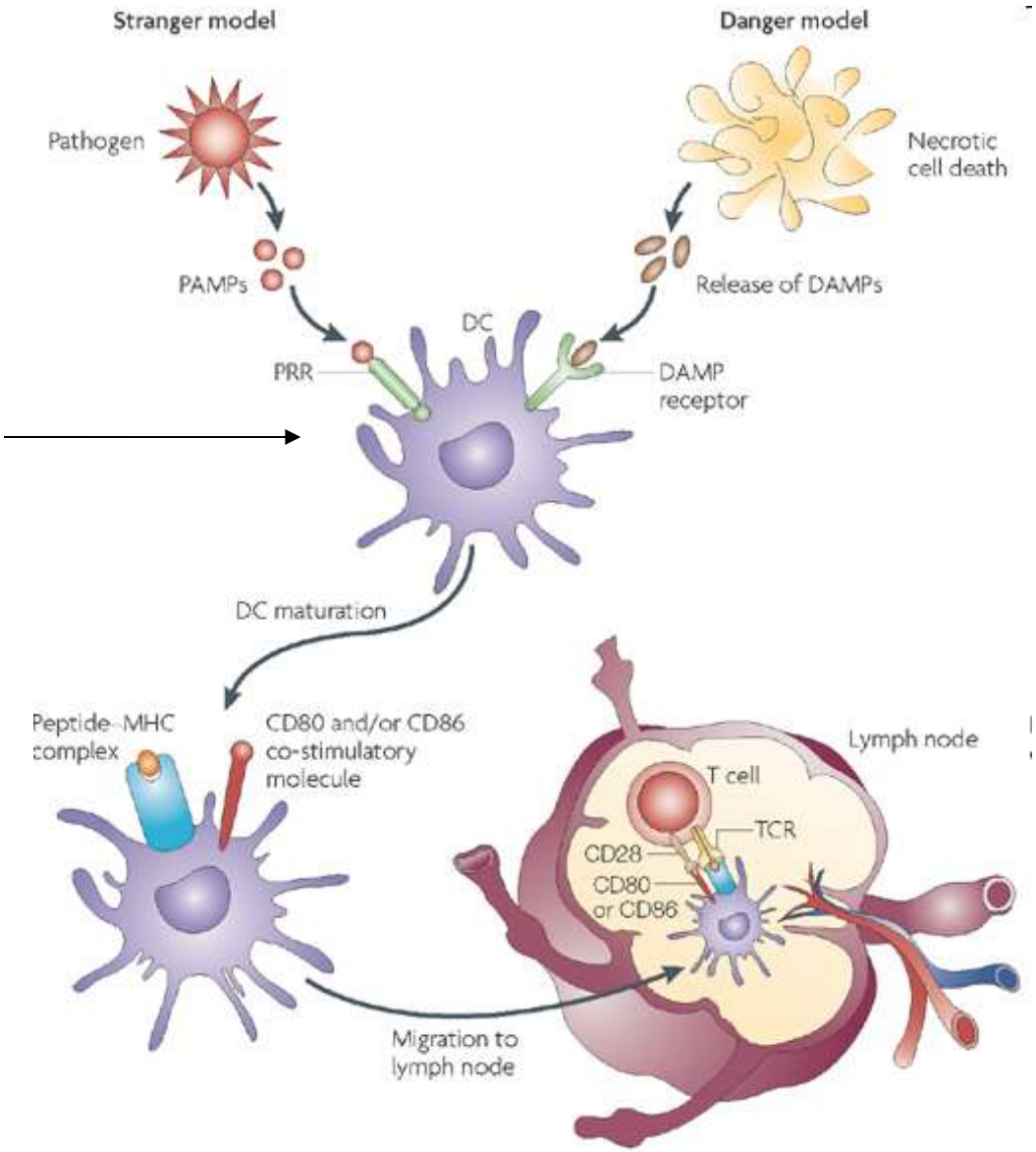
Differentiation of hematopoietic stem cell into sentinel cells of innate and adative immunity



(Janeway, 2001)

Innate immune mechanism - pathogen recognition

Stranger and danger model of dendritic cell activation



- recognition of P(D)AMPs (pathogen (danger)-associated molecular patterns) via PRRs (pattern recognition receptors)
- inflammation recruitment and activation of effector cells
- removal of infectious agent

(Hajime Kono & Kenneth L. Rock, 2008) Nature Reviews | Immunology

A key sentinel cell of innate imunity is the mononuclear phagocyte

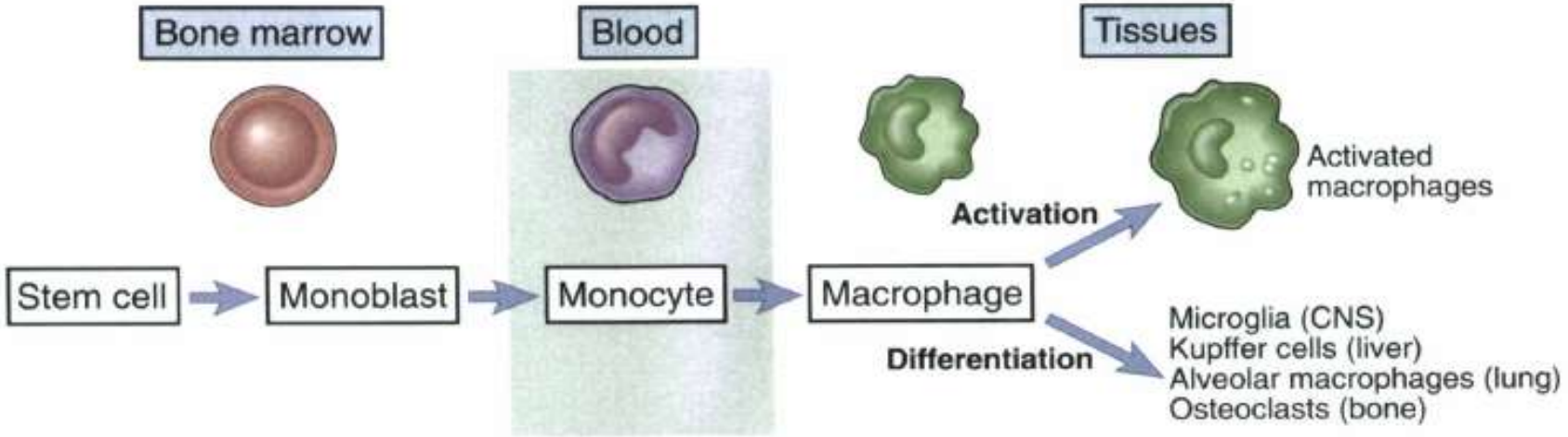


FIGURE 2–5 Maturation of mononuclear phagocytes. Mononuclear phagocytes develop in the bone marrow, circulate in the blood as monocytes, and are resident in all tissues of the body as macrophages. They may differentiate into specialized forms in particular tissues. CNS, central nervous system.

Monocytes are chemoattracted to infection sites to do the job

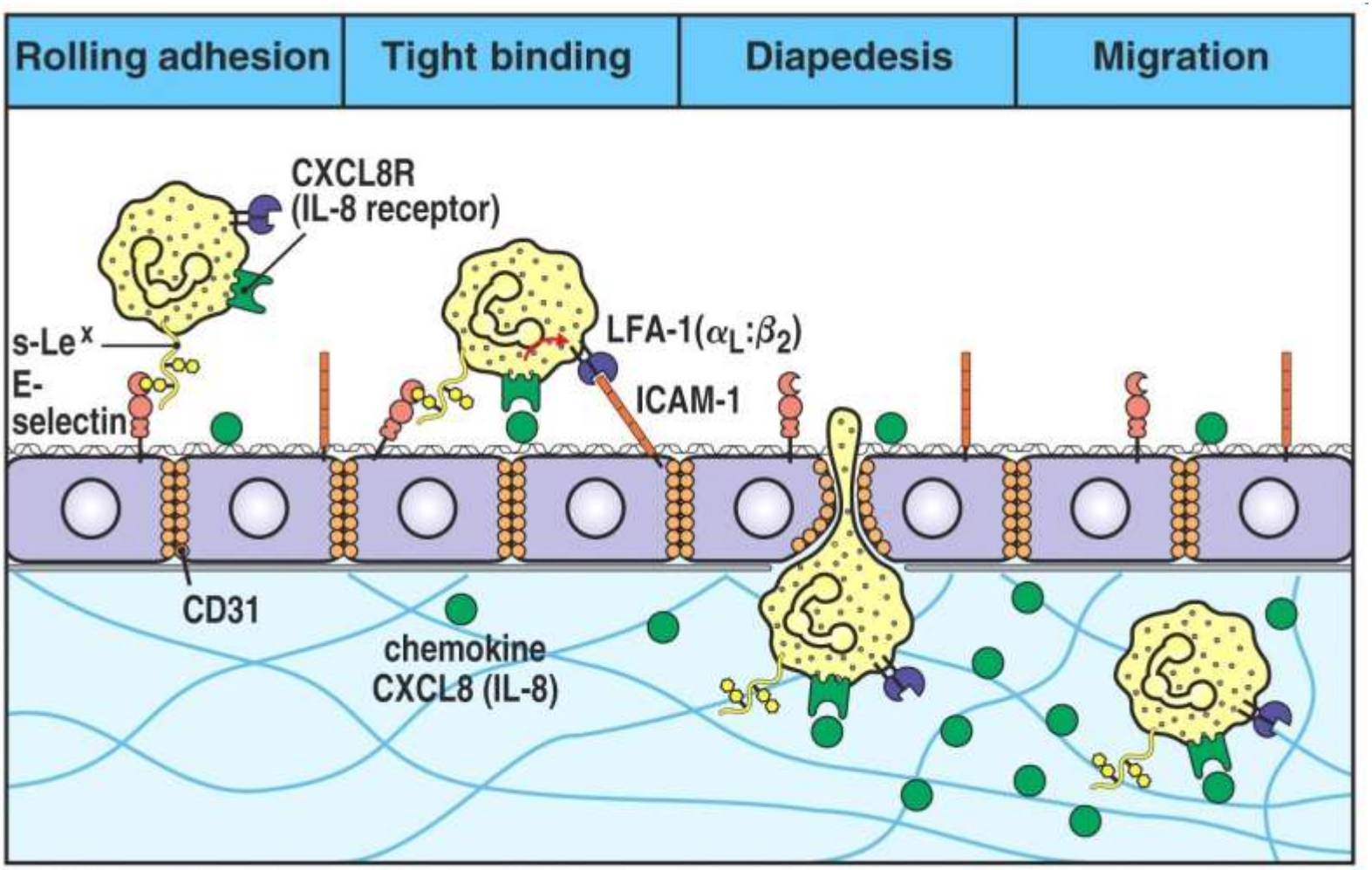
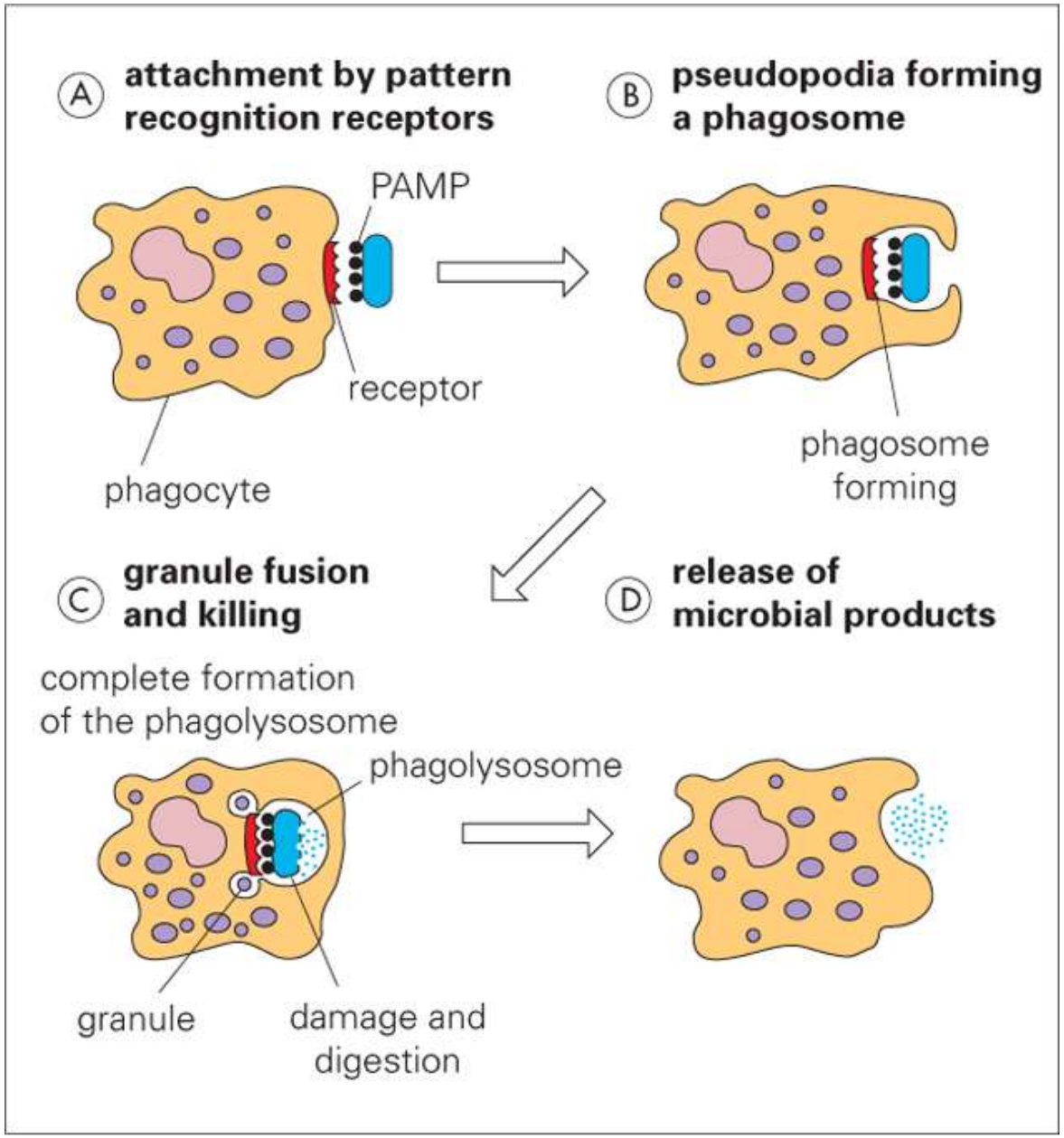
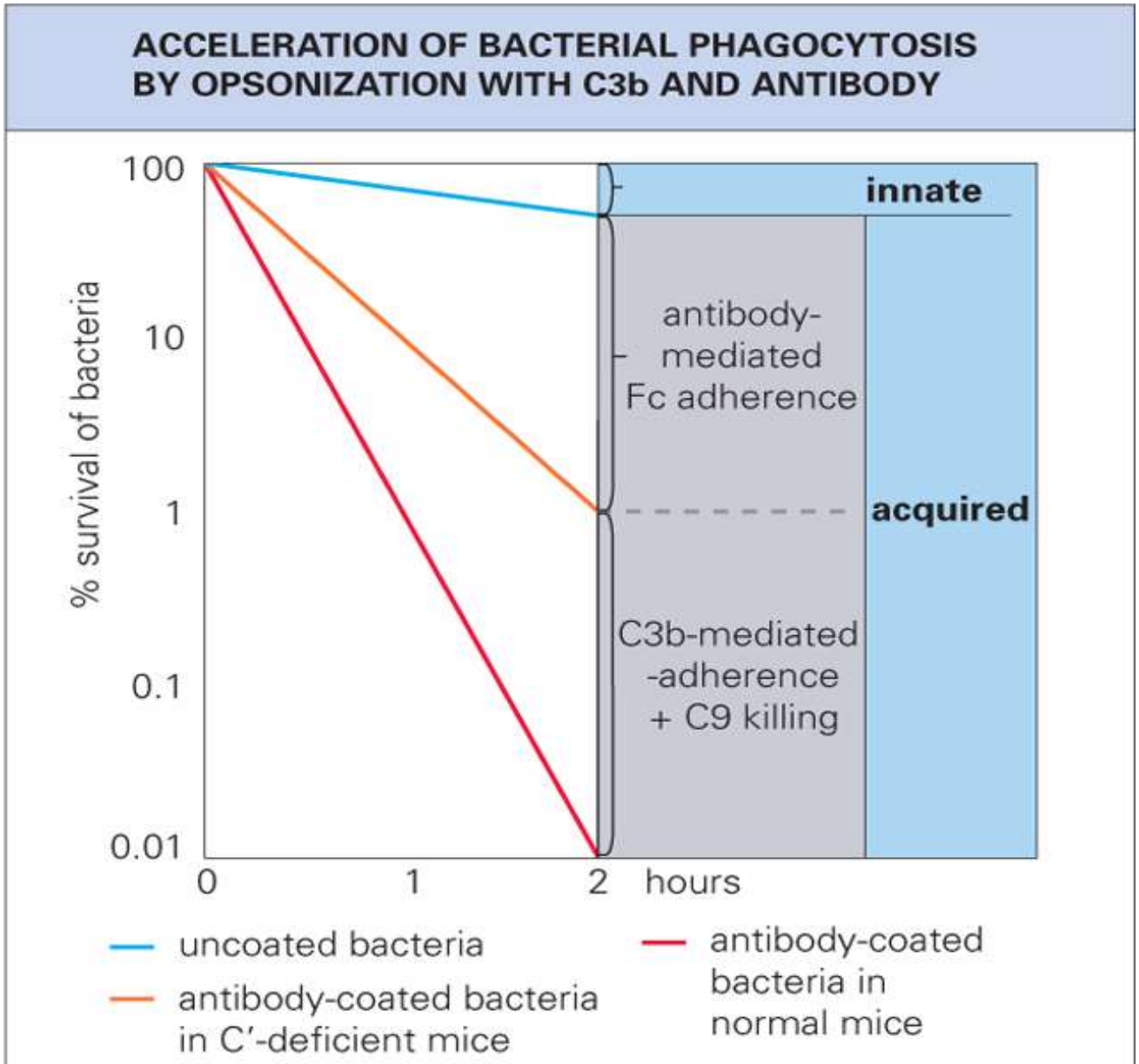


Figure 2-44 part 3 of 3 Immunobiology, 6/e. (© Garland Science 2005)

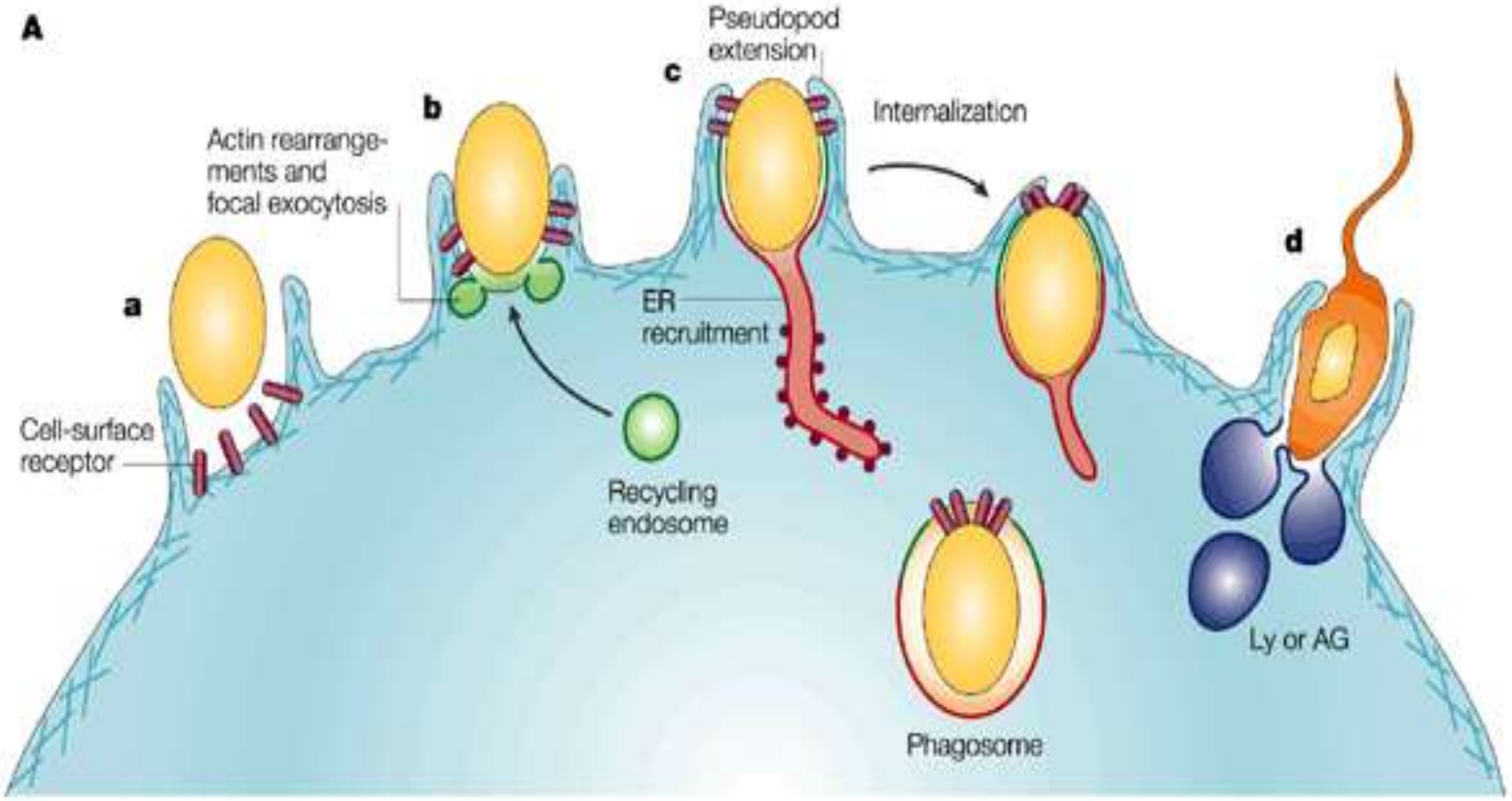
Phagocytes recognize PAMPs – pathogen associated molecular patterns



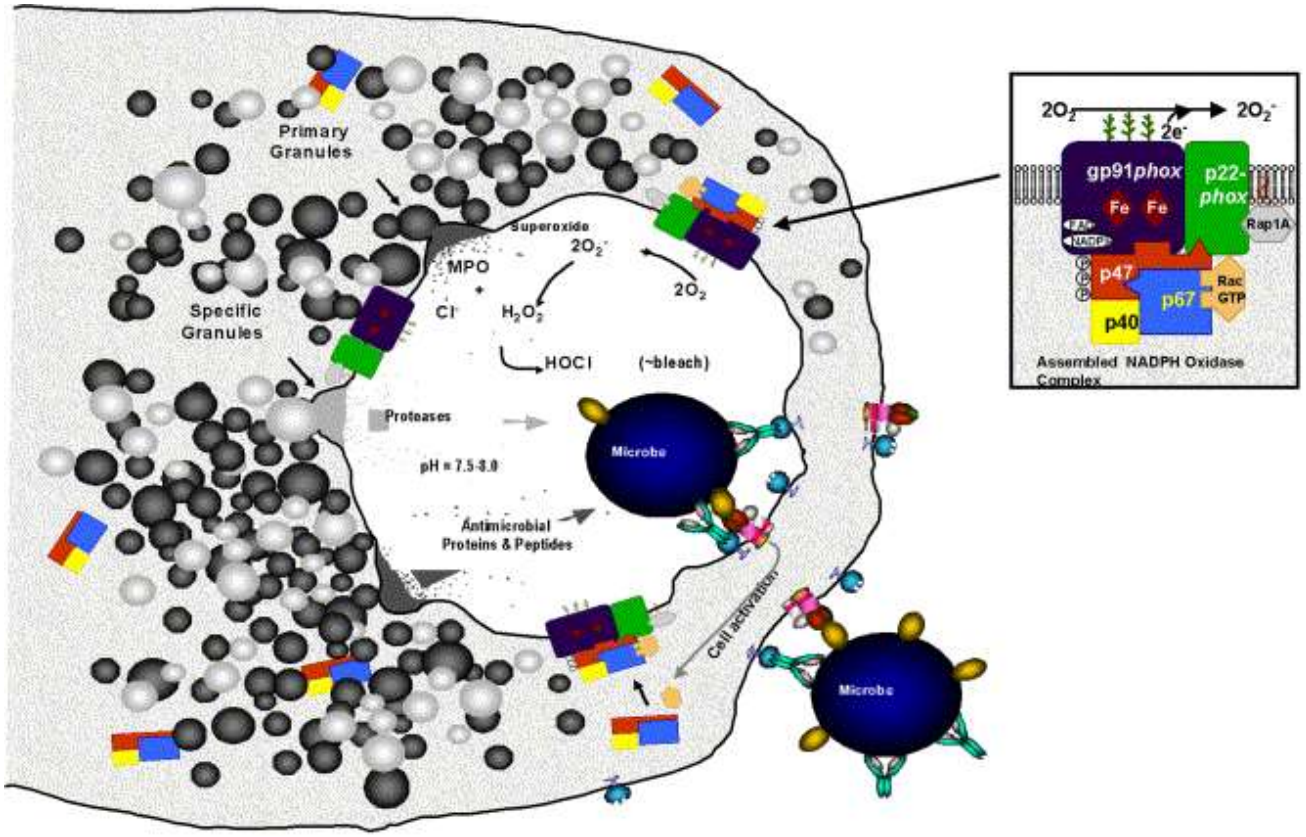
Opsonization by complement or antibodies is the key defense mechanism



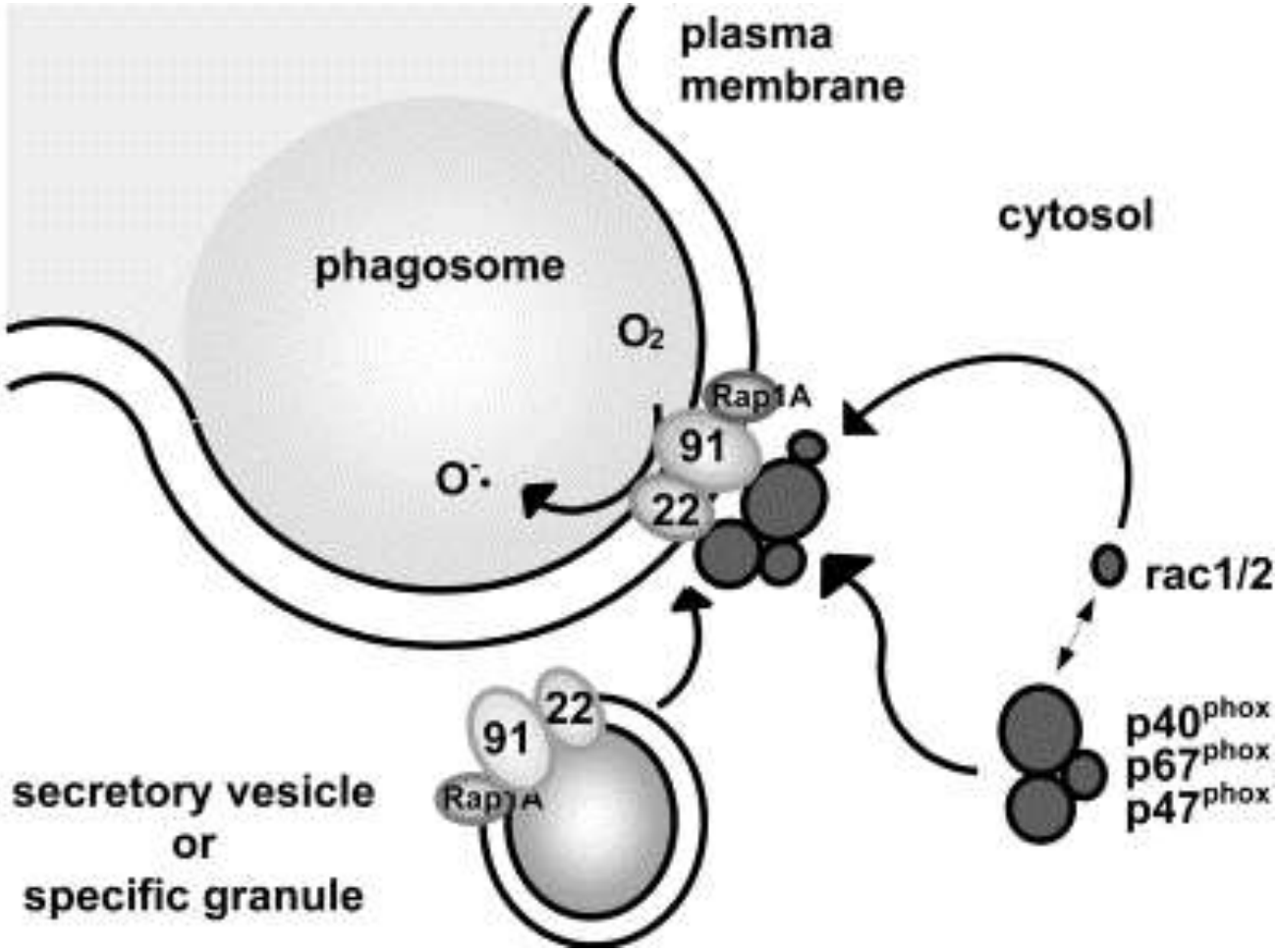
Phagocytosis



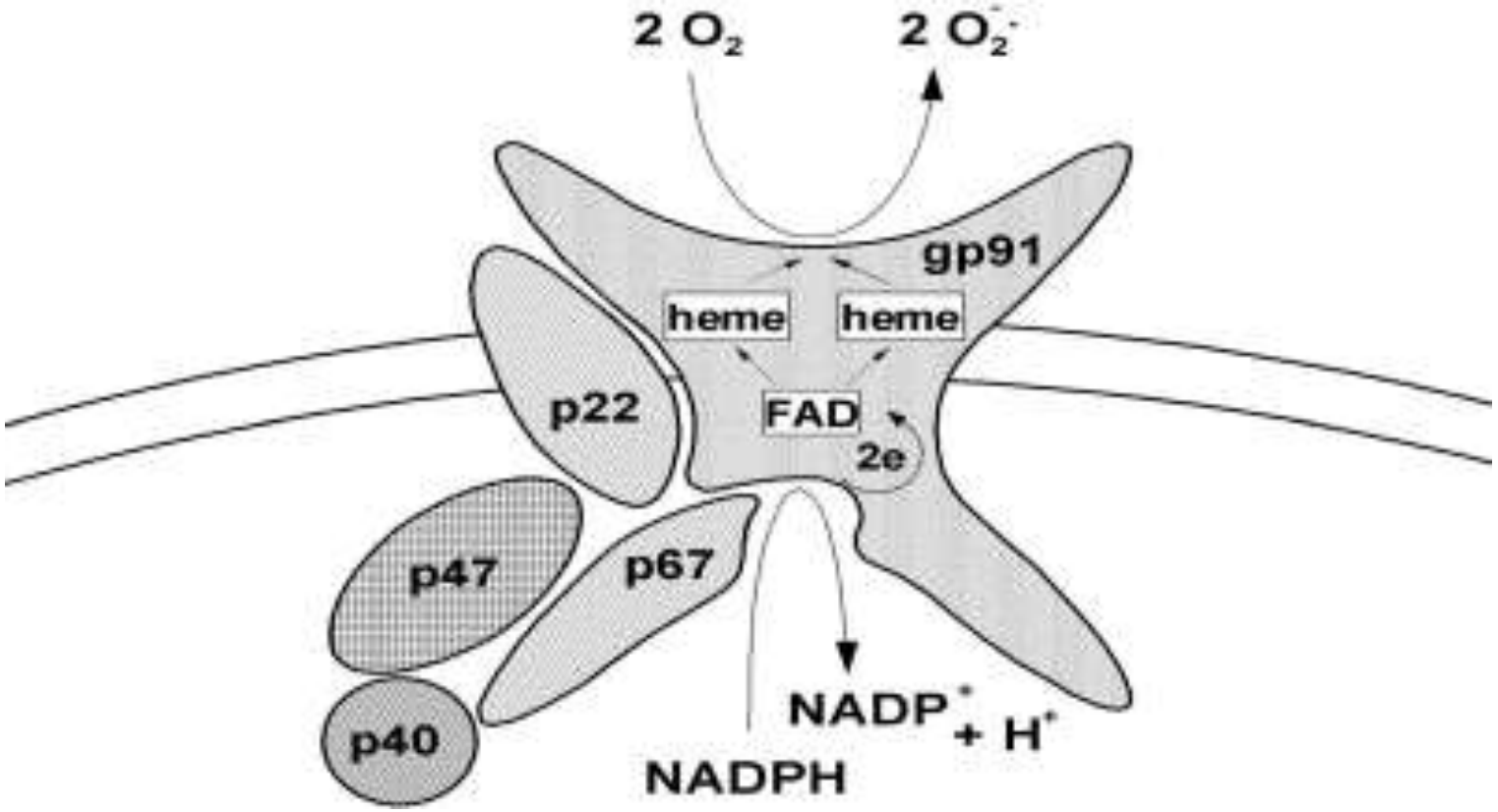
Complement activation promotes opsonophagocytosis, oxidative burst and killing of bacteria



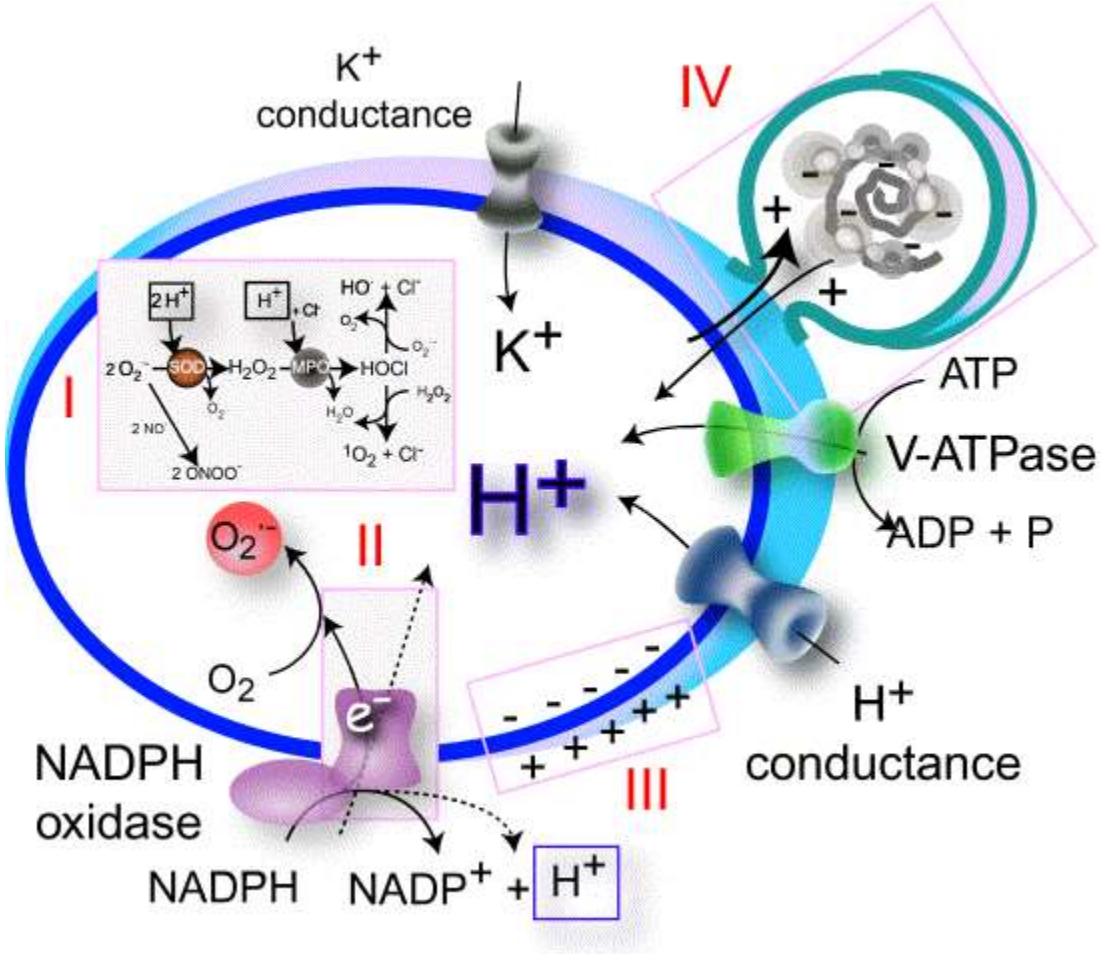
Neutrophil responses to phagocytic stimuli.
Neutrophil ingests an opsonized microbe. CR3 and Fc Rs are engaged by opsonins on the organism surface. **NADPH oxidase** complexes assemble at the phagosome membrane, and **superoxide anions** are generated in the phagosome lumen. Proteases and antimicrobial peptides are delivered into phagosomes by fusion with specific and azurophilic (primary) granules. Myeloperoxidase O released from azurophilic granules converts the primary oxidants into highly toxic HOCl.



Activation of the phagocyte NADPH oxidase. Assembly of the enzyme and phagosome formation are concomitant processes. Translocation of the cytosolic oxidase components is initiated by serine phosphorylation in p47phox and controlled by small Rho-like GTPases (Rac1, Rac2, Rap1A). This translocation leads to a conformational change in gp91phox that permits NADPH binding, thus activating the NADPH oxidase enzyme.



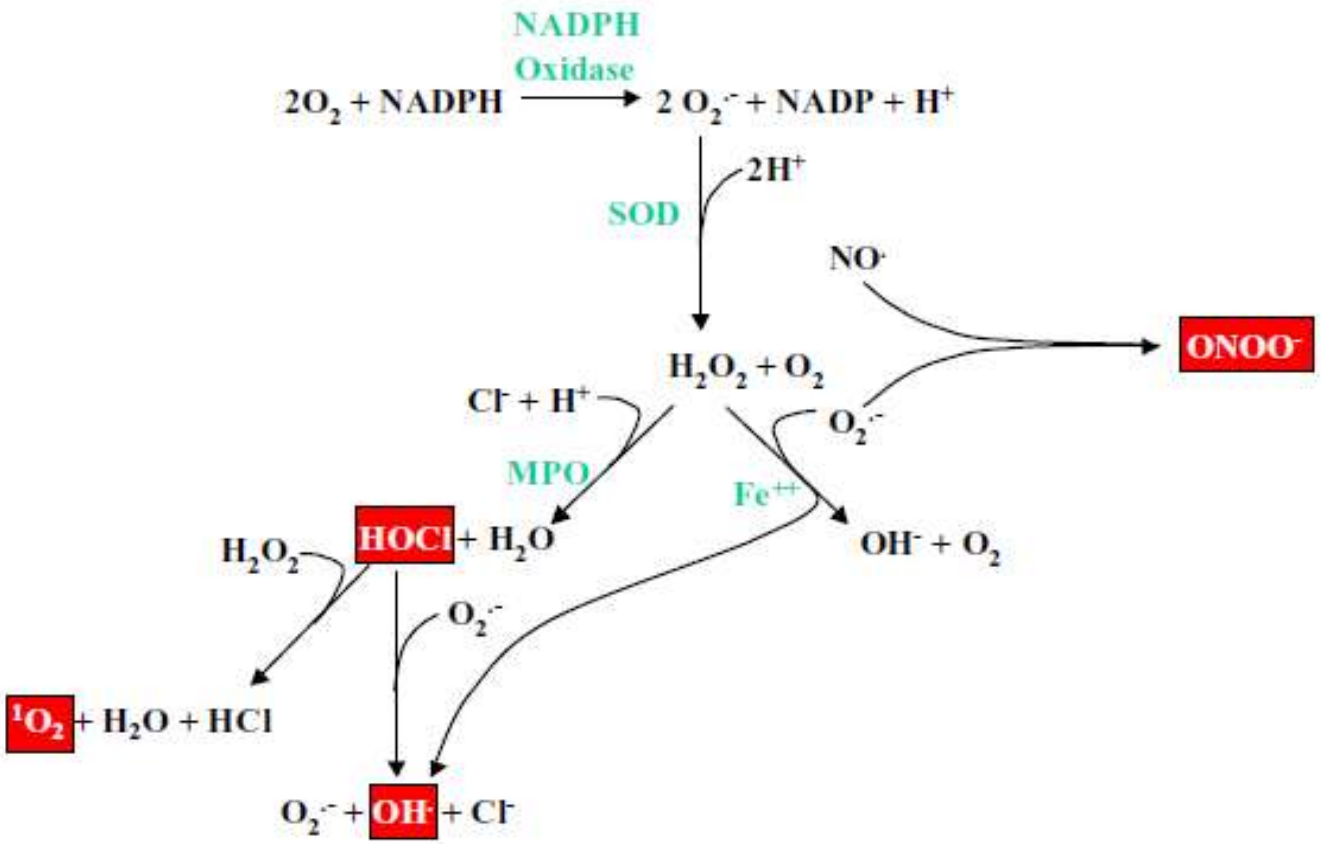
After assembly of the NADPH oxidase complex, NADPH from the cytosol can bind to the enzyme and donate its electrons. These electrons are then transmitted via FAD and two heme groups to molecular oxygen on the outside of the plasma membrane, thus generating superoxide in either the phagosome or in the extracellular environment.



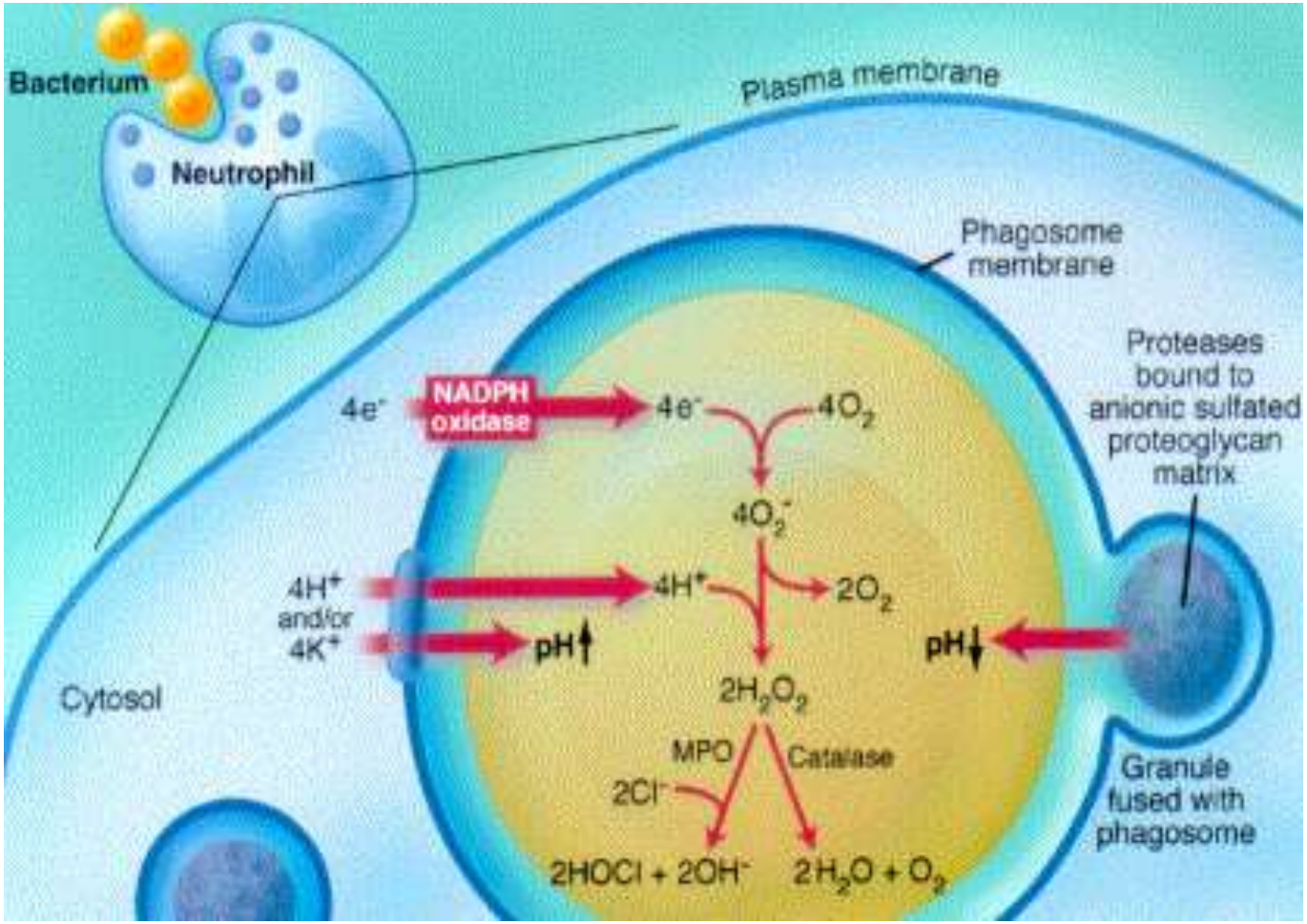
Oxidant generation and ionic homeostasis of the phagosome. (I) Production of superoxide anions results in consumption of protons during dismutation, leading to formation of other reactive oxygen species via superoxide dismutase (SOD) and myeloperoxidase (MPO). (II) The NADPH oxidase has been postulated to function as a proton channel (see text for alternative views). (III) The oxidation of NADPH and transport of electrons into the phagosome generates a membrane potential across the phagosomal membrane, which promotes proton influx, counteracting cytosolic acidification. (IV) Cations translocated in response to the electrical potential change (H+ and/or K+) enhance granule secretion by increasing the ionic strength of the phagosomal lumen

Reactive oxygen species

➢the generation of microbicidal molecules within mammalian neutrophile/macrophage

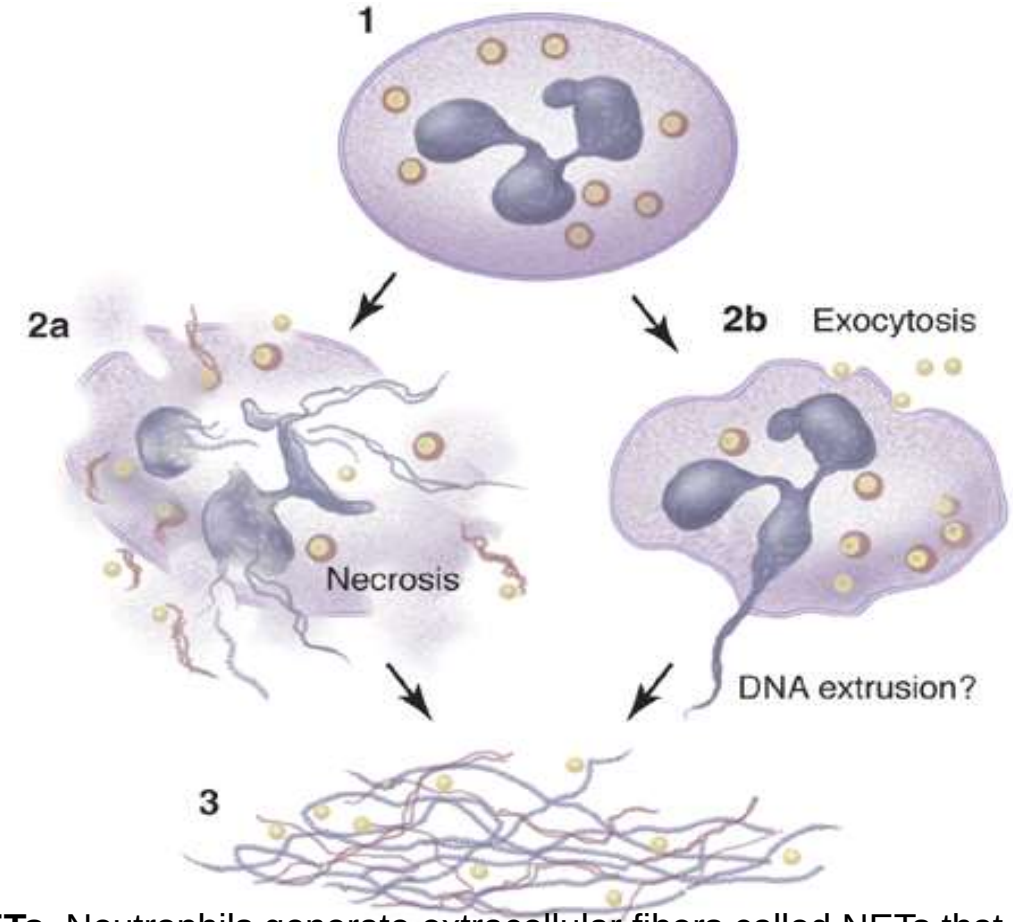


(Beutler, 2004)



Within the phagosome. In the small space between an ingested bacterium (shaded area) and the membrane of the phagosome, a number of chemical reactions take place. Molecular oxygen (O_2) is reduced to superoxide (O^{2-}) by phagocyte NADPH oxidase. This charge transfer is compensated by an influx of protons (H^+) or other cations. Protons are used to reduce superoxide to H_2O_2, which can be degraded to oxygen and water in a catalase-dependent reaction. Alternatively, H_2O_2 can combine with chloride (Cl–) to form hypochlorous acid (HOCl) in a reaction catalyzed by myeloperoxidase (MPO).

However, neutrophils also donate their life and eject DNA in the fight to capture and kill bacteria…



Making NETs. Neutrophils generate extracellular fibers called NETs that kill bacterial pathogens without the need for phagocytosis. A resting neutrophil becomes activated (1), leading to the release of DNA, histones, and granule proteins that assemble into NETs (3). The cellular constituents of neutrophils are released either through necrotic cell death (2a) or by exocytosis of granule contents coupled with active extrusion of DNA via an unknown mechanism (2b).

Science, Vol 303, Issue 5663, 1477-1478, 5 March 2004

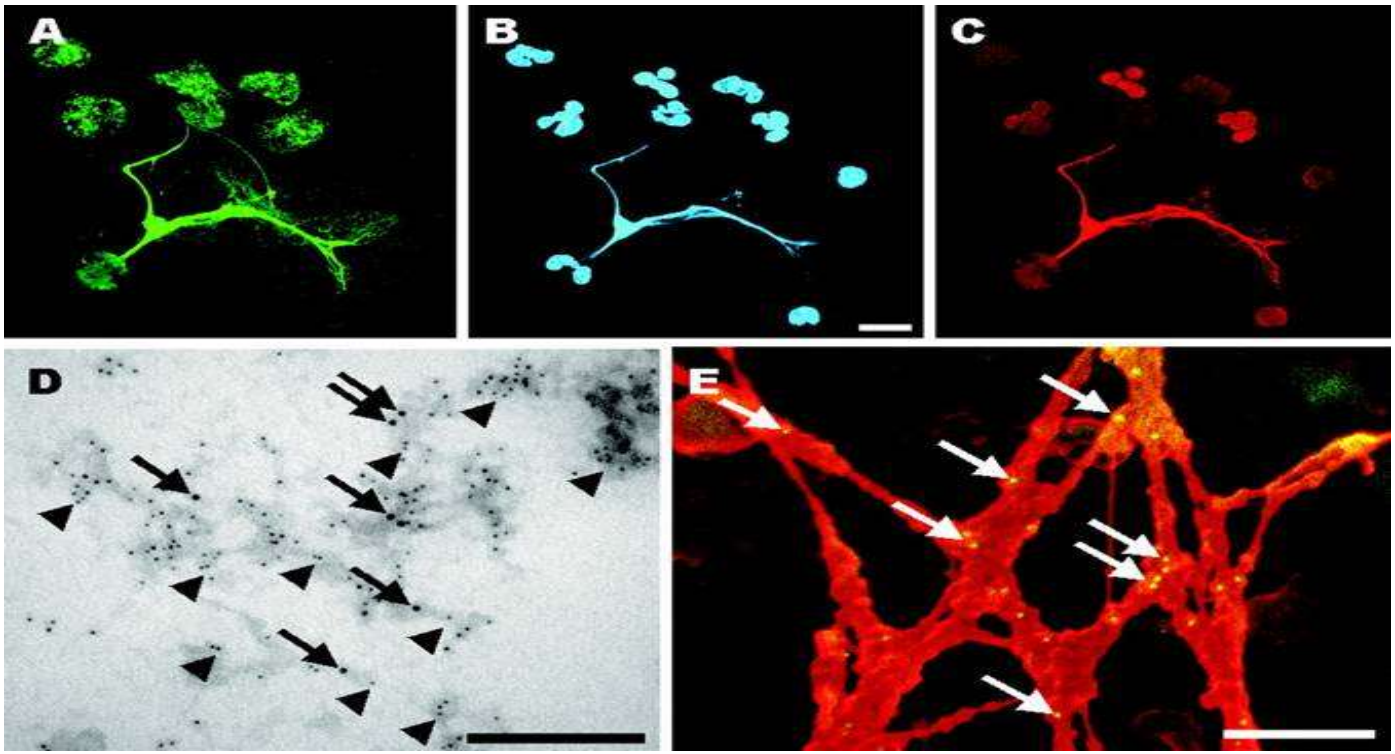


Fig. 2. Immunostaining of NETs. Neutrophils were activated with 10 ng of IL-8 for 30 min and stained for neutrophil elastase (**A**), DNA (**B**), and the complex formed by H2A-H2B-DNA (**C**). Extracellular fibrous material is stained brightly. As expected, we found granular staining for neutrophil elastase (A) and nuclear staining for histones and DNA [(B) and (C)]. Samples were analyzed with the use of a Leica TCS-SP (Beusheim, Germany) confocal microscope. The images are projections of a z stack (original dimensions: *x* and *y*, 85.5 μm; *z* = 6.3 μm). Bar, 10 μm. **(D) Immunodetection of histones (large gold particles, arrows) and neutrophil elastase (small gold particles, arrowheads**) in ultrathin cryosections of neutrophils stimulated with IL-8 (10 ng, 1 hour). Bar, 200 nm. (**E**) Immuno-SEM, pseudocolored, of neutrophils treated as in (A) to (C). Overlay of images from secondary electron detector (red, topography) and backscattered electron detector (green, element sensitive, most back-scattered electrons from the site of gold binding). **Bright yellow dots (arrows) show localization of 12-nm gold particles detecting neutrophil elastase. Bar, 200 nm**

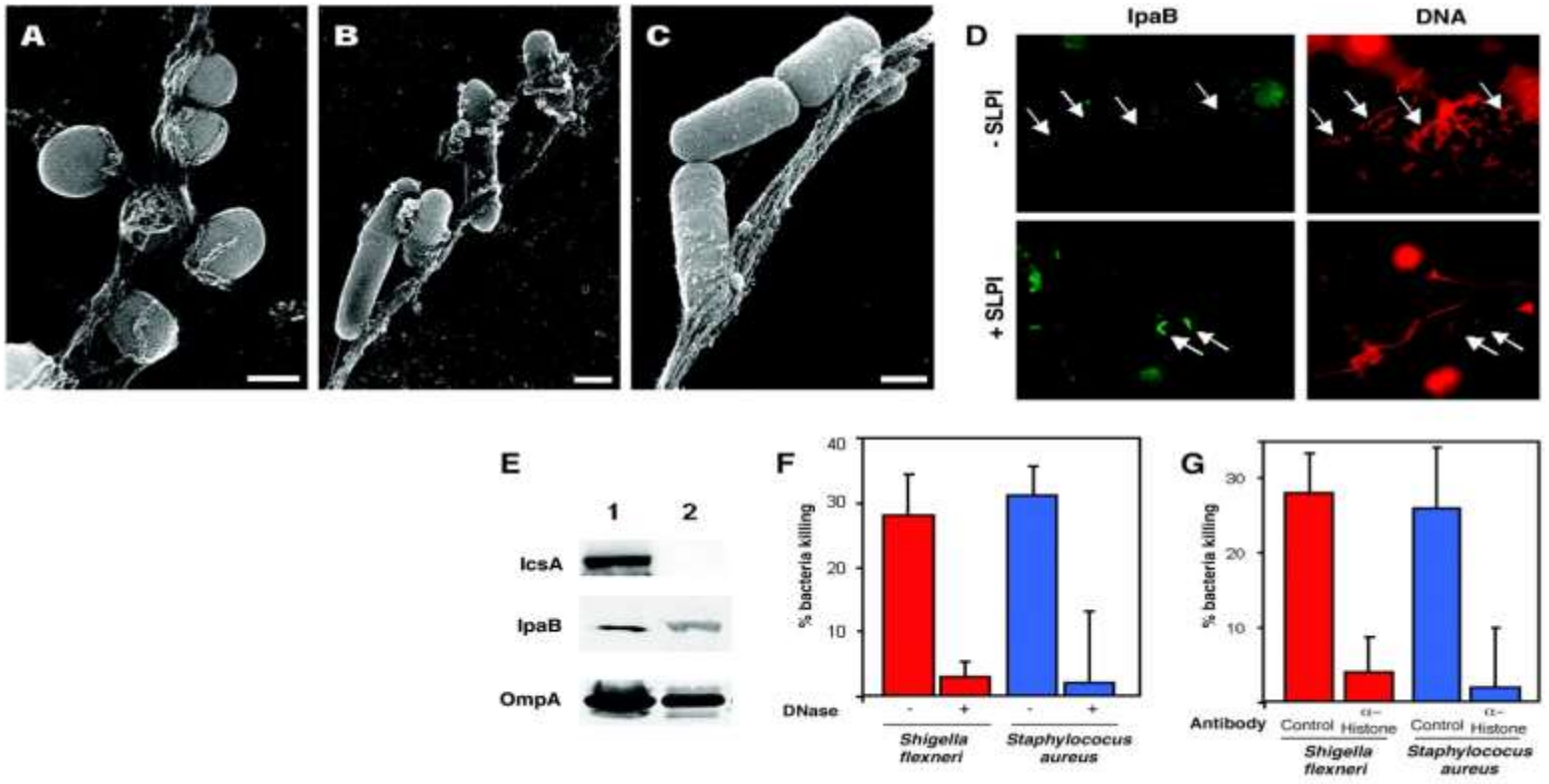
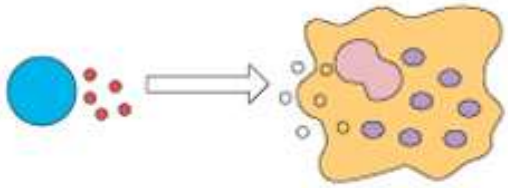


Fig. 3. Gram-positive and Gram-negative bacteria associate with neutrophil fibers. SEM of *S. aureus* (**A**), *S. typhimurium* (**B**), and *S. flexneri* (**C**) trapped by NETs. Neutrophils were treated with 100 ng of IL-8 for 40 min before infection. Bar, 500 nm. (**D**) Immunofluorescence of neutrophils infected with *S. flexneri* stained for the virulence factor IpaB and DNA. **IpaB is degraded by neutrophil elastase and is only detectable on the bacteria (arrows) when neutrophil elastase is blocked with SLPI.** DNA staining shows NETs and bacteria (arrows). (**E**) Western blot showing that the virulence factors IcsA and IpaB but not OmpA were degraded by cytochalasin D–treated neutrophils incubated with *S. flexneri*. Lane 1, bacteria alone. Lane 2, bacteria incubated with cytochalasin D–treated neutrophils. (**F**) Extracellular bactericidal activity was greatly reduced in both *S. flexneri* and *S. aureus* infections after incubation with DNase, which dissociates NETs. (**G**) Extracellular bacterial killing by neutrophils was reduced by addition of antibodies against histones. Neutrophils were treated with cytochalasin D to prevent phagocytosis and infected with *S. flexneri* or *S. aureus*. In the presence of antibody against H2A, bacterial killing was abrogated.

Avoiding death from phagocytosis

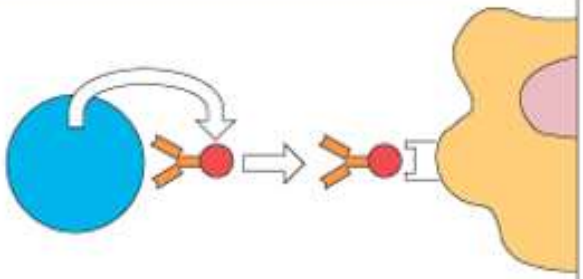
toxin release



organism releases toxin, e.g. staphylococci, streptococci, amebae

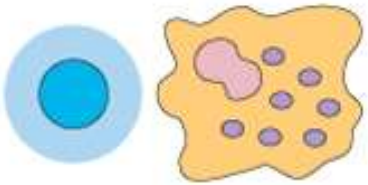
phagocyte killed by toxin

opsonization prevented



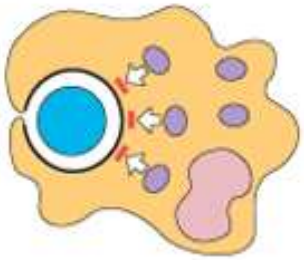
organism (e.g. staphylococci) produces a protein (e.g. protein A) which prevents interaction between opsonizing antibody and phagocyte, so preventing phagocytosis

contact with phagocyte prevented



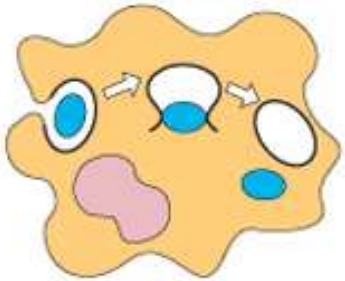
organism possesses a capsule which prevents contact with the phagocyte, e.g. *Streptococcus pneumoniae,* haemophilus, *Bacillus anthracis*

phagolysosome fusion inhibited



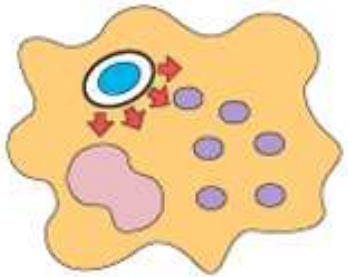
fusion of phagosome and lysosome inhibited by organism, e.g. *Mycobacterium tuberculosis,* toxoplasma, chlamydia

escape into the cytoplasm

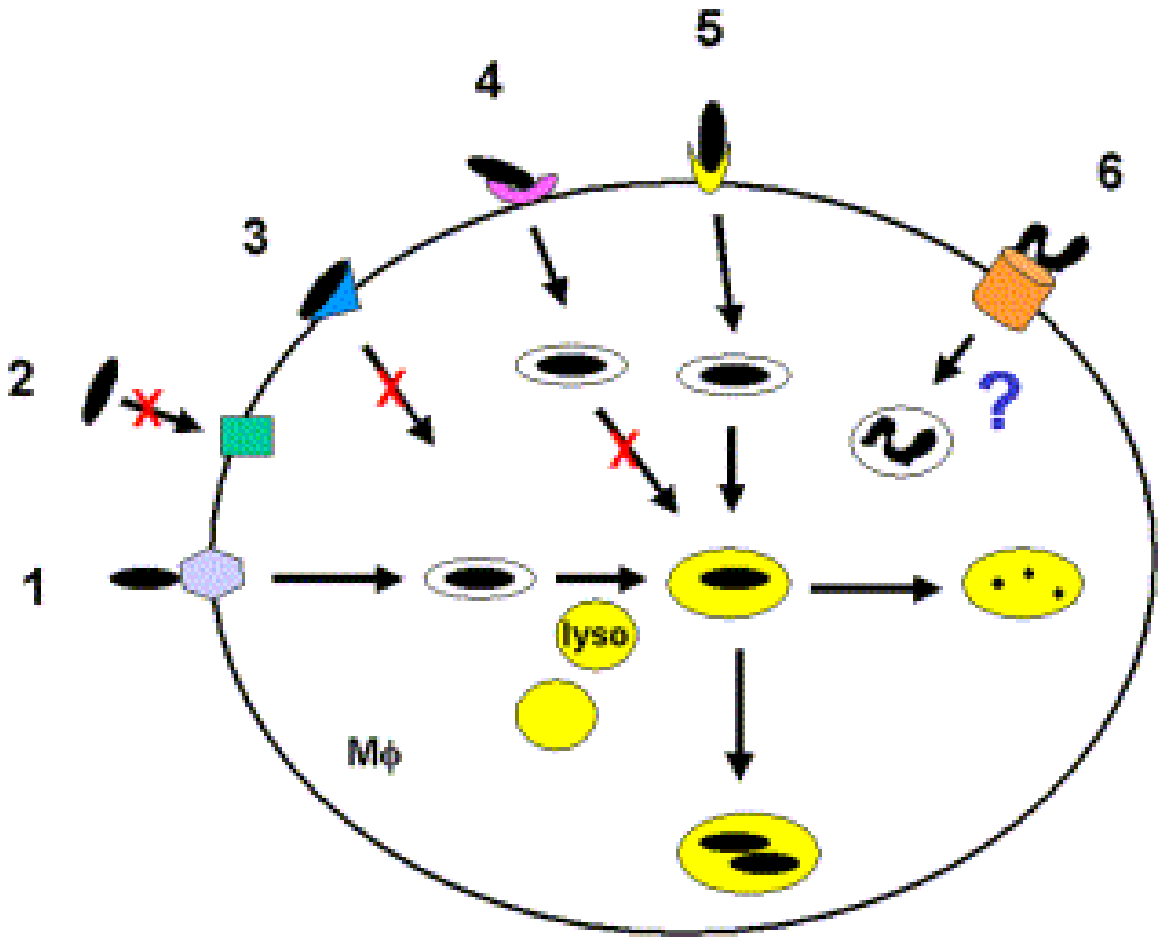


organism escapes from the phagolysosome into the cytoplasm and replicates within the phagocyte, e.g. leishmania, *T. cruzi*

resistance to killing



organism resists killing by producing antioxidants, e.g. by catalase in staphylococci, or by scavenging free radicals, e.g. by phenolic glycolipid of *M. leprae*



Evasion of phagocytic killing by macrophages and neutrophils. Under normal circumstances, adherent microbes are phagocytosed, the nascent phagosome matures via fusion with endolysosomal membranes, and internalized organisms are killed and degraded (1). Pathogens can perturb this system at several points by evasion of binding (2); **blockade of phagocytosis** (3); **interference with phagosome maturation** (4); or **survival inside phagolysosomes** (5). For some pathogens, mechanisms of resistance are undefined (6). Lyso, lysosome

Shigella flexneri can **be ingested by macrophages and kills them** by an unusual mechanism of bacteria-induced apoptosis - IpaB triggers apoptosis by binding to and activating caspase-1. Caspase-1 in turn activates interleukin (IL)-1 and IL-18. Genes needed for killing are are part of the Mix-Spa locus encoding a **type III secretion system (TTSS).**

Legionella pneumophila internalization occurs via a novel **coiling phagocytic process**, and virulence factors encoded by ***dot–icm* genes (a type IV secretion system (TFSS))** are utilized very early in infection to divert the nascent phagosome away from the endosomal pathway. Like type III secretion apparati, TFSSs of *Legionella, Brucella* and *Helicobacter pylori* appear to **transport bacterial proteins directly into host cells.**

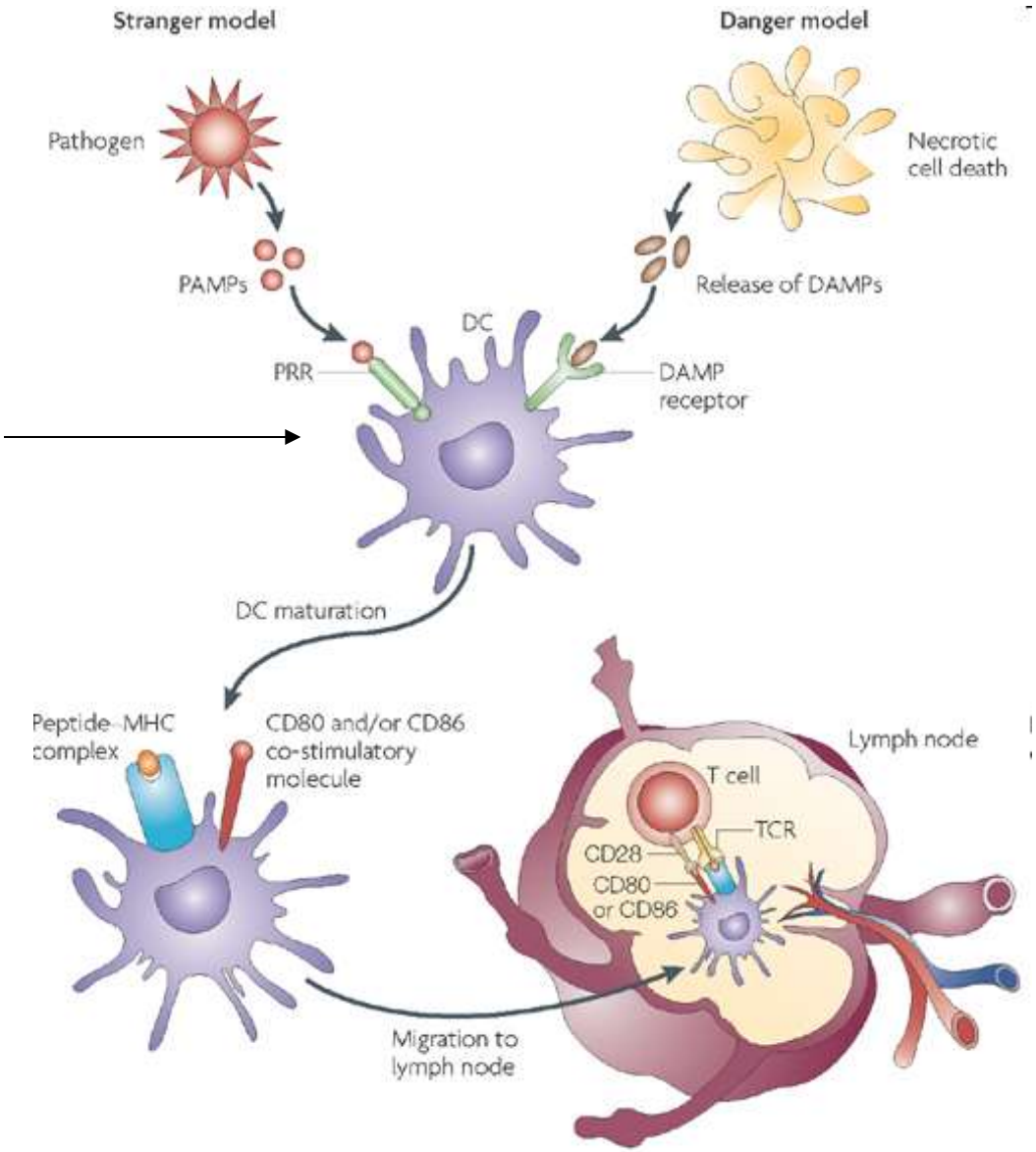
Yersinia enterocolitica crosses the Peyer's patches of the gut. Bacteria replicate extracellularly in the spleen and liver. Proteins encoded on the 70-kb virulence plasmid are critical for pathogenesis and include specific **adhesins and a TTSS** that act in concert to **block phagocytosis in both macrophages and PMNs**

Group A *streptococci* resist phagocytosis and killing by PMNs. **Surface C5a peptidase and hyaluronic acid capsule retard phagocyte recruitment and prevent phagocytosis of unopsonized bacteria.** GAS secrete **MAC**, a protein with homology to CD11b that binds to Fc RIIIB on PMNs and sequesters CR3–Fc R complexes at the cell surface. Consequently, phagocytosis of opsonized GAS is impaired.

Mycobacterium tuberculosis **inhibits phagosome-lysosome fusion** and phagosome acifdification and maturation, can survive for long time

Innate immune mechanism - pathogen recognition

Stranger and danger model of dendritic cell activation



- recognition of P(D)AMPs (pathogen (danger)-associated molecular patterns) via PRRs (pathogen recognition receptors)
- inflammation recruitment and activation of effector cells
- removal of infectious agent

(Hajime Kono & Kenneth L. Rock, 2008) Nature Reviews | Immunology

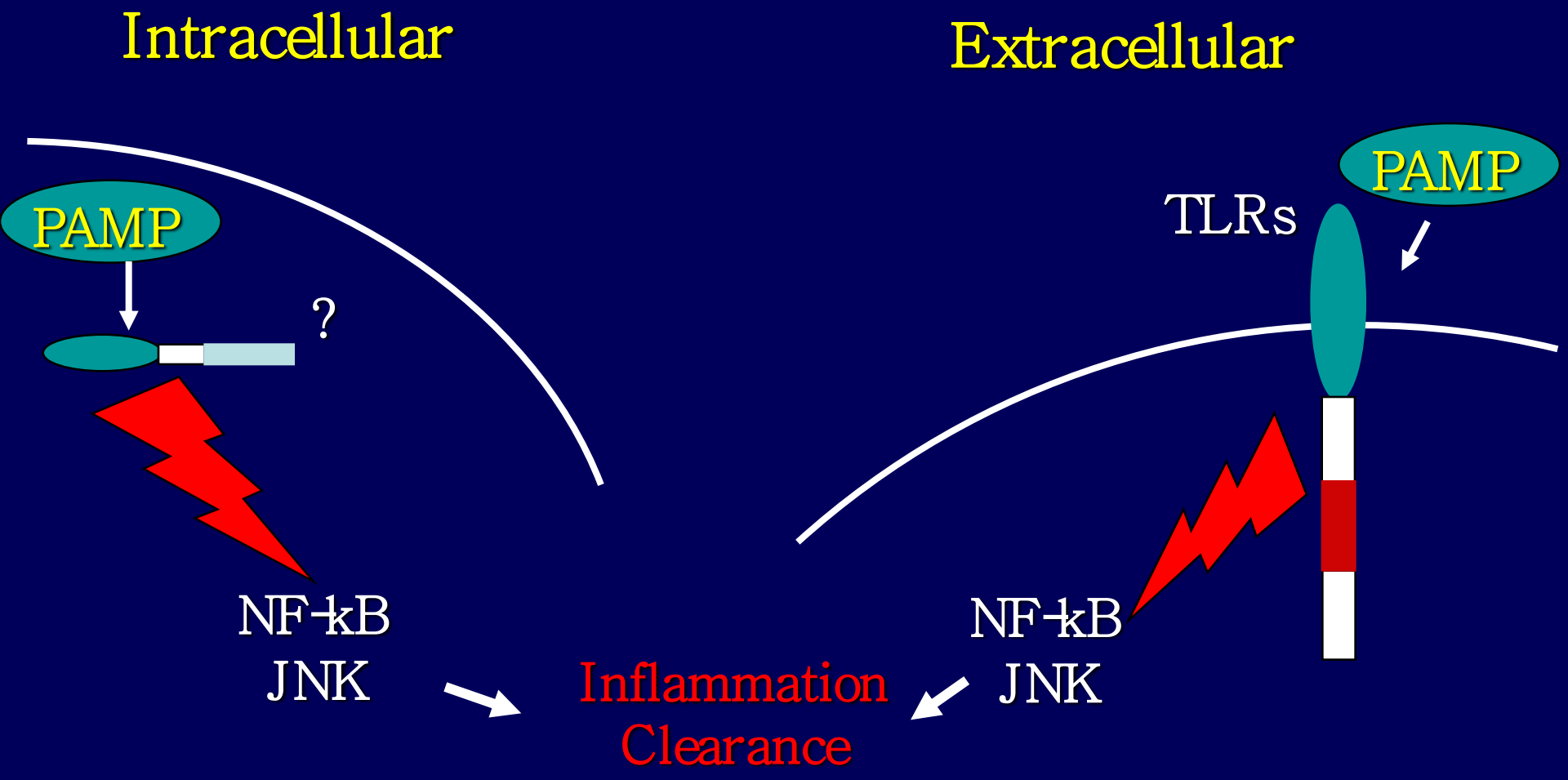
Pathogen-associated molecular patterns (PAMPs)

- general patterns shared by a large group of pathogens
- essential for the survival or pathogenicity of the microorganism
- unique for microbial organism x not represent in host organism

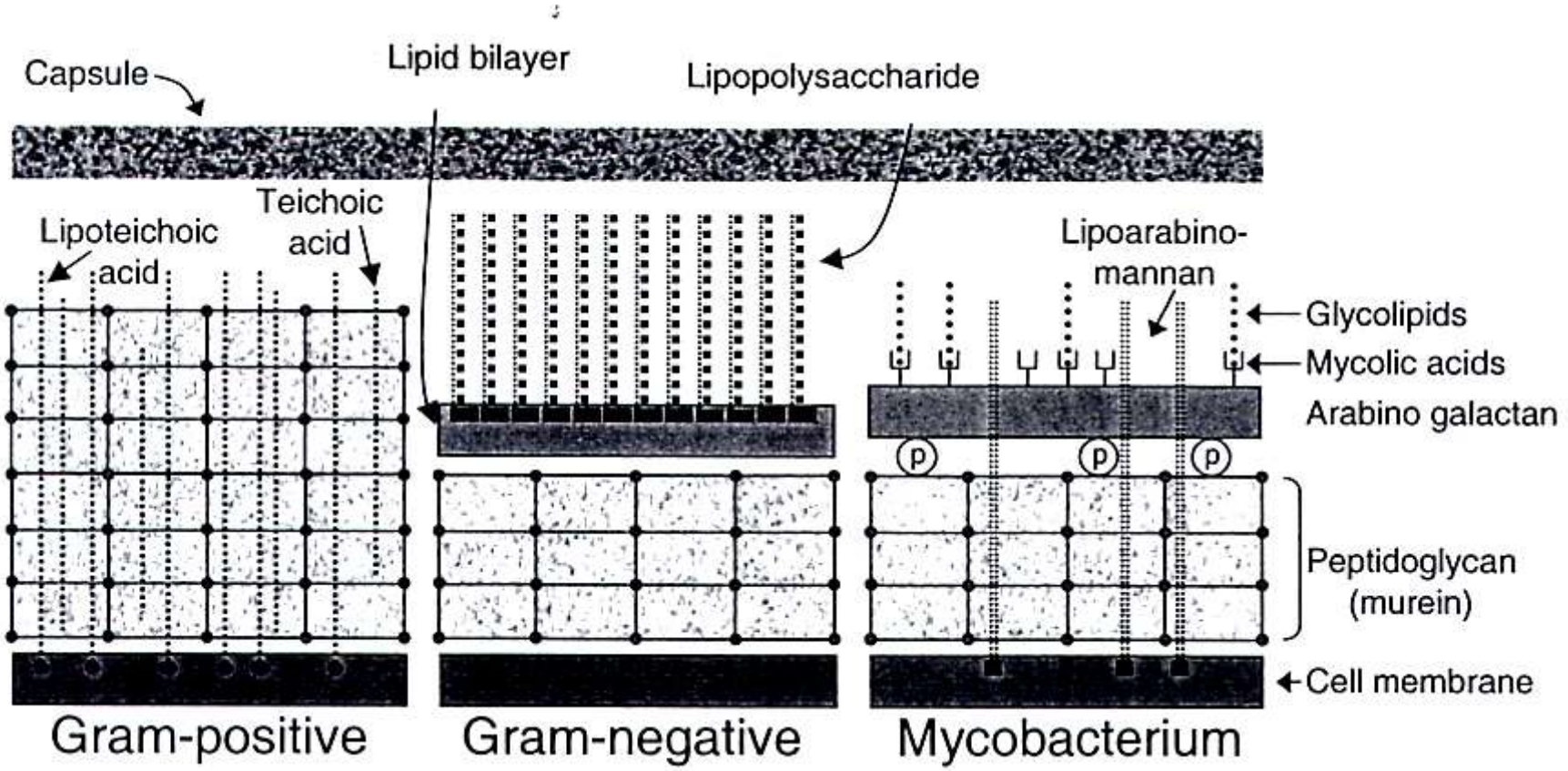
Pathogen recognition receptors (PRRs)

secreted / intracellular / cell surface

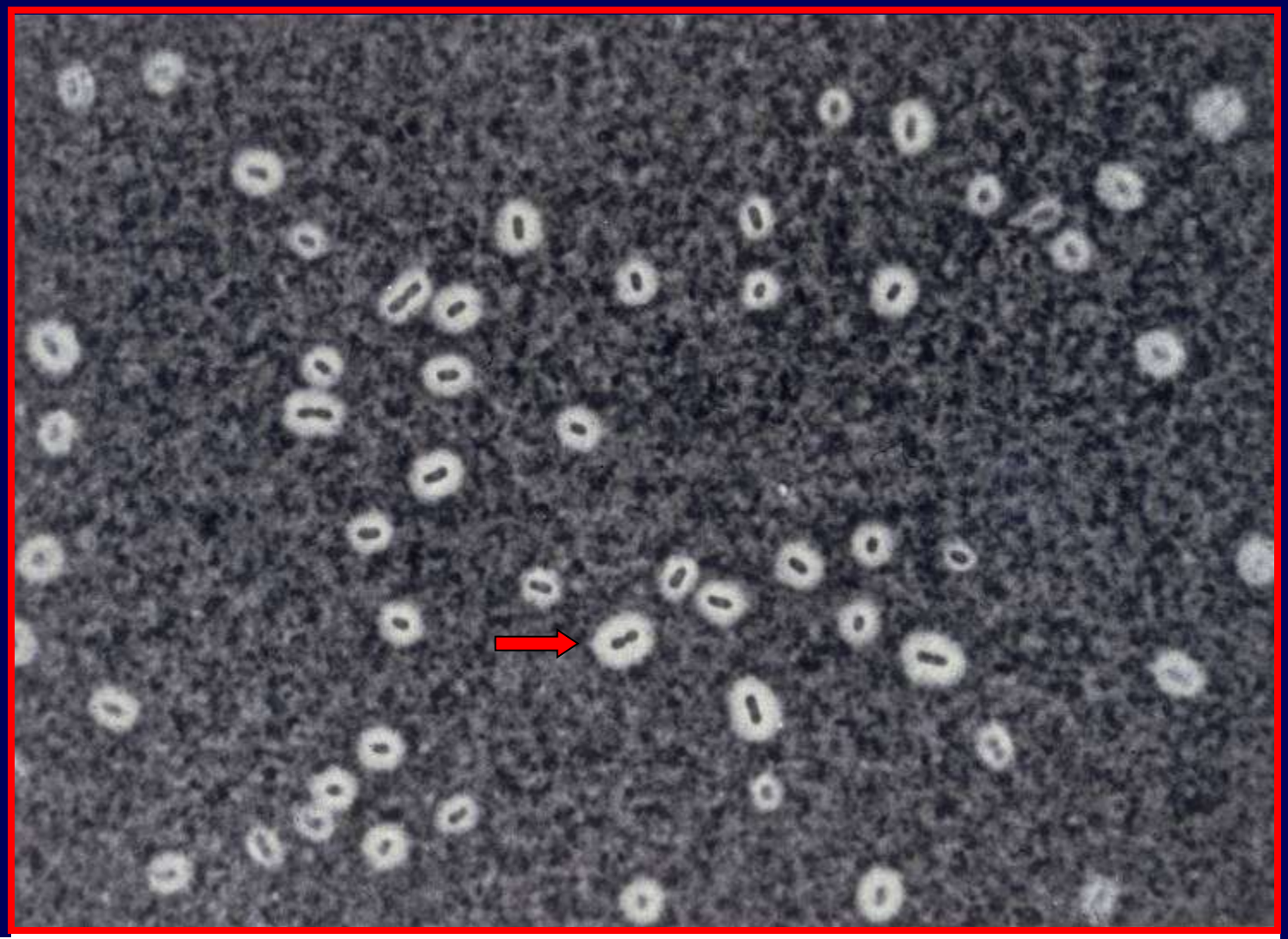
Two distinct systems of pathogen recognition: ouside-in versus inside-in?



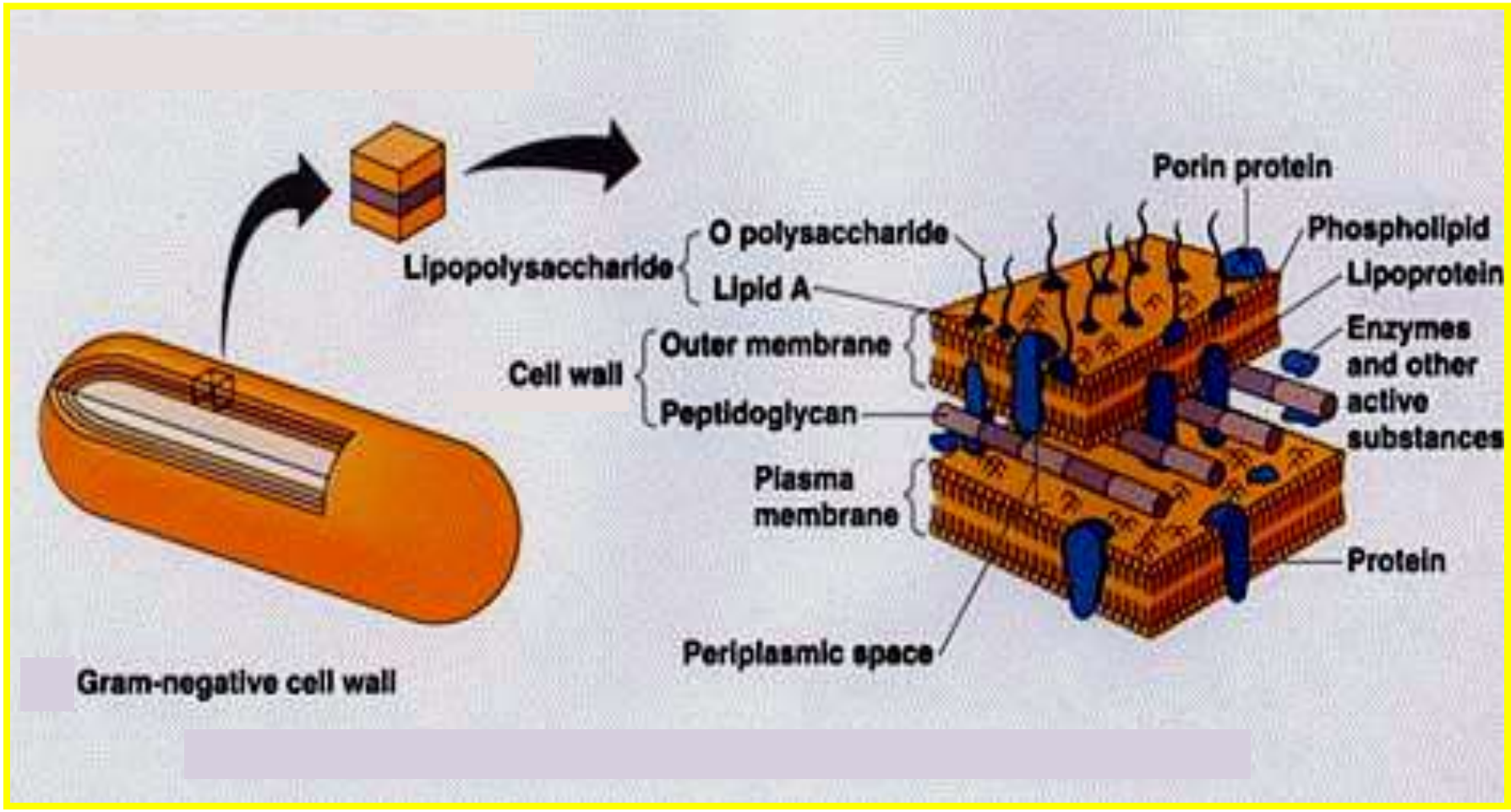
Bacterial cell wall structure



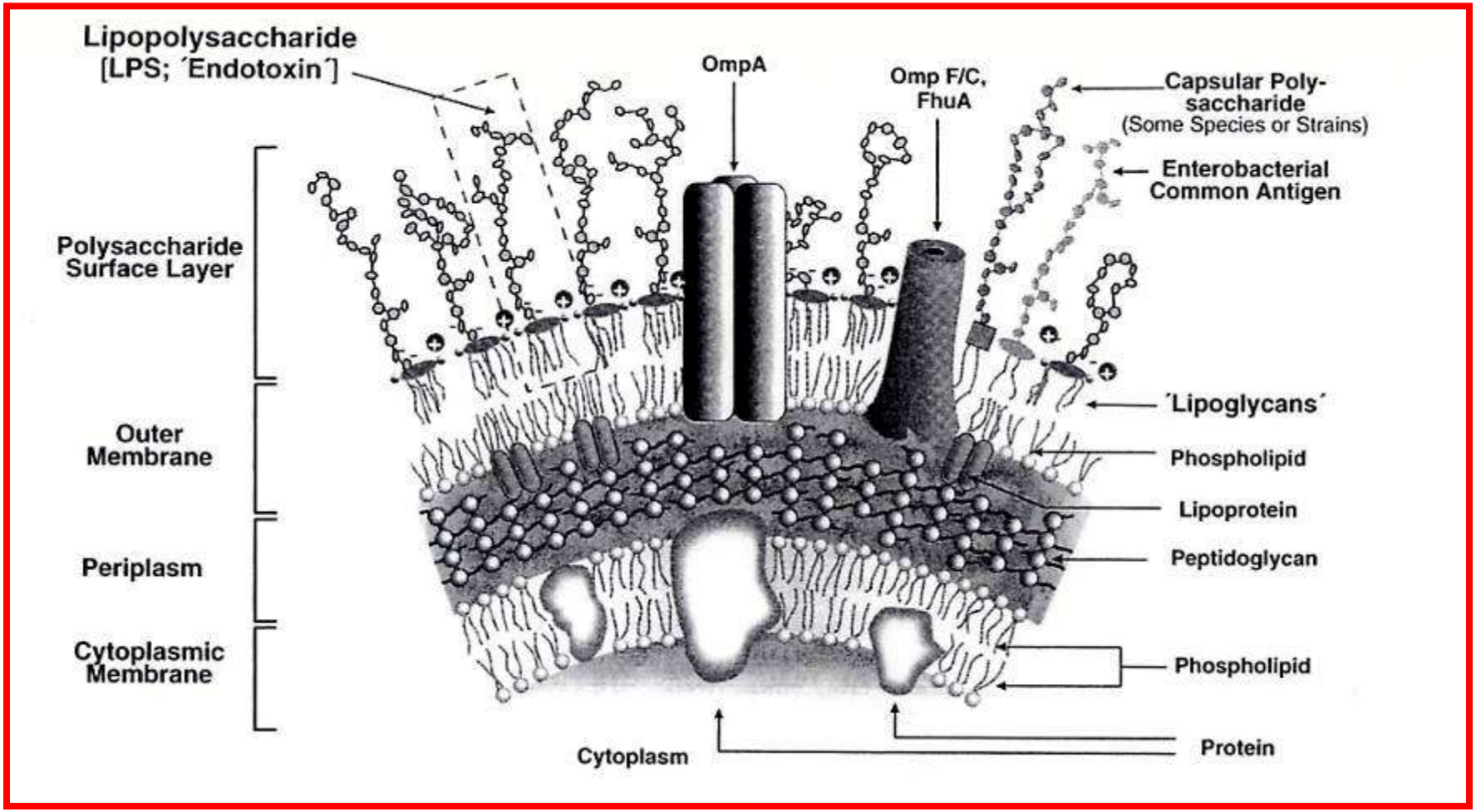
Klebsiella pneumoniae CAPSULE (China ink)



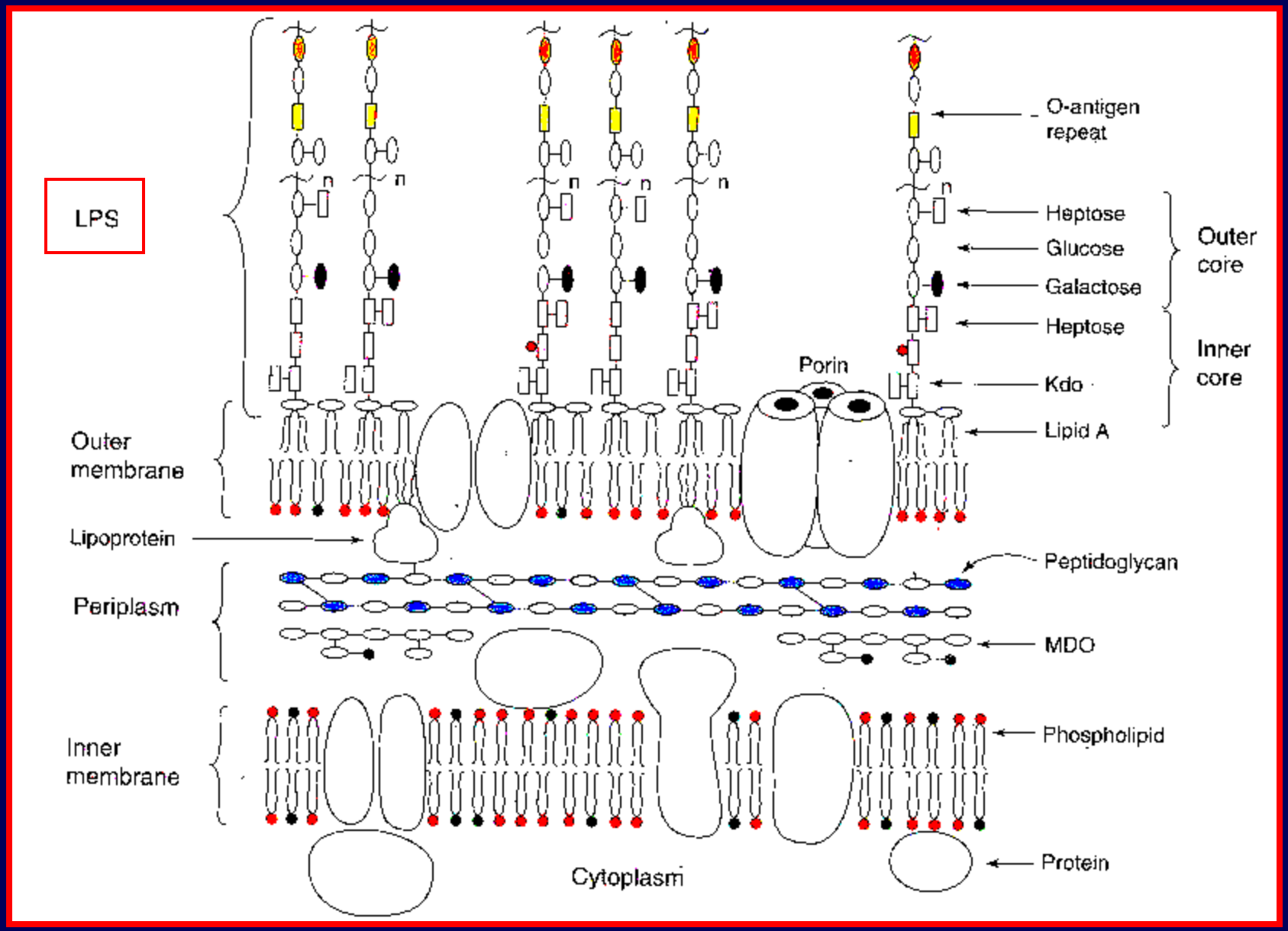
Cell wall of Gram-negative bacteria



Organization of surface in gram-negative bacteria

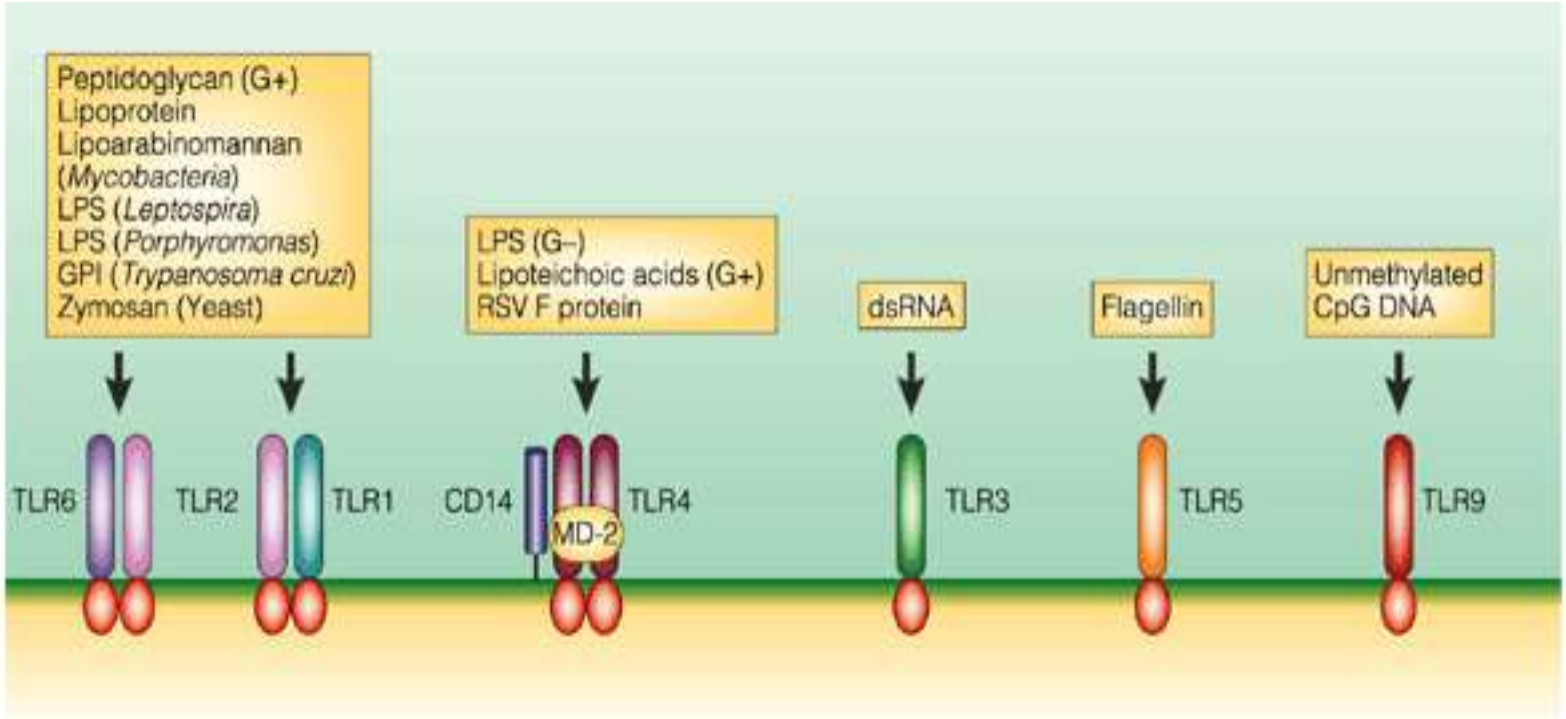


SURFACE COMPOSITION OF GRAM-BACTERIA



Toll-like receptors (TLRs)

- a family of transmembrane receptors homologous to *Drosophila* Toll (role in development and immunity)
- cell surface signalling complexes
- induction of immune response – transcription of genes involved in host defence, dendritic cell maturation
- different tissue expression – way of regulation of immune response



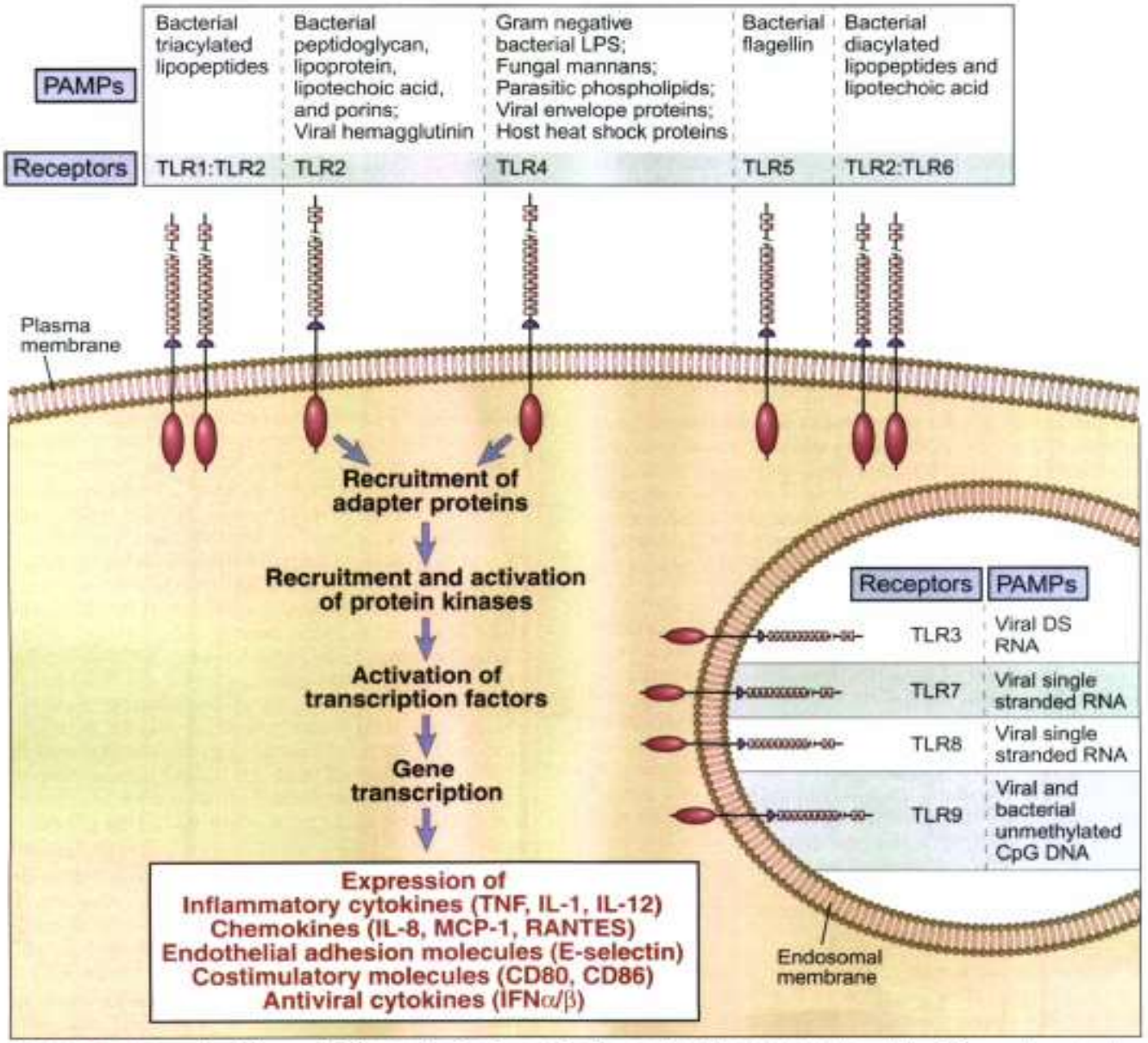
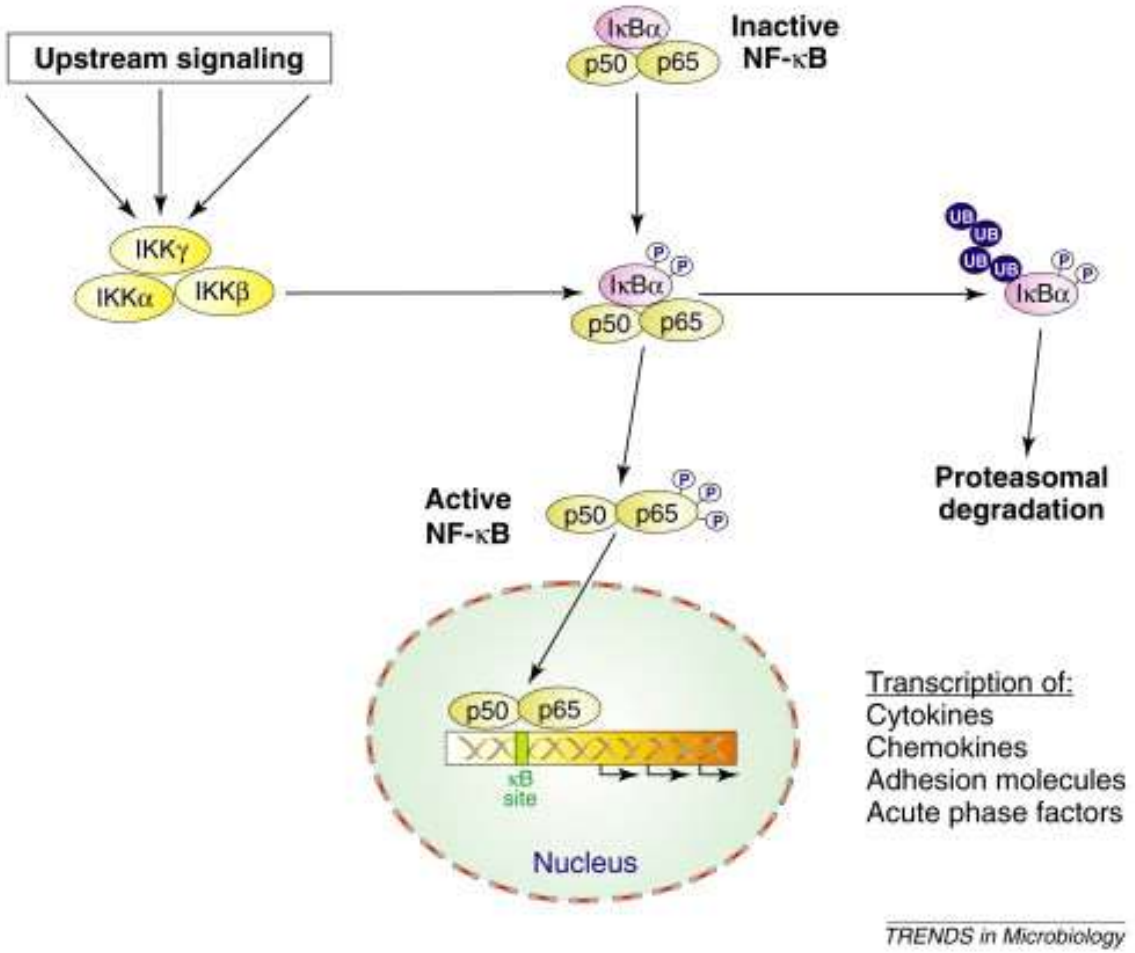


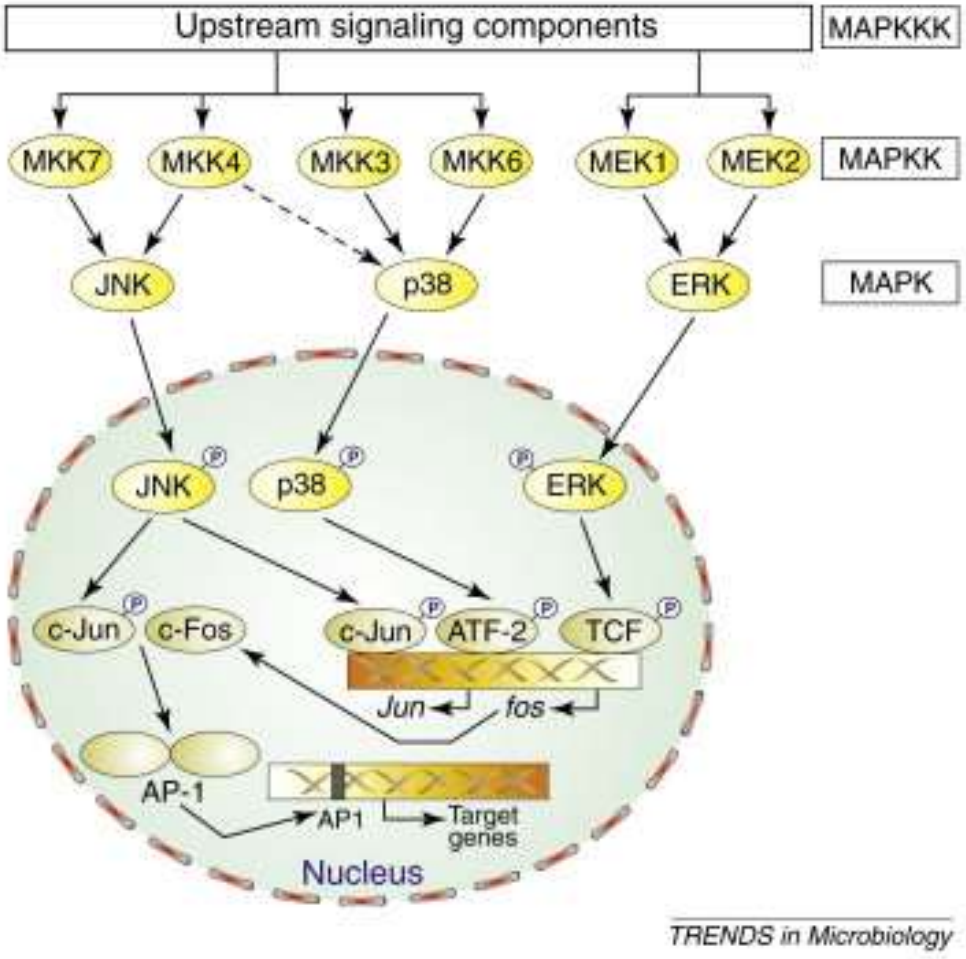
FIGURE 2–2 Mammalian TLRs: specificities, basic signaling mechanisms, and cellular responses. Ligands for TLRs are shown together with dimers of the TLRs that specially bind them. Note that some TLRs are expressed in endosomes and some on the cell surface (see Fig. 2–7). The basic steps in TLR signaling, illustrated only for TLR3 and TLR4, are applicable to all TLRs. Further details about the signaling pathways are described in Box 2–1.

Regulation of NF-κB activity

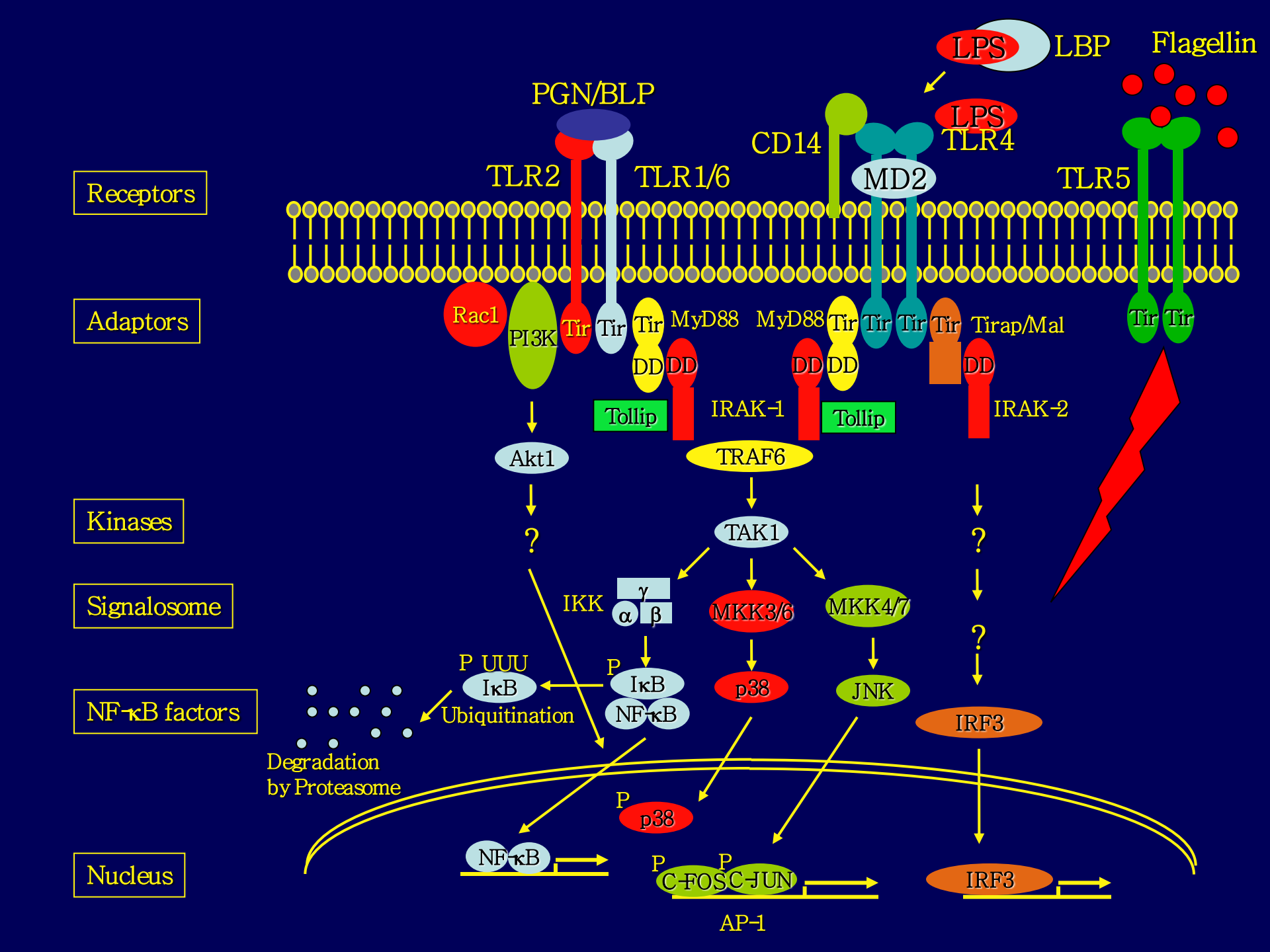


Upon stimulation by multiple signaling processes, the active complex of the p50/p65 subunits translocates into the nucleus and binds to the NF-κB consensus DNA sequence. Various mechanisms can trigger the nuclear activity of NF-κB as indicated. Post-translational modification of proteins, including phosphorylation (P) and ubiquitinylation (UB), regulate NF-κB activity.

Molecular pathways leading to AP-1 activation

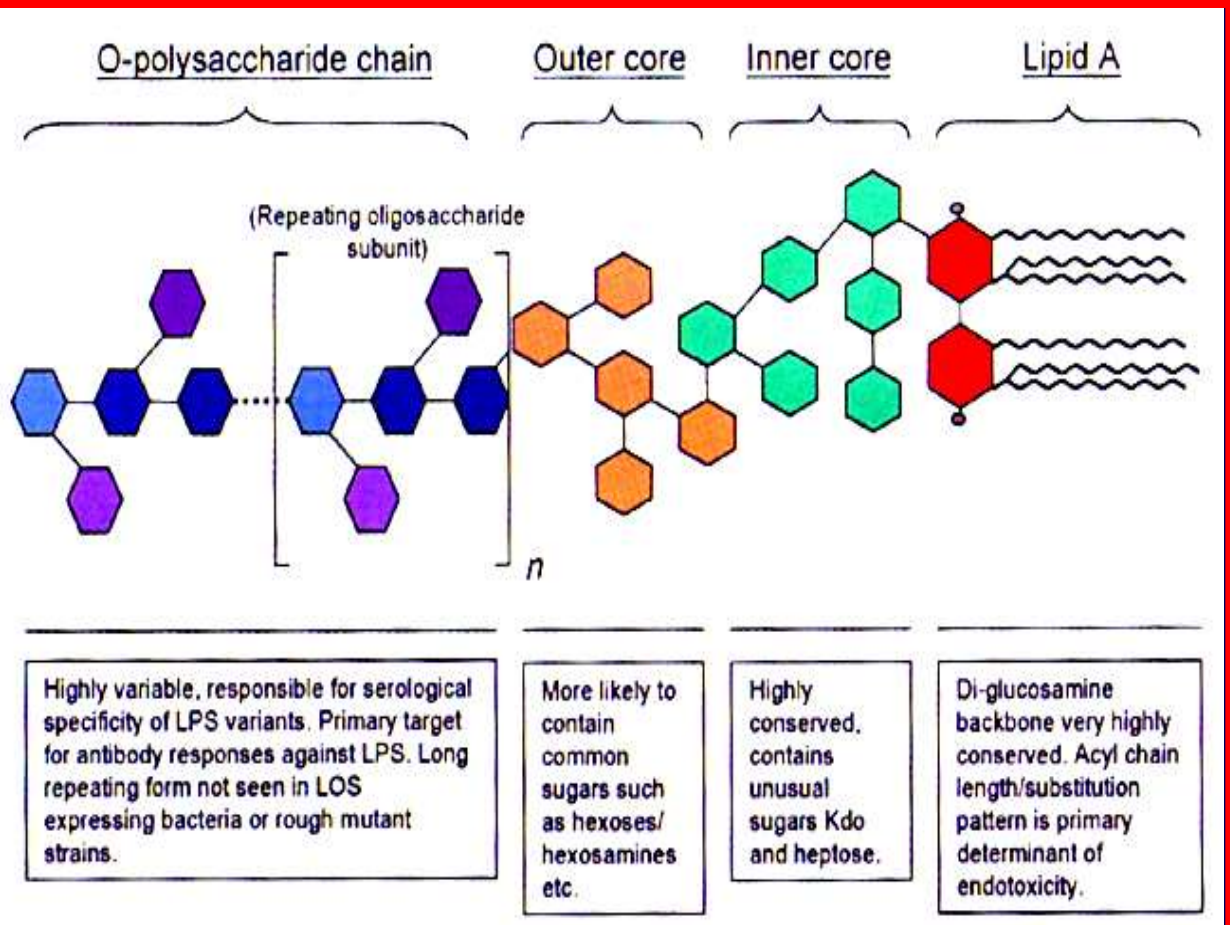


AP-1 activation is induced by major MAPK pathways as indicated. These include JNK, p38 and ERK, which can translocate in the nucleus, phosphorylate and activate diverse transcription factors including c-Jun, ATF-2 (activating transcription factor 2) and TCF (ternary-complex factor). p38 phosphorylates ATF-2 and contributes together with c-Jun to the activation of the Jun promoter. Activation of TCF is regulated by ERK kinase leading to the expression of Fos. Expression of c-Jun and c-Fos leads to the assembly of the AP-1 transcription factor complex and regulation of AP-1 target genes.

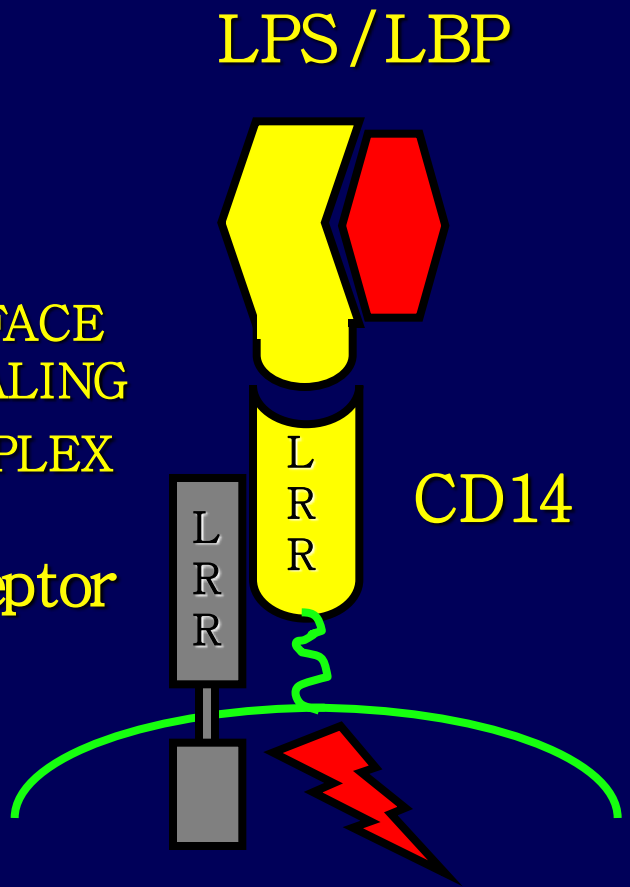

LPS
LBP
Flagellin
PGN/BLP
LPS
CD14
TLR4
TLR2
TLR1/6
MD2
TLR5
Receptors
Adaptors
Kinases
Signalosome
NF-κB factors
Nucleus
Rac1
PI3K
Tir
Tir
Tir
MyD88
MyD88
Tir
Tir
Tir
Tir
Tirap/Mal
Tir
Tir
DD
DD
DD
DD
DD
Tollip
IRAK-1
Tollip
IRAK-2
Akt1
TRAF6
?
TAK1
?
?
IKK
γ
α
β
MKK3/6
MKK4/7
P
UUU
IκB
Ubiquitination
P
IκB
NF-κB
p38
JNK
IRF3
Degradation
by Proteasome
P
p38
NF-κB
P
P
C-FOS
C-JUN
AP-1
IRF3

Lipopolysaccharide (LPS) receptor signaling

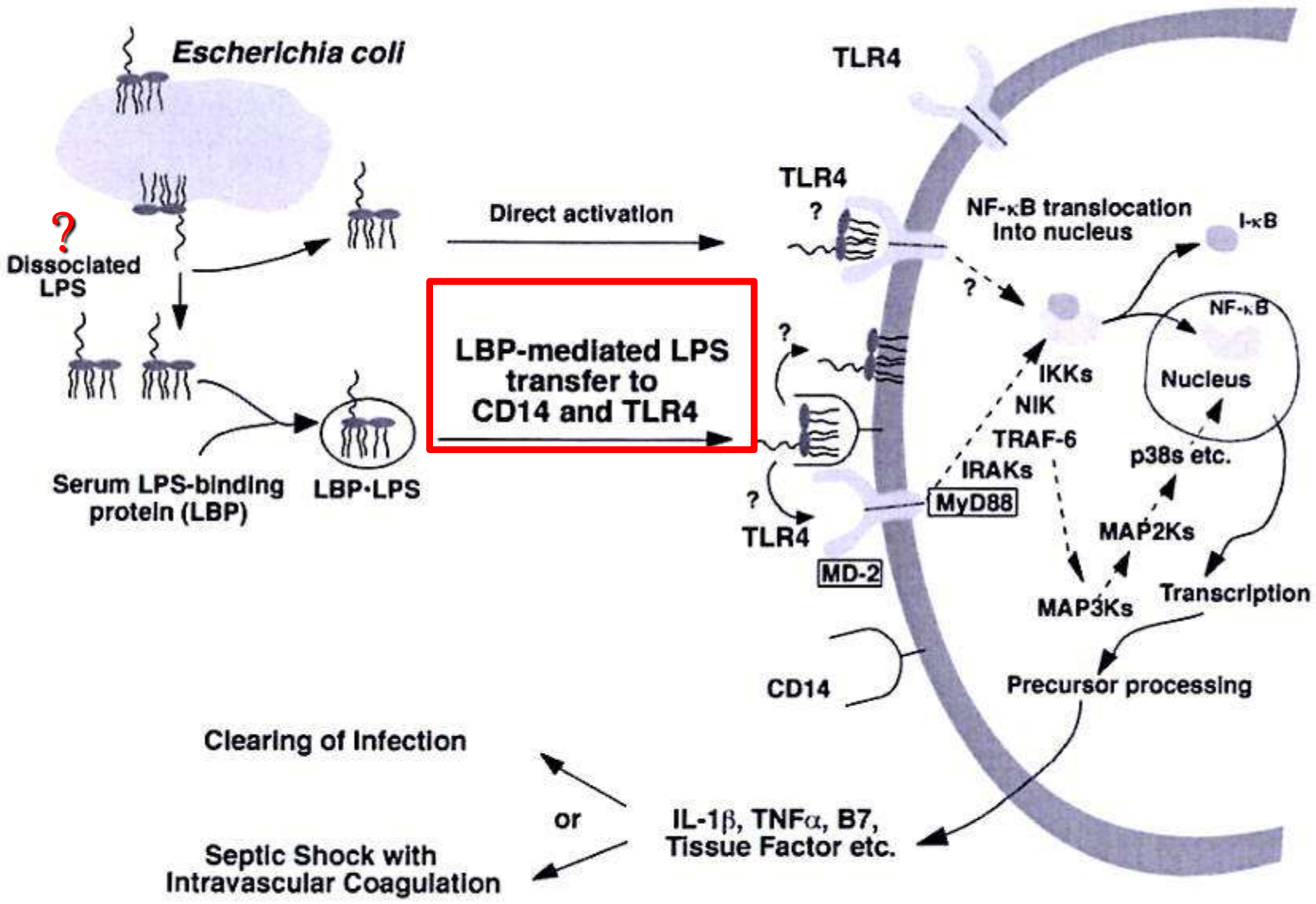
COMPOSITION OF LIPOPOLYSACCHARIDE IN ENTEROBACTERIACEAE

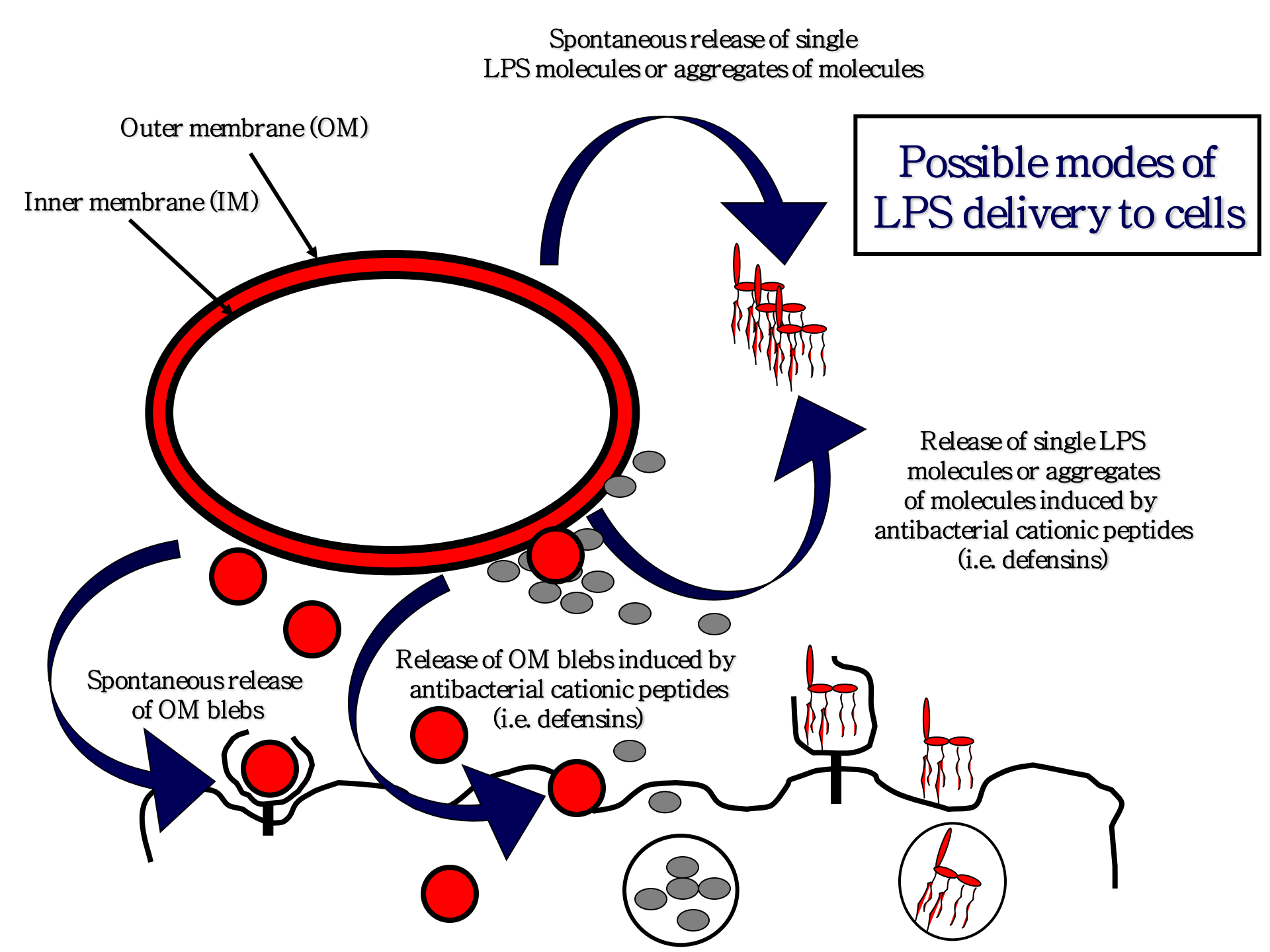


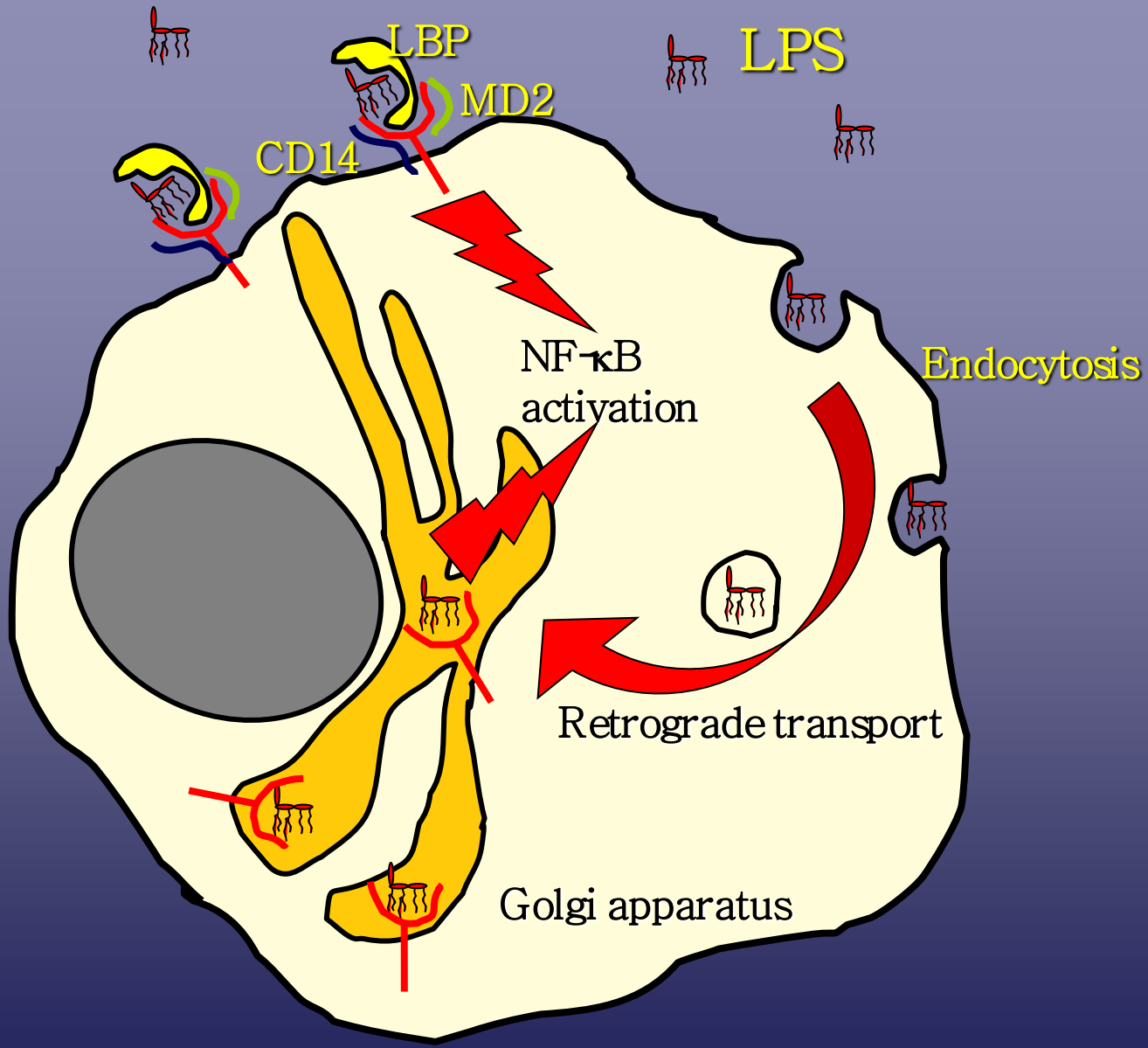
SIGNALIZATION IN RESPONSE TO PAMPs



C3H/HeJ mice that have a mutation in
the cytoplasmic domain of TLR4
are resistant to LPS-induced inflammation and shock.

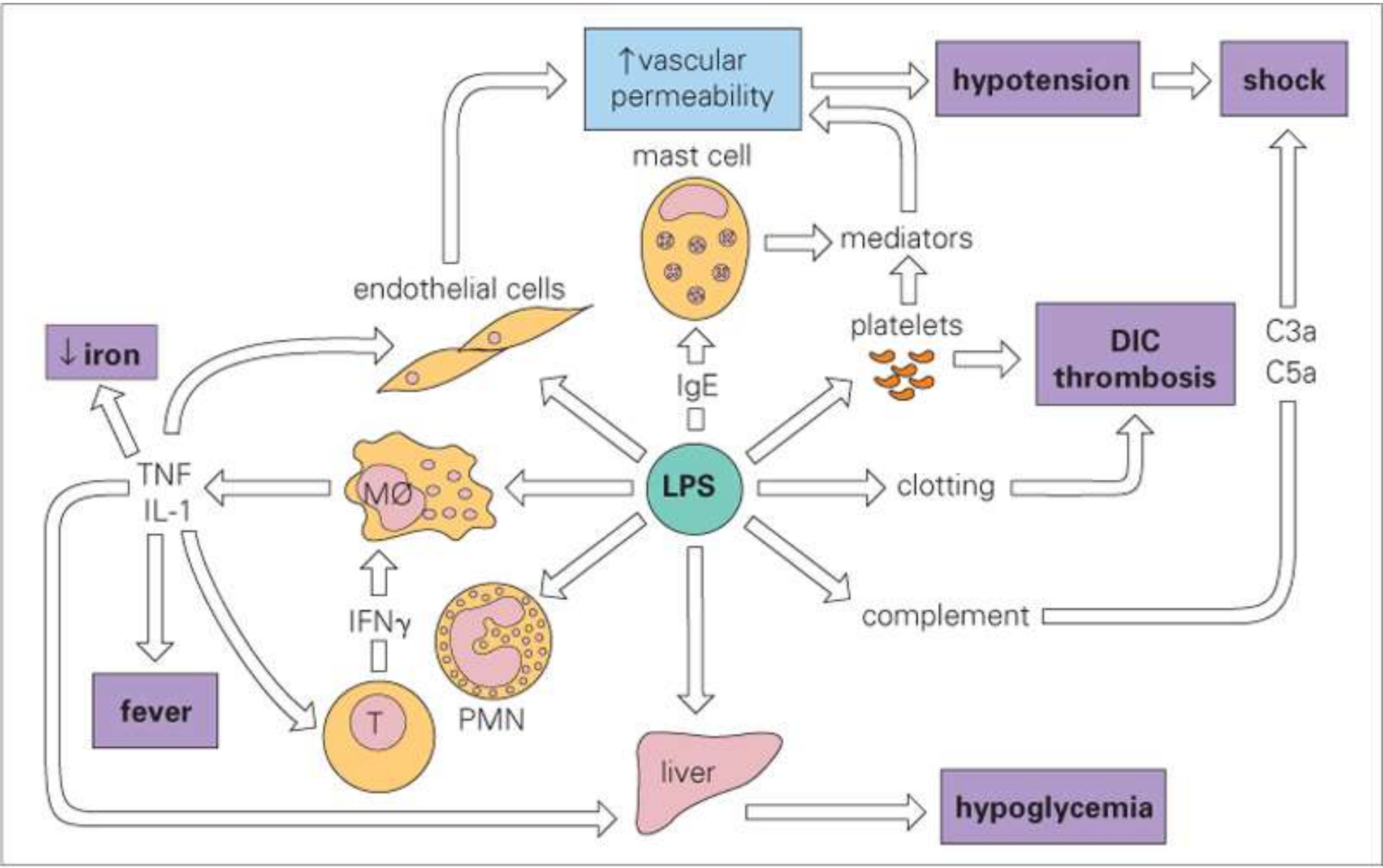

Escherichia coli
?
Dissociated LPS
Serum LPS-binding protein (LBP)
LBP·LPS
Direct activation
LBP-mediated LPS transfer to CD14 and TLR4
TLR4
TLR4
?
NF-κB translocation into nucleus
I-κB
NF-κB
Nucleus
IKKs
NIK
TRAF-6
IRAKs
p38s etc.
MyD88
MAP2Ks
MAP3Ks
Transcription
Precursor processing
TLR4
MD-2
CD14
Clearing of Infection
or
Septic Shock with Intravascular Coagulation
IL-1β, TNFα, B7, Tissue Factor etc.


Spontaneous release of single
LPS molecules or aggregates of molecules
Outer membrane (OM)
Inner membrane (IM)
Possible modes of
LPS delivery to cells
Release of single LPS
molecules or aggregates
of molecules induced by
antibacterial cationic peptides
(i.e. defensins)
Spontaneous release
of OM blebs
Release of OM blebs induced by
antibacterial cationic peptides
(i.e. defensins)

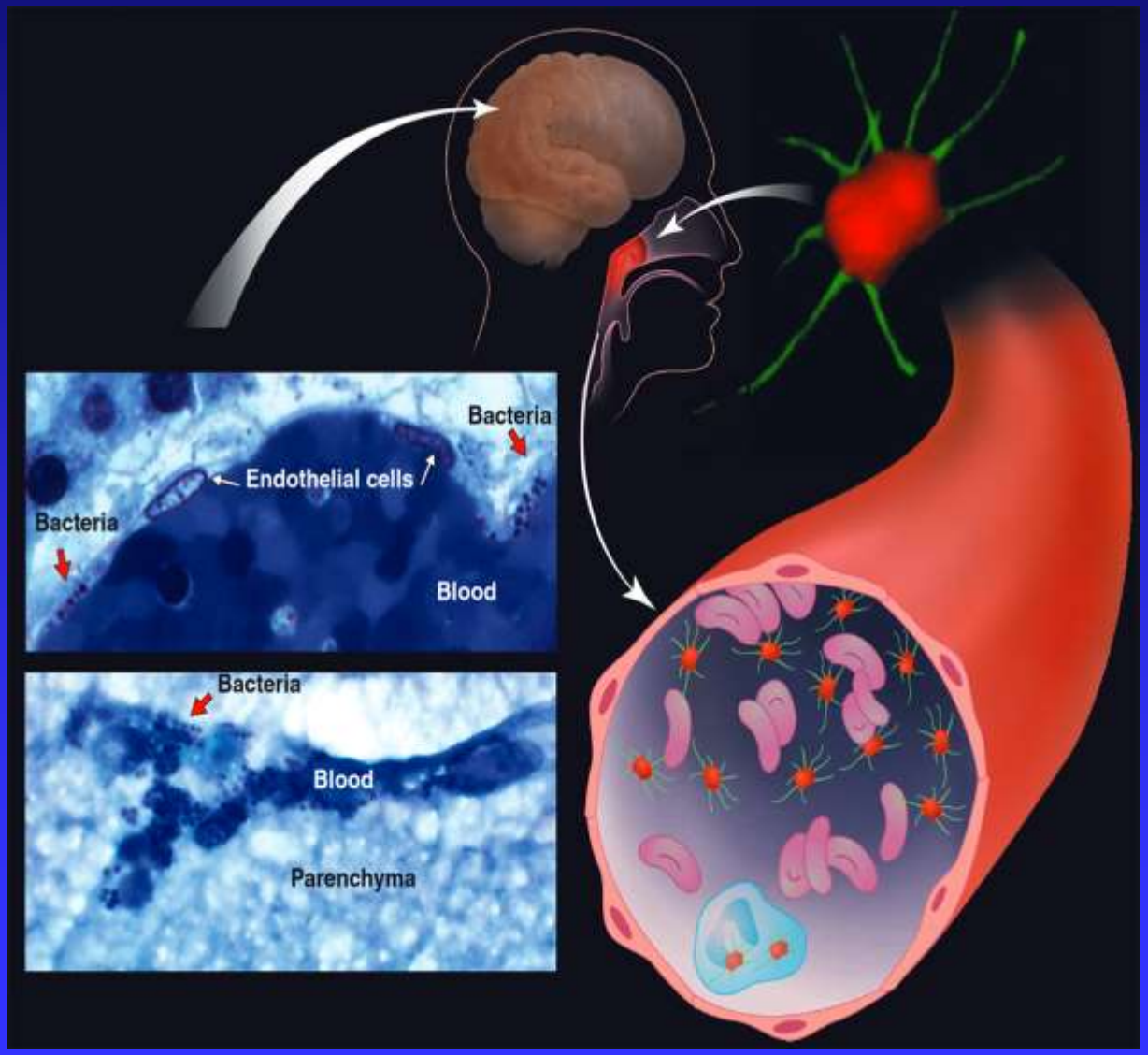


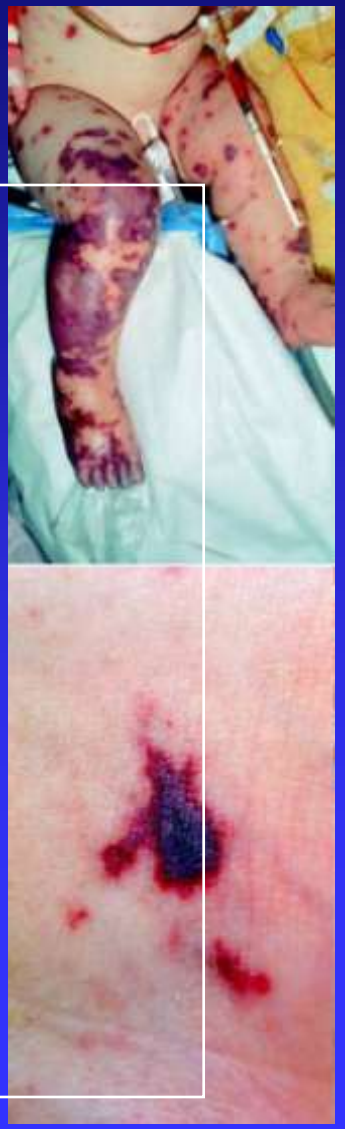
Possible sites of TLR4-mediated recognition of LPS.

Endotoxin signaling and septic shock



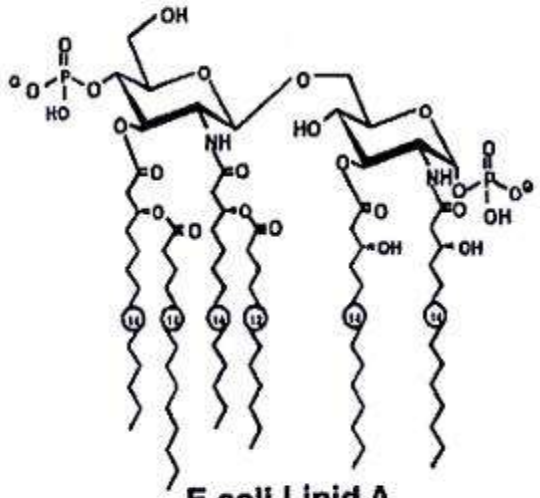
However, occasionally „traffic accident“ occurs , due to environmental factors, viral co-infection, unmatching genotypes of bug and host .... **invasive meningococcal disease, can develop and kill...- dead-end for meningococcus as well**





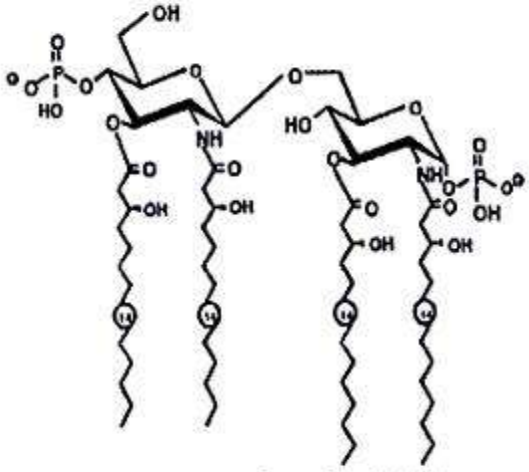
Agonistic vs. Antagonistic Lipid A

Agonistic Lipid A

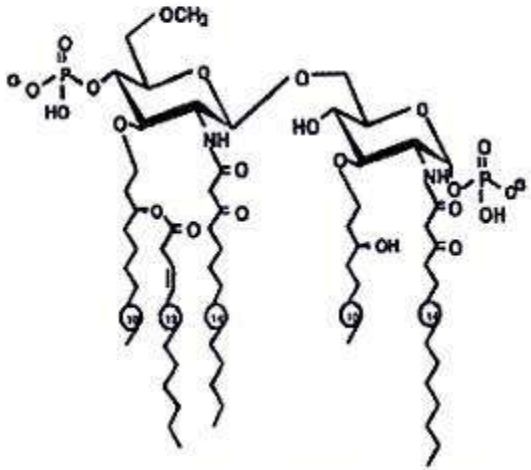


E.coli Lipid A
Compound 506

Antagonistic Lipid A Analogs

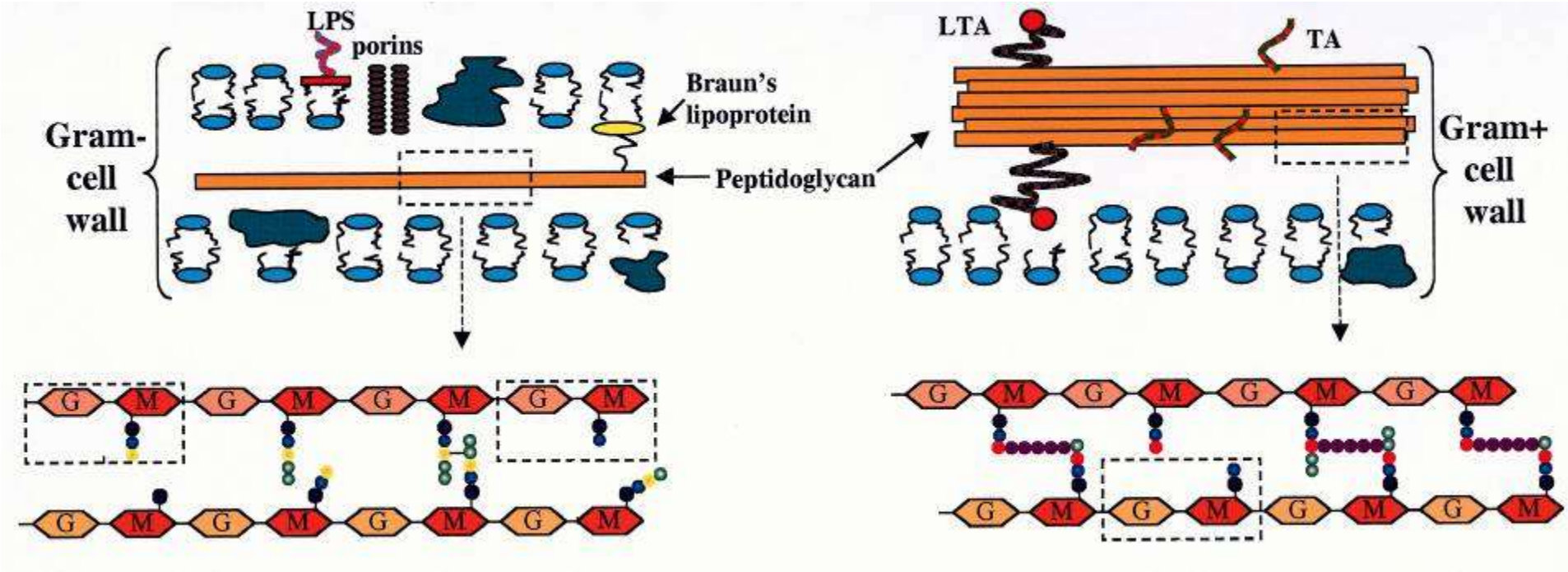


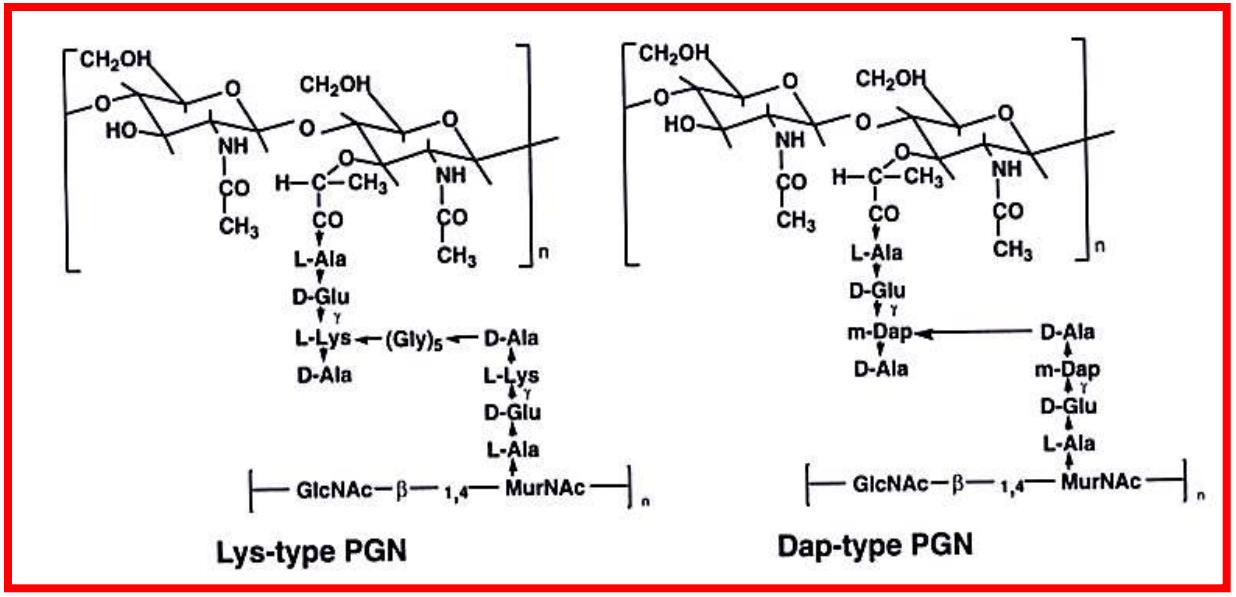
Precursor Ia Lipid IV_A
Compound 406

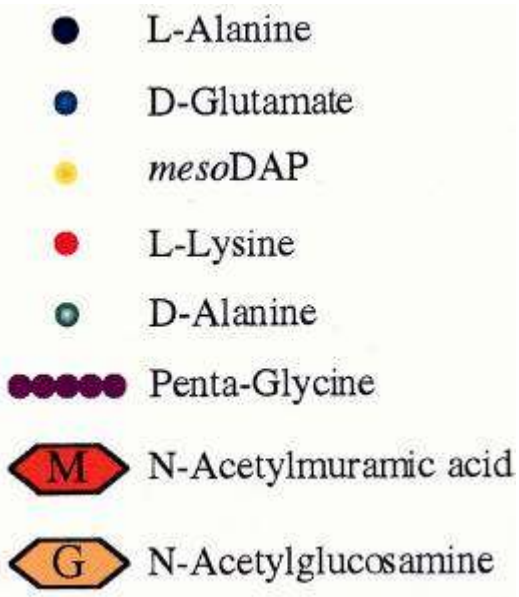


R.spharoides Lipid A Analog
Compound E5531

Peptidoglycan (PGN)

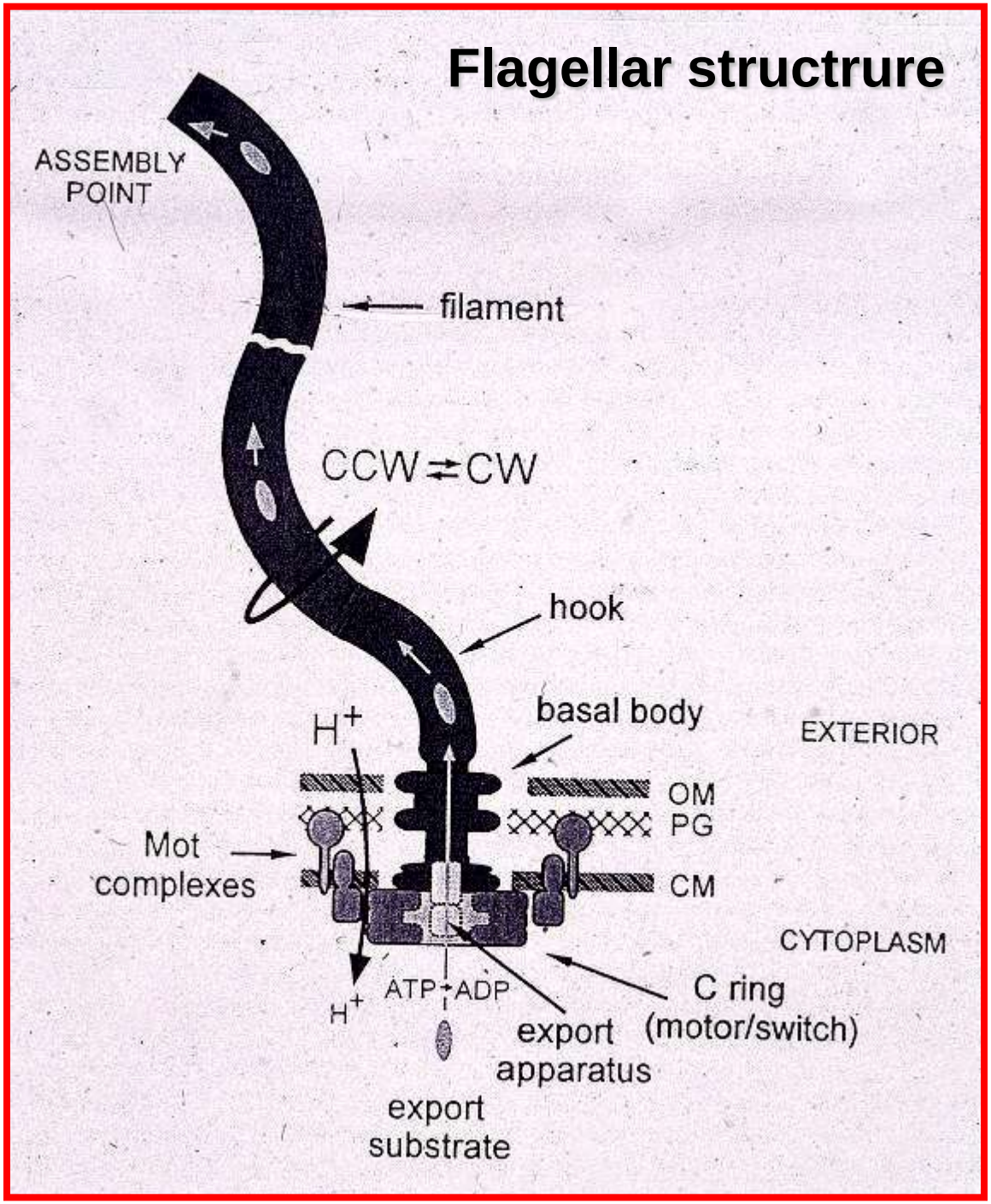

LPS
porins
Braun's lipoprotein
Gram- cell wall
Peptidoglycan
LTA
TA
Gram+ cell wall
G
M

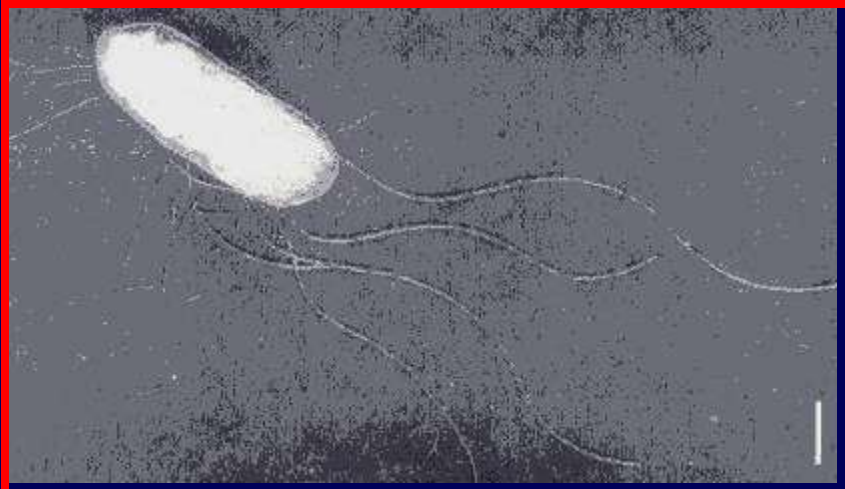

L-Ala
D-Glu
L-Lys
(Gly)5
D-Ala
GlcNAc
MurNAc
Lys-type PGN
m-Dap
Dap-type PGN

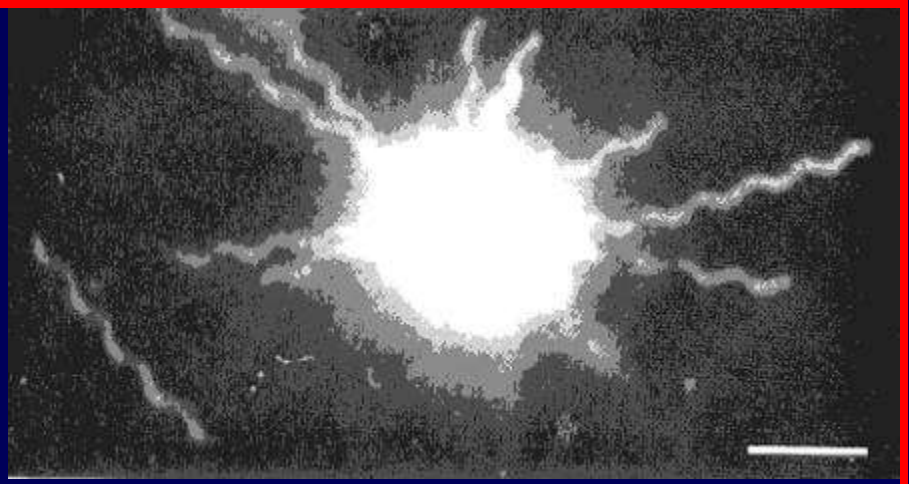

L-Alanine
D-Glutamate
mesoDAP
L-Lysine
D-Alanine
Penta-Glycine
N-Acetylmuramic acid
N-Acetylglucosamine

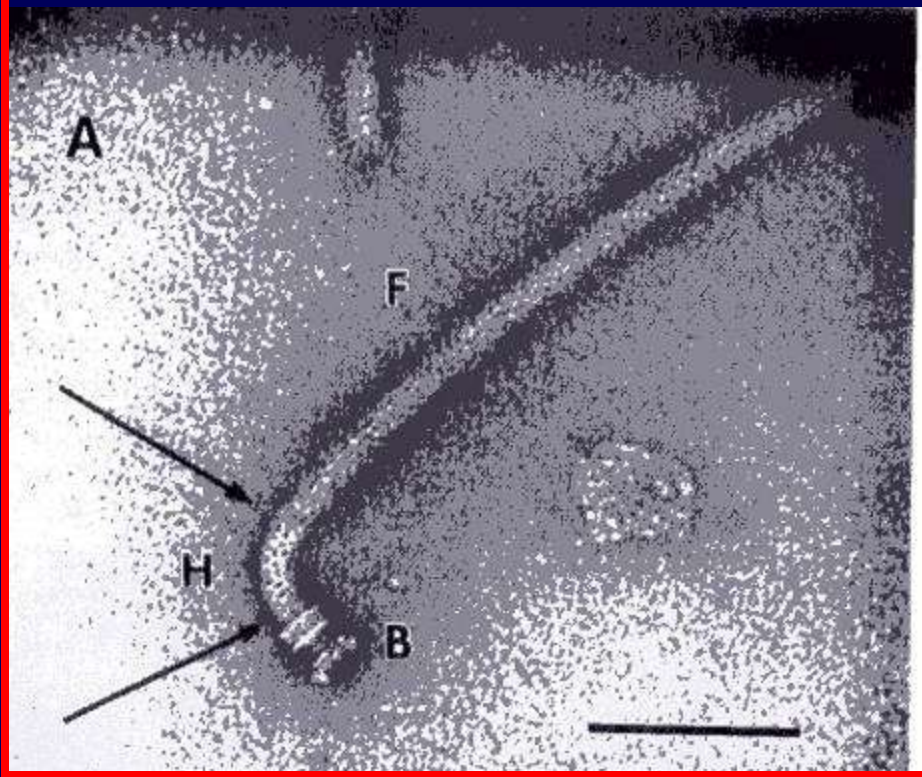
Flagellin

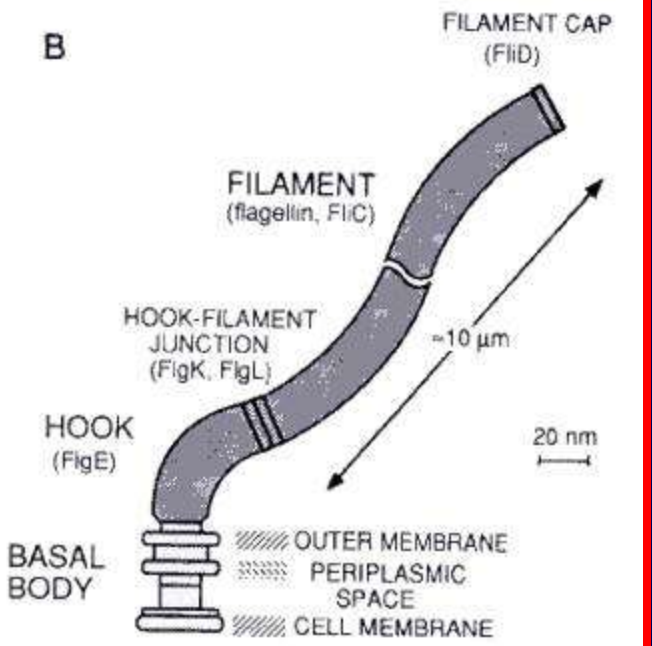
Flagellar structrure








A
F
H
B

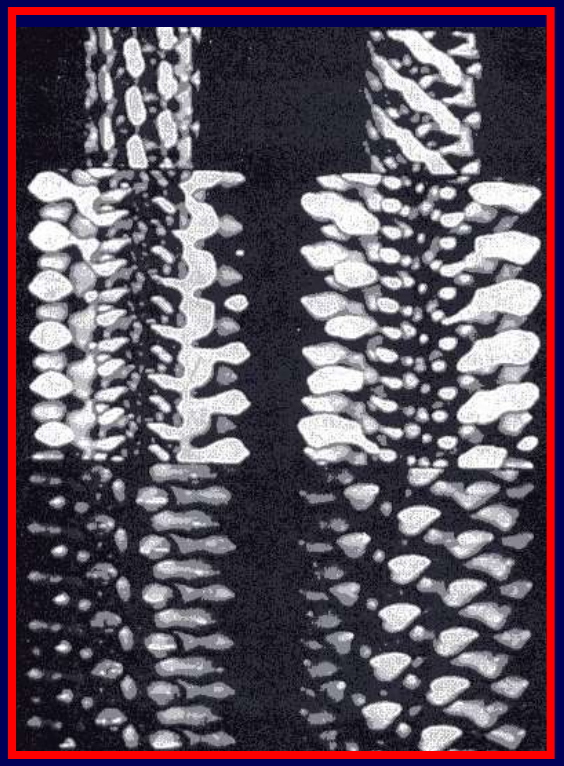

B
FILAMENT CAP
(FliD)
FILAMENT
(flagellin, FliC)
HOOK-FILAMENT
JUNCTION
(FlgK, FlgL)
~10 µm
HOOK
(FlgE)
20 nm
BASAL
BODY
OUTER MEMBRANE
PERIPLASMIC
SPACE
CELL MEMBRANE

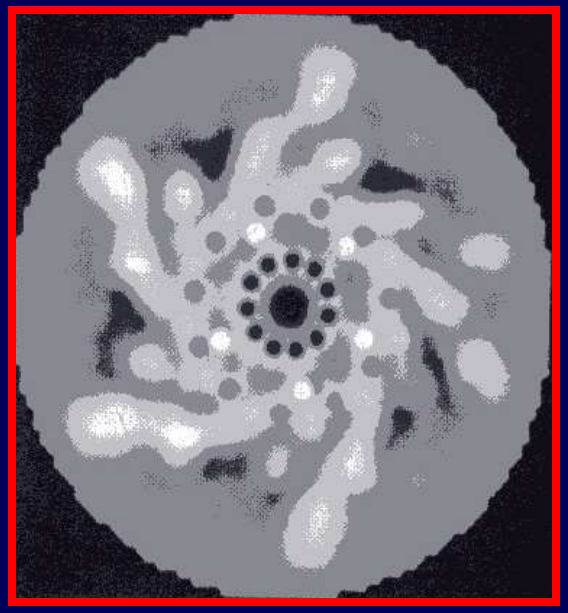
Flagellum

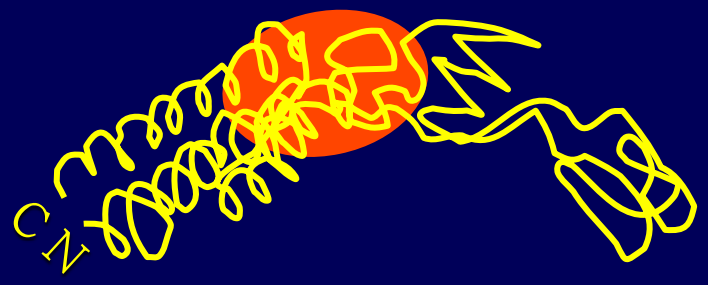
direct interaction between individual molecules of flagellin and LRR of TLR5

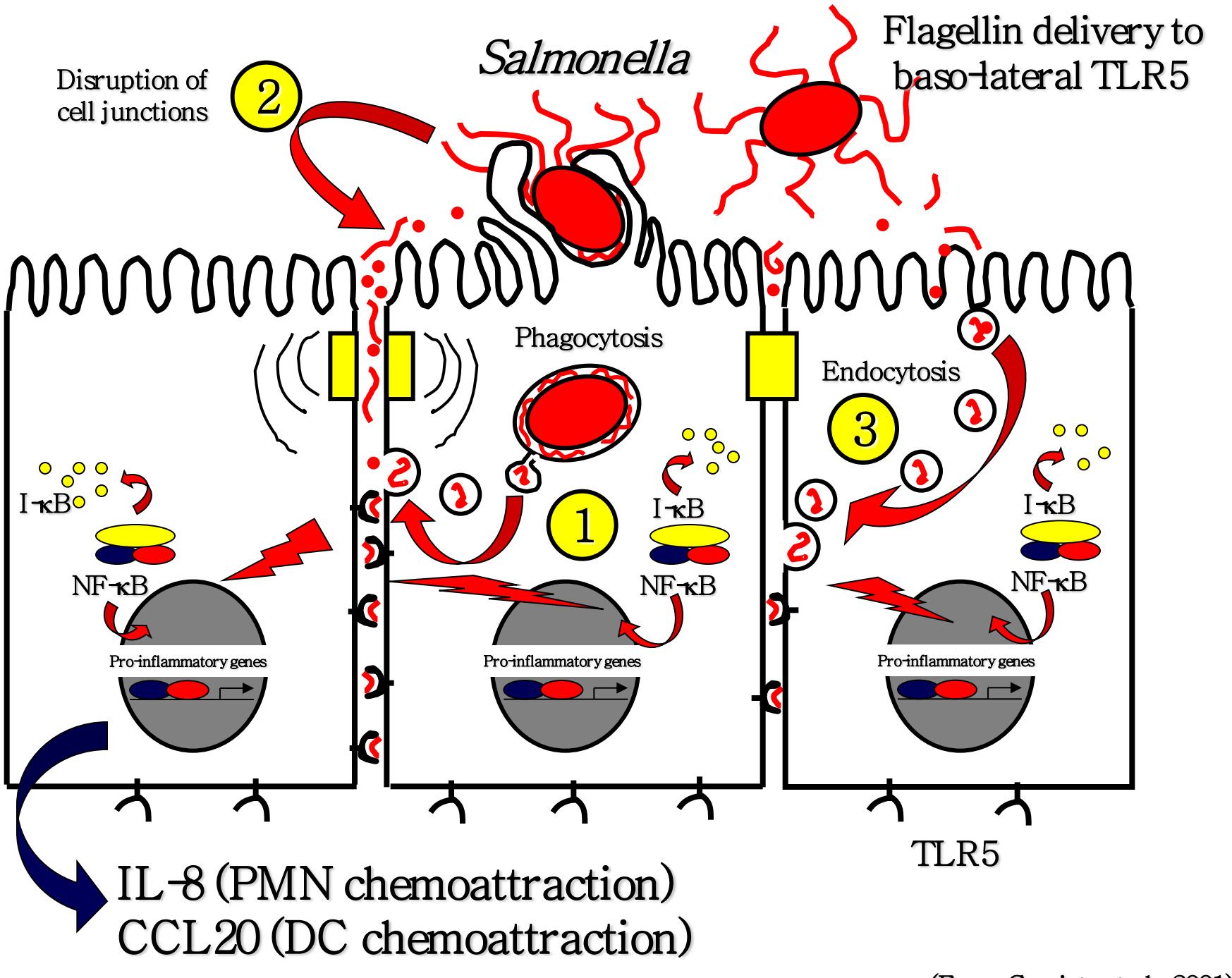
Detection of flagellin by TLR5 occurs at 100 fM !

Specificity of recognition involves a highly conserved structure



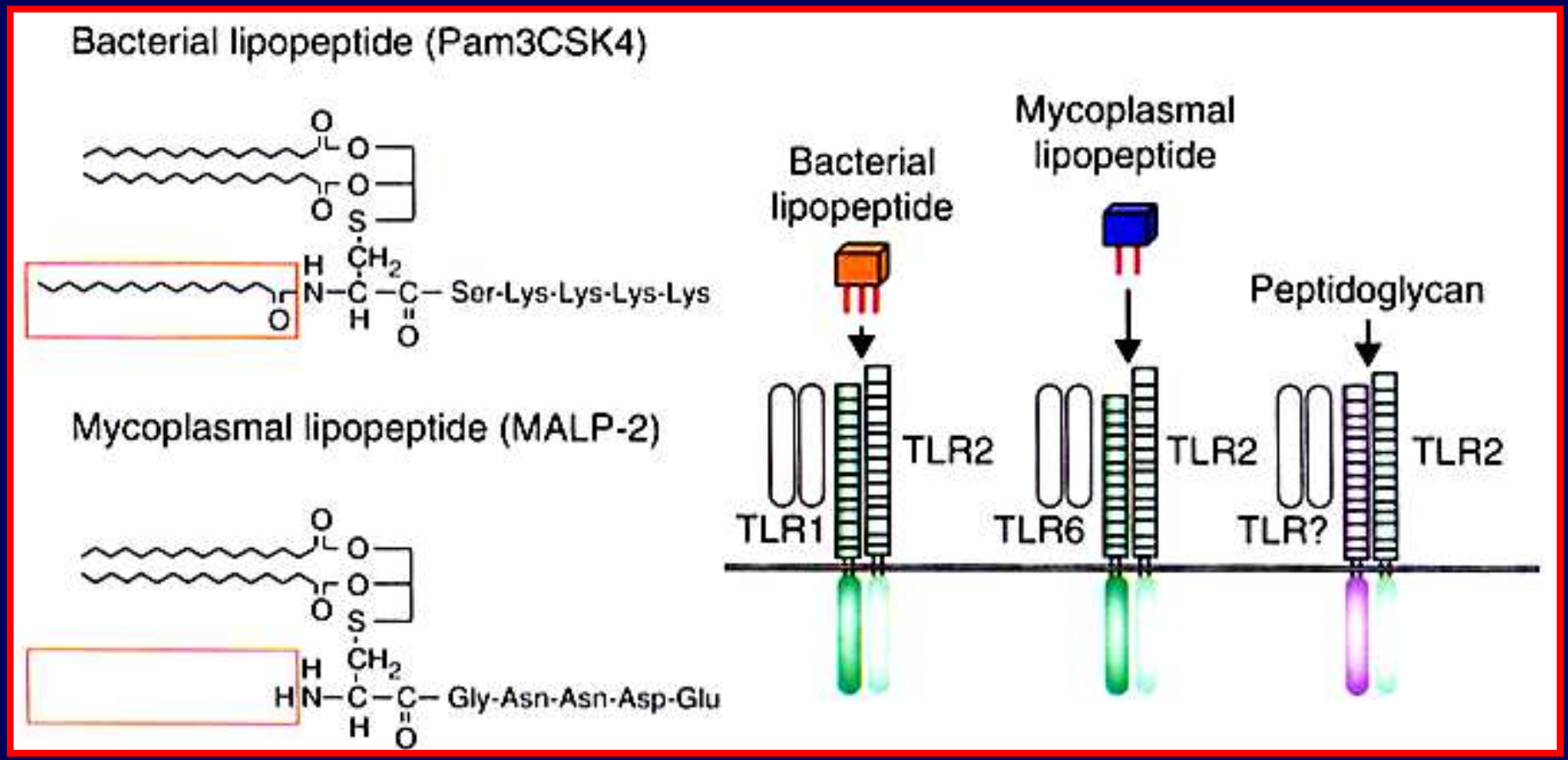






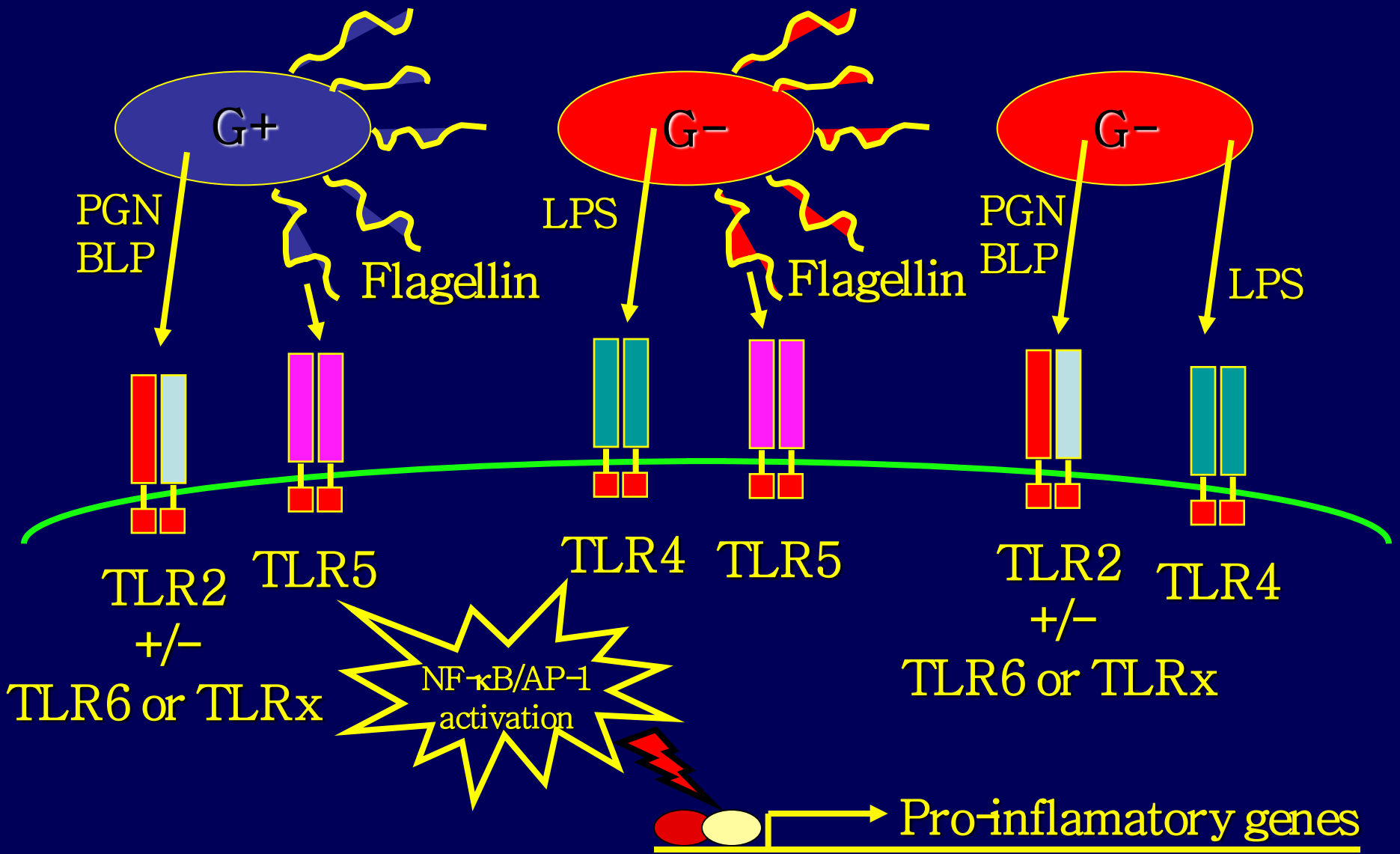
(From Gewirtz et al., 2001)

Bacterial lipoproteins (BLP)



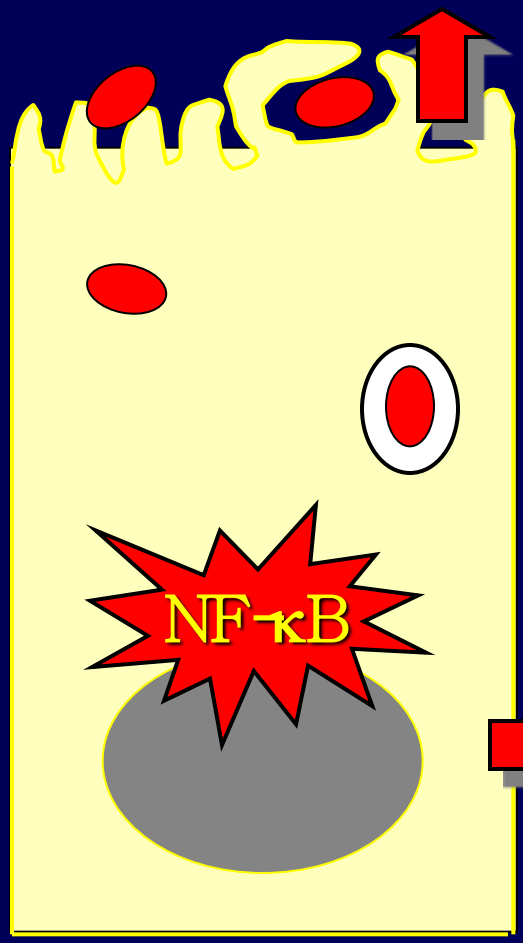
-Lipoylation of amino-terminal cysteinyl residue by DAG moiety via a thioeter bond is common to all bacterial lipopeptides (BLP).
-Immunostimulating activity is attributed to lipid moiety.
-Some BLP undergo further acylation at cysteinyl residue via an amide bond (e.g. Braun's lipoprotein in *E.coli*).

INTEGRATION OF BACTERIAL DETECTION PROCESSES THROUGH A COMBINATION OF TOLL-LIKE RECEPTORS (TLR)



EPITHELIAL CELL RESPONSES TO PATHOGENS

PEEC
TNFα



INV−: H.pylori, UPEC, EPEC, EAEC
INV+ : Shigella, Salmonella, Yersinia, N.gonorhoeae, L.monocytogenes

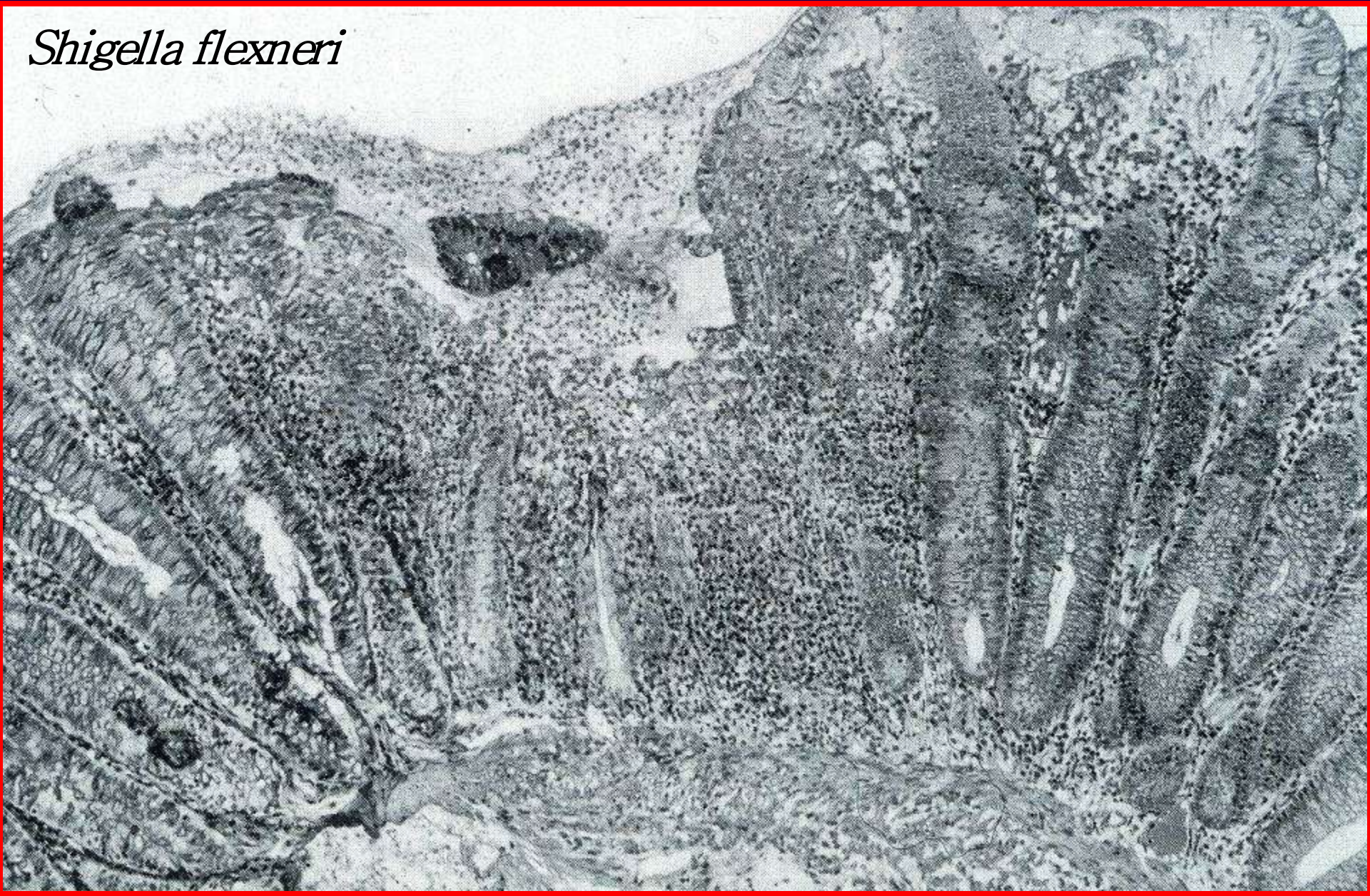
CXC chemokines (chemoattraction)
IL-8, CXCL1-3, ENA78: PMN
IP10, Mig, I-TAC: CD4+ Th1 memory T cells

CC chemokines (chemoattraction)
MCP-1, MIP-1b: Macrophages, T cells
CCL20: Immature Dendritic Cells
RANTES: T cells, Eosinophils, Macrophages

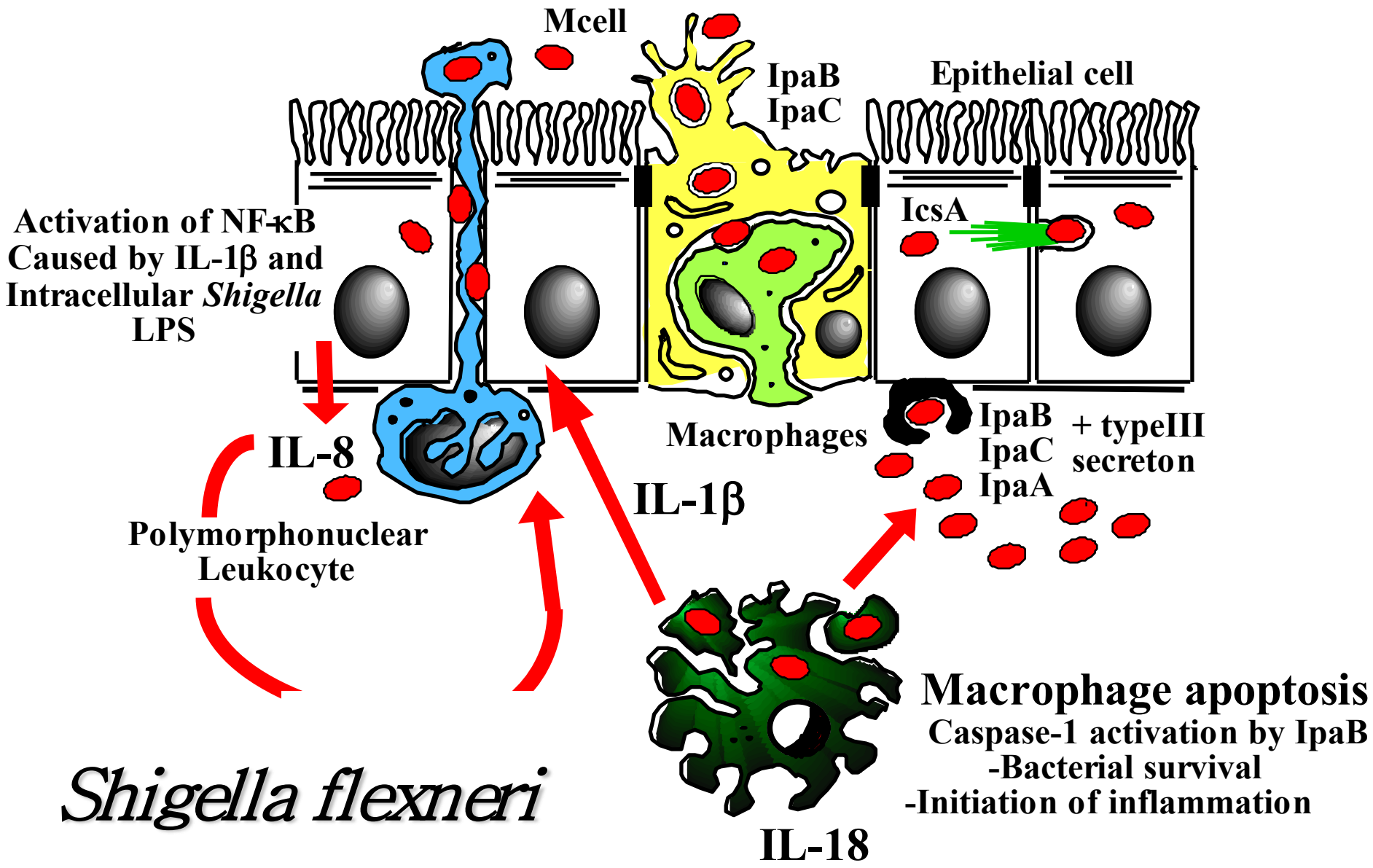
Cytokines (differenciation, proliferation, activation)
G-CSF: PMN & Precursors
GM-CSF: PMN, Macrophages
IL-6: Macrophages, B cells, Fibroblasts
TNFα, Macrophages, Fibroblasts, T cells

Eckmann & Kagnoff
Svanborg et al.

Bacillary dysentery: massive PMN infiltrate, abscess/ulcer



How are intracellular bacteria sensed by innate immunity ?



Intracellular pattern recognition receptors

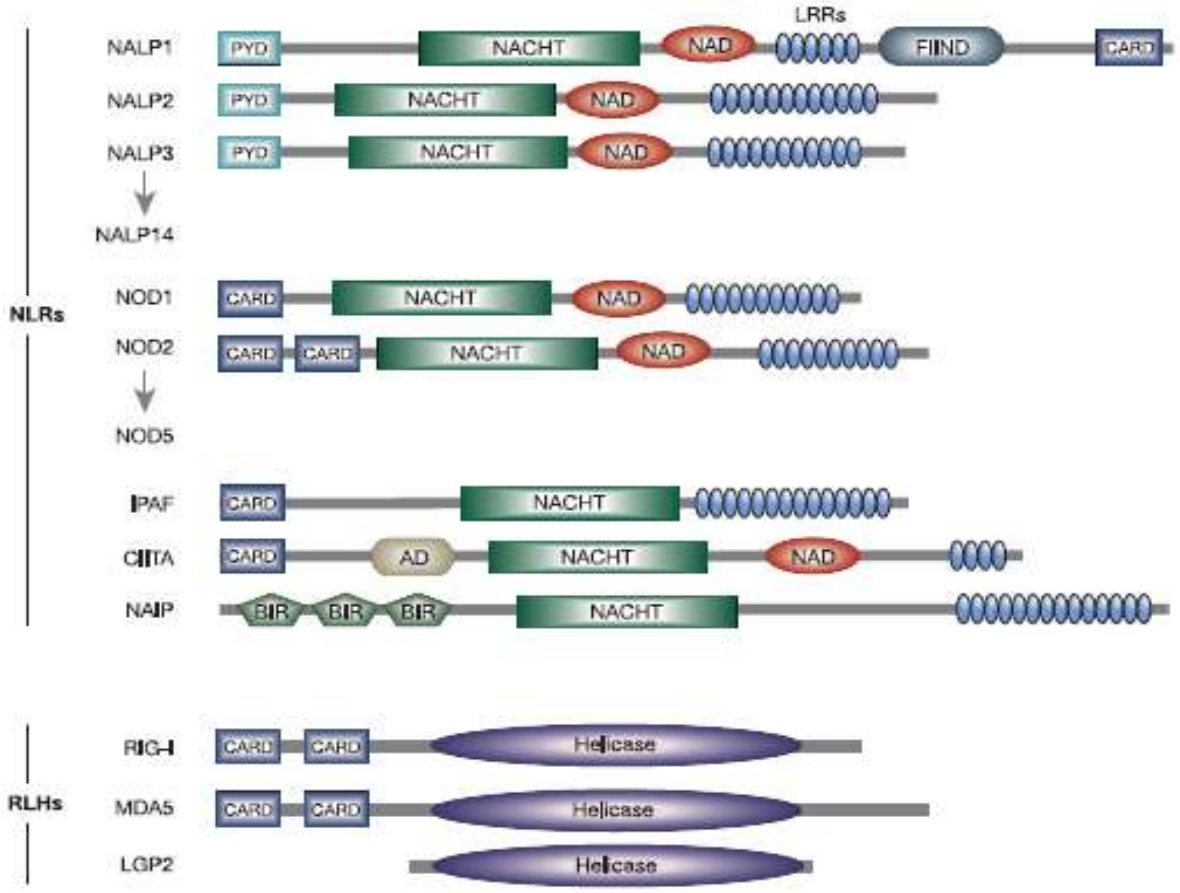


Figure 1 | Intracellular sensors of bacteria, viruses and danger signals. NOD-like receptors (NLRs) are characterized by three distinct domains: the putative ligand-sensing leucine-rich repeats (LRRs); the NACHT domain, which mediates oligomerization; and an effector domain, which can be a pyrin domain (PYD), a CARD (caspase recruitment domain) or a BIR (baculovirus IAP repeat) domain. Most of the NLRs also contain a NACHT-associated domain (NAD). NLRs comprise two large subfamilies: 14 members of the PYD-containing NALP clan and five members of the mostly CARD-containing NODs (NOD5 does not contain a typical CARD domain). The two CARD-containing proteins CIITA and IPAF, and the BIR-containing NAIP protein, constitute the remaining NLR members. RIG-like helicases (RLHs) contain a helicase and two CARD domains, except for LGP2 where the CARDs are absent. Additional abbreviations: FIIND, function to find; AD, activation domain.

(Meylan et al., 2006)

Intracellular pattern recognition receptors

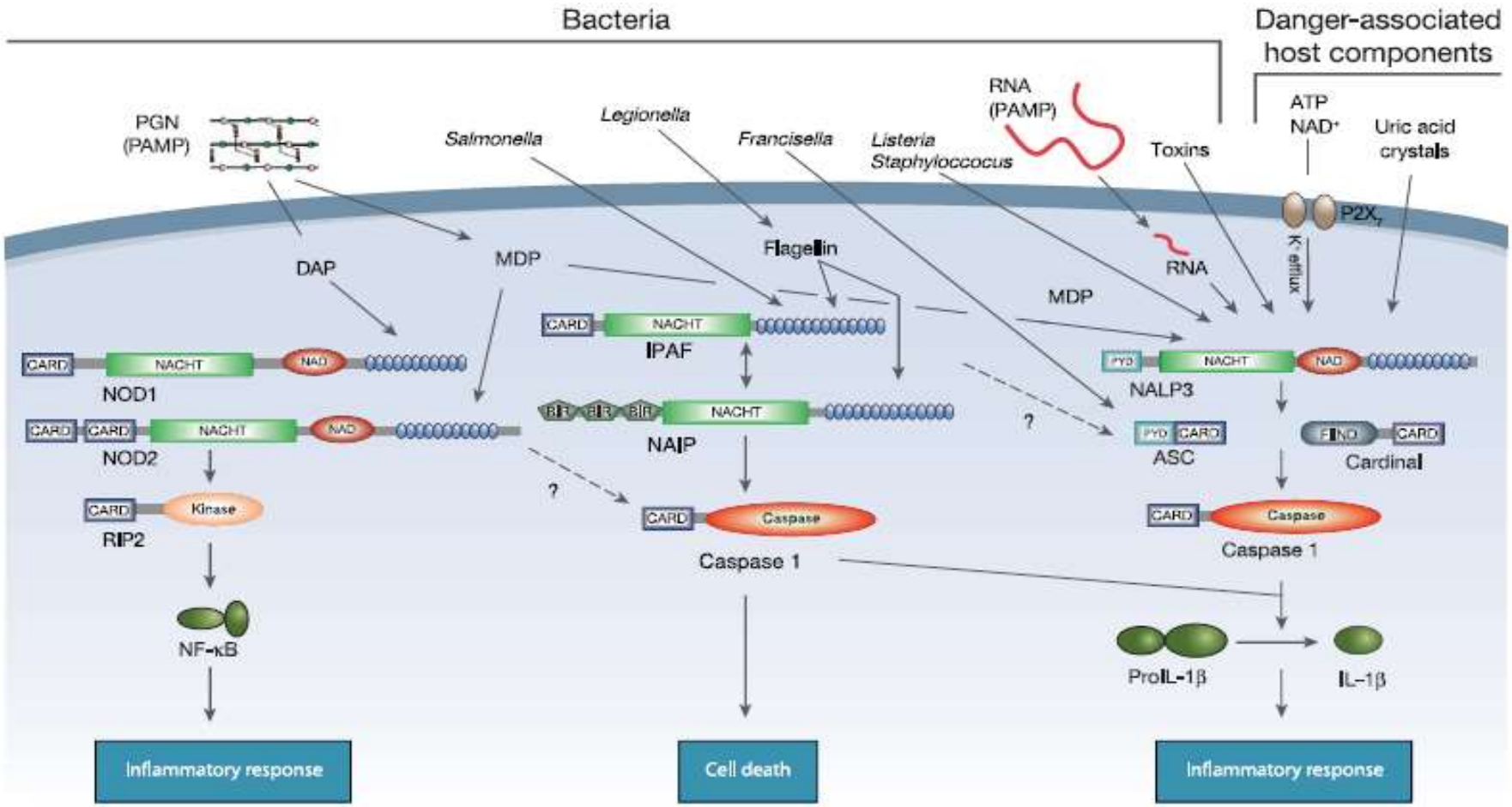


Figure 2 | Activation of NLRs by bacterial and host-derived components. On recognition of peptidoglycan (PGN)-derived molecules (meso-diaminopimelic acid (DAP) and muramyl dipeptide (MDP), respectively), NOD1 and NOD2 recruit RIP2, which, in turn, activates NF-κB. NOD2 activation can lead to caspase 1 activation through a mechanism that remains to be identified. Muramyl dipeptide (and bacterial RNA) also activates the NALP3 inflammasome, which is formed by NALP3, Cardinal, ASC and caspase 1, resulting in the processing of proIL-1β. Endogenous danger signals that activate NALP3 include uric acid crystals and the efflux of K^+, triggered by ATP, NAD^+ or bacterial toxins. Various bacteria trigger activation of distinct NLR pathways. Whereas caspase 1 activation by *Salmonella* and *Legionella* requires IPAF, NAIP (and ASC), but not NALP3, *Listeria* and *Staphylococcus* triggers caspase 1 activation via the NALP3 inflammasome.

(Meylan et al., 2006)

Type I interferon response to viral infection (nucleic acid)

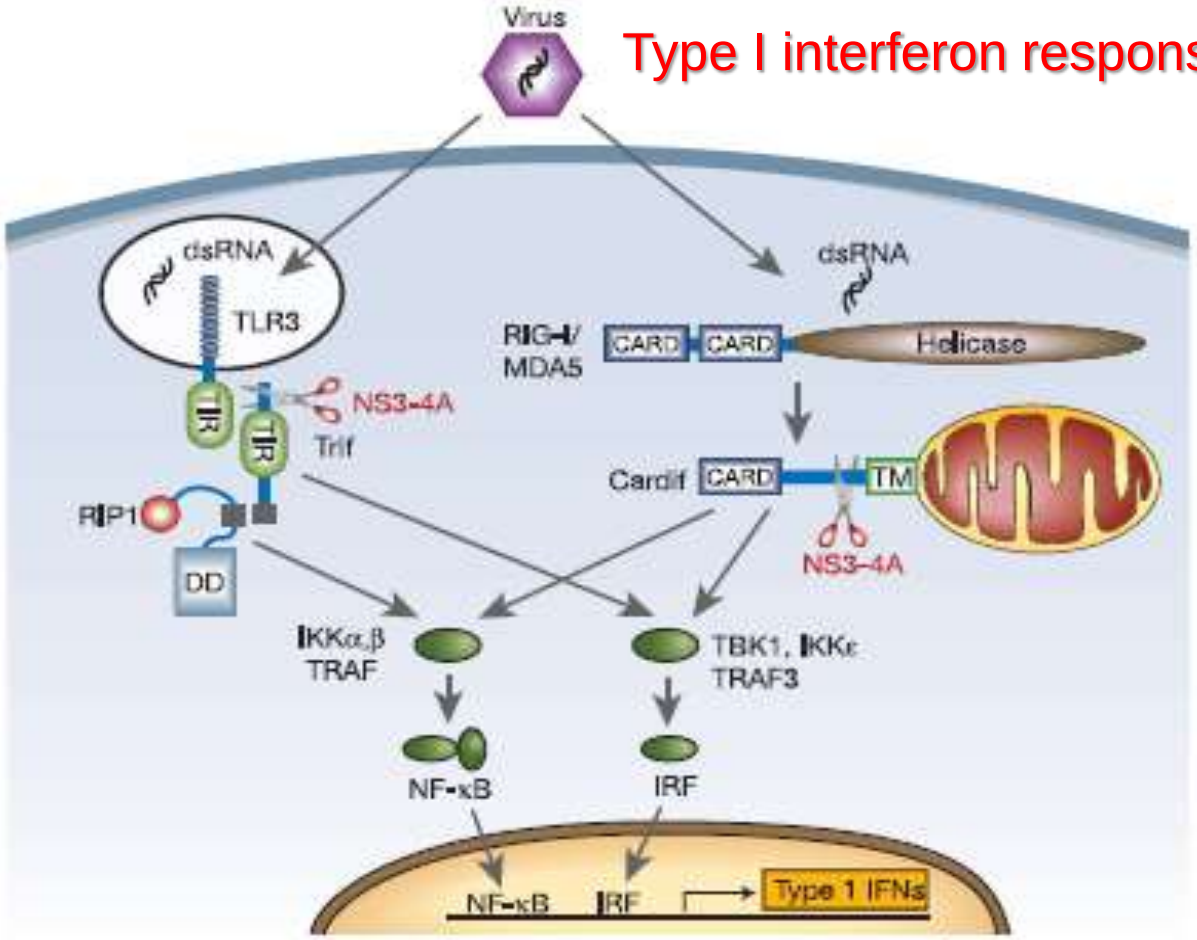
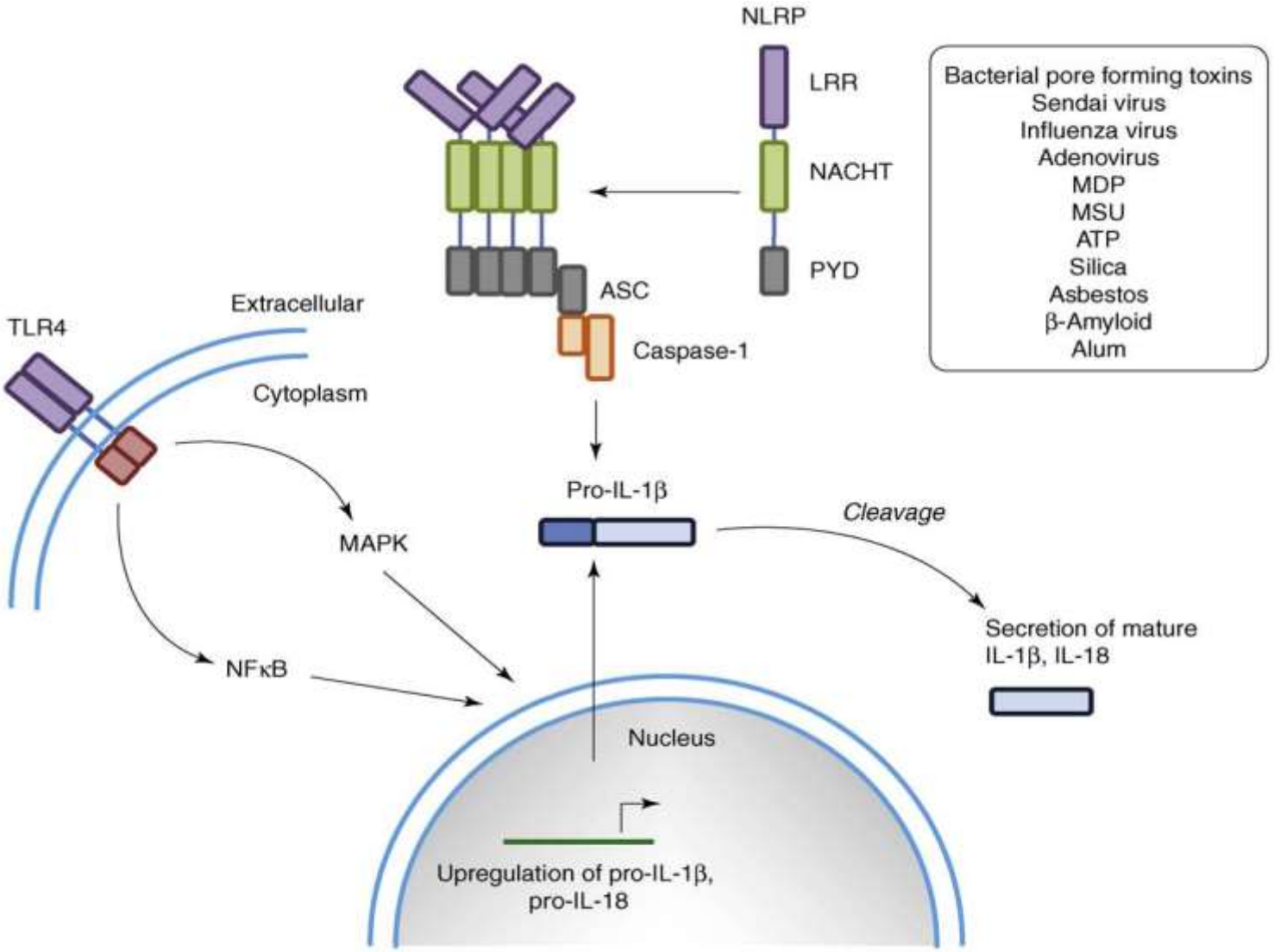
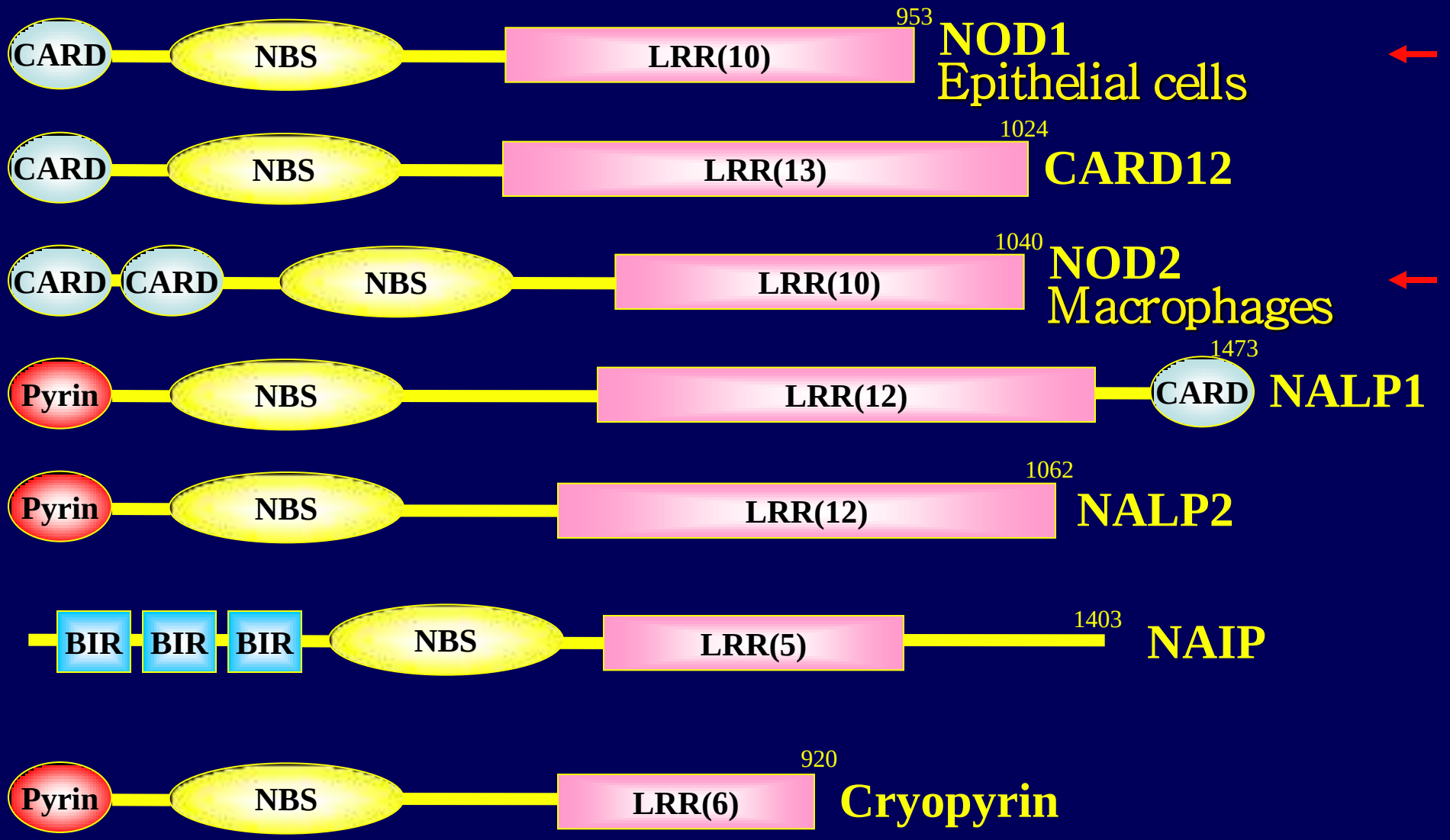


Figure 3 | Sensing of viral dsRNA by TLR3 and RLH. Left: On dsRNA binding within endosomes, TLR3 recruits the adaptor Trif through a TIR–TIR interaction. Trif, in turn, recruits RIP1 to activate NF-κB, via TRAFs and the IKK (IκB kinase) complex. Trif also recruits TBK1/IKKε and TRAF3 to activate IRF3/7. Right: On dsRNA binding, RIG-I (and also MDA5) recruits mitochondrially anchored Cardif, which, in turn, recruits appropriate IKKs to activate NF-κB and IRF, resulting in the induction of type I IFN. During an HCV infection, both Trif and Cardif are cleaved and inactivated by the HCV NS3-4A protease. Additional abbreviations: DD, death domain; TIR, Toll/IL-1 receptor; TM, transmembrane region.

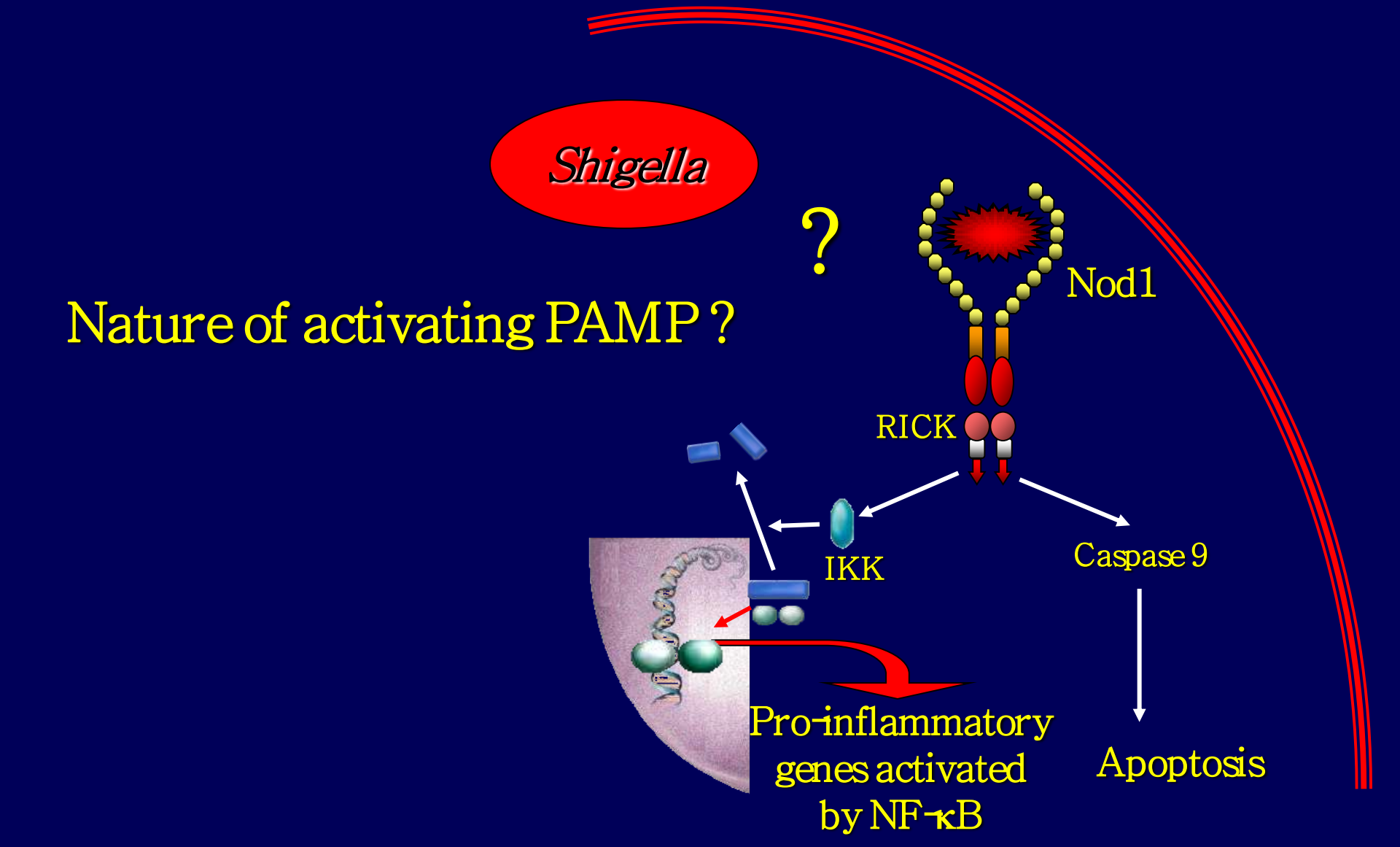
(Meylan et al., 2006)

Inflammasome activation



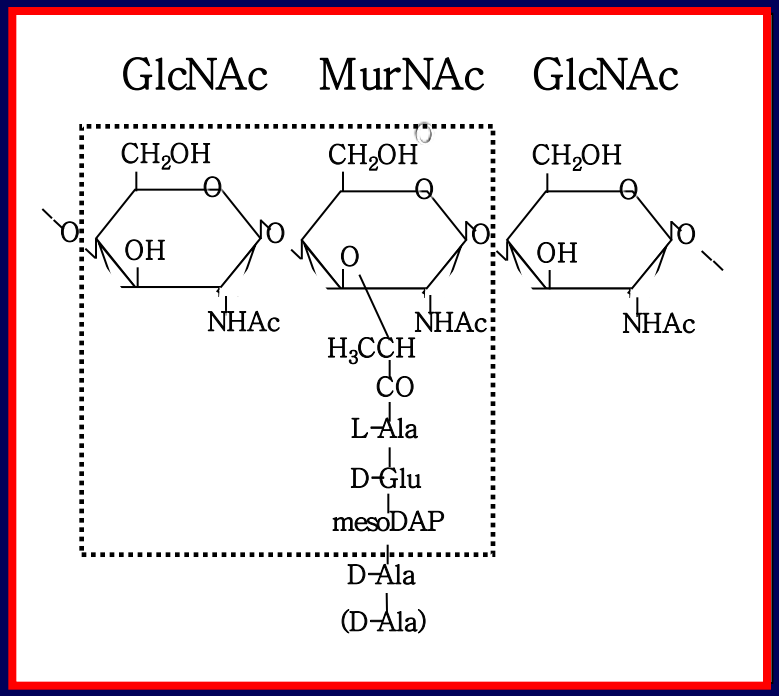

CARD
NBS
LRR(10)
953
NOD1
Epithelial cells
CARD
NBS
LRR(13)
1024
CARD12
CARD
CARD
NBS
LRR(10)
1040
NOD2
Macrophages
Pyrin
NBS
LRR(12)
CARD
1473
NALP1
Pyrin
NBS
LRR(12)
1062
NALP2
BIR
BIR
BIR
NBS
LRR(5)
1403
NAIP
Pyrin
NBS
LRR(6)
920
Cryopyrin

Functions of Nod1

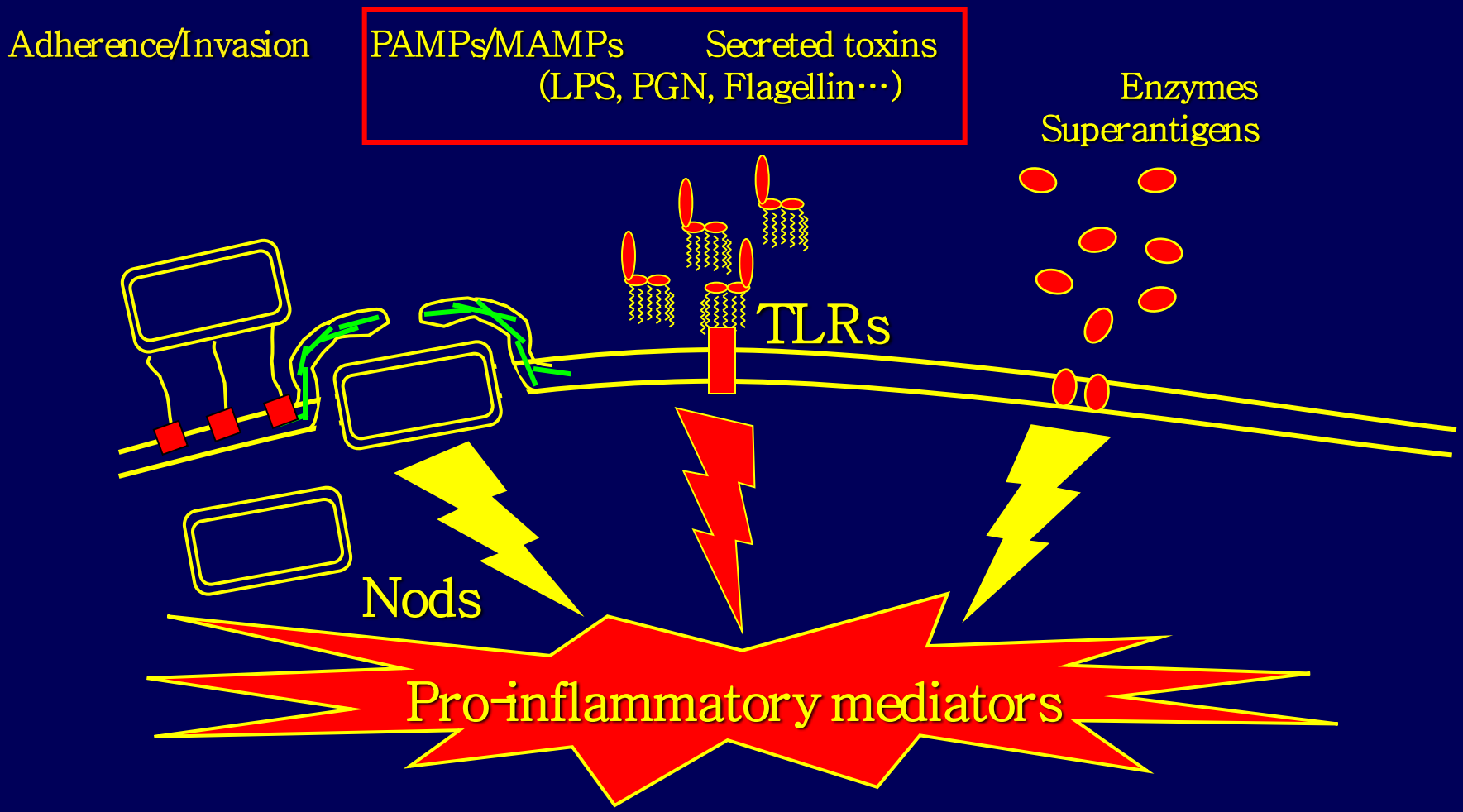


Nature of activating PAMP ?

Nod1-agonist is Muramyl-tripeptide



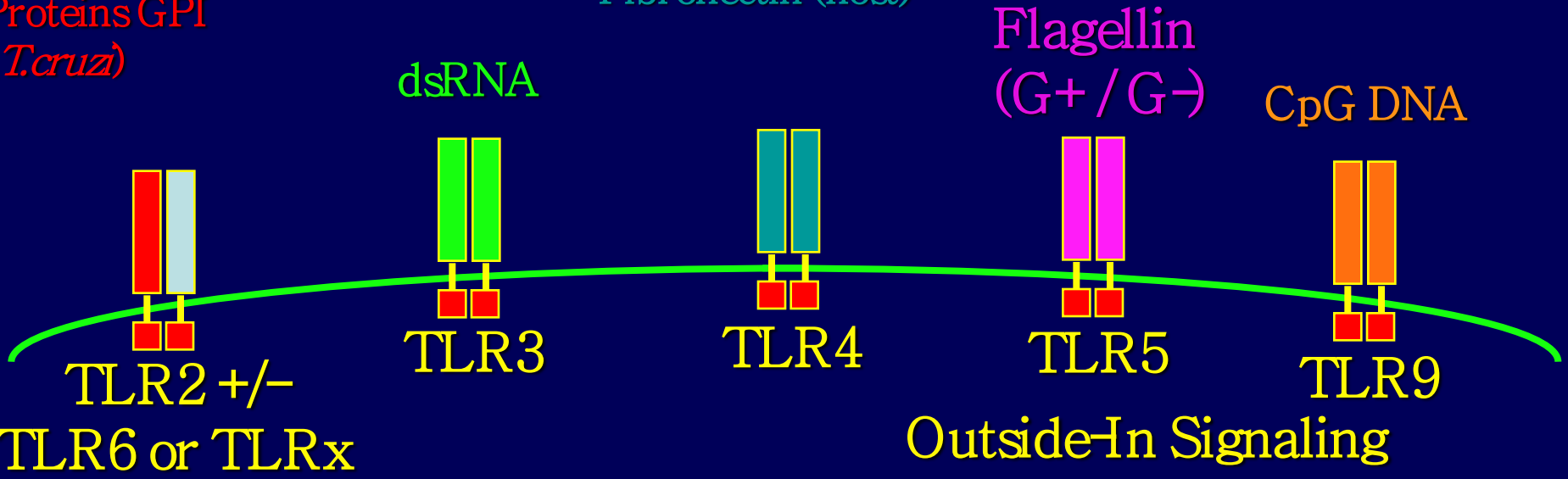
(1) The host recognizes pathogens
by several pathways



TOLL-LIKE RECEPTORS (TLR) Intracellular NOD SENSORS AND THEIR KNOWN AGONISTS

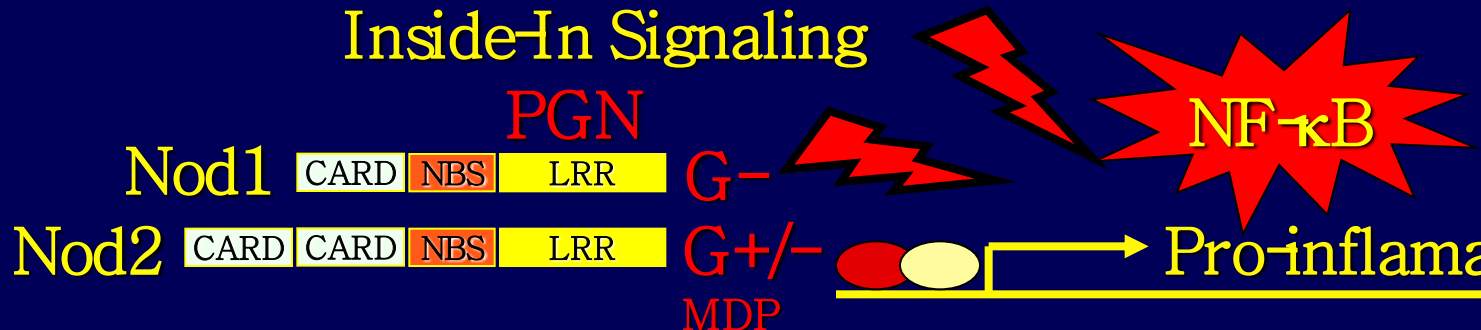
(Peptidoglycan-PGN of G+/G-)
Lipoproteins (bacteria)
Lipoarabinomannan
(*M.tuberculosis*)
Phospatidylinositol dimanoside
(*M.tuberculosis*)
LPS (*Leptospira*)
LPS (*P.gingivalis*)
Zymosan (*S.cerevisiae*)
Proteins GPI
(*T.cruzi*)

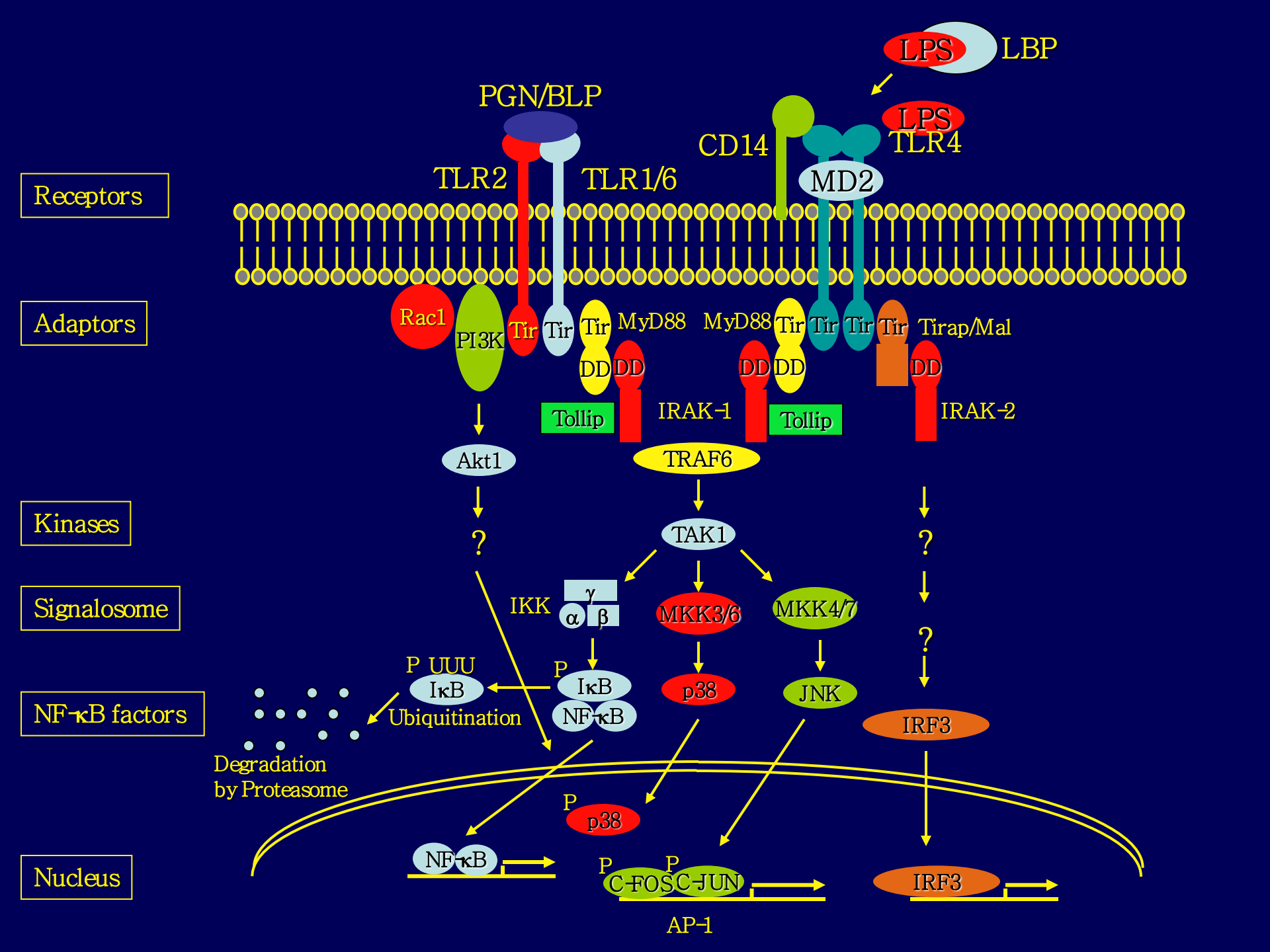
LPS (G-)
Taxol (plants)
F-protein (VRS)
HSP60 (host & *Chlamydia*)
Fibronectin (host)



Outside-In Signaling

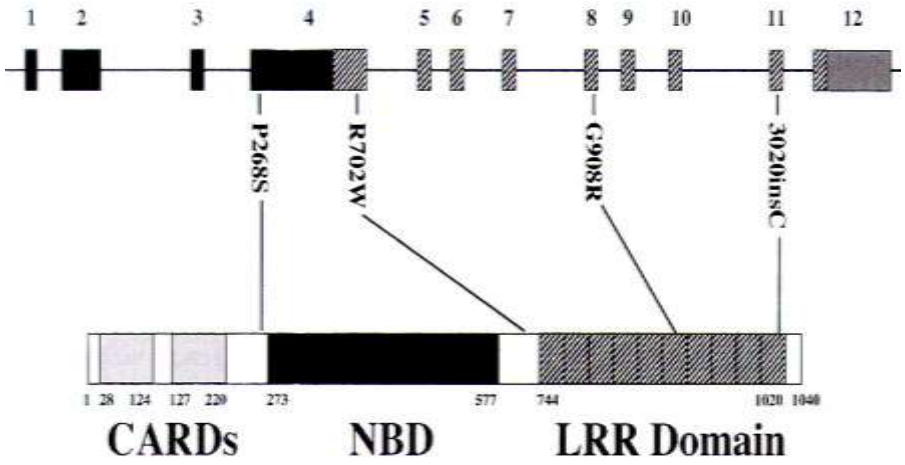
Inside-In Signaling




LPS
LBP
PGN/BLP
LPS
CD14
TLR4
TLR2
TLR1/6
MD2
Receptors
Adaptors
Rac1
PI3K
Tir
Tir
Tir
MyD88
MyD88
Tir
Tir
Tir
Tir
Tirap/Mal
DD
DD
DD
DD
DD
Tollip
IRAK-1
Tollip
IRAK-2
Akt1
TRAF6
Kinases
?
TAK1
?
Signalosome
IKK
γ
α
β
MKK3/6
MKK4/7
?
P UUU
P
IκB
IκB
NF-κB
p38
JNK
IRF3
NF-κB factors
Ubiquitination
Degradation
by Proteasome
P
p38
NF-κB
P
P
C-FOS
C-JUN
IRF3
Nucleus
AP-1

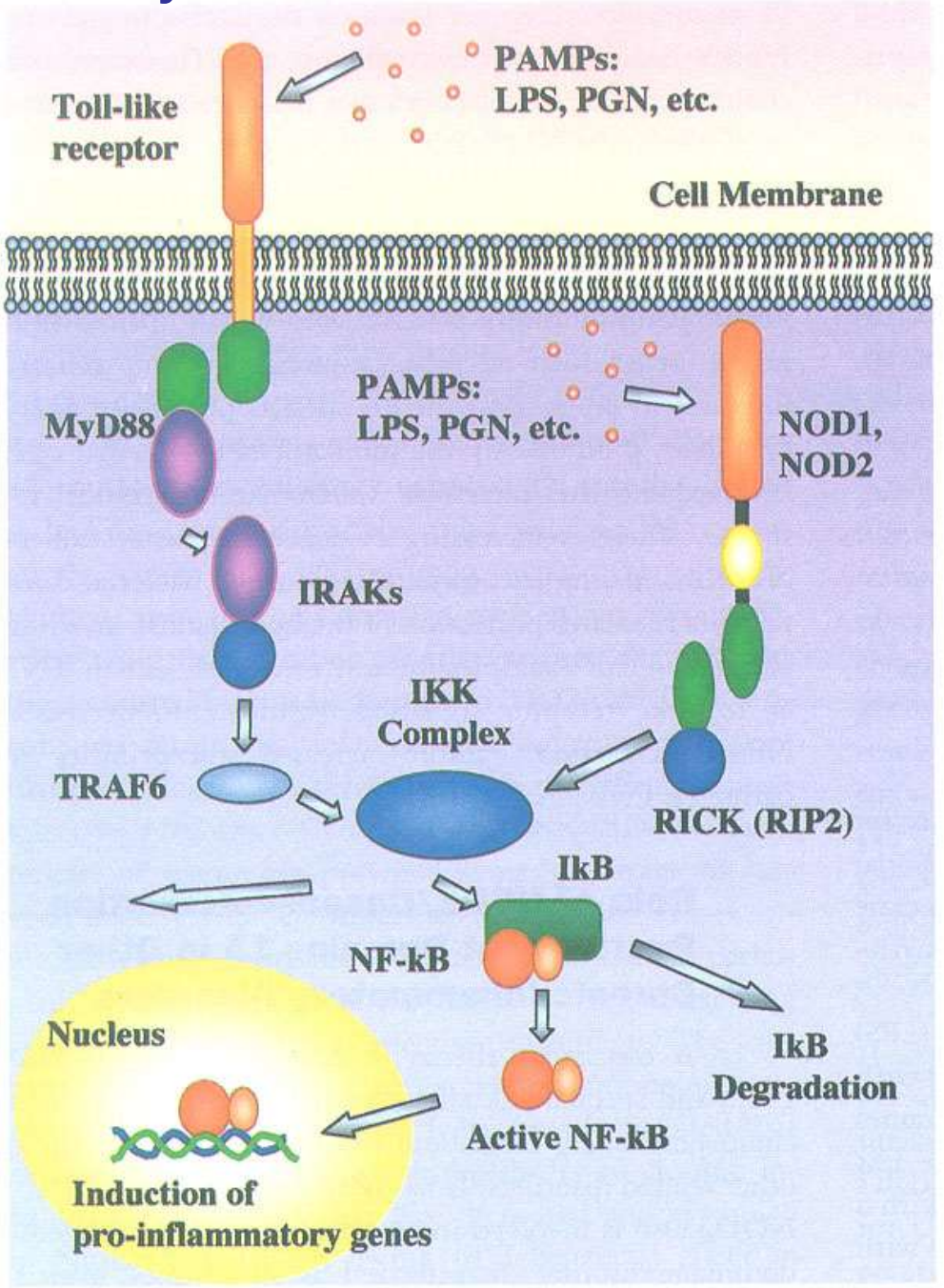
Signaling pathways of microbial - host interaction

Genomic structure of the NOD2/CARD15 (Caspase-Activation Recruitment Domains 15)



Mutations in LRR of NOD2 lead to increased risk of Crohn´s ileitis. NOD2 gene product is most abundant in Paneth cells in terminal ileum

[Bonen and Cho 2003, Lala et al. 2003]



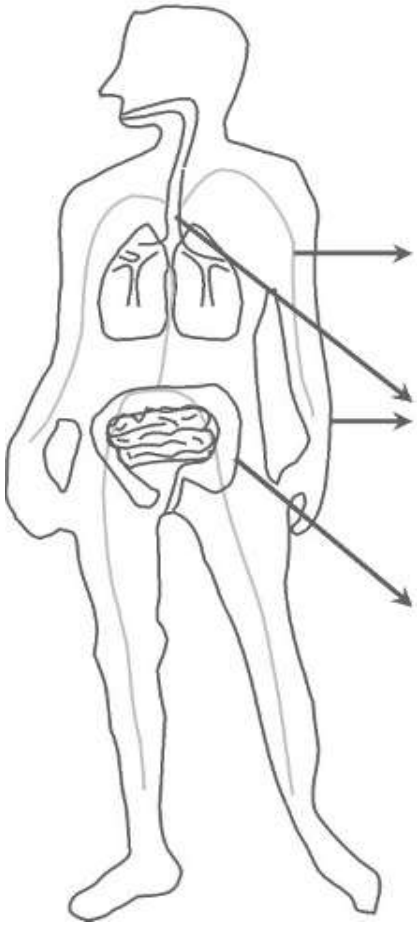
One has to survive the action of the immune system…

Organ-specific regulation of immune responses

graded immune responses:

each organ senses infecious danger in a different way

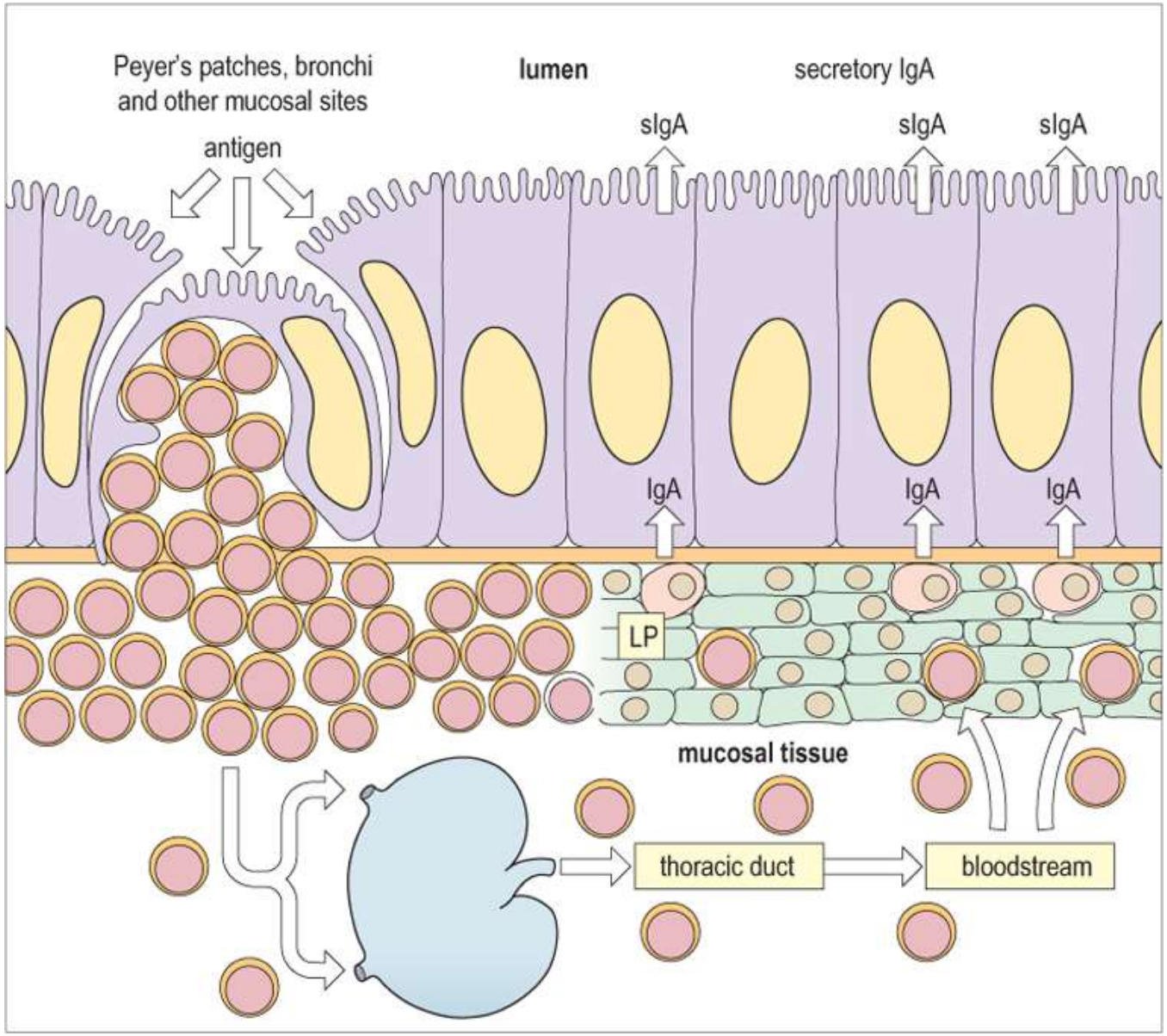
(Raz et al., 2007)



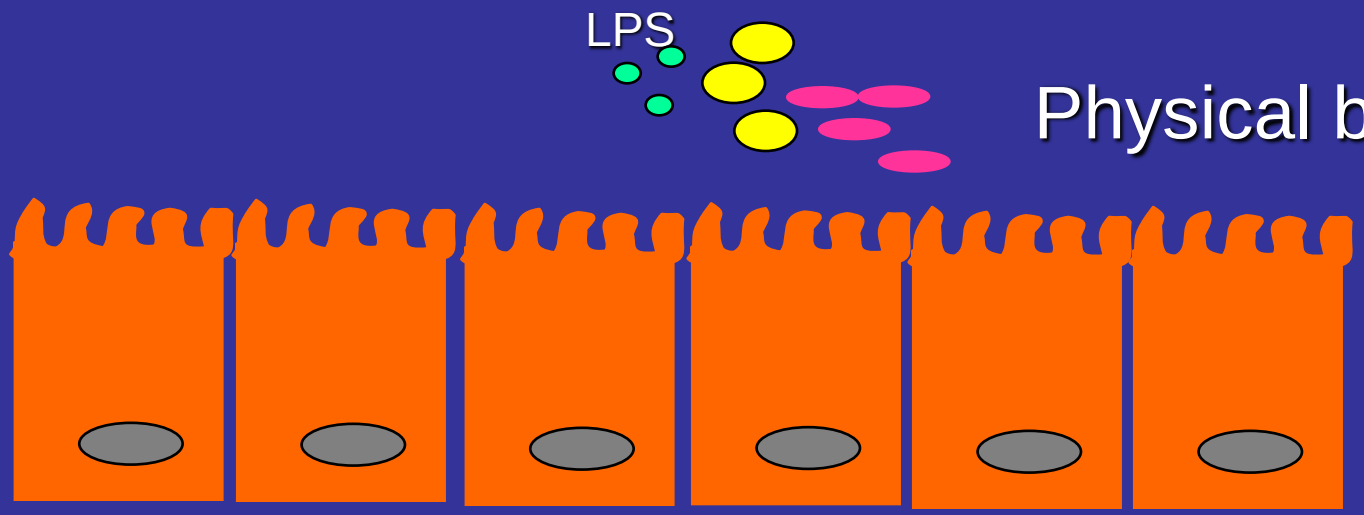
compartment	microbial contact	PRR sensitivity
blood	none	high every microbial contact indicates danger
airways, skin	frequent	regulated certain microbial load might be tolerated
gut	permanent	suppressed tolerance is dominating

(Mayer et al., 2007)

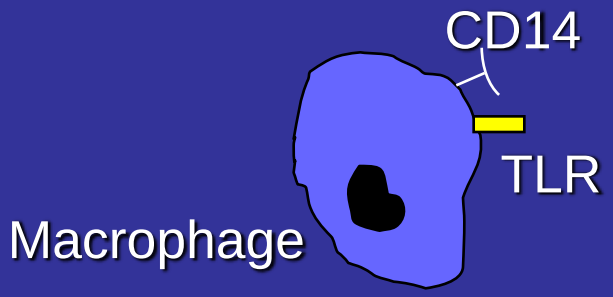
The mucosa-associated immune system



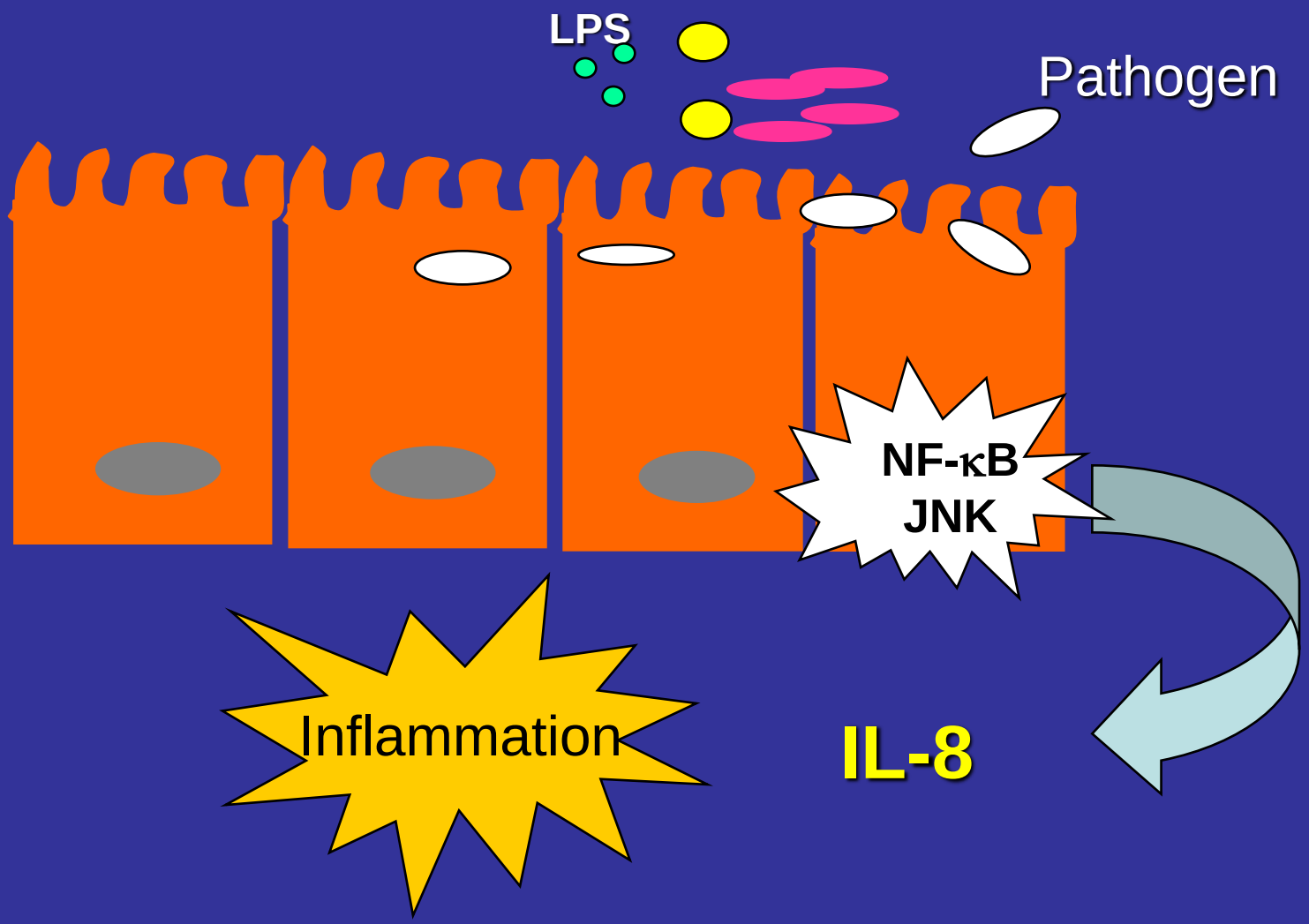
Mucosal epithelial cells



-normally insensitive to PAMPs
-usually absence of TLRs or associated co-factors

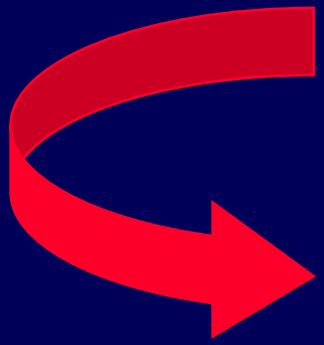


Infection by a pathogenic microbe



Microflora versus Pathogen

Presentation of bacterial products to the intracellular compartment is sufficient to initiate a defensive response



What is the “PRR” or intracellular sensor?

Mechanisms by which pro-inflammatory signaling of commensals on mucosal surfaces is limited

- Mucus layer separates commensals from epithelial cells

- **Epithelial cells and innate immunity cells in mucosal tissues are hyporeactive to stimulation by bacterial producs**
  - **EPIGENETIC REPROGRAMING!!!**

1)negative feedback regulation - reduction of TLR expression – through IL-10 a TGFß signaling inducing inhibitory SMAD6 a SMAD7 molecules (*i.e*. unless inflammation is induced and upregulates TLR4)

2)resting *lamina propria* macrophages lack CD14 expression IκB degradation and NFκB activation (inflammatory signaling)

3) **Commensal and probiotic bacteria induce SOCS expression (supressor of cytokine signaling)**

Nasopharynx and intestinal epithelium-specific adaptations in pattern recognition needed to face bacterial colonization

- reduced sensitivity to PAMPs
- the proposed mechanisms that control TLRs sensitivity are:

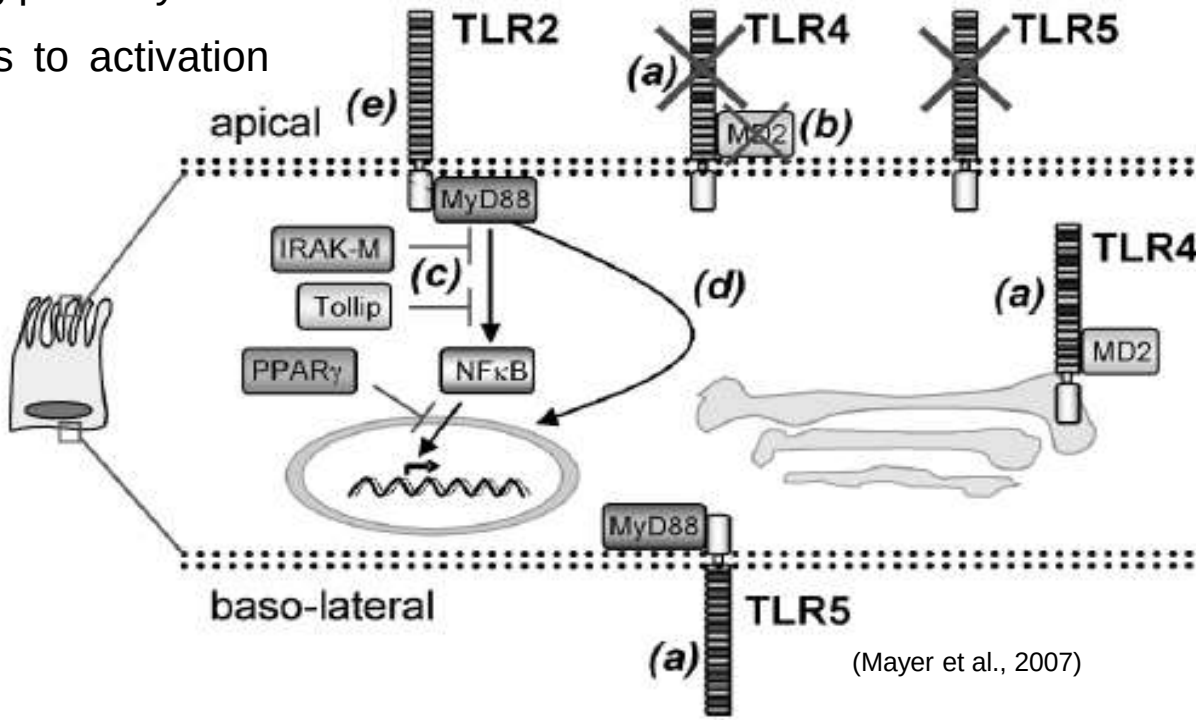
a) restriction of apical TLR expression (TLRs could be intracelular and only after activation they are expressed on cells surface)

b) missing co-receptors which are needed for proper interaction

c) presence of inhibitory signalling molecules

d) epithelium-specific signaling pathways

e) reduced sensitivity of TLRs to activation by microbial compounds



(Mayer et al., 2007)

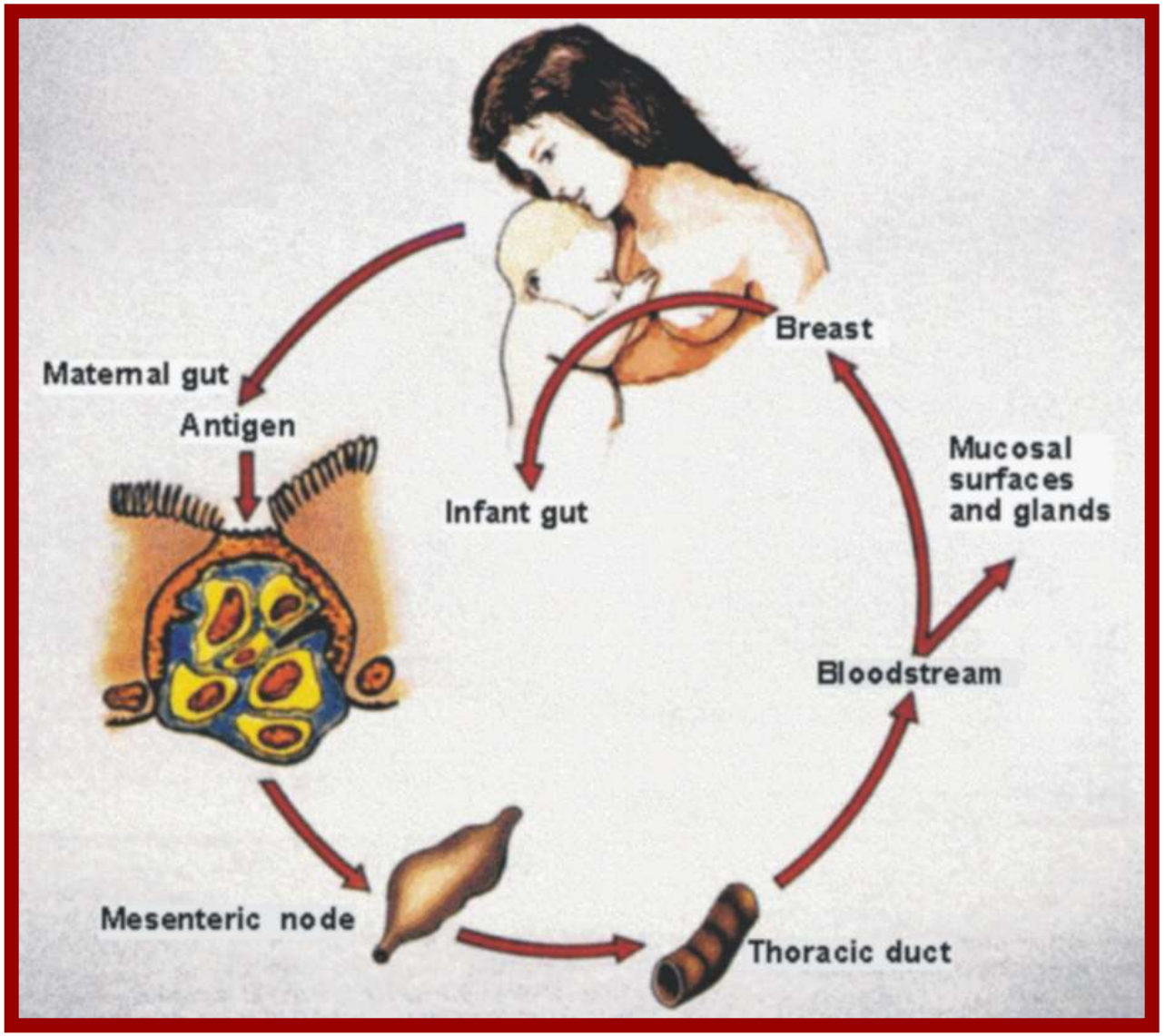
Oral (mucosal) tolerance

Inhibition of systemic immune response to antigens contacting mucosal surfaces (orally, intranasally …), such as:

Food antigens, components of microflora

Mucosal immune system

The common mucosal immune system
(ther largest organ with a surface of a soccer field)



How can airway epithelial cells combat a bacterial infection and modify immune response

innate

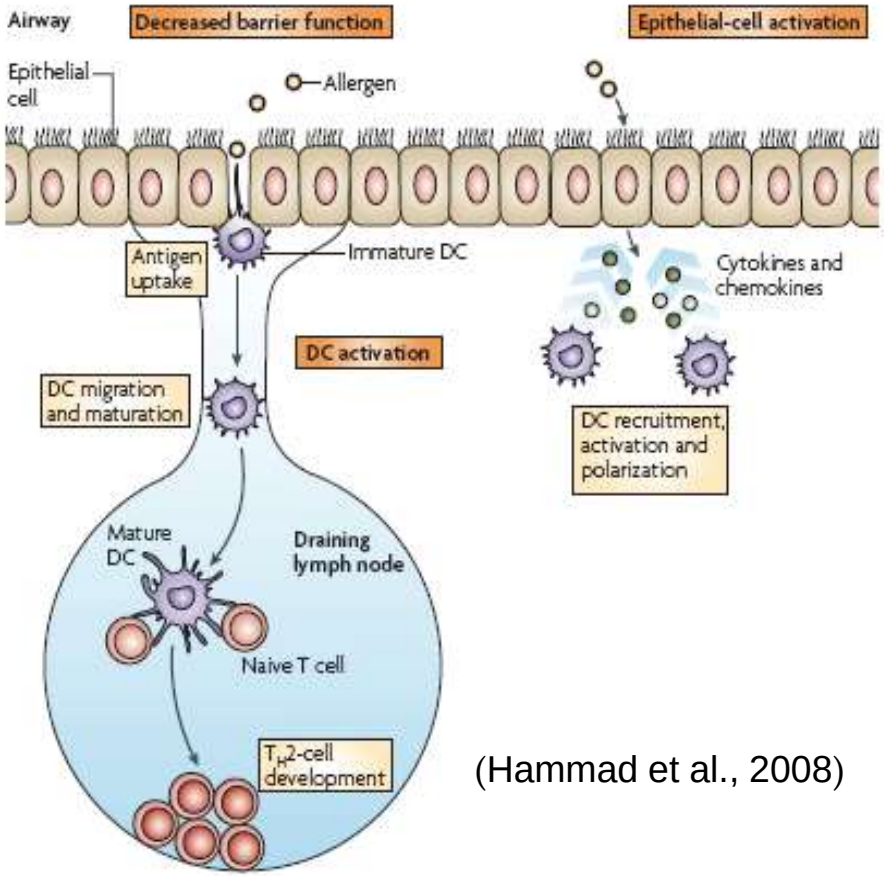
- *the physical removal by ciliary clearence and cough*
- *the presence of broad spectrum antimicrobial agents in the mucus*
- *the recruitment of phagocytic cells and an inflammatory response*

immunity

- *modulate phenotype of DC*
  → type of T cell response
- *modulate effector T cell functio*

adaptive

- *priming naive T cells ?*



(Hammad et al., 2008)

Bacterial strategies for overcoming host immune response

• microbial organism have coevolved with their hosts to overcome protective host barriers

• avoid host recognition or dampen the subsequent immune activation
x some pathogens benefit from stimulation of inflammatory reactions

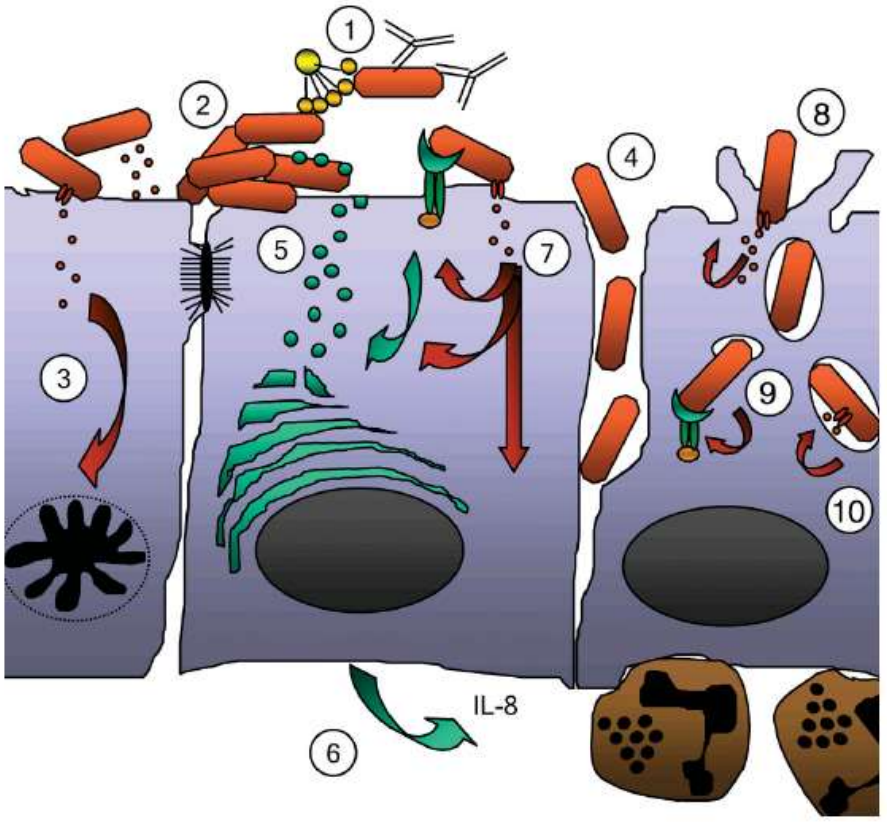
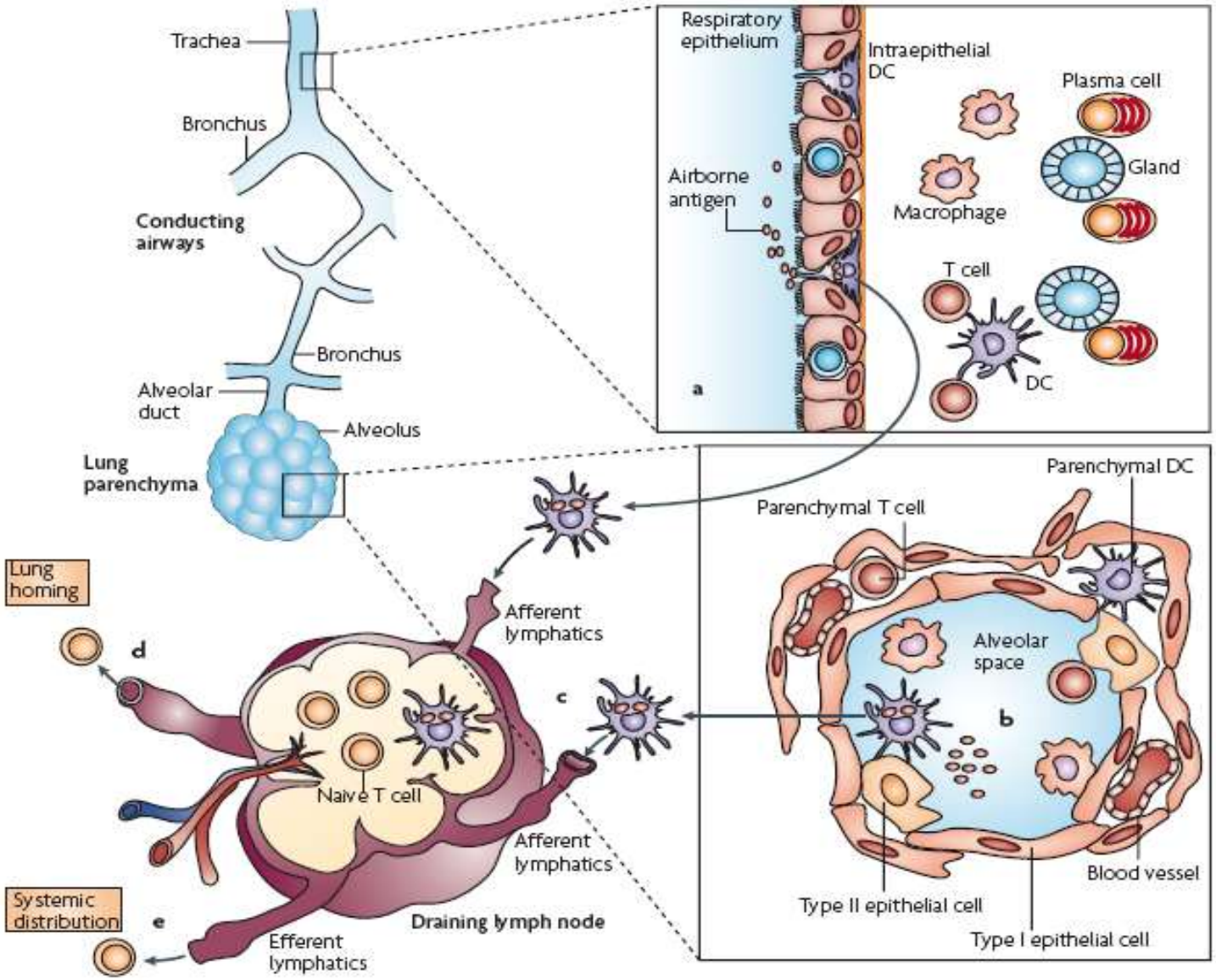


Figure 1. Strategies for bacterial escape from epithelial defense mechanisms. Prevention of opsonization (1) is required to facilitate colonization of host surfaces (2). Toxin secretion can paralyze the host's defenses (3) and disrupt its mucosal integrity (4). Microbial recognition and host responses—such as the secretion of antimicrobial peptides (5) or chemokine production (6)—can be impaired by modification of pattern molecule presentation or interference with intracellular signaling or cell trafficking (8). Microbe-induced self-uptake (7) and escape from the phagosome along with inhibition of intracellular recognition (9) or persistence in modified endosomes (10) can then impede removal by host defense mechanisms. Green, host responses; orange, bacterial components and interference with host defense strategies.

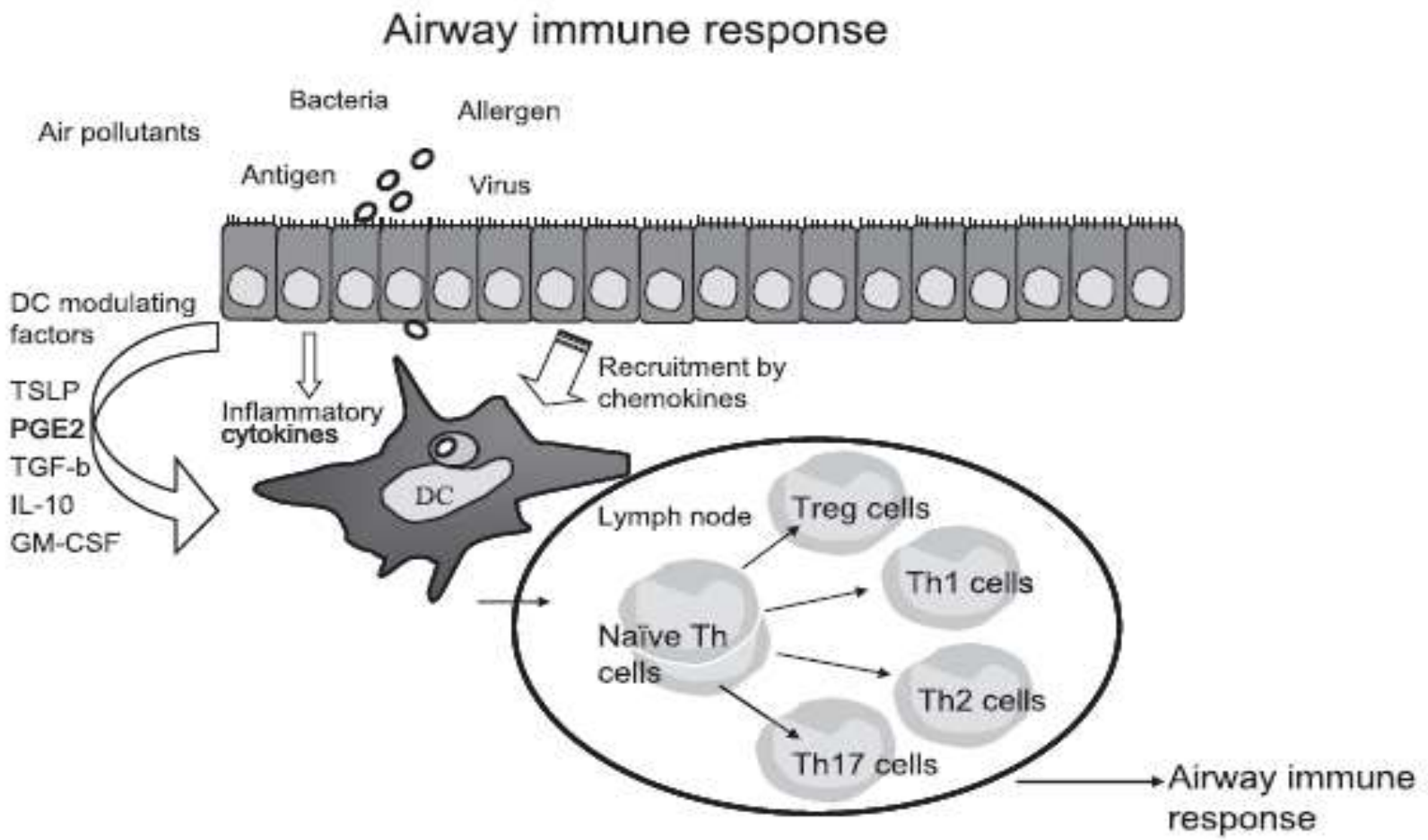
(Hornef et al., 2002)

Airway epithelial cells in interaction with immune cells



(Holt et al., 2008)

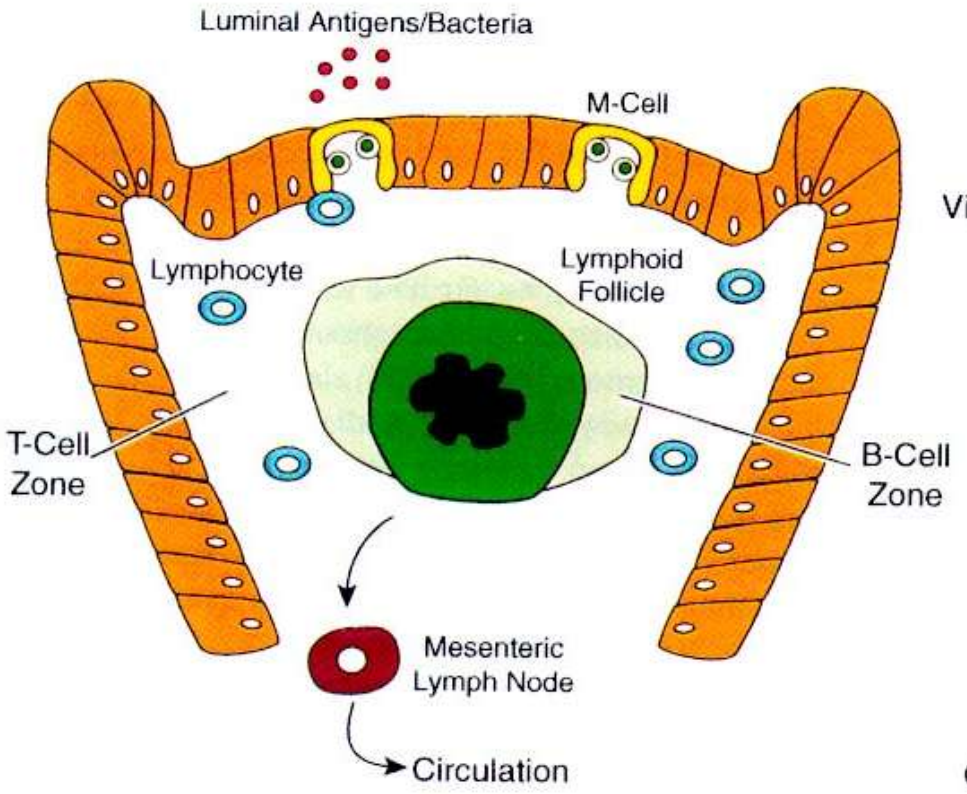
Interaction between epithelial cells and dendritic cells in airway immune responses



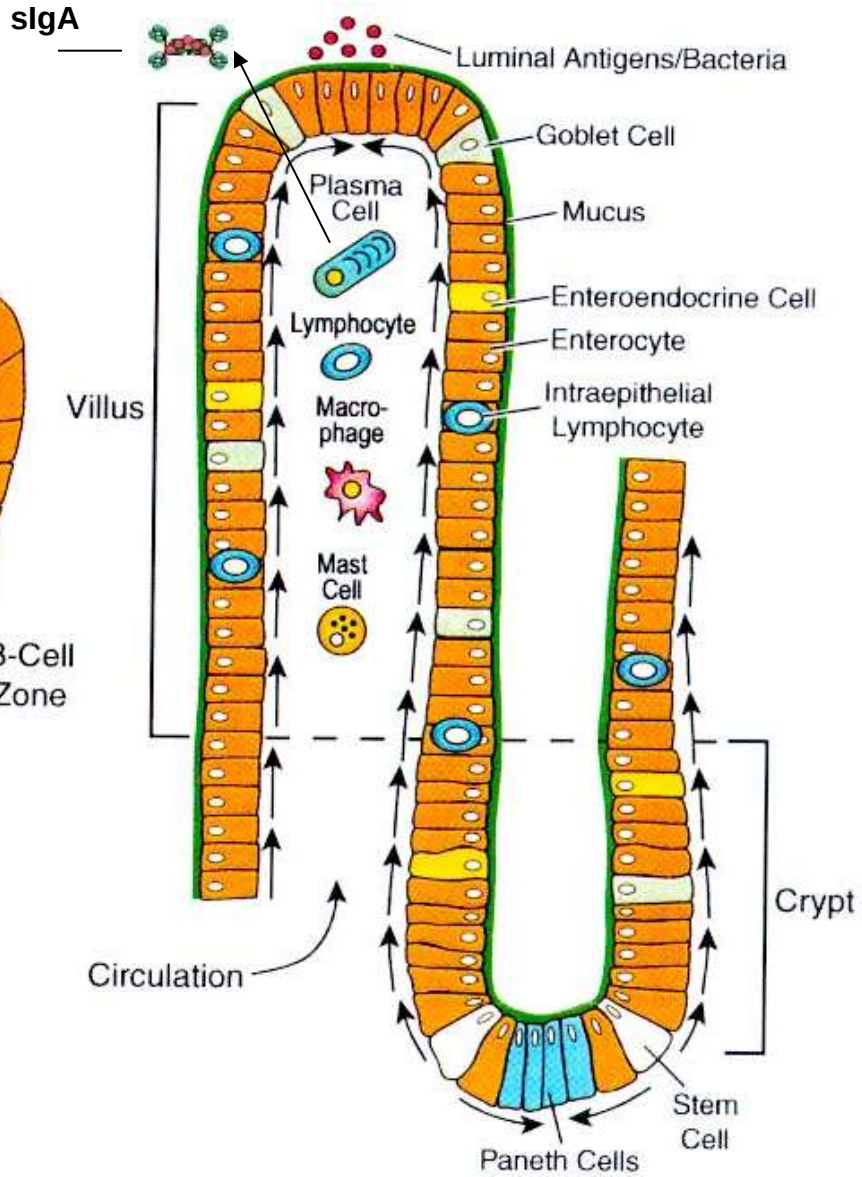
(Tongeren et al., 2008)

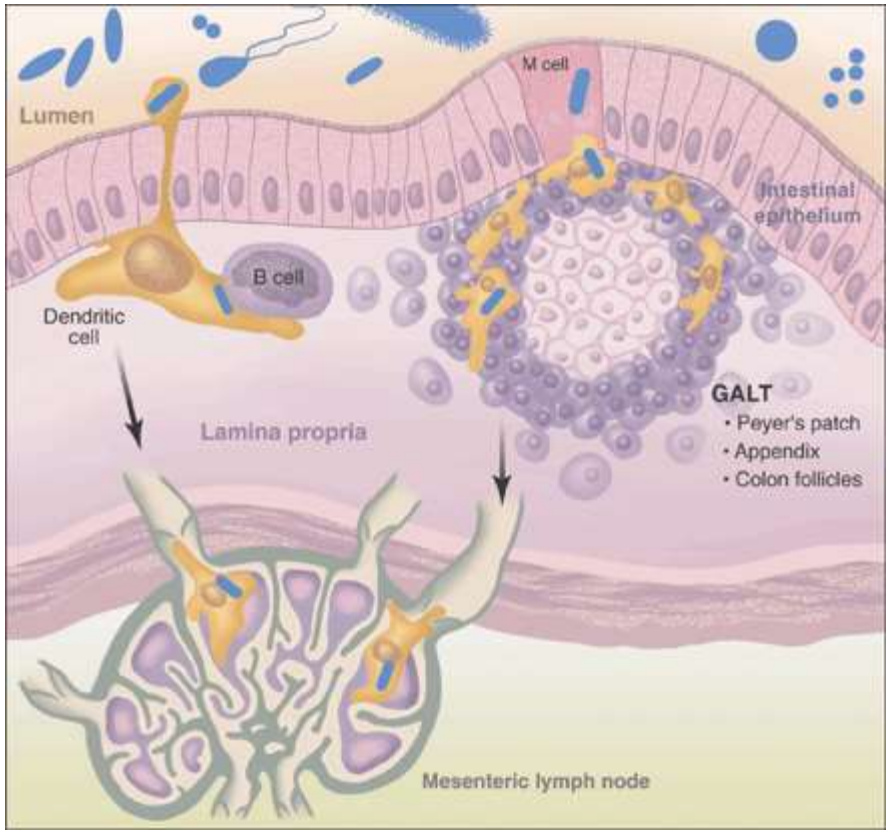
GALT - Gut Associated Lymphatic Tissue

INDUCTIVE SITE (Peyer´s Patch)



EFFECTOR SITE (Lamina Propria)





Dendritic cell-mediated transport of commensal bacteria in the gut. Commensal bacteria in the gut lumen are continuously sampled by cells of the innate immune system, such as dendritic cells (DCs) and M cells. M cells of the gut epithelium may import bacteria into the dome region of the GALT where DCs engulf the microbes. Alternatively, DCs may sample commensals directly by prying open the tight junctions between epithelial cells and projecting their dendrites into the gut lumen. DCs present antigenic peptides from captured microbes to B and T lymphocytes either locally in the GALT, or within the mesenteric lymph nodes that drain the gut submucosa. Presentation of microbial antigens to B cells triggers production of a commensal-specific IgA response that prevents the commensals from straying beyond the gut mucosa where they could elicit a systemic inflammatory response.

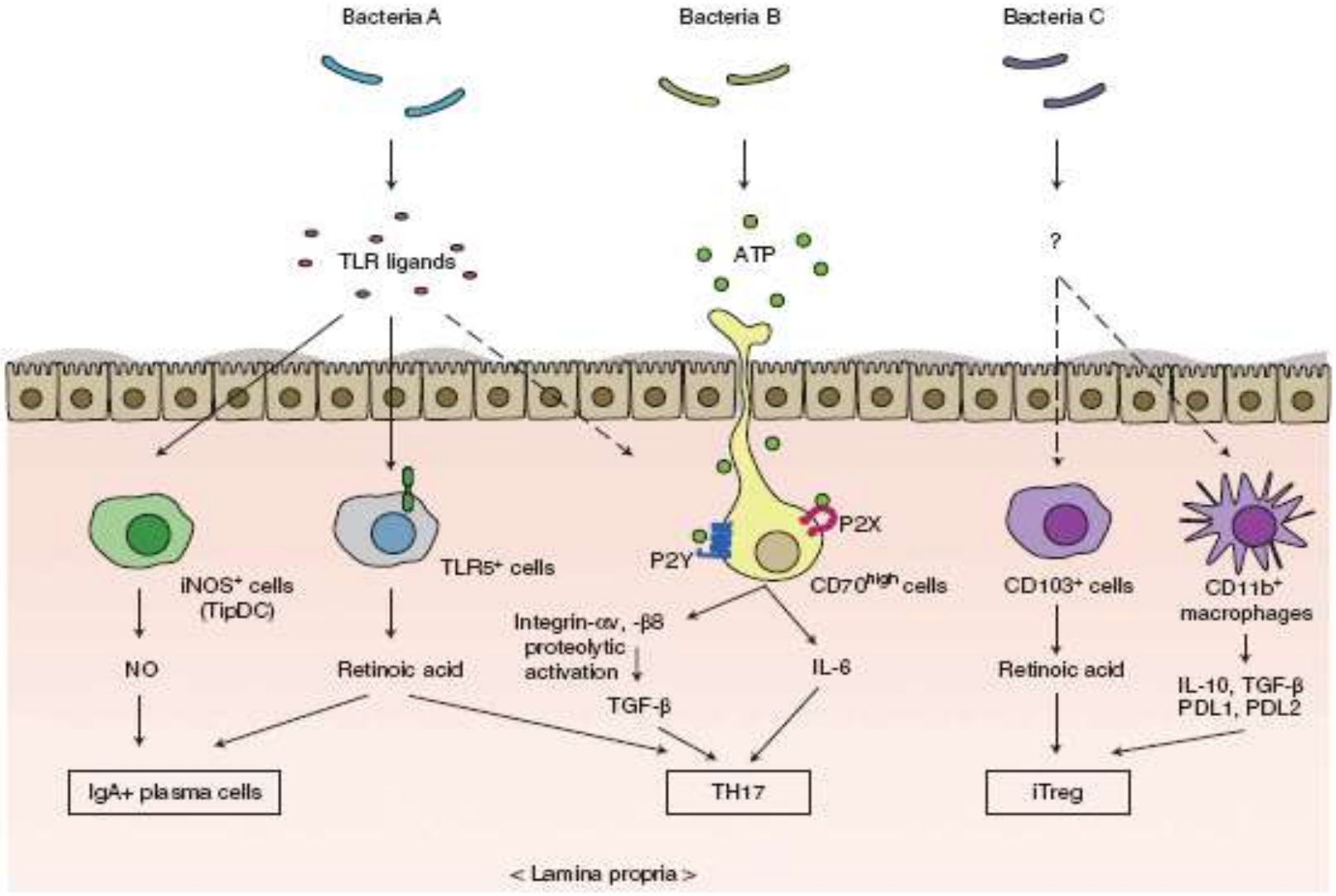
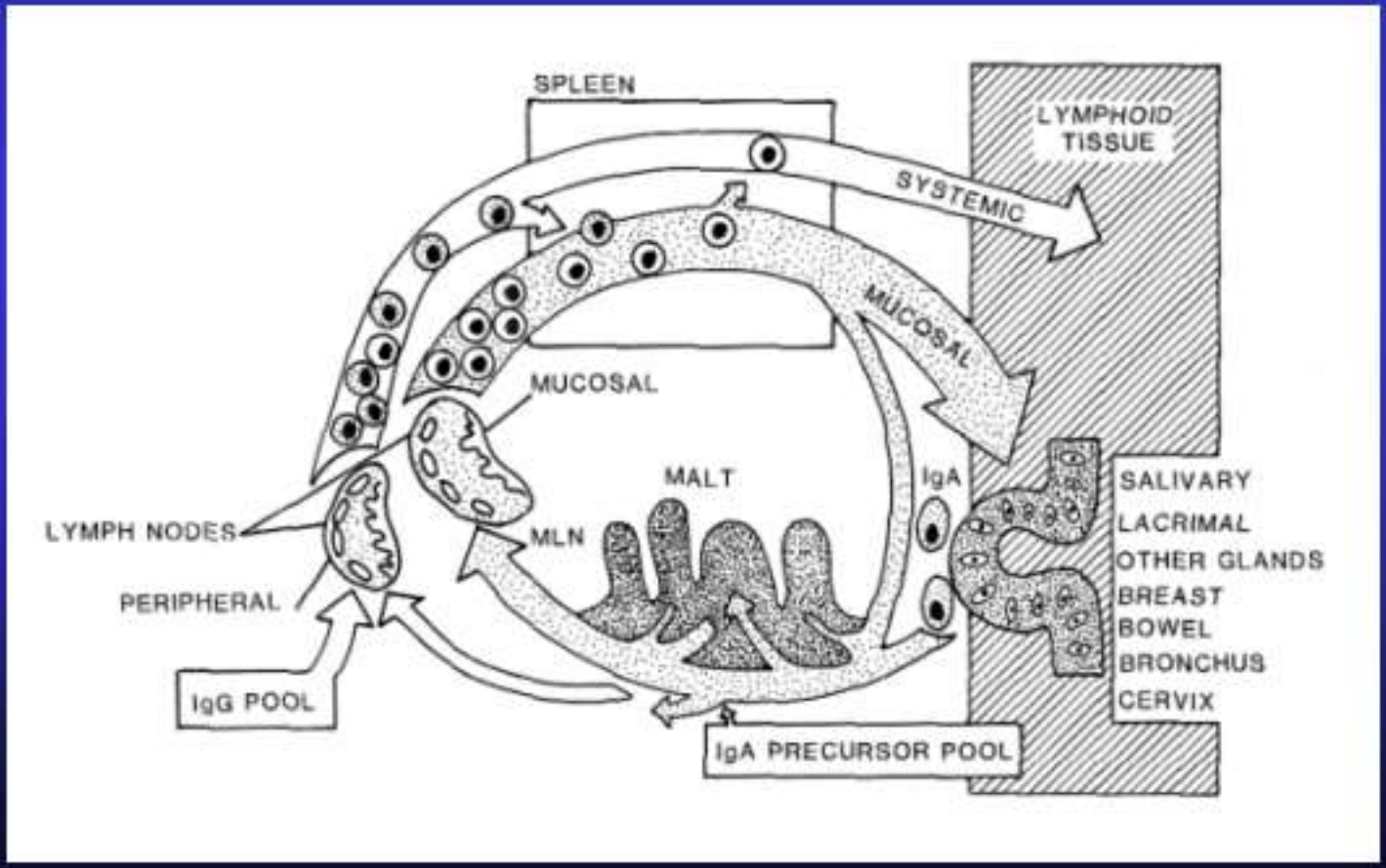


Figure 1 A hypothetical model for intestinal bacteria-mediated differentiation of adaptive immune cells in the lamina propria. The intestinal microflora is composed of a variety of bacteria with different characters; each of them produces stimulatory factors for host immune system. A subset of commensal bacteria (Bacteria A) produces TLR ligands, which activate specific subsets of lamina propria APCs, TipDCs, and TLR5$^+$ DCs, thereby inducing the differentiation of IgA$^+$ plasma cells and TH17 cells. Another subset of intestinal bacteria (Bacteria B) produces and secretes high amounts of extracellular ATP. CD70^{high}CX3CR1$^+$ dendritic cells extend their dendrites into the lumen, sense the extracellular ATP through P2X and P2Y receptors, and produce IL-6 and TGF-β-activating enzymes to promote differentiation of TH17 cells. A (Honda and Takeda, 2009) commensal bacteria (Bacteria C) produce an as-yet unknown factor that activate CD103$^+$ DCs or CD11b$^+$ macrophages to produce retinoic acid and other factors to induce differentiation of iT_{reg}s. TLR, Toll-like receptor; APC, antigen-presenting cell; IgA, immunoglobulin A; TipDC, TNF-α/iNOS-producing dendritic cell subset; IL-6, interleukin-6; TGF-β, transforming growth factor-β; DC, dendritic cell; iT_{reg}, inducible T_{reg}

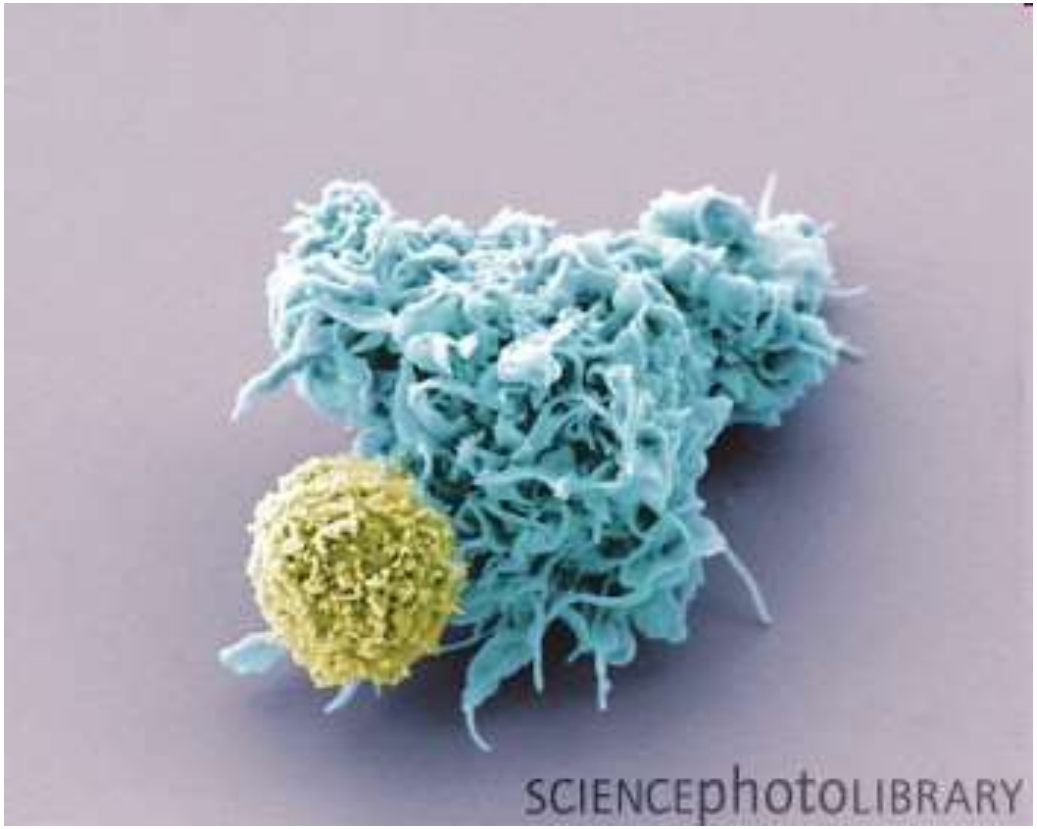
The Common Mucosal Immune System

MALT – Mucosa Associated Lymphoid Tissue



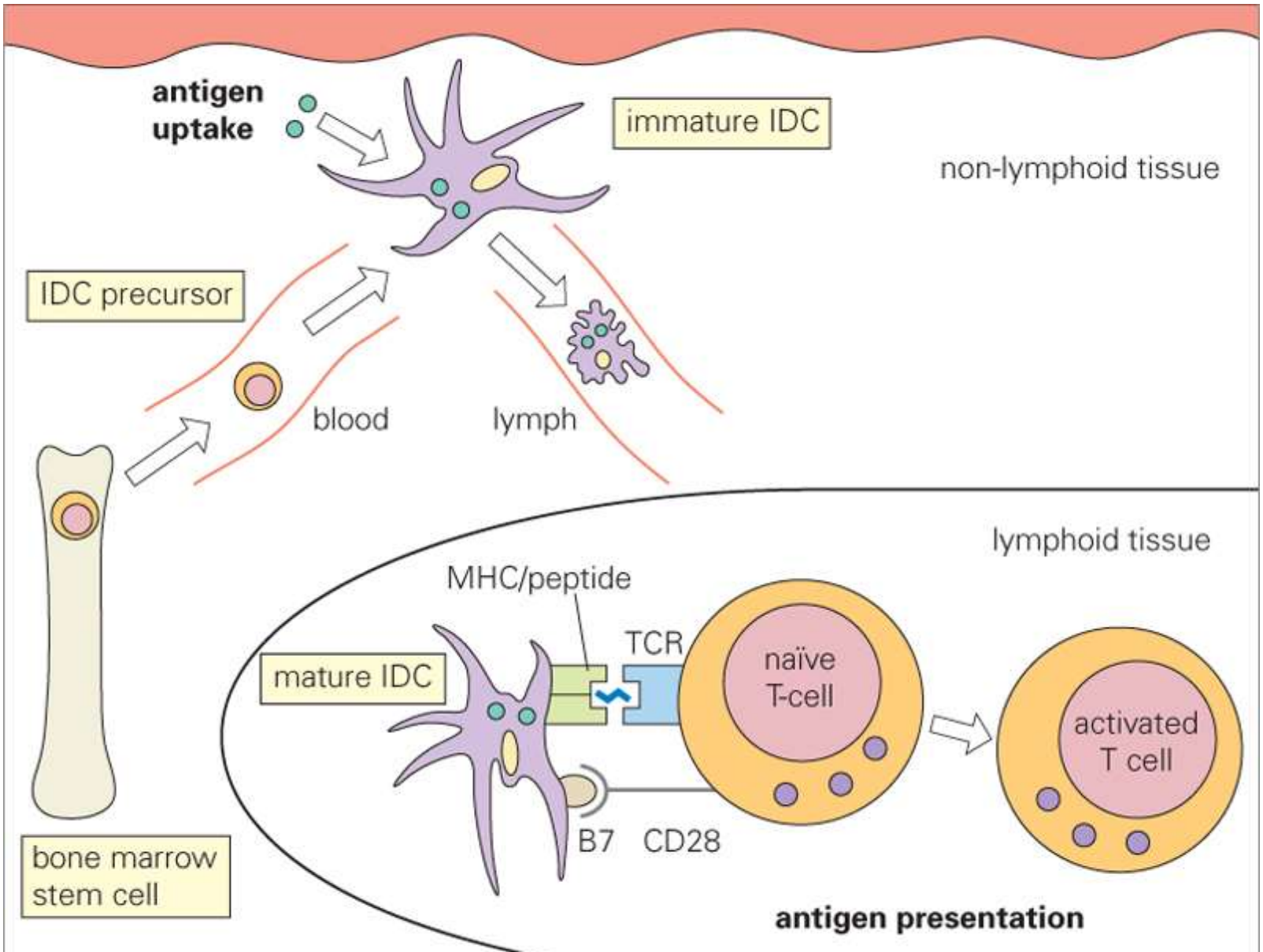
Dendritic cells (DCs)

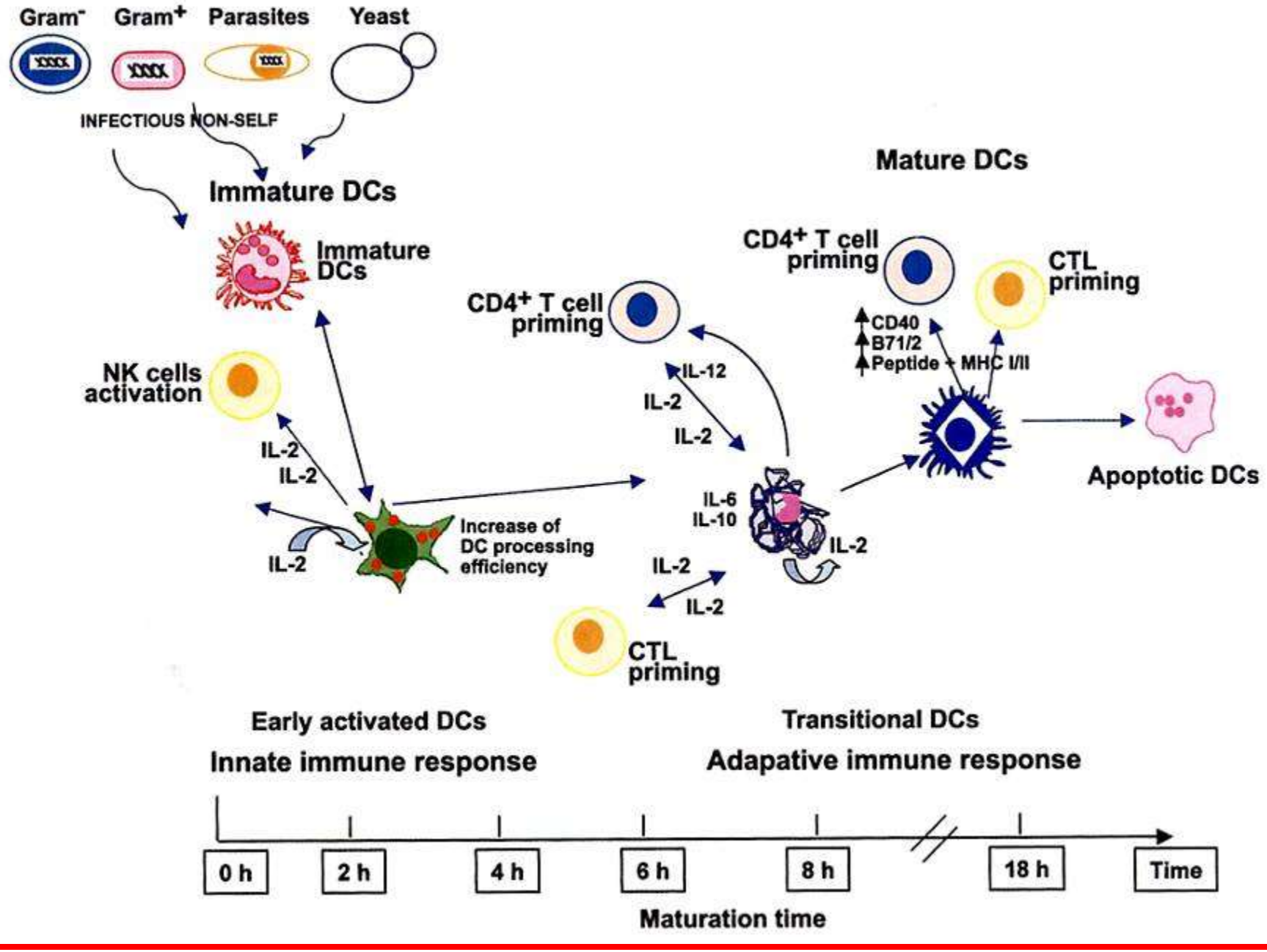
- essential bridge linking innate nad adaptive immunity
- professional antigen presenting cells
- need to be activated for creating the effective bridge
- Numerous subpopulations of dentritic cells – function and tissue specific



A Dendritic cell (blue) interacting with a T-cell (gold)

Dendritic cells in adaptive immunity

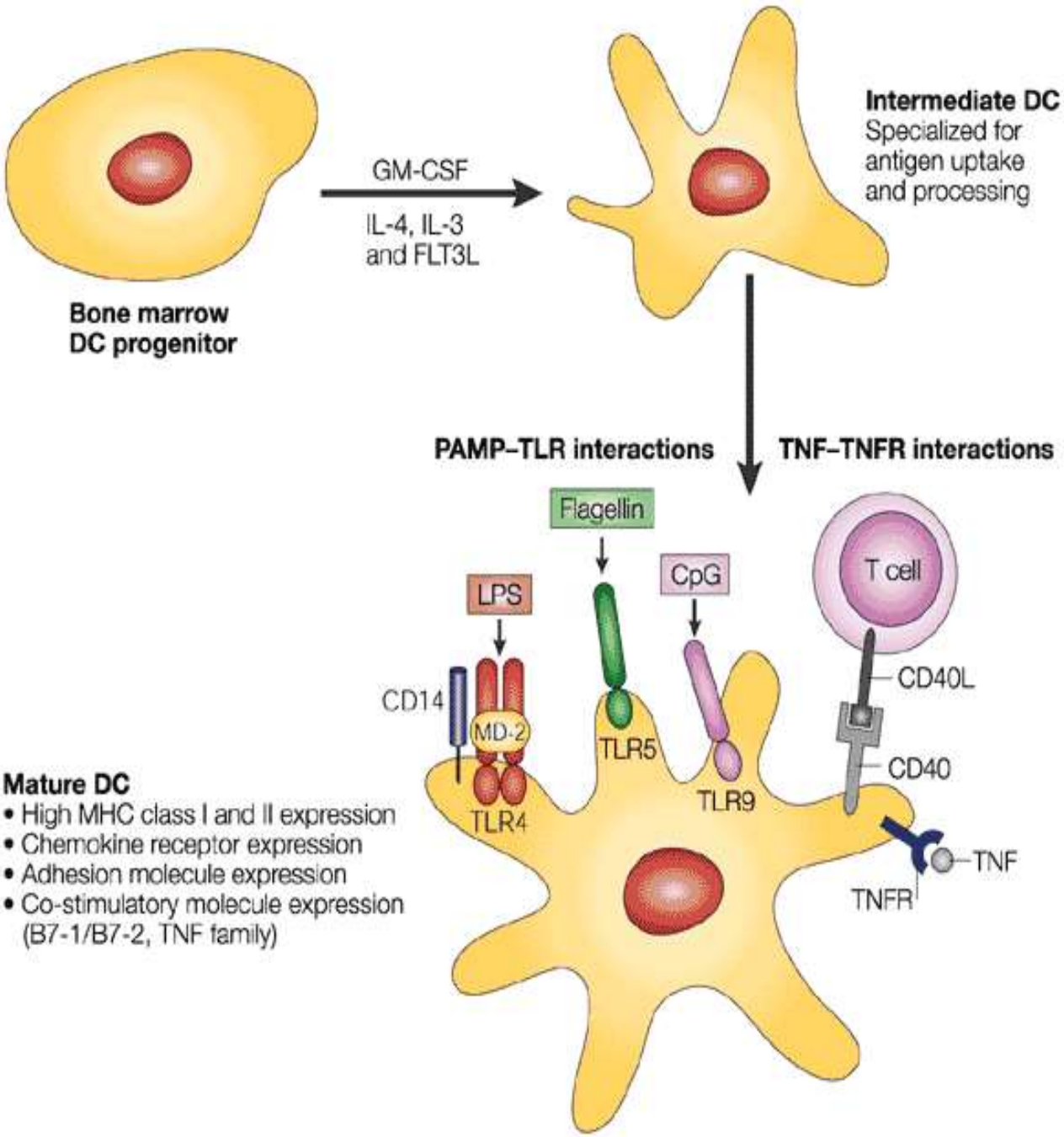



Gram⁻
Gram⁺
Parasites
Yeast
INFECTIOUS NON-SELF
Immature DCs
Immature DCs
Mature DCs
CD4⁺ T cell priming
CTL priming
CD40
B71/2
Peptide + MHC I/II
CD4⁺ T cell priming
NK cells activation
IL-12
IL-2
IL-2
IL-2
IL-2
Increase of DC processing efficiency
IL-6
IL-10
IL-2
Apoptotic DCs
IL-2
IL-2
CTL priming
Early activated DCs
Transitional DCs
Innate immune response
Adapative immune response
0 h
2 h
4 h
6 h
8 h
18 h
Time
Maturation time

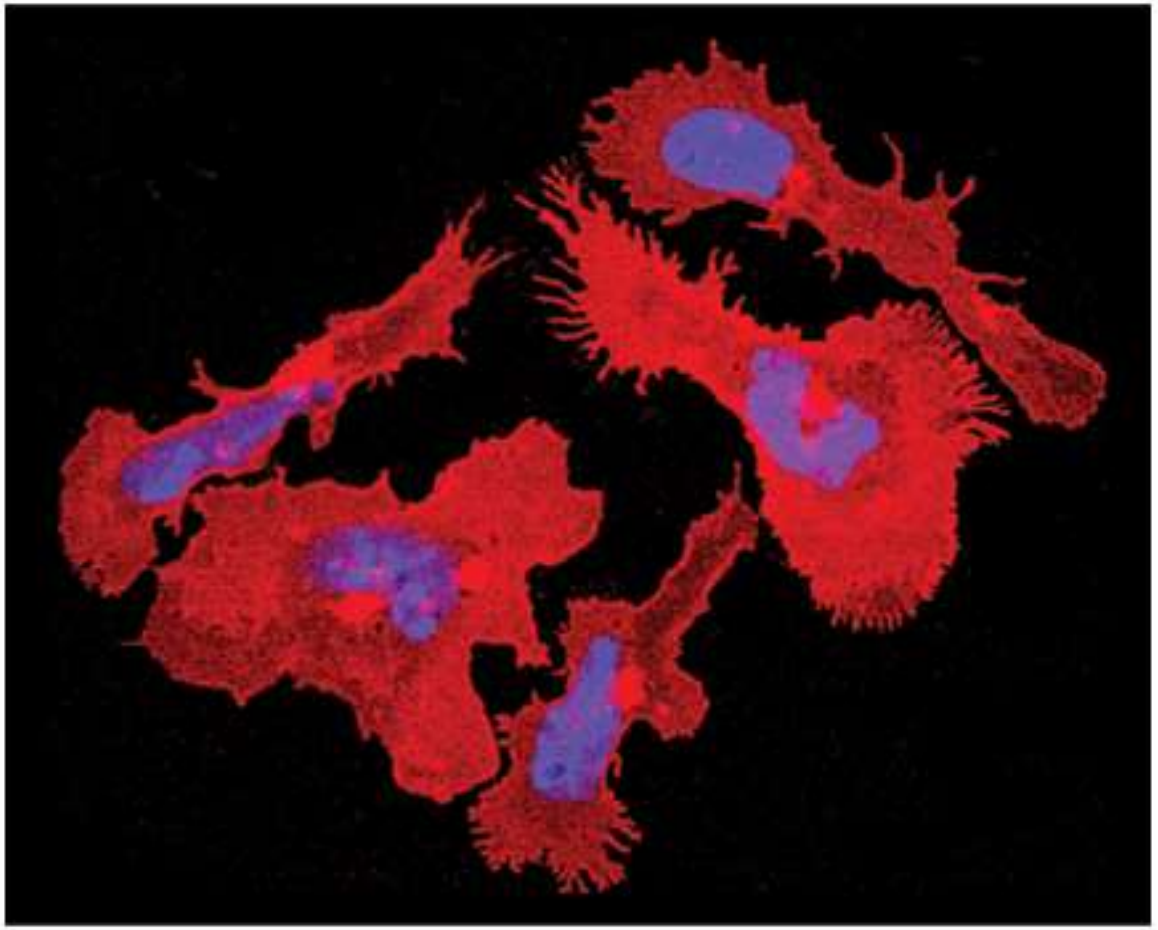
DANGER SIGNALS:

- EXOGENOUS (PAMPs)

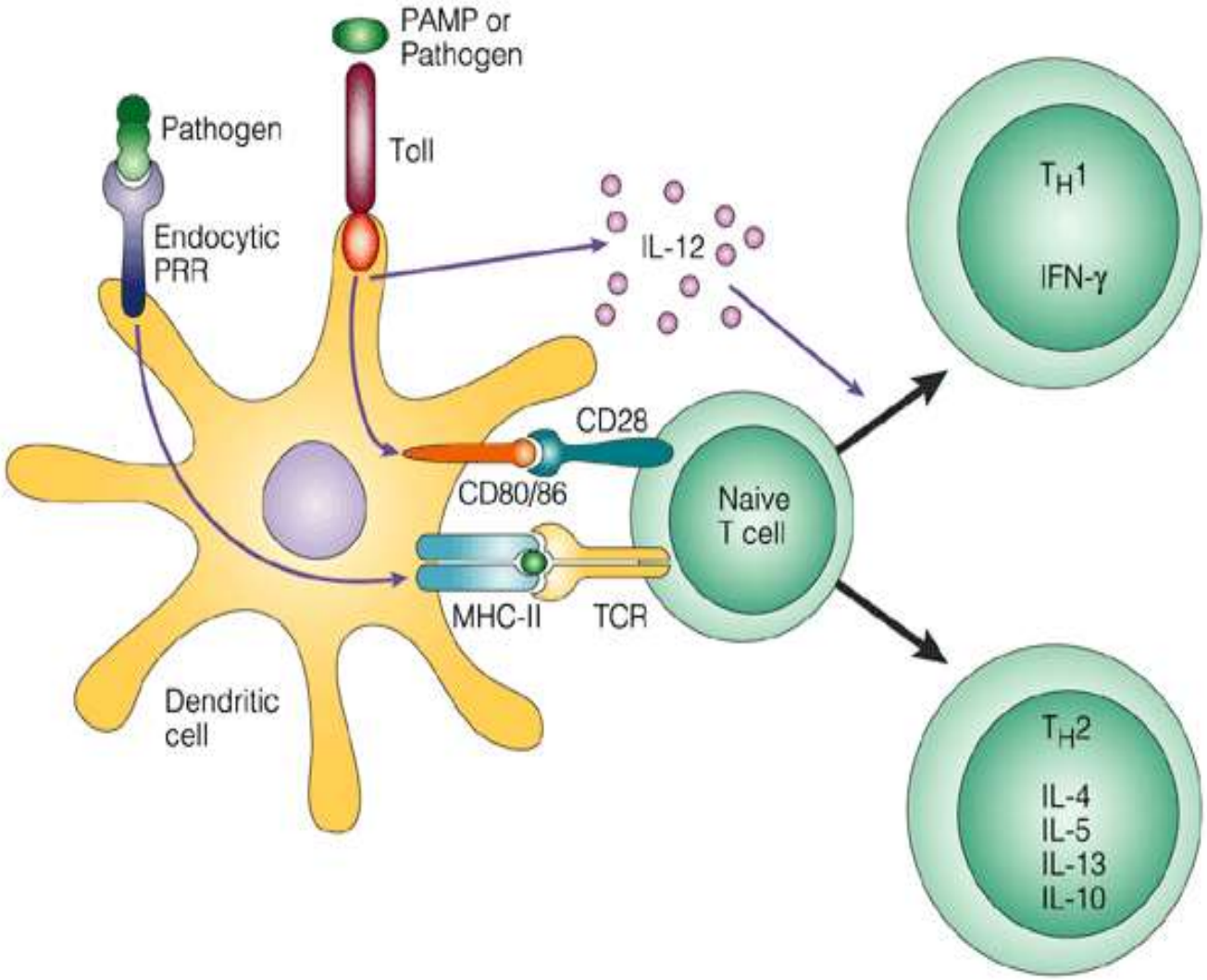
- ENDOGENOUS (e.g. STRESS PROTEINS RELEASED FROM NECROTIC CELLS)


GM-CSF
IL-4, IL-3
and FLT3L
Bone marrow
DC progenitor
Intermediate DC
Specialized for
antigen uptake
and processing
PAMP–TLR interactions
TNF–TNFR interactions
Flagellin
LPS
CpG
T cell
CD14
MD-2
TLR5
TLR9
TLR4
CD40L
CD40
TNF
TNFR
Mature DC
• High MHC class I and II expression
• Chemokine receptor expression
• Adhesion molecule expression
• Co-stimulatory molecule expression
(B7-1/B7-2, TNF family)

THE BEAUTY OF DENDRITIC CELLS…

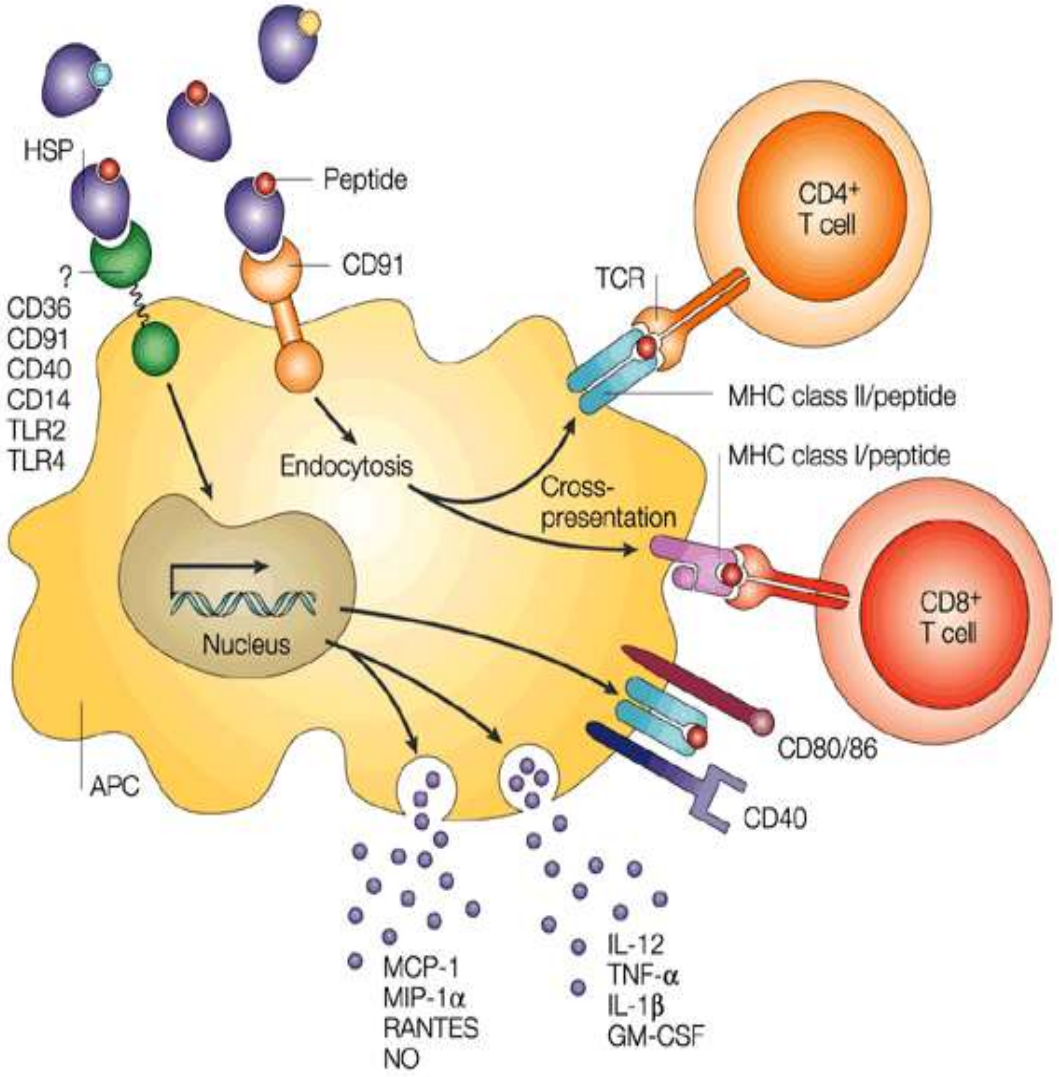


TLR or NOD signaling primes Th cell differentiation



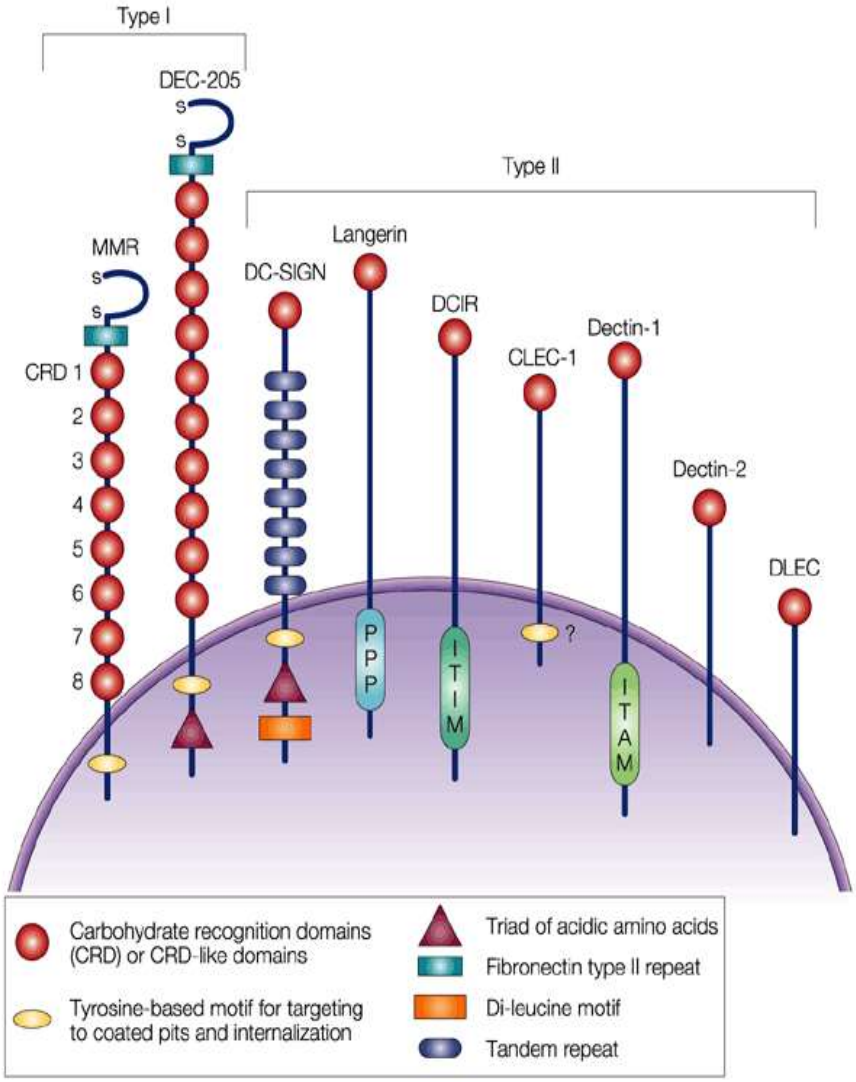
Nature Reviews | Immunology

Crosspresentation of antigens released from lyzed cells



Nature Reviews | Immunology

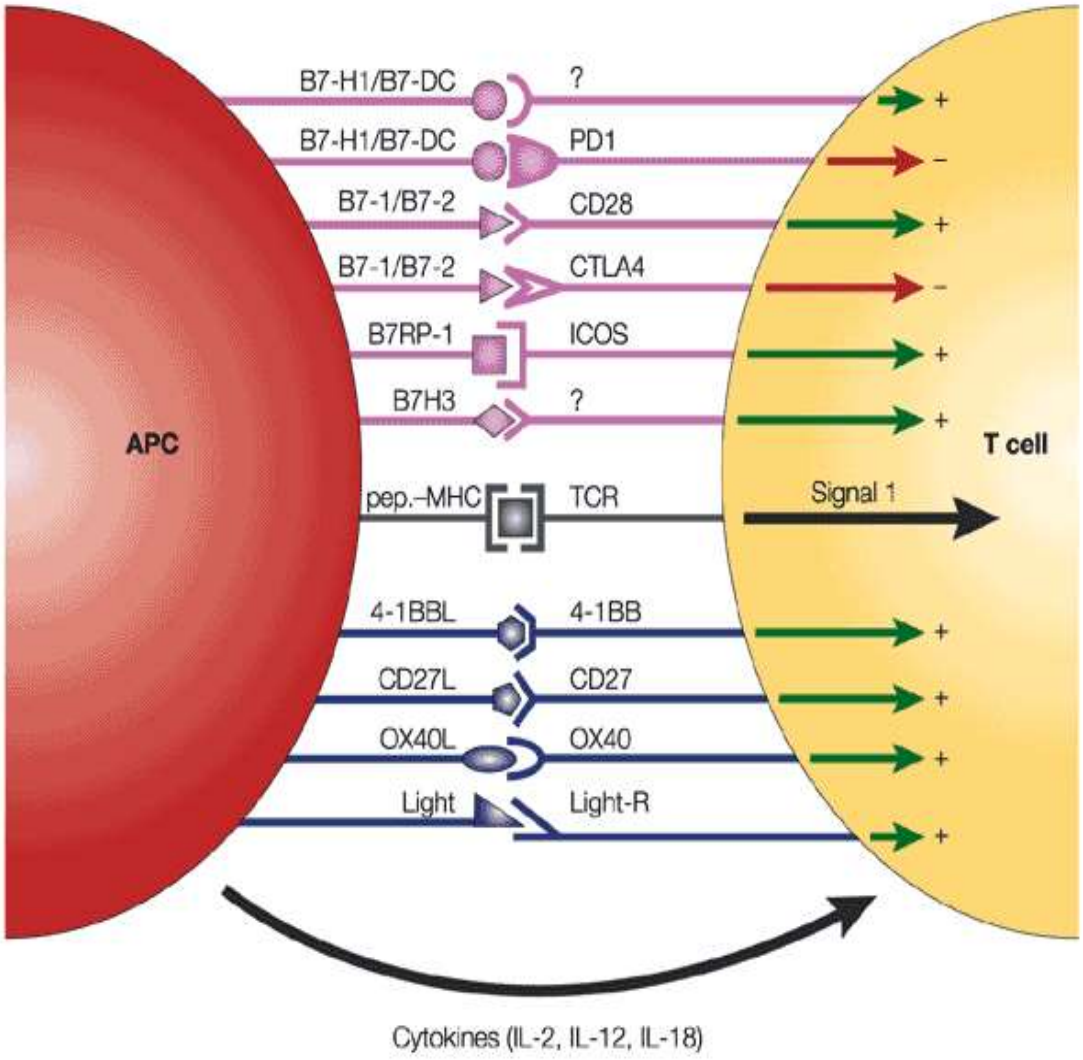
SURFACE LECTINS



Nature Reviews | Immunology

Dendritic cell as a bridge

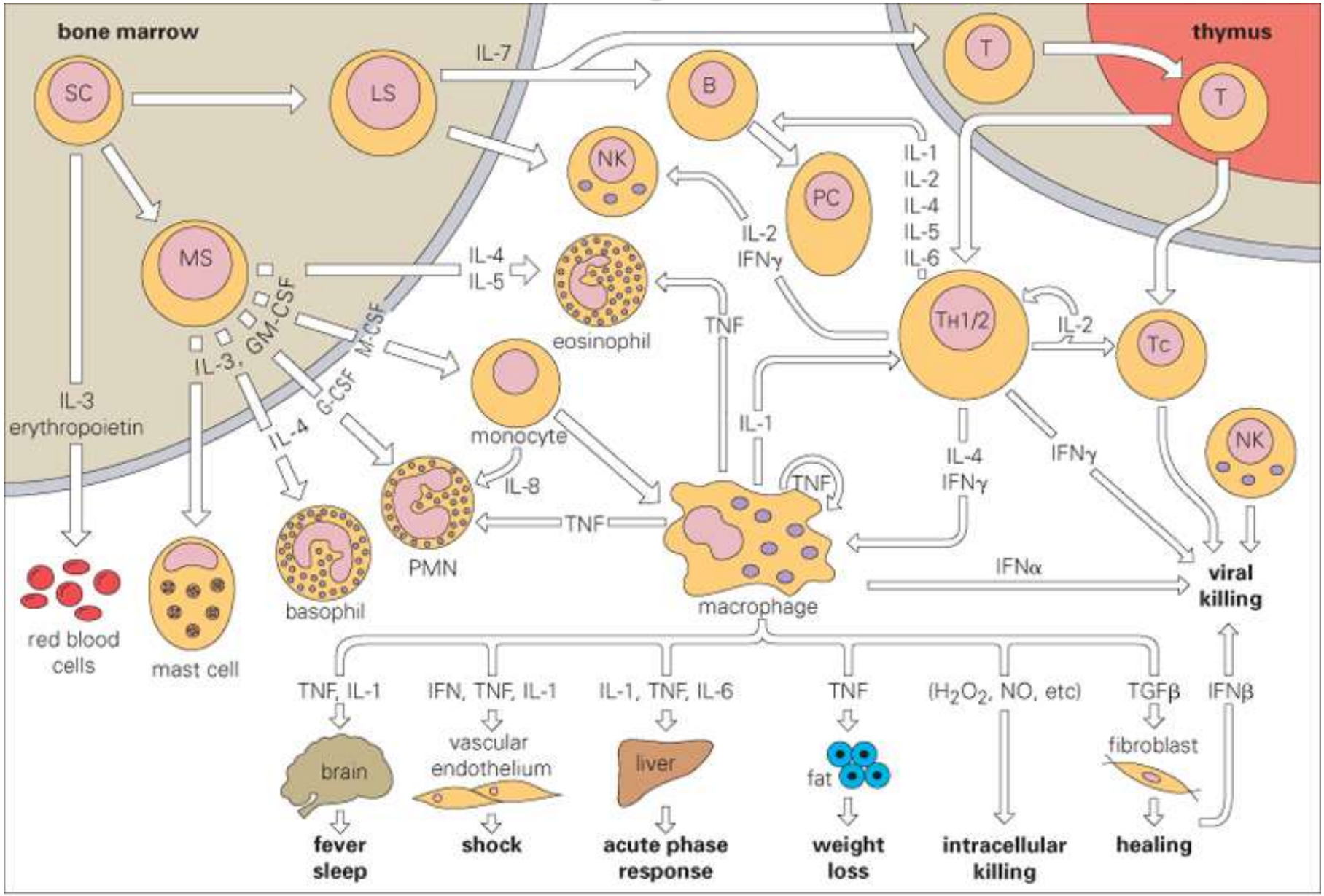
innate



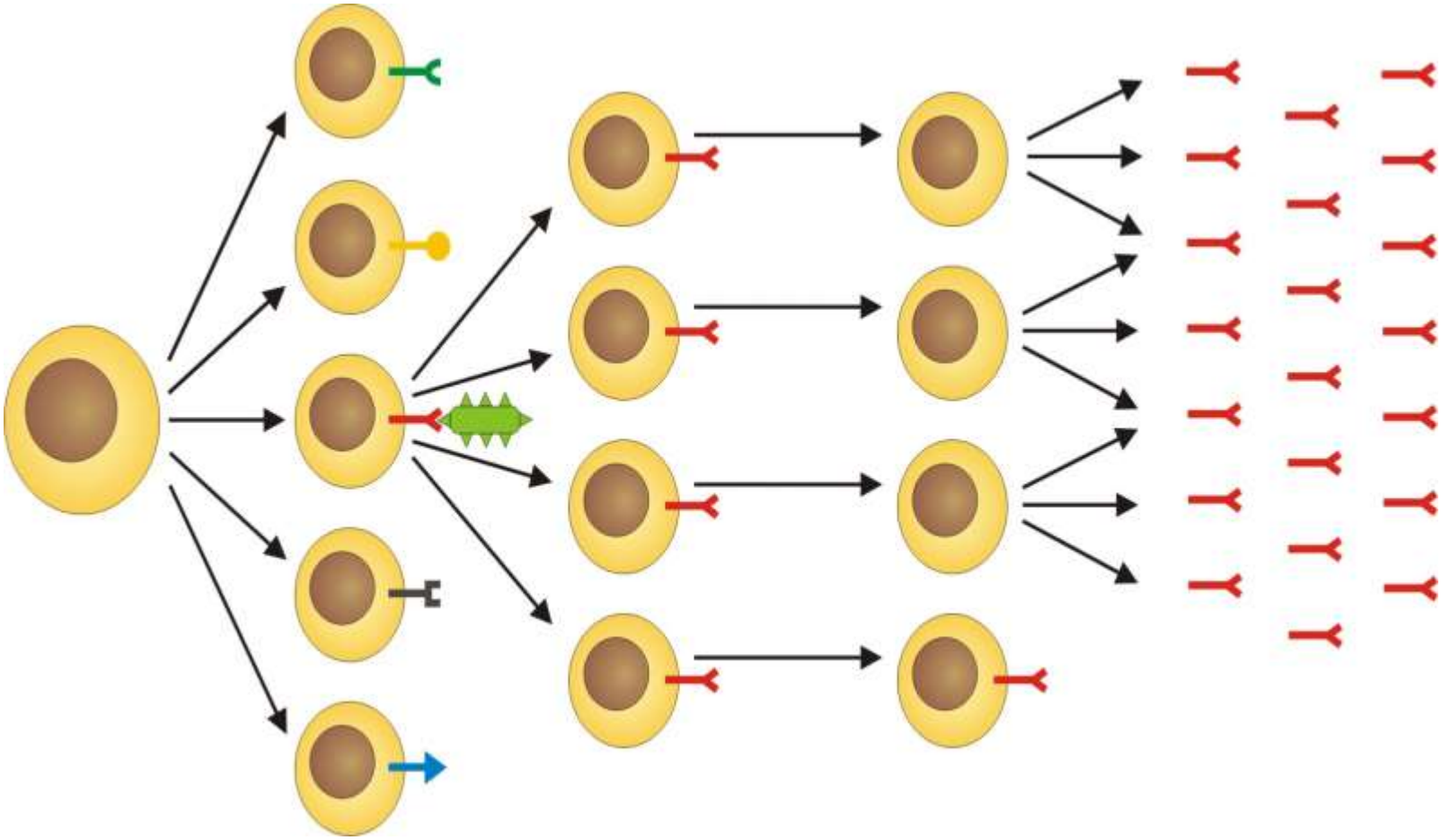
adaptive

Nature Reviews | Immunology

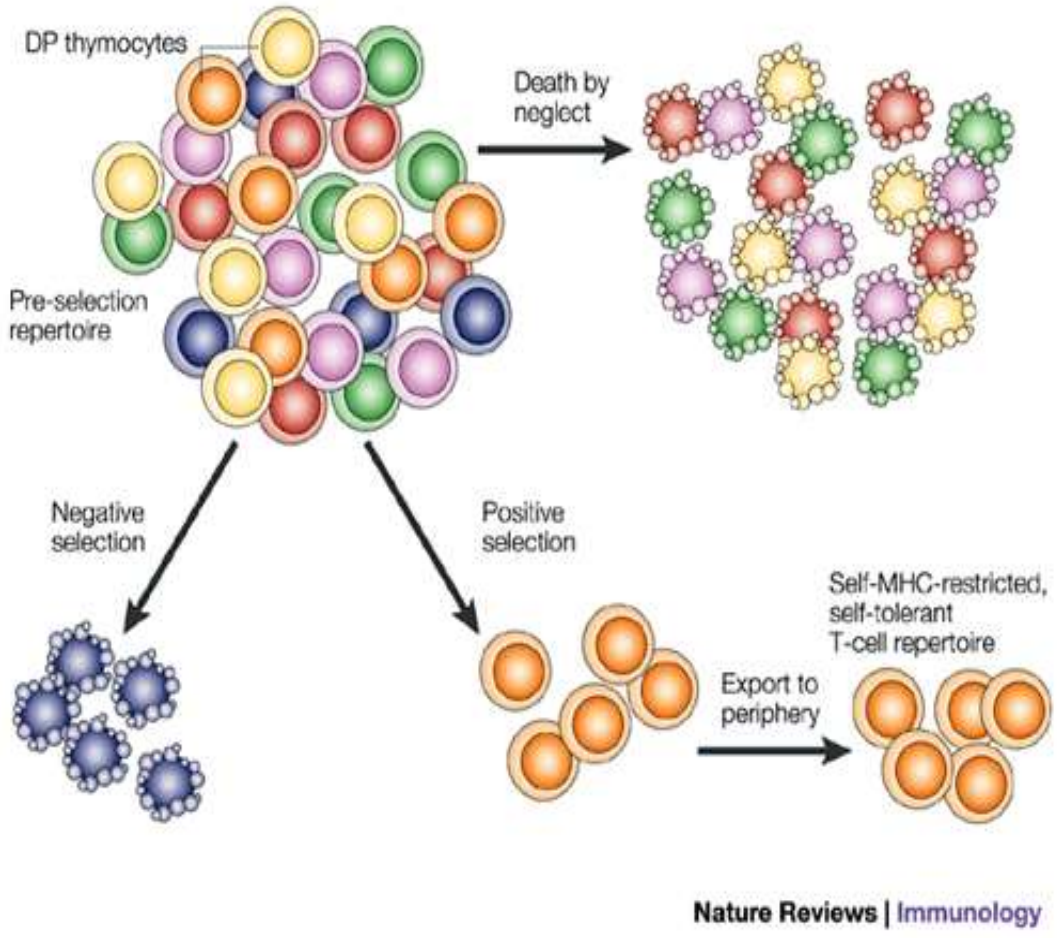
The immune system cell ZOO



B CELL DIFFERENTIATION



T LYMPHOCYTE DEVELOPMENT AND SELECTION IN THYMUS

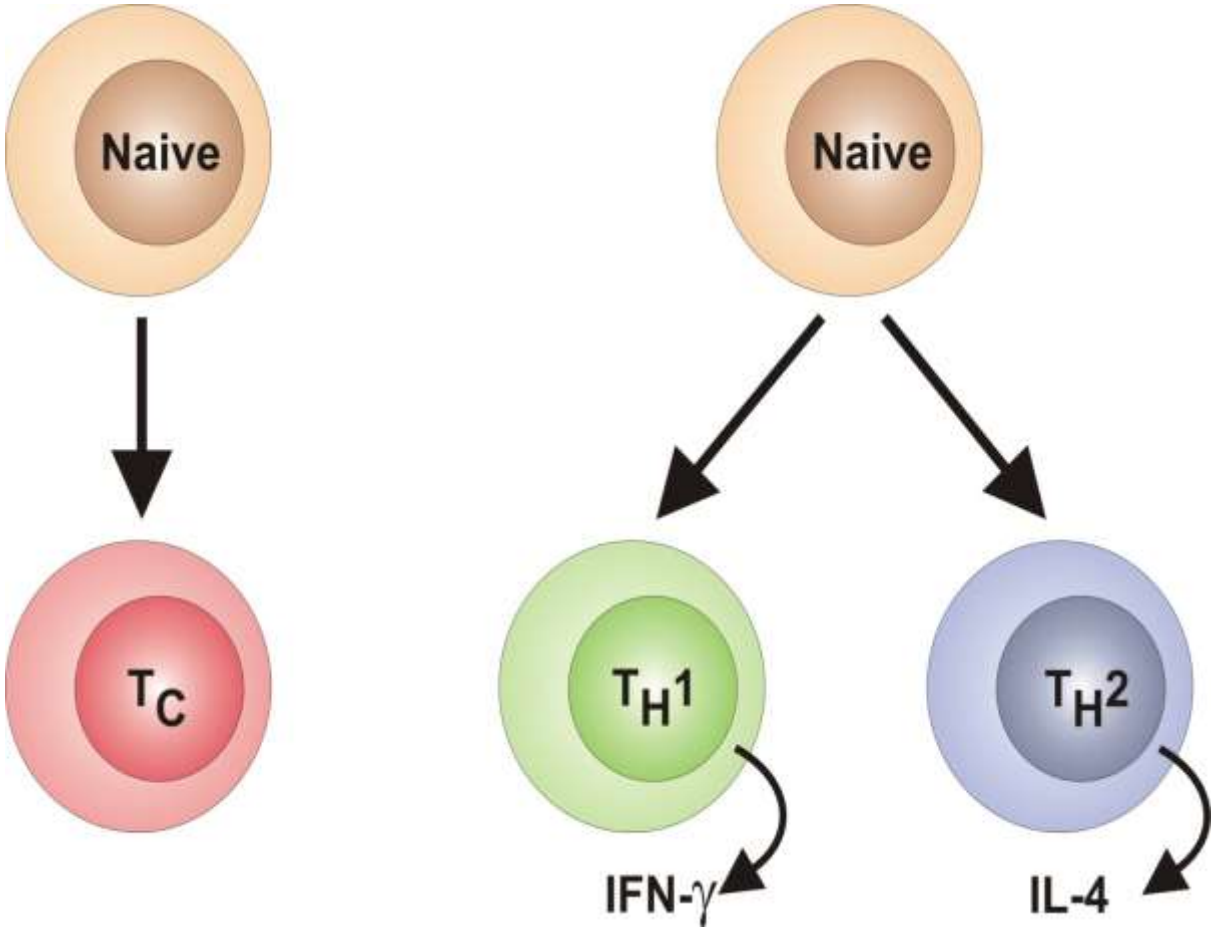


T-CELL RECEPTORS:

MAINLY RECOGNITION OF MHC-PEPTIDE COMPLEXES ON OTHER CELL'S SURFACE

PURPOSE: DETECTION OF CELLS INFECTED BY "HIDDEN" INTRACELLULAR PARASITES (e.g. VIRUSES)

T LYMPHOCYTES:
IMPORTANT FUNCTIONAL SUBSETS



THERE ARE MANY OTHER T CELL SUBSETS, SOME OF THEM HAVE HOMOGENOUS TCR AND THUS ARE RATHER OF "INNATE" CHARACTER

BASIC DOGMA FOR THE ADAPTIVE RESPONSES:

ANTIBODY RESPONSES (B, Th2) – EFFECTIVE FOR EXTRACELLULAR PARASITES

INFLAMMATORY RESPONSES (Th1, Tc) – EFFECTIVE FOR INTRACELLULAR PARASITES

MUTUAL INHIBITION Th1 vs. Th2 (POSITIVE FEEDBACK REGULATION)

WRONG CHOICE Th1 vs. Th2 CAN BE FATAL (LEPROSY…)

Th17 (IL-17) involved in autoim munity as well as infection clearance

Th1 x Th2

Bacterial strategies for overcoming host immune response

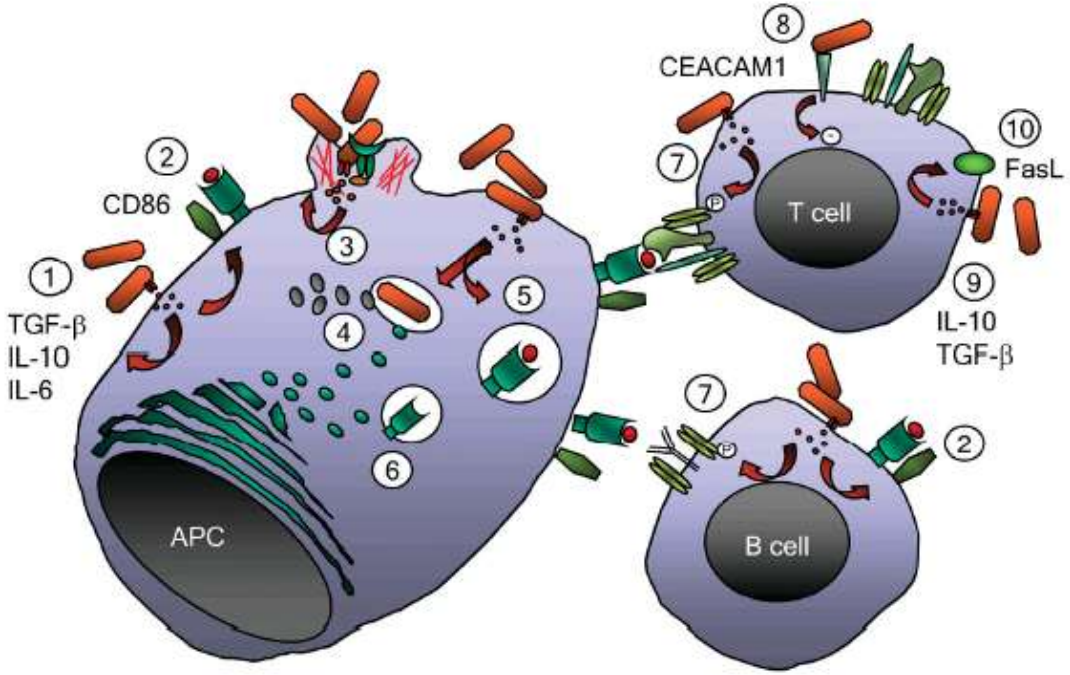
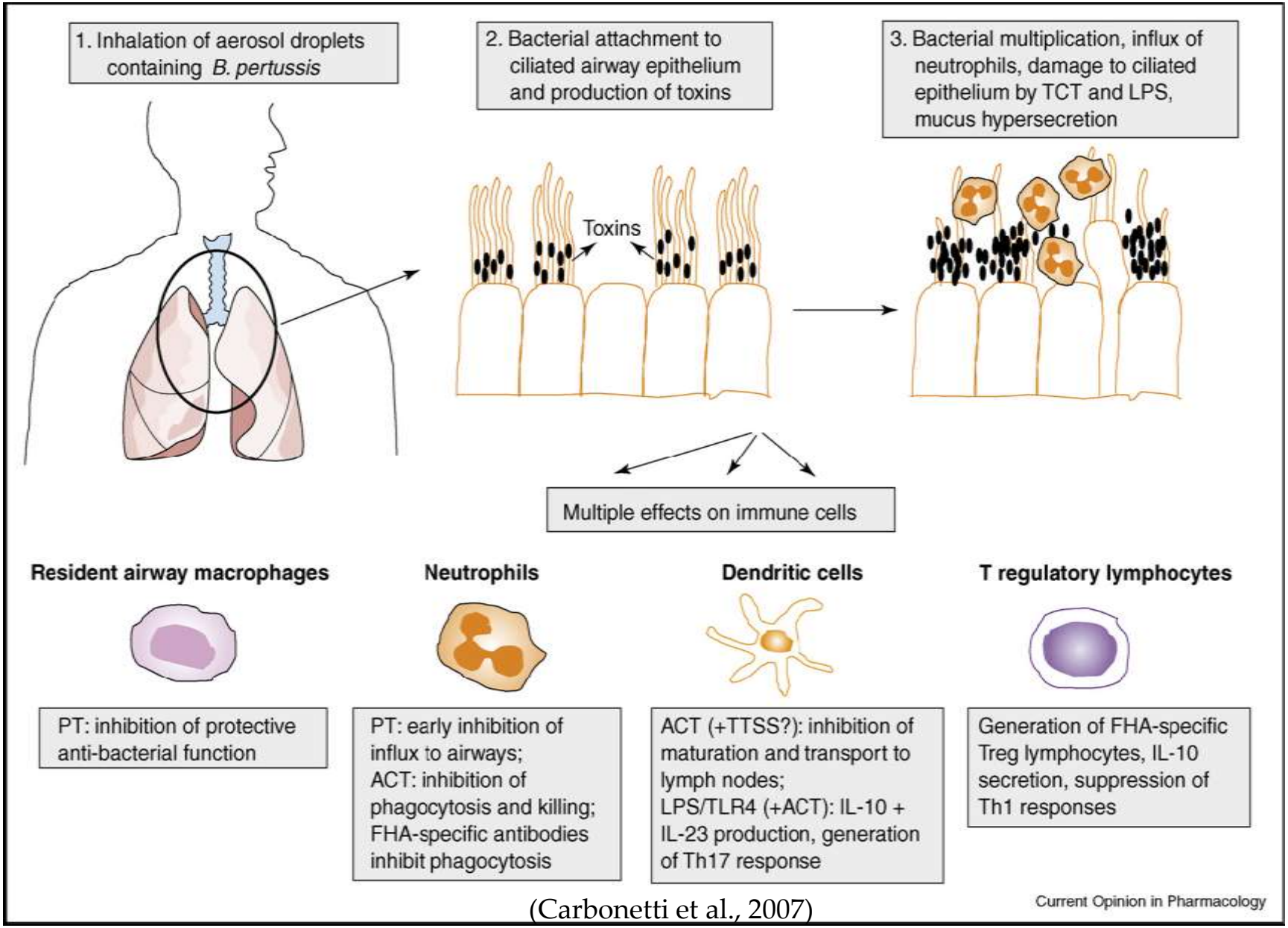


Figure 3. Bacterial defense strategies against the adaptive immune response. Strategies include the induction of immunosuppressive cytokines, such as IL-10, IL-6 and TGF-β (1); inhibition of pro-inflammatory cytokine production; and surface expression of costimulatory molecules such as CD86 (2) by antigen presenting cells (APC). Interference with bacterial uptake (3), phagosome maturation (4) and antigen processing (5) as well as MHC class I and II expression (6) also lead to diminished antigen presentation. Inhibiting tyrosine phosphorylation of the T and B cell receptors (7) and activating the inhibitory CEACAM1 receptor on T cells (8) further decreases effector cell function. Certain bacteria can also induce regulatory T cells (formerly called suppressor T cells) that dampen the immune response (9) or induce T cell apoptosis by enhancing FasL expression on T cells (10).

(Hornef et al., 2002)

Bordetella pertussis infection: immunomodulatory function of virulence factors

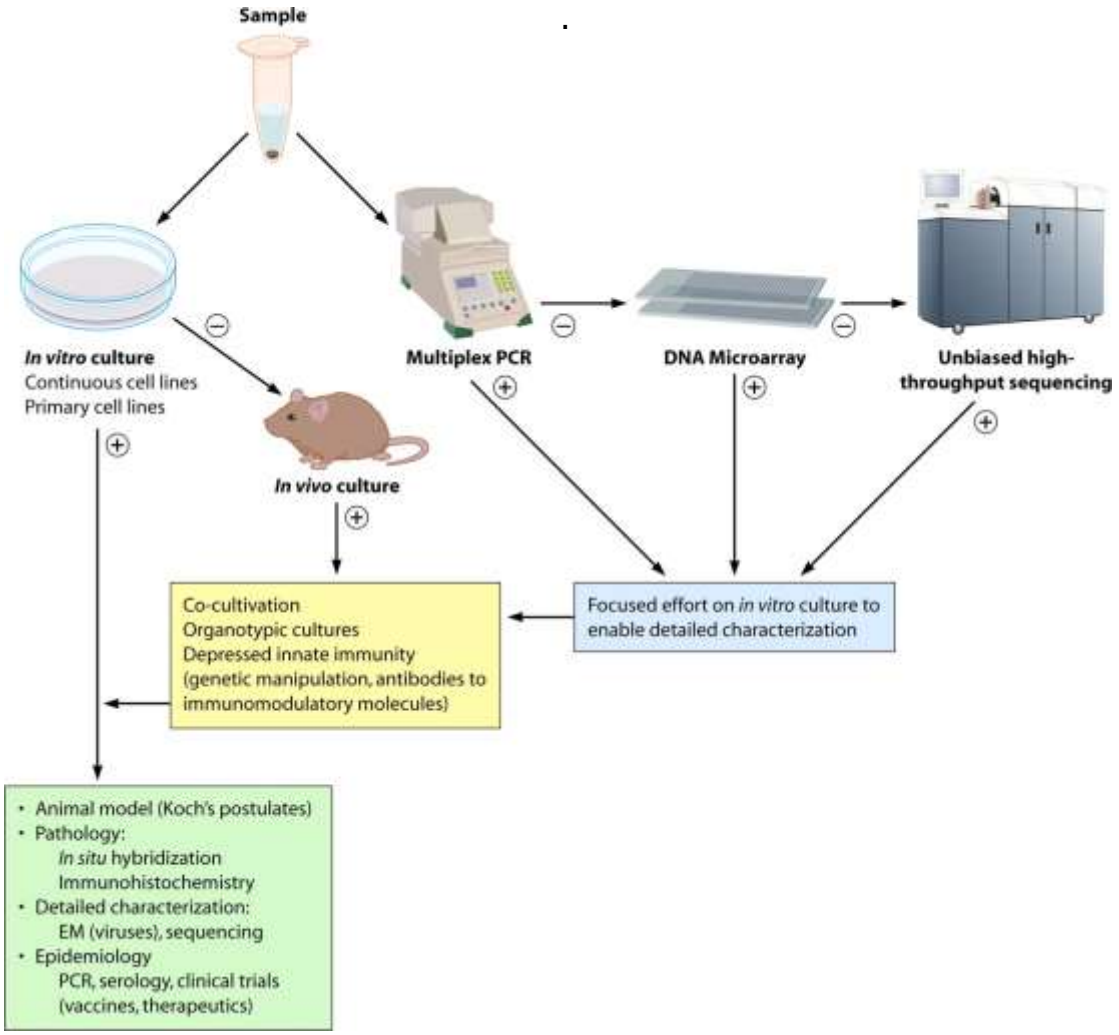


(Carbonetti et al., 2007)

2. Principles of virulence factor action.

Staged strategy for pathogen discovery and proof of causation:

In the molecular era of pathogen discovery, culture and molecular methods are pursued in parallel until an agent is detected, isolated, and characterized. +, positive result; –, negative result.



Lipkin, W. I.. 2010. Microbiol. Mol. Biol. Rev. 74(3):363-377

As an ideal, implication of an infectious agent can be viewed as a four-step process.

- ***Step 1. Detect an agent or its footprints in association with disease***
  - Isolate and propagate the agent in culture or in animal models
  - Image the agent or its gene products *in situ* by using immunological (immunohistochemistry) or molecular (*in situ* hybridization) methods
  - Demonstrate an agent-specific adaptive immune response (e.g., IgM or an increase in IgG titer, cell-mediated immunity)
- ***Step 2. Provide a plausible mechanism for an explanation of disease***
  - Demonstrate the presence of the agent, a gene product of the agent (e.g., a toxin), or a host product attributable to infection (e.g., a cross-reactive antibody or cytotoxic T cell) at site(s) of pathology
  - Demonstrate that the same or a similar agent can induce disease in a model system
- ***Step 3. Demonstrate that modulation of the agent concentration, or of a factor that can be attributed to the presence of the agent (e.g., an antibody), influences the presence or severity of disease***
- ***Step 4. Demonstrate that preventing infection prevents disease***

The Nature of Bacterial Host-Parasite Relationships in Humans

- Bacteria are consistently associated with the body surfaces of animals (Bacteria > Animal cells
- association with an animal = normal flora (symbiosis or **indigenous microbiota**)

Bacterial Pathogenesis

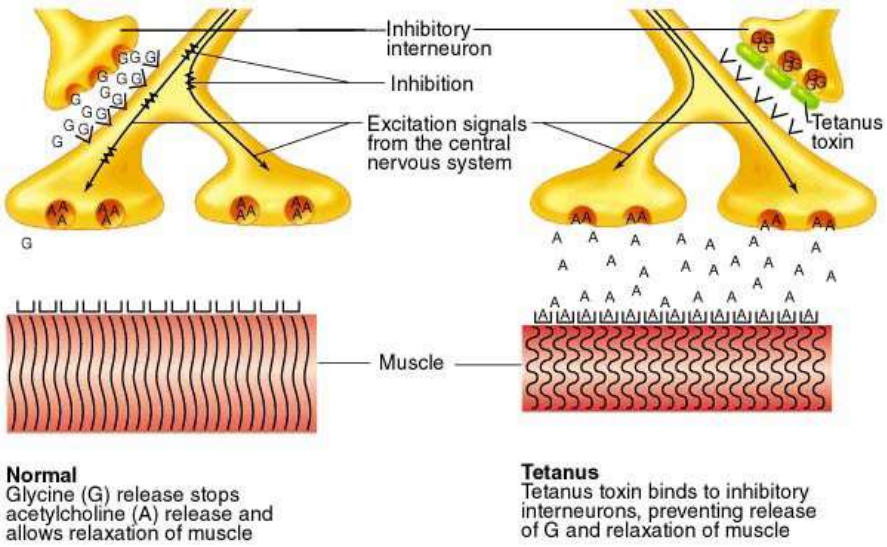
Koch (1890)	Rivers (1936)	Fredericks and Relman (1996)
The bacterium should be found in all people with the disease, and the bacterium or its products should be found in parts of the body affected by the disease	A virus must be associated with a disease with a degree of regularity	Candidate sequences should be present in most cases of disease and at sites of disease pathology
The bacterium should be isolated from the lesions of an infected person and maintained in pure culture	The association cannot be incidental	Few or no sequences should be present in host or tissue without disease
The pure culture, inoculated into a susceptible human volunteer or experimental animal, should produce the symptoms of the disease	Rivers predicted that methods for proving causal relationships would evolve with improvements in technology; at the time of his writing, he invoked seroconversion and experimental inoculations but acknowledged the limitations of both	Sequences should diminish in frequency with resolution of disease and increase with relapse
The same bacterium should be reisolated in pure culture from the intentionally infected animal or human		Sequences should be present prior to the onset of disease

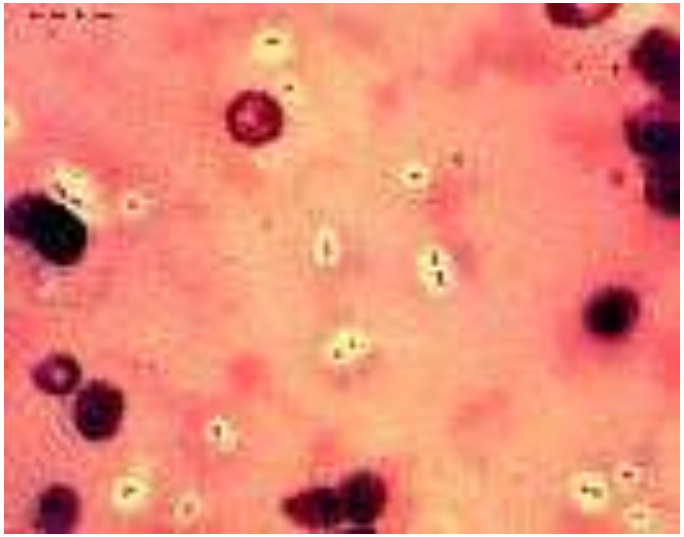
Basic terms in bacteriology

- **infection** – bacterium capable of causing disease becomes established in the body
- **disease** – infection that produces symptoms
- colonization – persistence in a particular body site of bacteria that do not cause disease (resident microflora)
- **asymptomatic carriag**e – if the host defences are adequate, a person can be infected without any sign of symptoms (→ spreading the infection)

Invasiveness and toxogenicity

- **Inavsinevess: Ability of bacteria to infiltrate the host organism and spread out**
- **Toxogenicity: Ability of bacteria to produce metabolic substances toxic for host organism**





Clostridium tetani – toxin tetanospasmin, neinvazivní bakterie

Streptococcus pneumoniae – bez toxinu, invazivní bakterie

Virulence (pathogenicity)

- ability of a bacterium to cause infection

- Virulence assessment:
  - LD50 (lethal dosis – number of bacterial cells/animal → 50 % killed
  - ID50 (infection dosis – number of bacterial cells/animal → 50 % infected

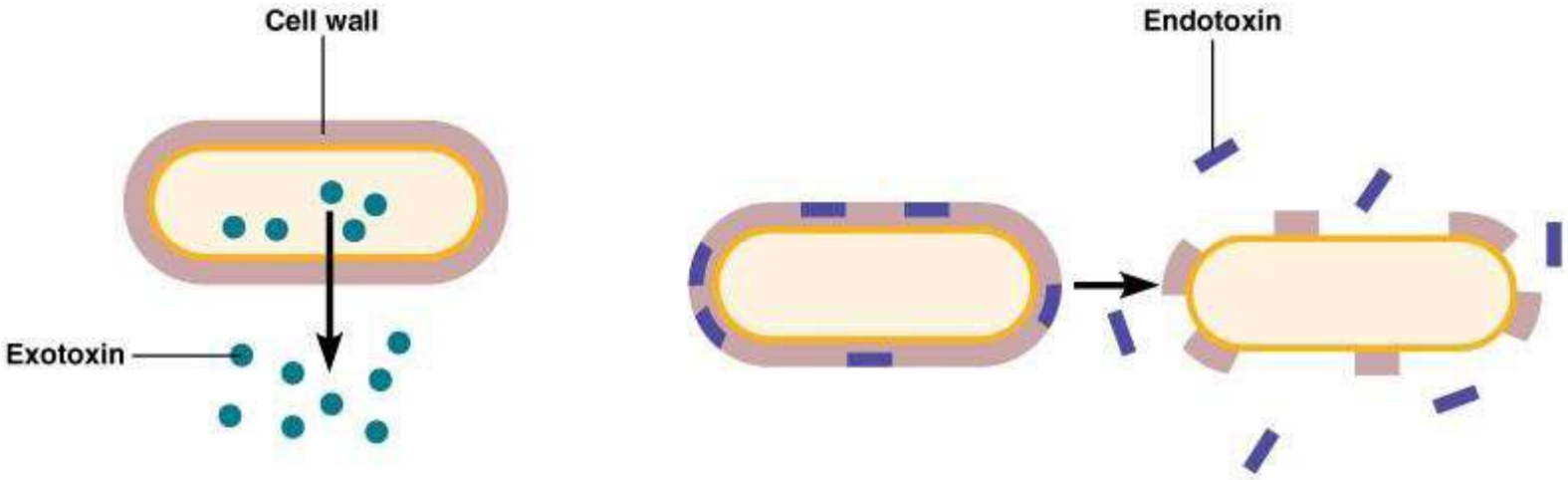
Virulence factor

- **a bacterial product or strategy that contributes to virulence or pathogenicity**

- What should be considered as a virulence factor?
  - some bacterial traits and products (ability to adhere, toxic proteins) with direct conection with the infection process
  - some traits (energy from sugar fermentation) – housekeeping functions, but still essential for the infection
  - virulence factors can be used as a target of a vaccine or therapeutic strategy

Virulence factor	Function
Pili	Adherence to mucosal surfaces
Nonfimbrial adhesins	Tight binding to host cell
Bacterial triggering of actin rearrangement in host cells	Forced phagocytosis of bacteria by normally nonphagycytic host cells; movement of bacteria within host cells or from one host cell to another
Binding to and entry of M cells	M cells used as natural port of entry into underlying tissue
Motility and chemotaxis	Reaching mucosal surfaces (especially areas with fast flow)
sIgA proteases	Prevent trapping of bacteria in mucin
Siderophores, surface proteins that bind transferrin, lactoferrin, ferritin, or hemin	Iron acquisition
Capsules (usually polysaccharides)	Prevent phagocytic uptake; reduce complement activation
Altered LPS O antigen	MAC not formed; serum resistance
C5a peptidase	Interferes with signaling function of complement
Toxic proteins	Kill phagocytes; reduce strength of oxidative burst
Variation in surface antigens	Evade antibody response

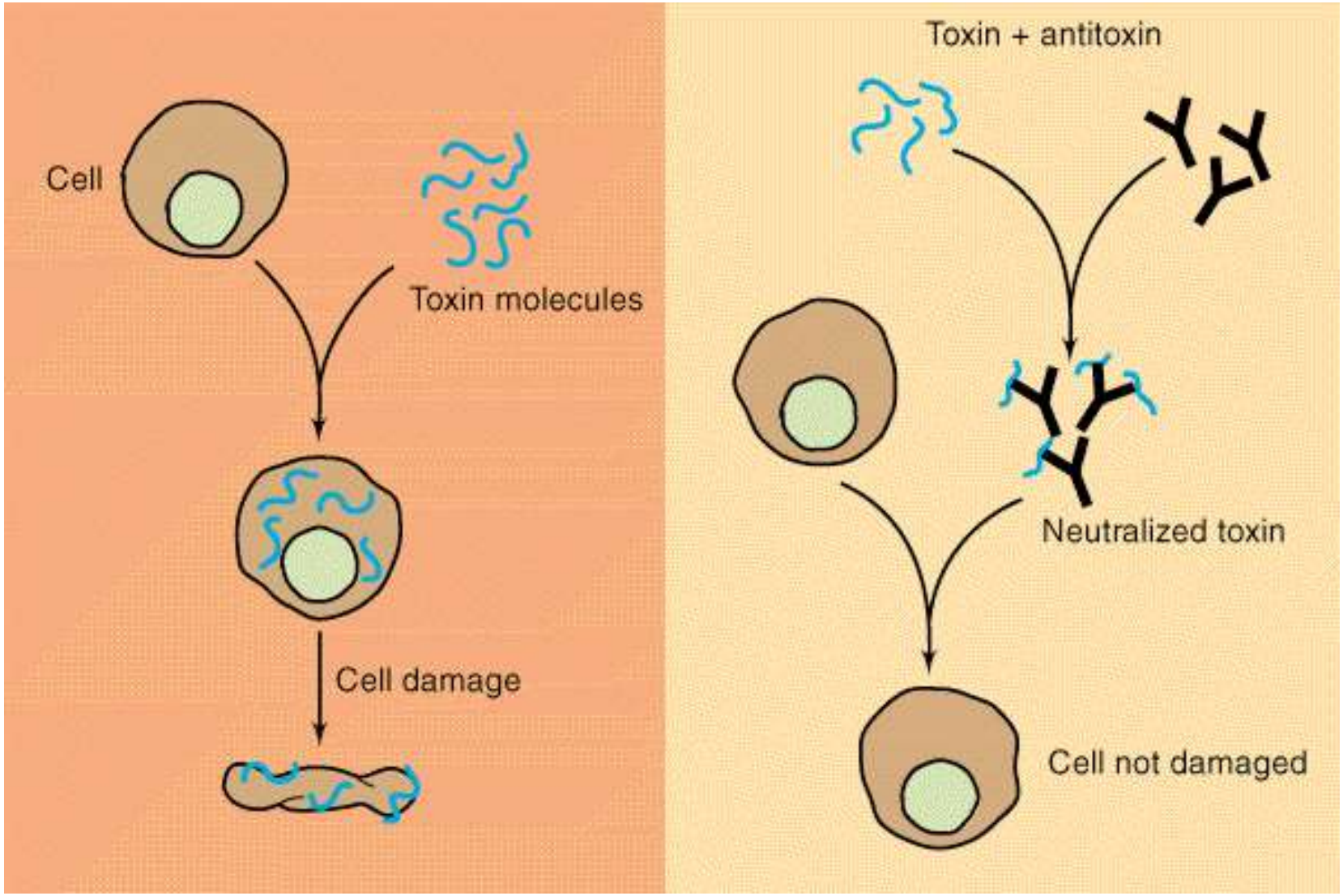
Exo- and Endotoxins



(a) **Exotoxins** are produced inside mostly gram-positive bacteria as part of their growth and metabolism. They are then secreted or released following lysis into the surrounding medium.

(b) **Endotoxins** are part of the outer portion of the cell wall (lipid A; see Figure 4.12c) of gram-negative bacteria. They are liberated when the bacteria die and the cell wall breaks apart.

Antibody mechanism against bacterial toxin



Mechanisms of bacterial toxin action

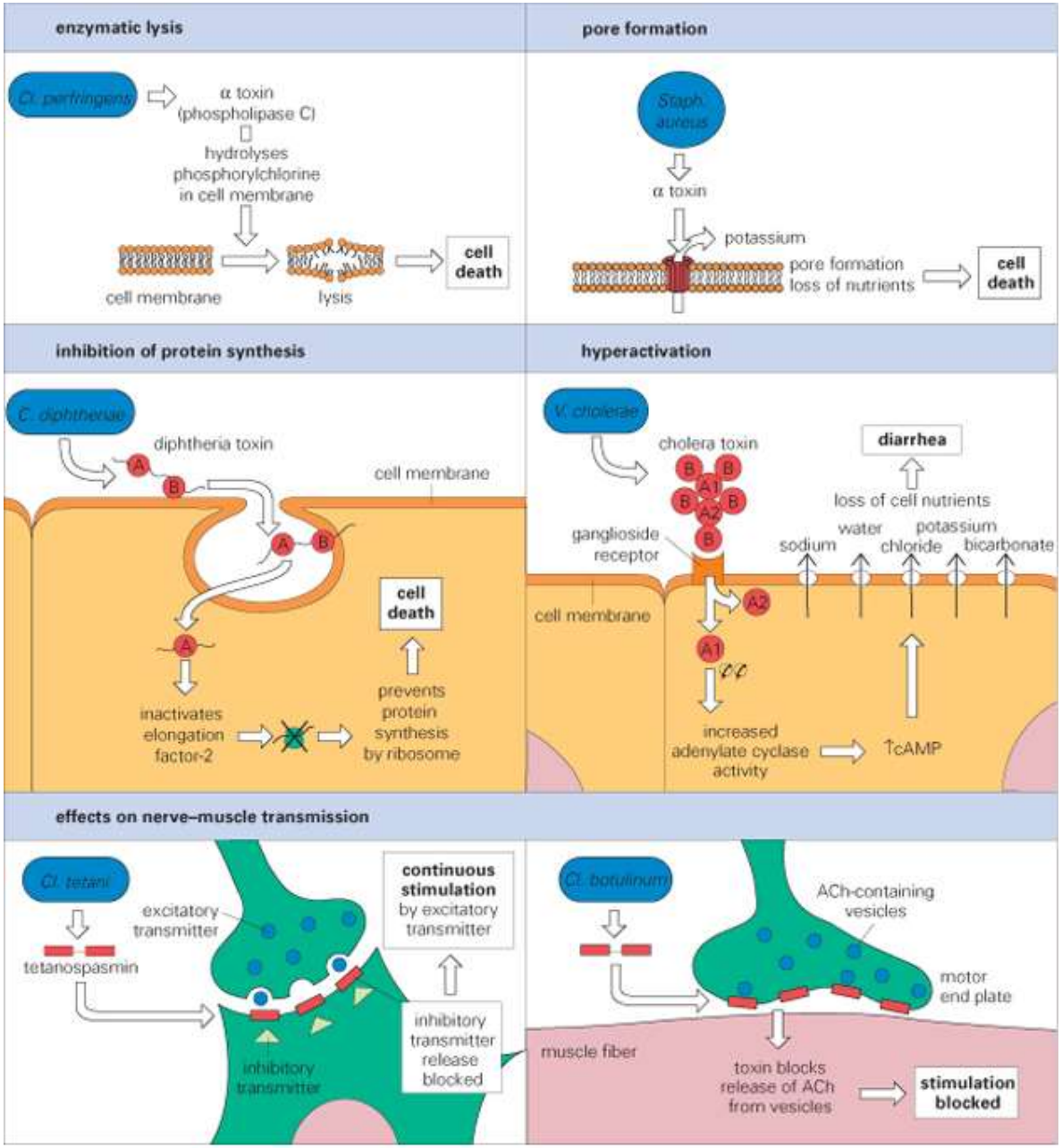


Table 1.5 Examples of Bacterial Toxins; Mechanisms of Action and Contribution to Clinical Picture

Toxin	Cell specificity	Molecular effect	Contribution to clinical picture
AB toxins			
Diphtheria toxin (*Corynebacterium diphtheriae*)	Many different cell types	ADP-ribosyl transferase. Inactivation of ribosomal elongation factor eEF2 resulting from ADP-ribosylation during protein synthesis; leads to cell death.	Death of mucosal cells. Damage to heart musculature, kidneys, adrenal glands, liver, motor nerves of the head.
Cholera toxin (*Vibrio cholerae*)	Enterocytes	ADP-ribosyl transferase. ADP-ribosylation of regulatory protein G_s of adenylate cyclase, resulting in permanent activation of this enzyme and increased levels of cAMP (second messenger) (see Fig. 4.**20**, p. 298). Result: increased secretion of electrolytes.	Massive watery diarrhea; severe loss of electrolytes and water.
Tetanus toxin (*Clostridium tetani*)	Neurons (synapses)	Metalloprotease. Proteolytic cleavage of protein components from the neuroexocytosis apparatus in the synapses of the anterior horn that normally transmit inhibiting impulses to the motor nerve terminal.	Increased muscle tone; cramps in striated musculature.

AB exotoxins

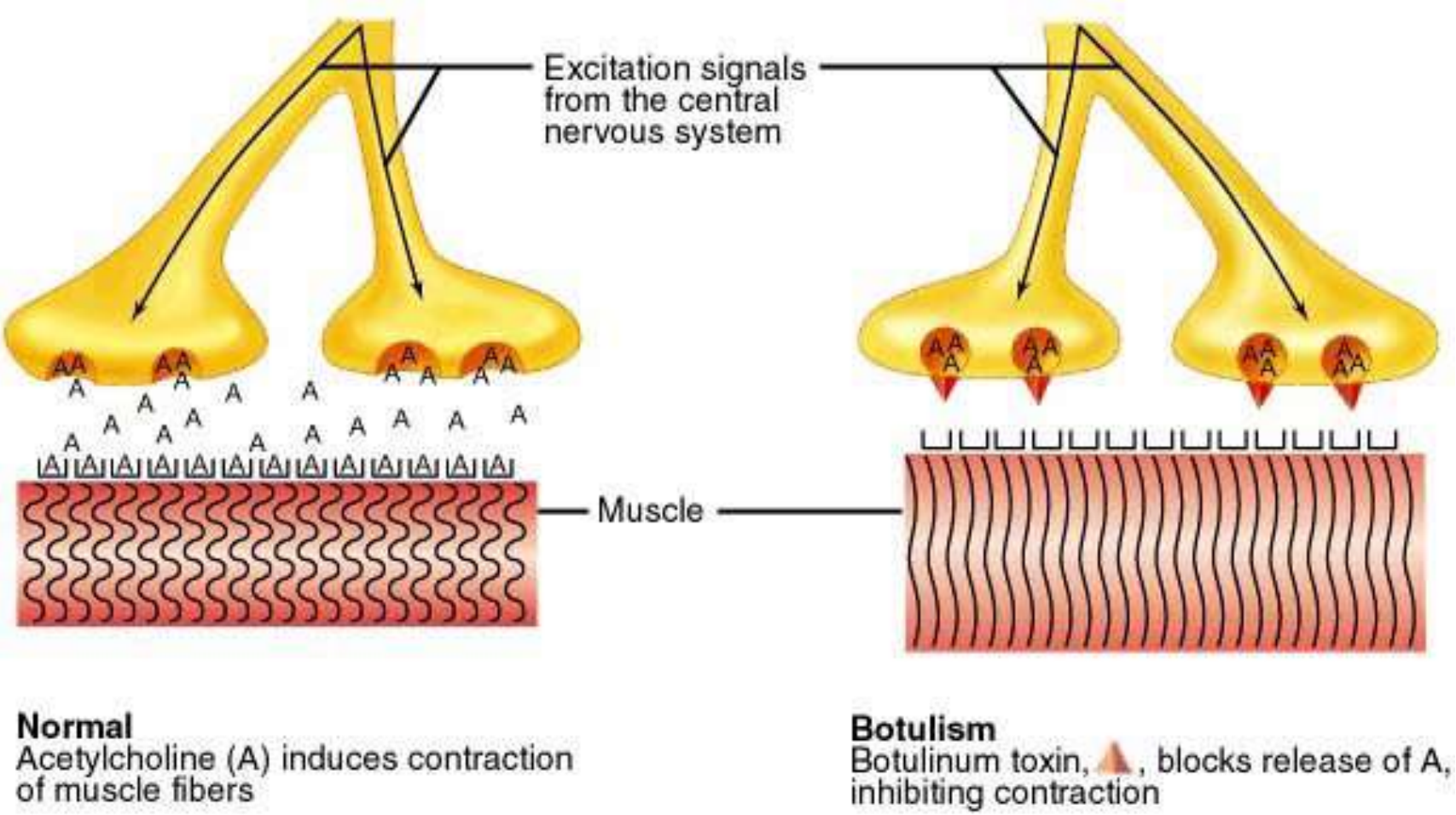
Membrane toxins

Alpha toxin (*Clostridium perfringens*)	Many different cell types	Phospholipase.	Cytolysis, resulting tissue damage.
Lysteriolysin (*Listeria monocytogenes*)	Many different cell types	Pore formation in membranes.	Destruction of phagosome membrane; intracellular release of phagocytosed listeriae.

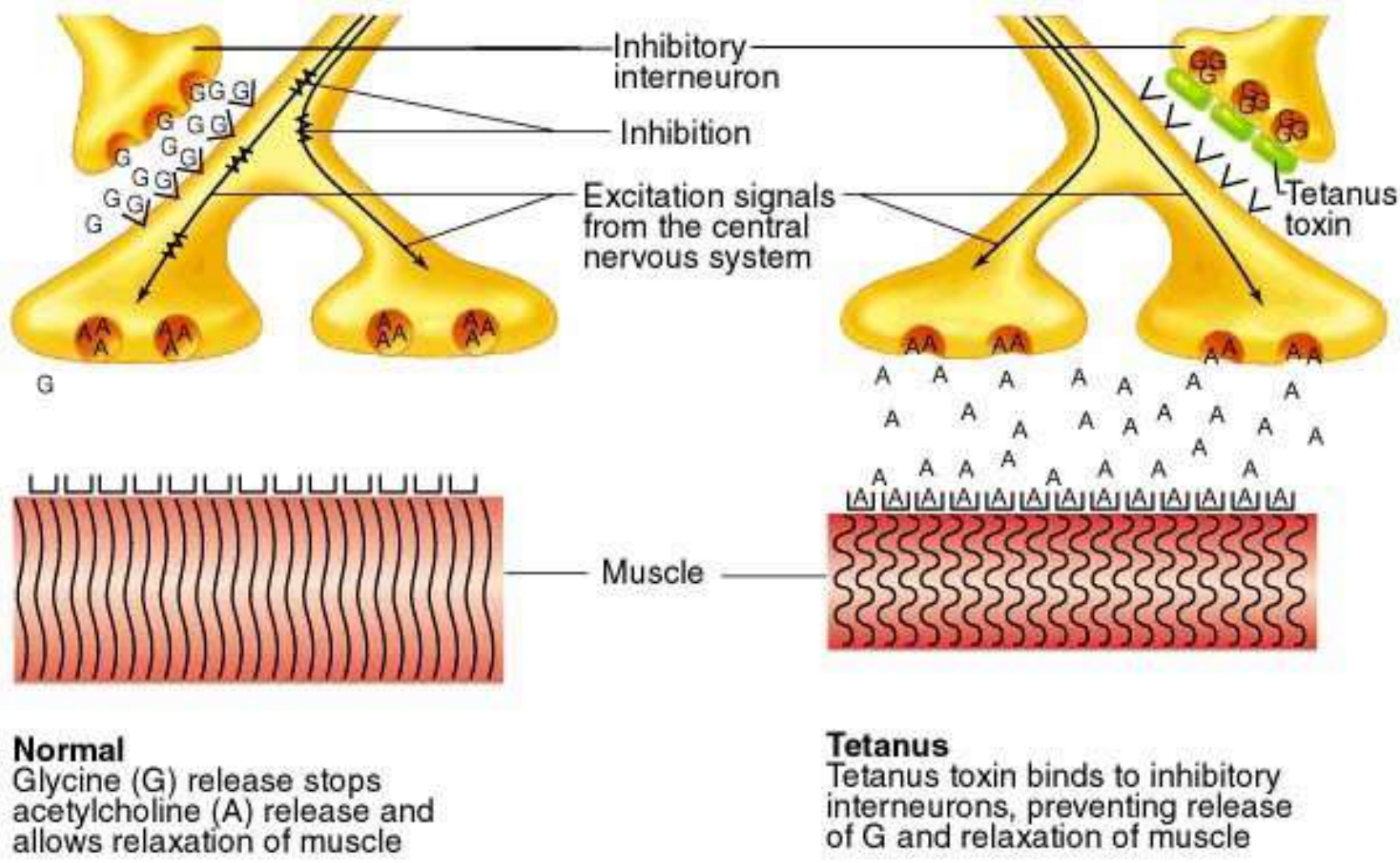
Superantigen toxins

Toxin	Cell specificity	Molecular effect	Contribution to clinical picture
Superantigen toxins			
Toxic shock syndrome toxin-1 (TSST-1) (*Staphylococcus aureus*)	T lymphocytes; macrophages	Stimulation of secretion of cytokines in T cells and macrophages.	Fever; exanthem; hypotension.

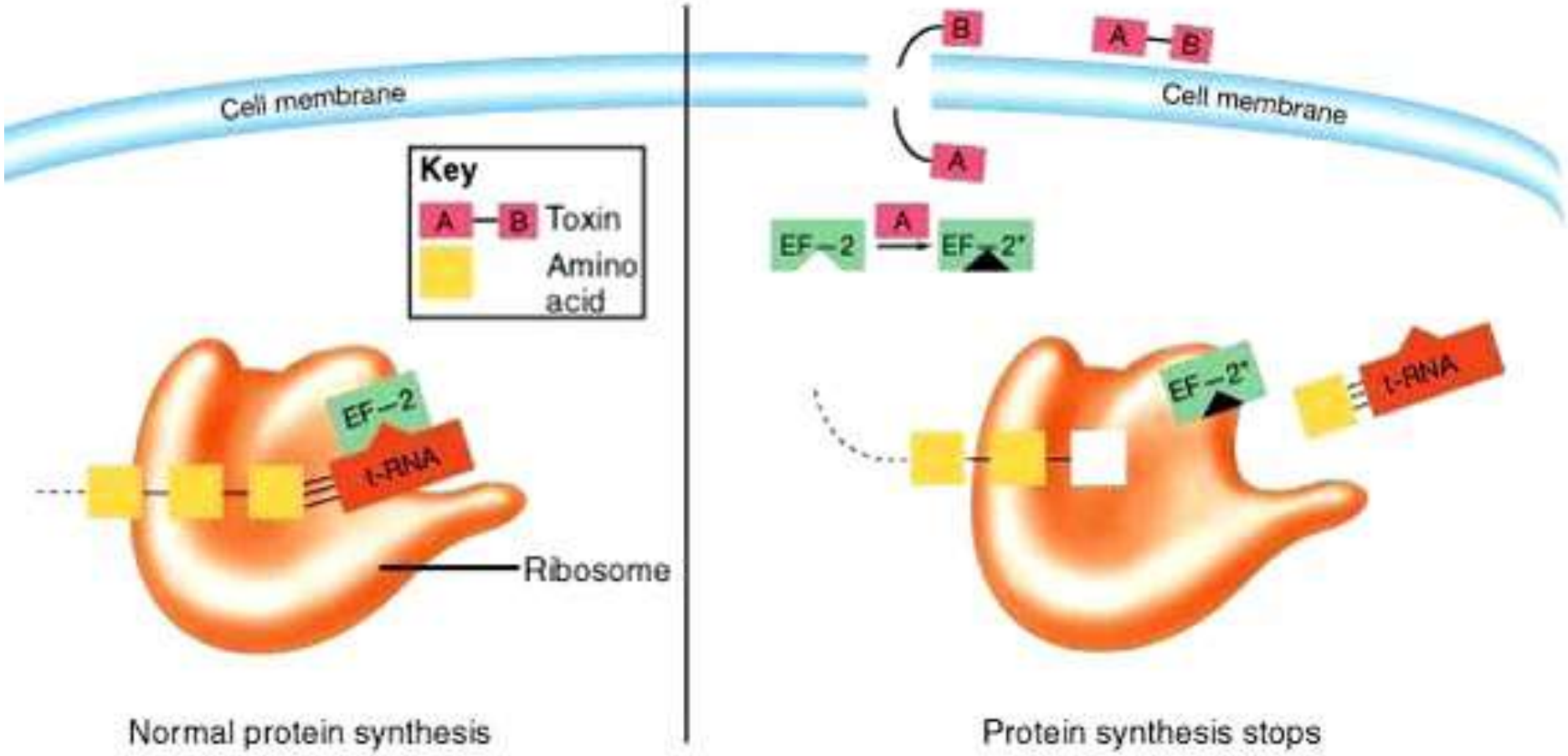
Clostridium botulinum (botulismus)



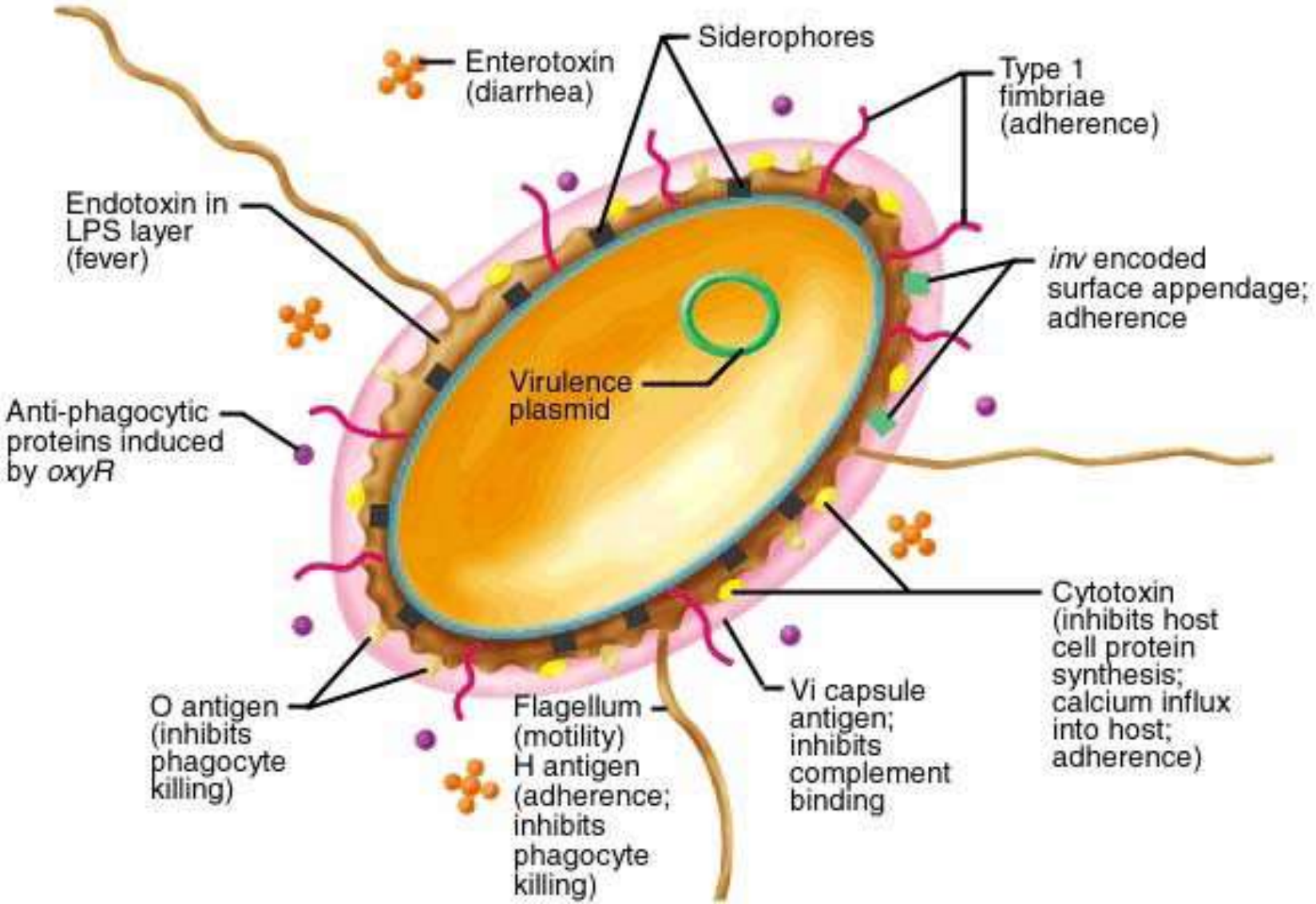
Clostridium tetani (tetanus)



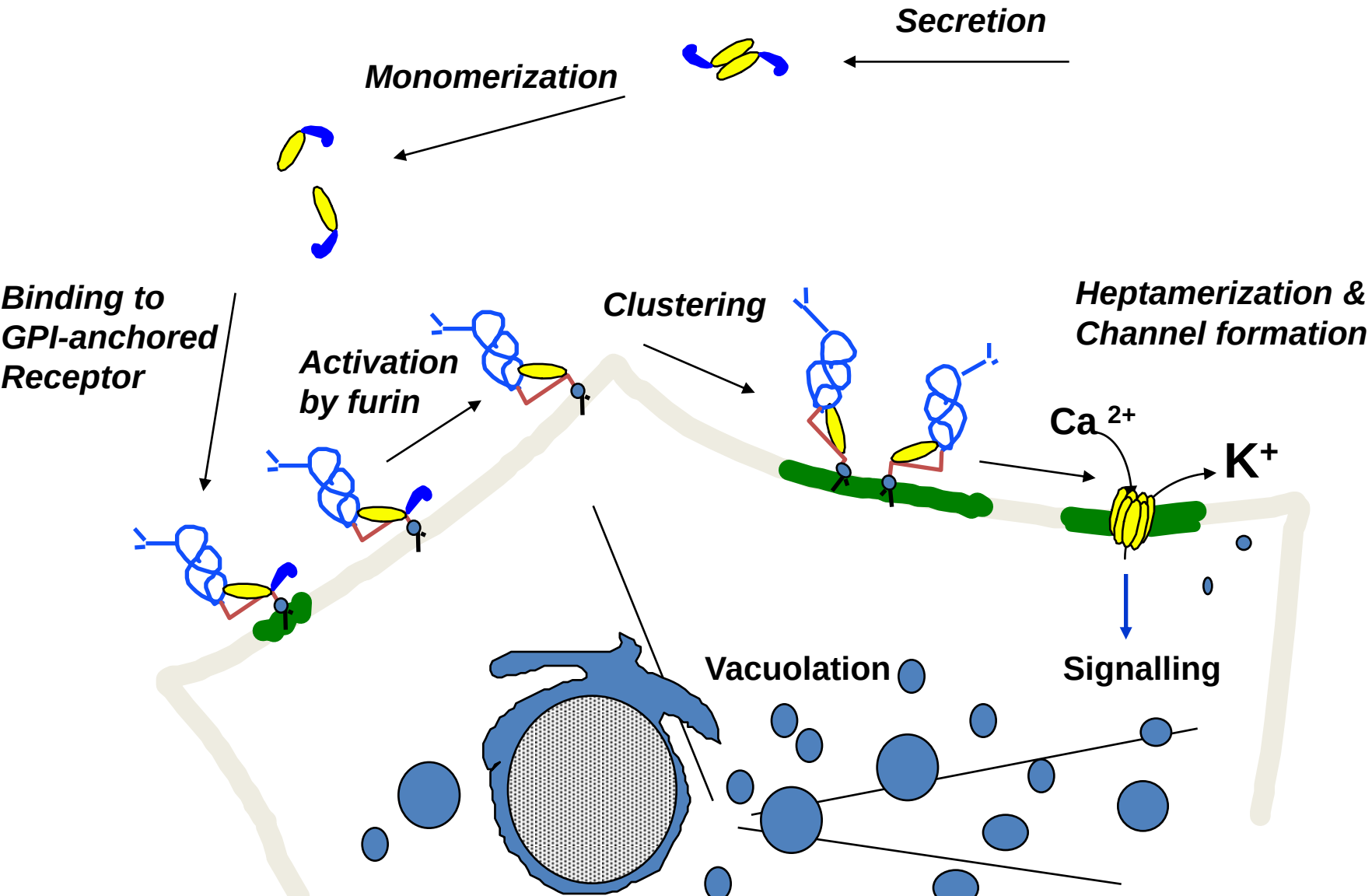
Corynebacterium diphteriae (záškrt)



Virulence factors of *Salmonella*

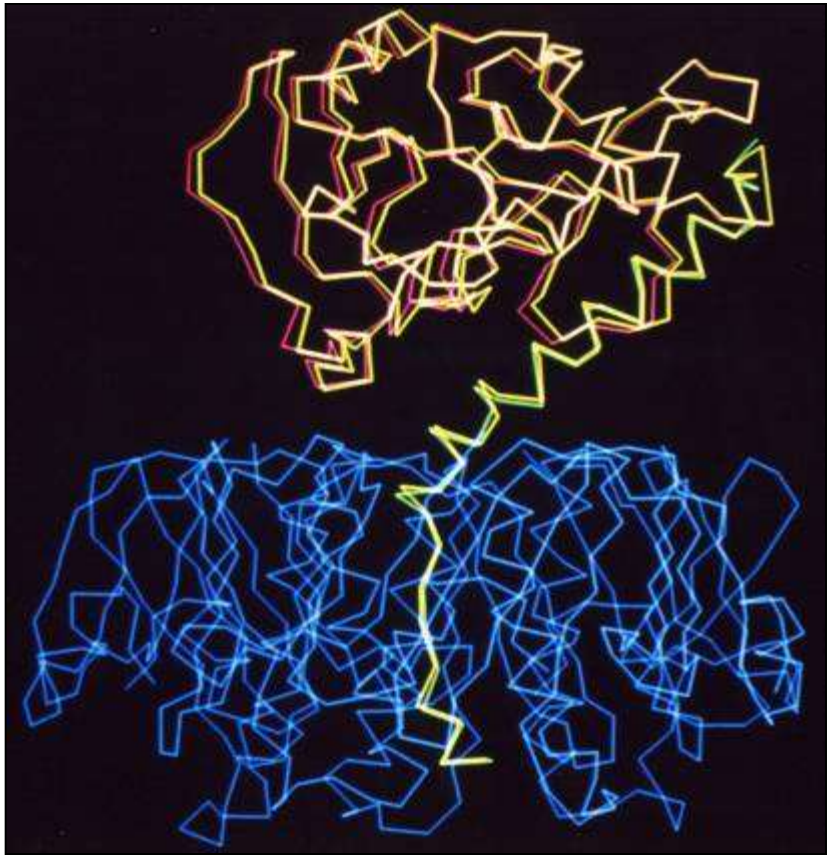


Proaerolysin: from secretion to pore formation

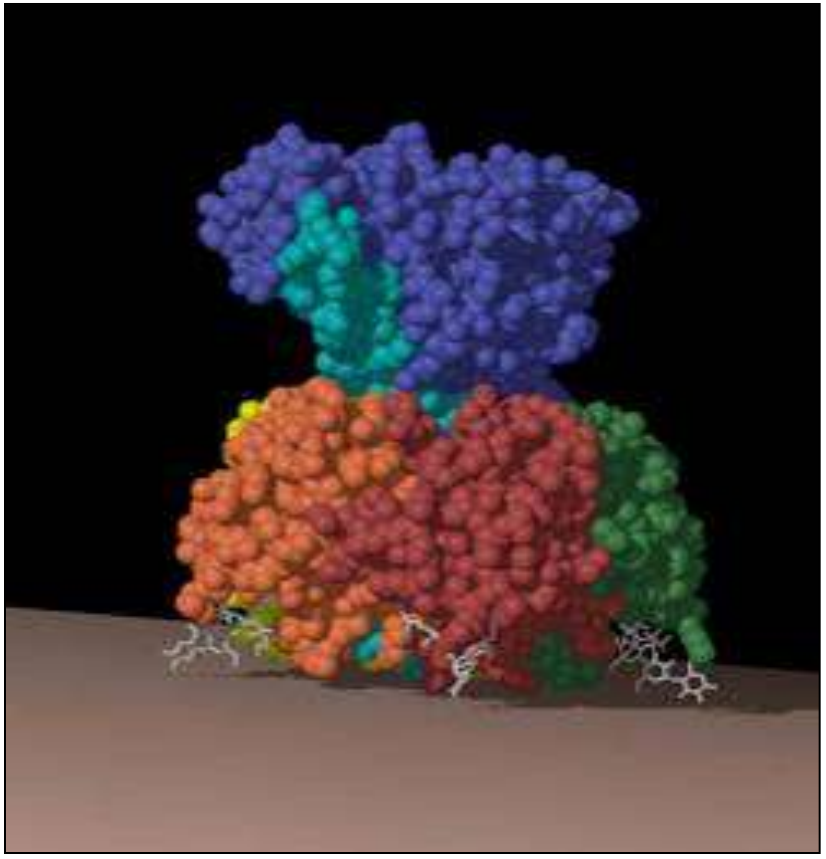


Toxiny nám pomáhají …

ve vakcínách



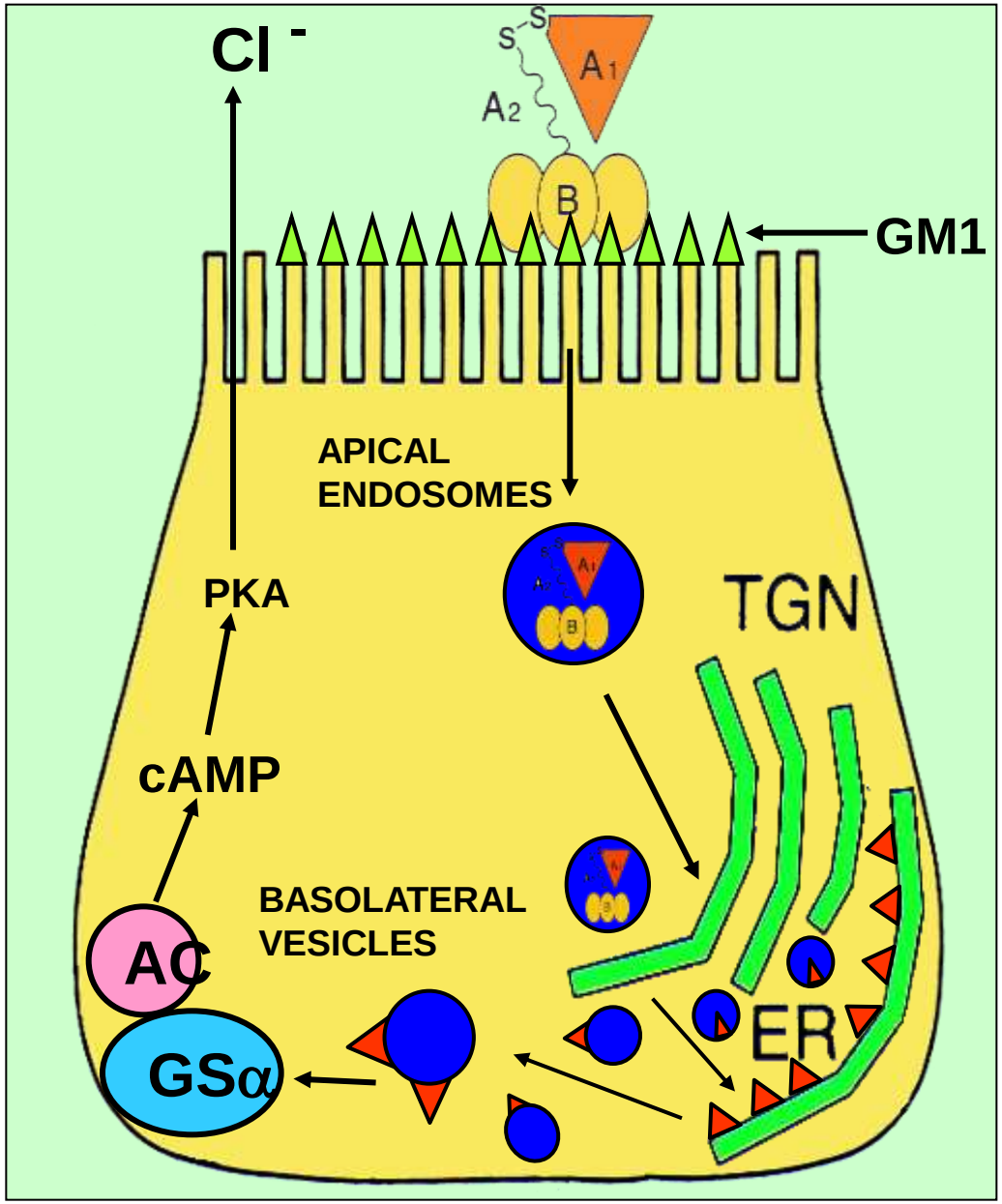
Sixma *et al.*, Nature 351: 371-377, 1991



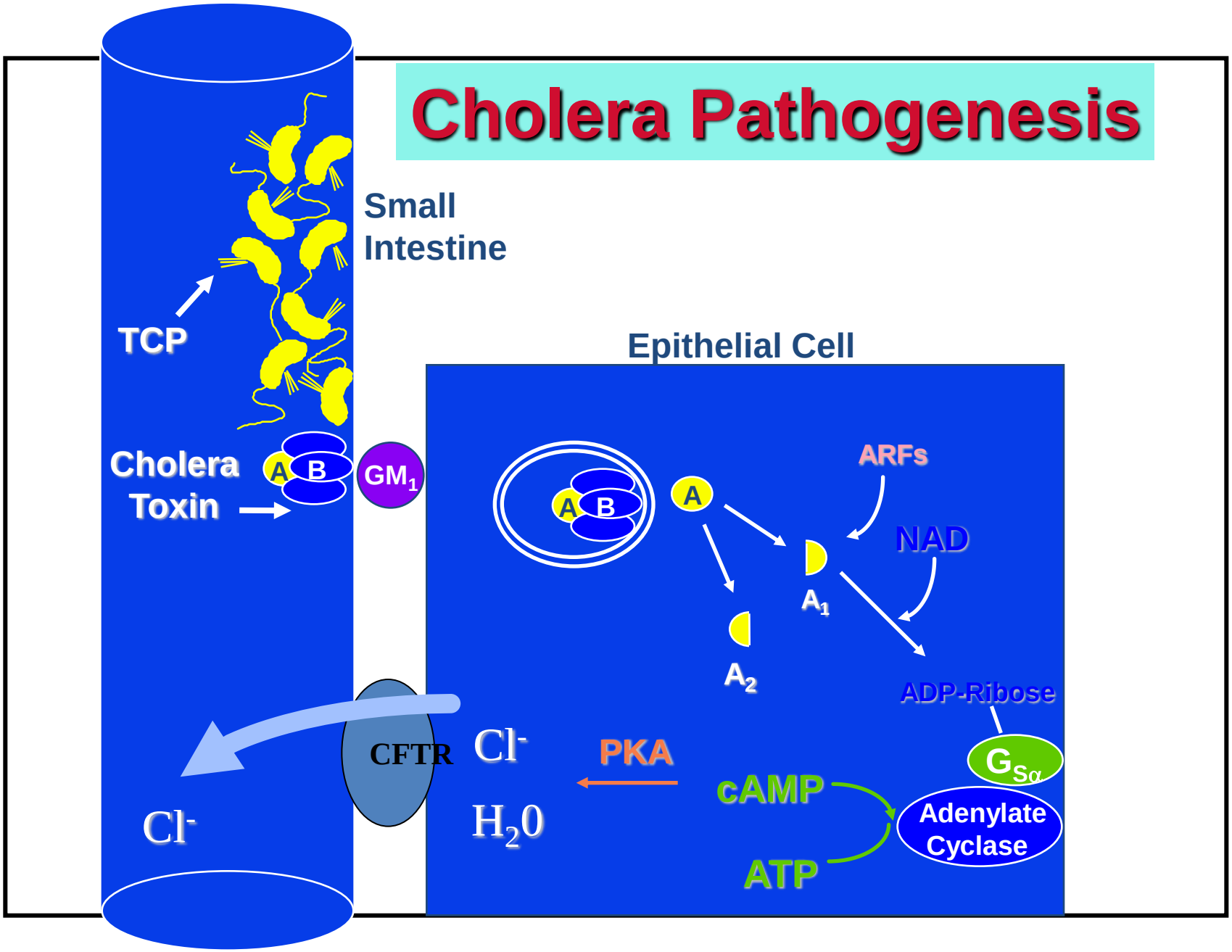
Merritt *et al.*, Prot. Sci. 3: 166-175, 1994

Termo-labilní toxin *V. cholerae* a *E. coli* jsou mimořádně účinná adjuvans

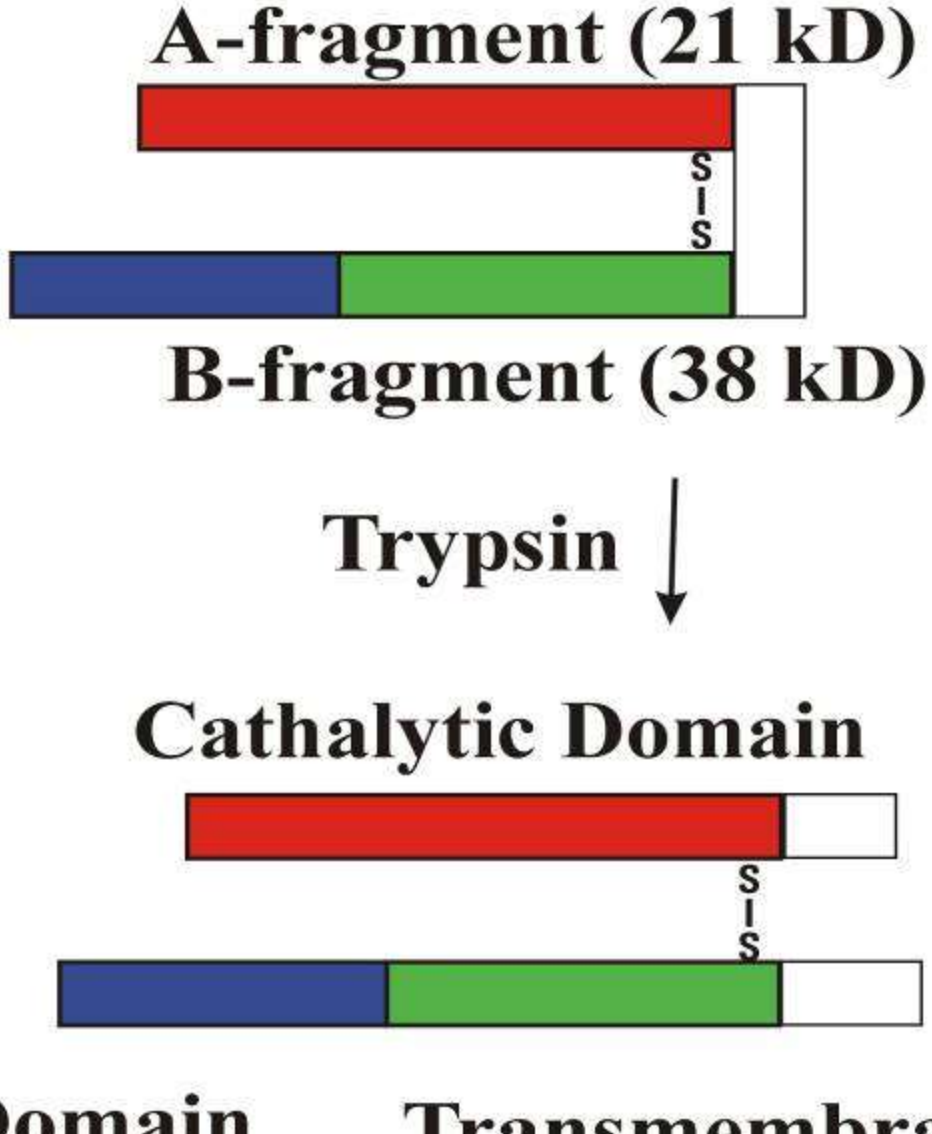
Action of cholera toxin and related enterotoxins

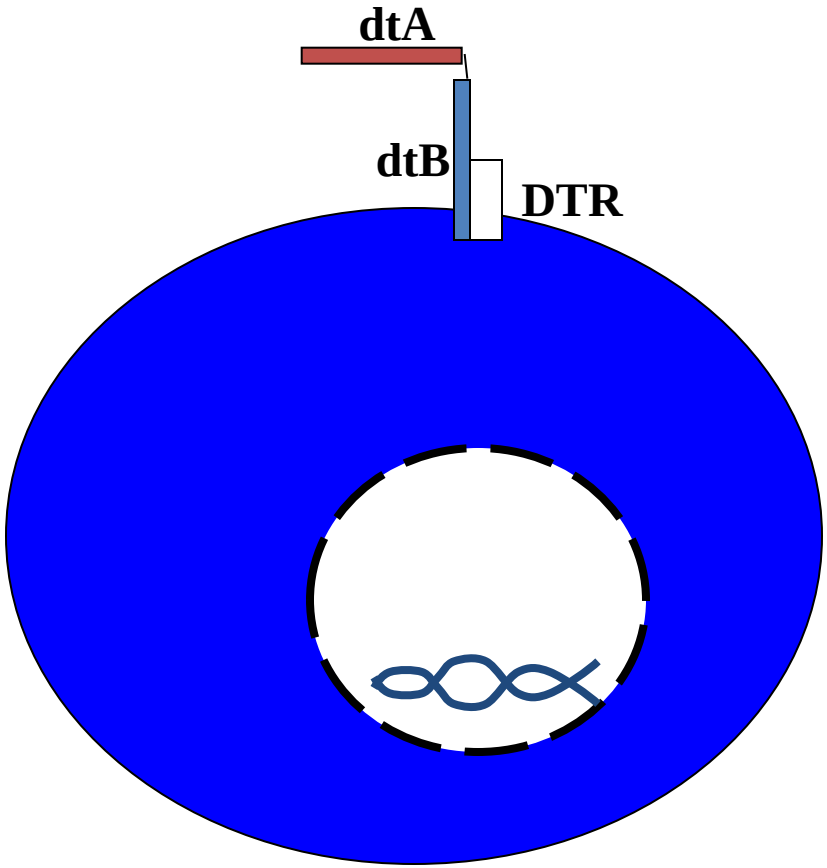


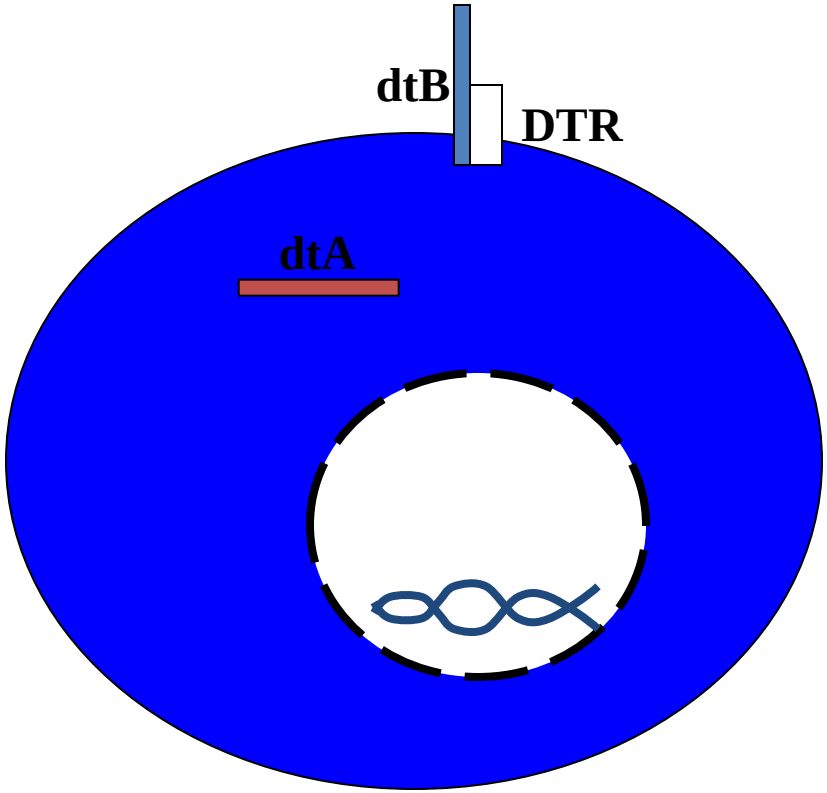
Cholera Pathogenesis



Diphtheria Toxin



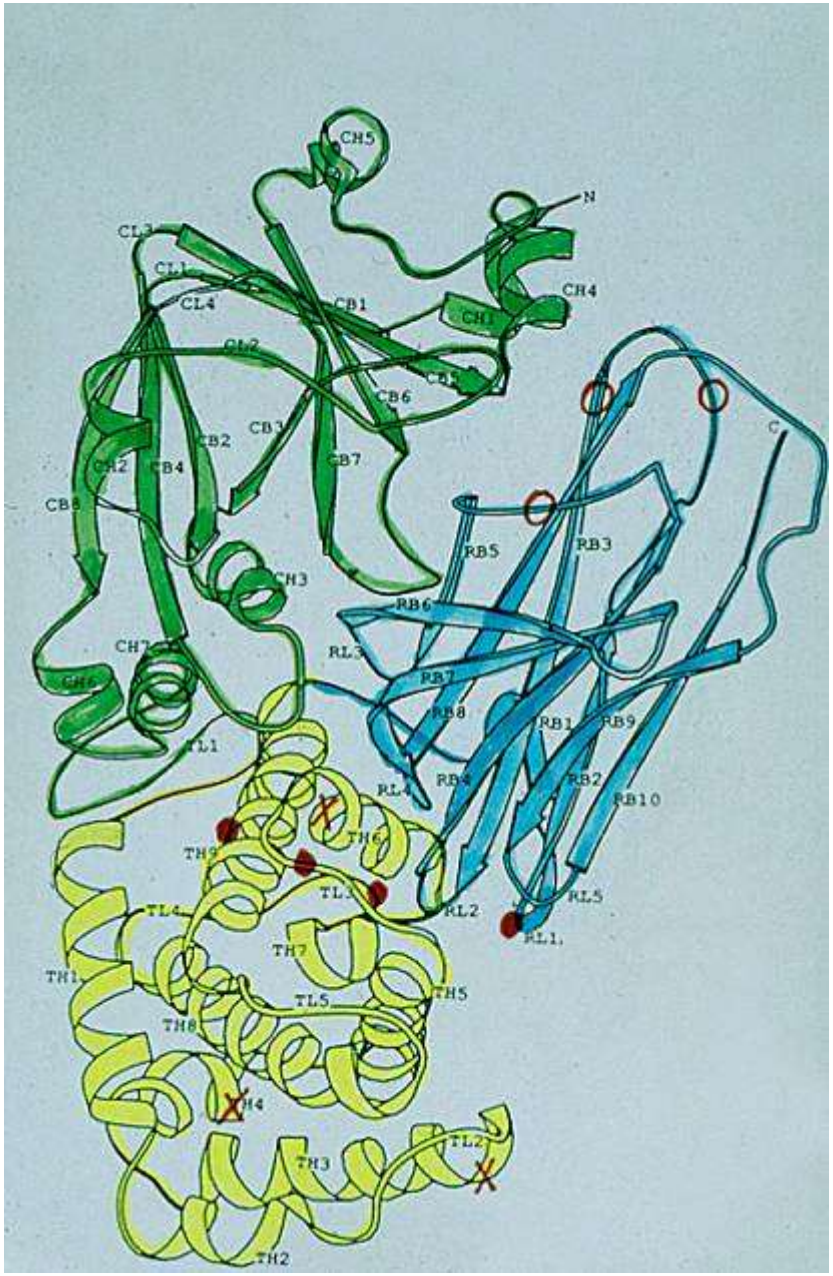

dtA
dtB
DTR


dtB
DTR
dtA

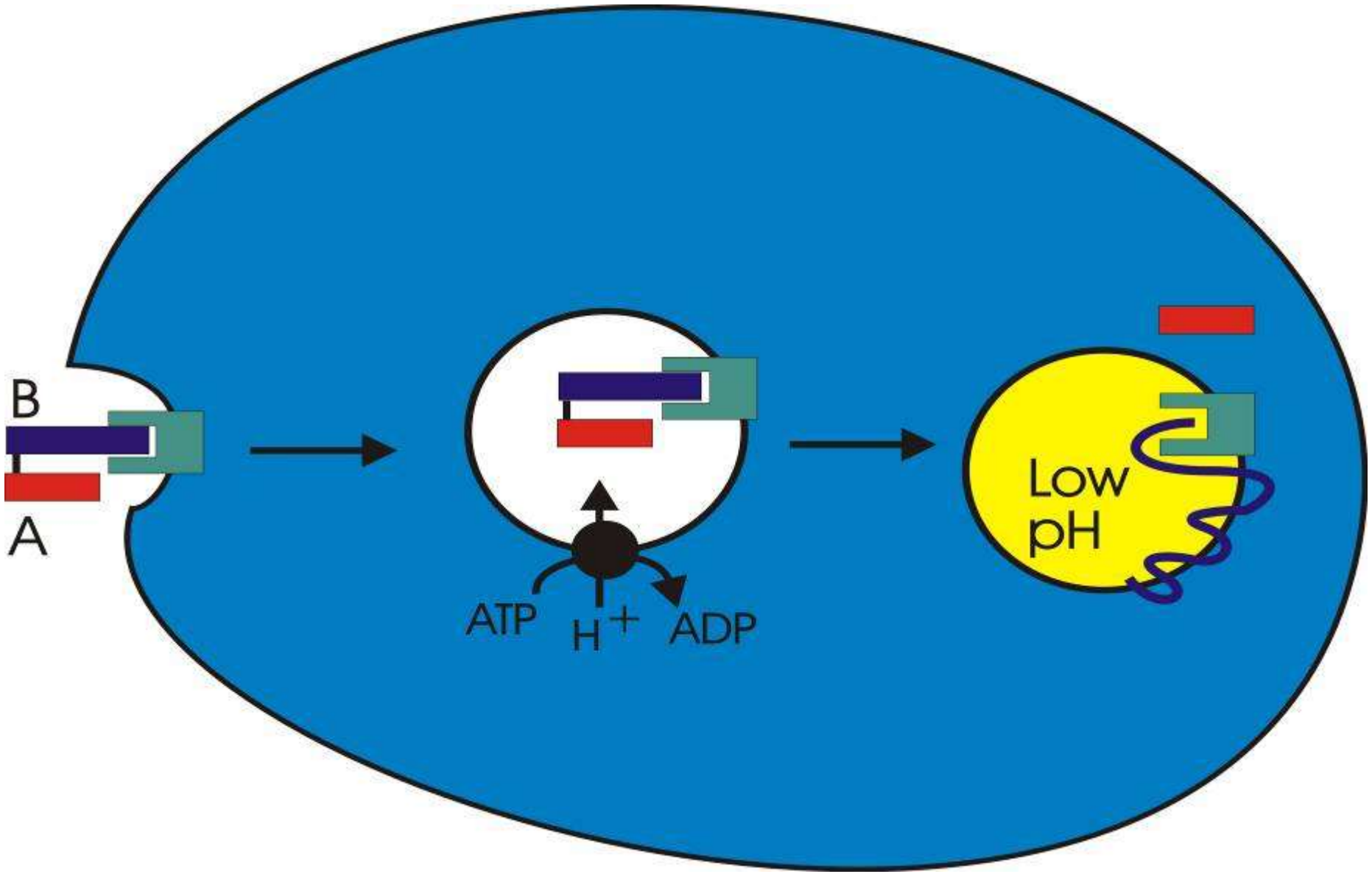
Structure of diphtheria toxin

A-fragment

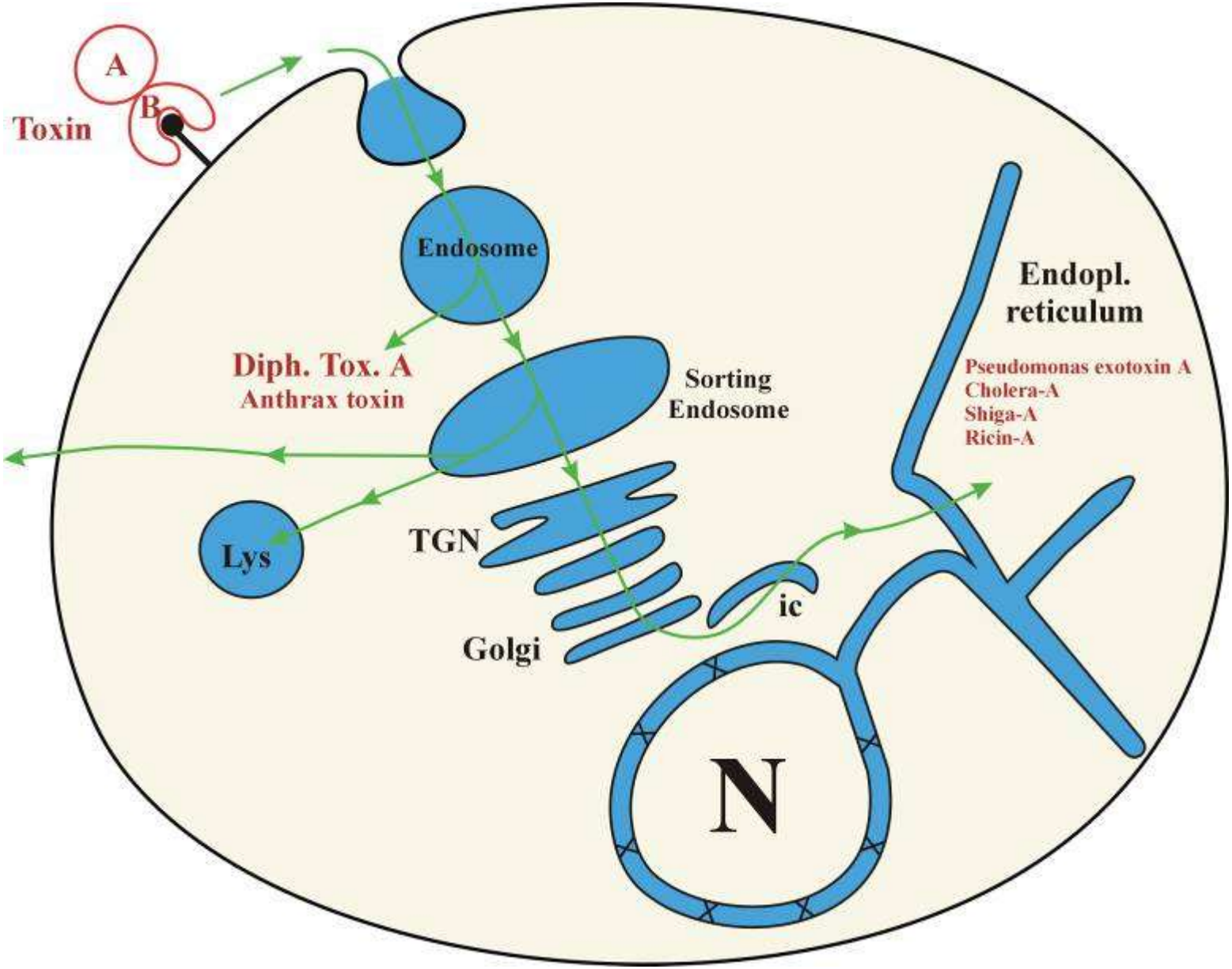
T-domain



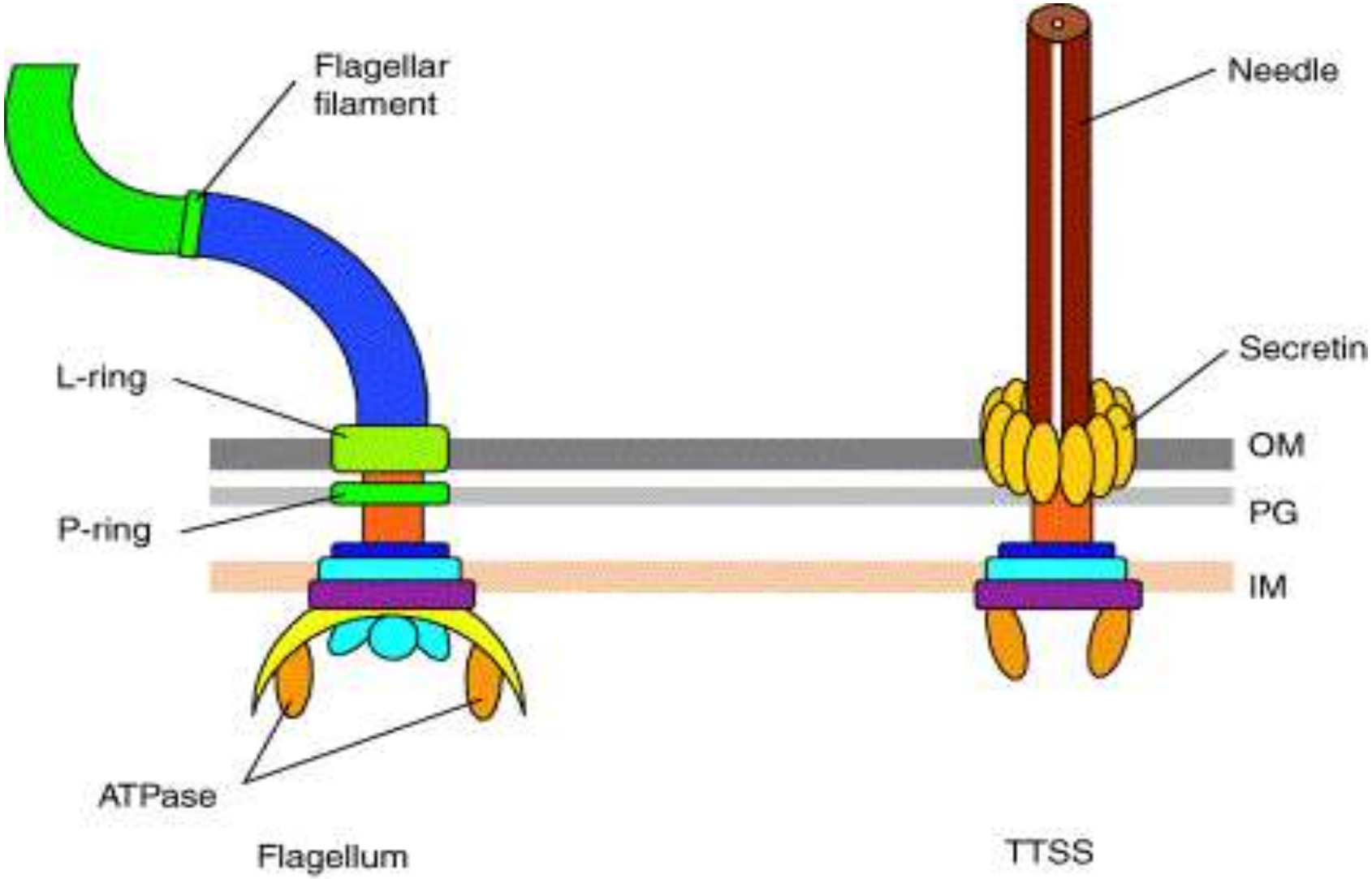
R-domain


B
A
ATP
H+
ADP
Low
pH

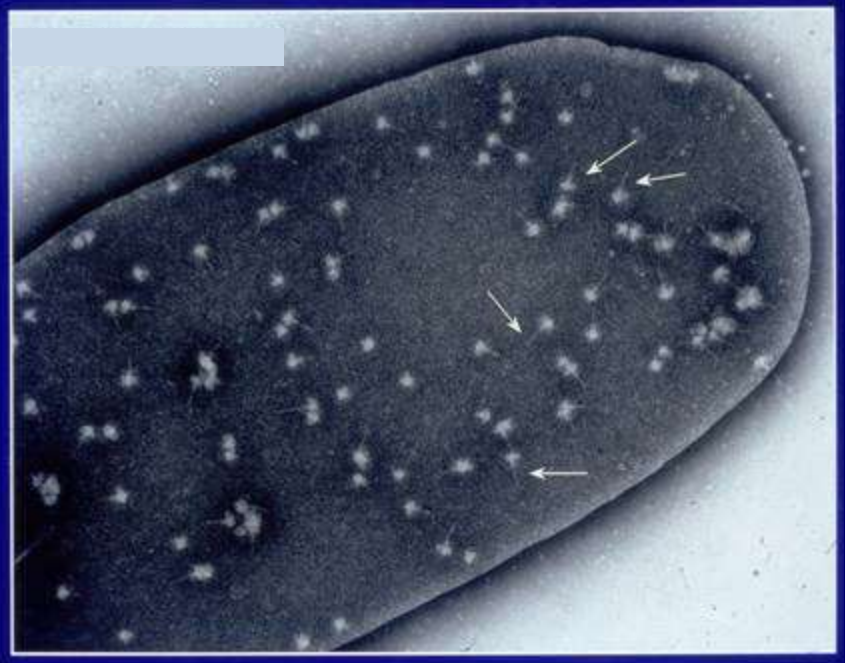
Intracellular transport and translocation of toxins to the cytosol

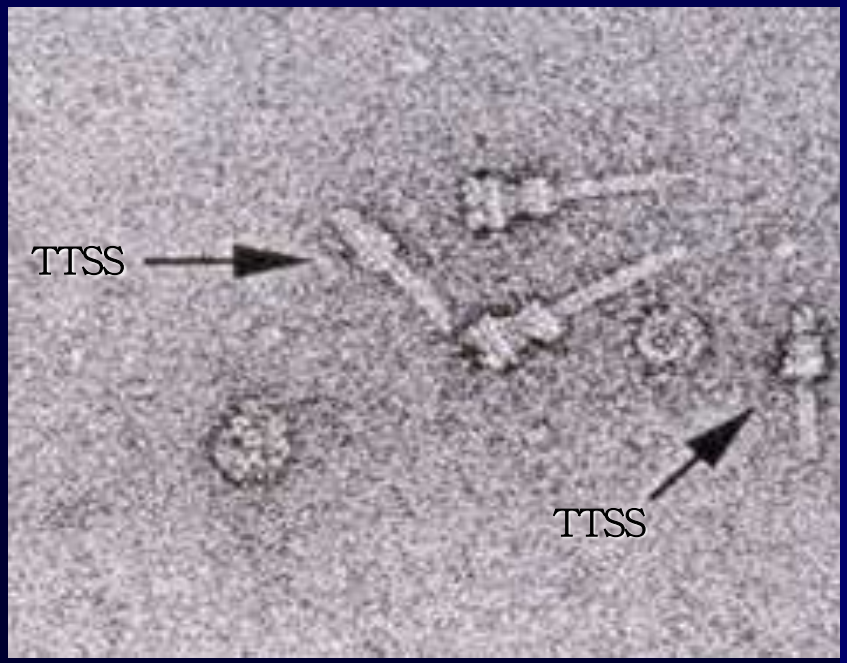


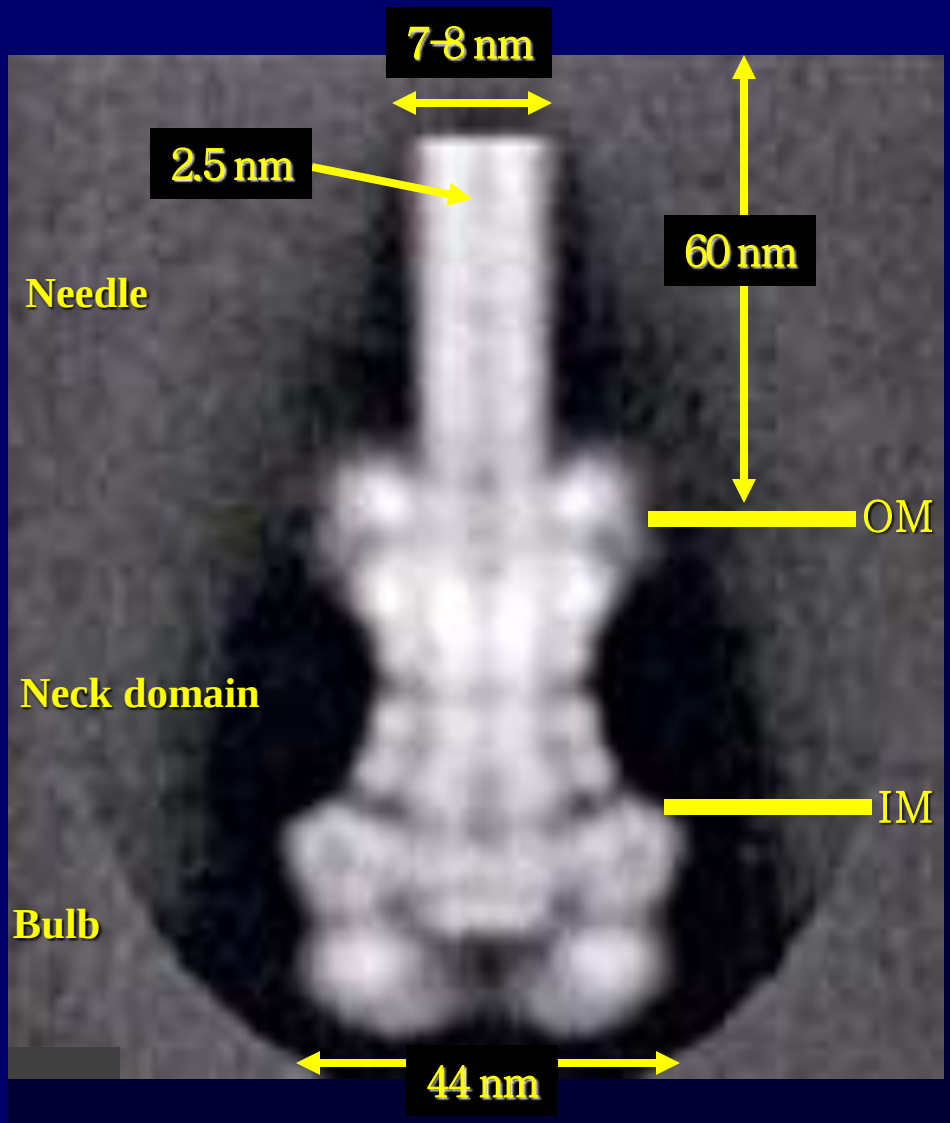
Bakterie jsou sofistikované organizmy a vynalezly toxinové nanotechnologie…



Shigella Type Three Secretory System TTSS

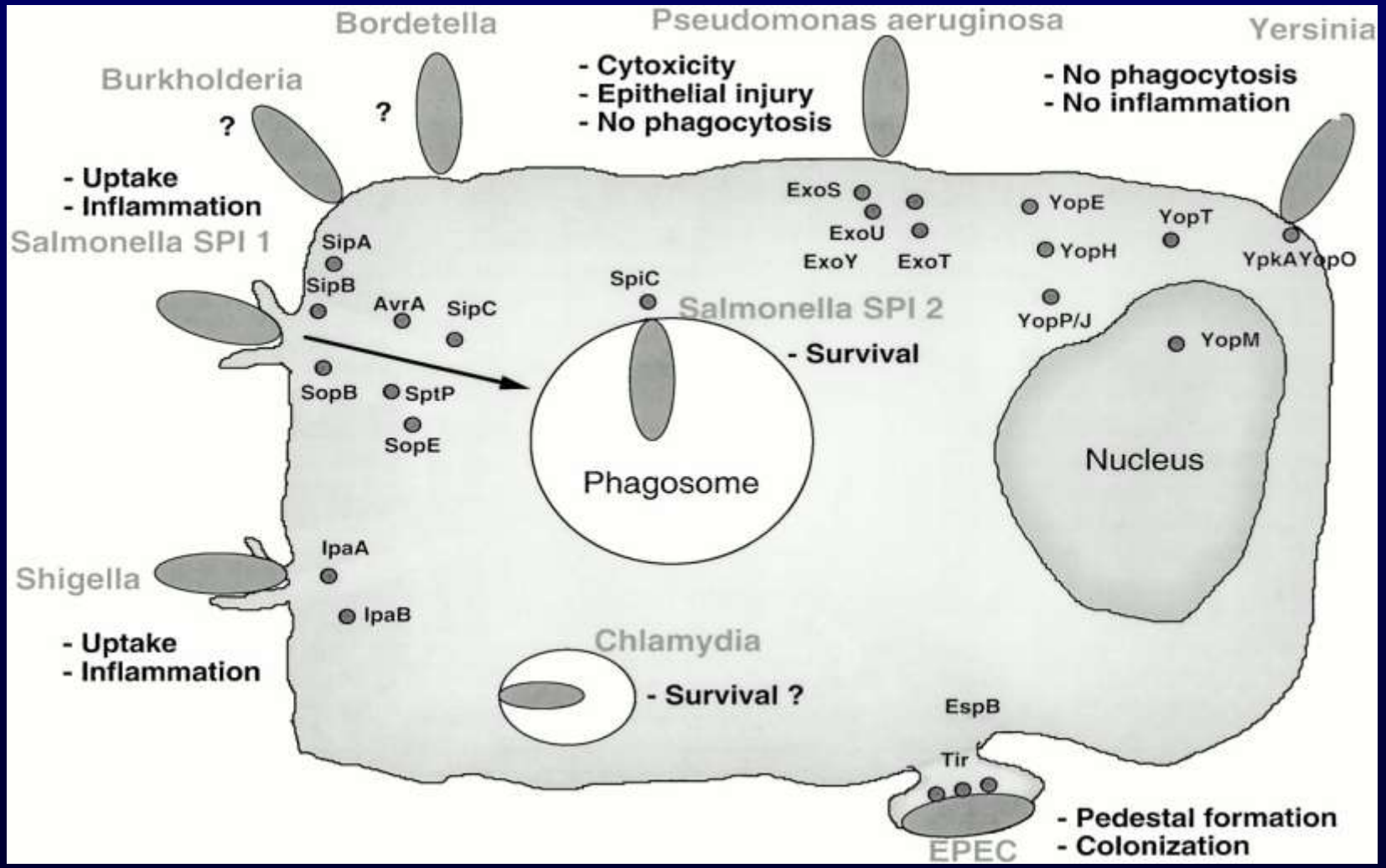






A true molecular injection syringe…

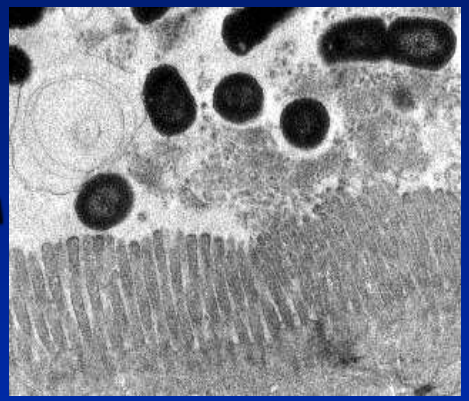
Type III secretion systems inject into cells protein kinases, phosphatases, actin remodelling enzymes and dozens of other effector types to re-program host cells homeostasis for the benefit of the bug….

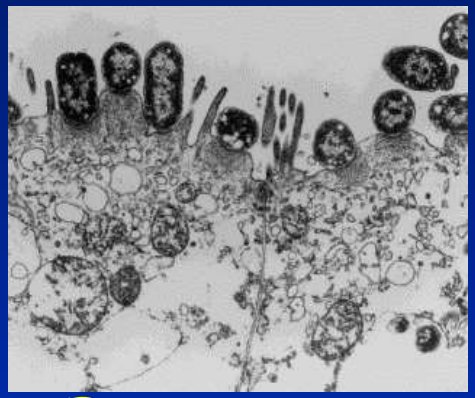


Adapted from GR Cornelis and F Van Gijsegem Annu. Rev. Microbiol. 2000. 54:735-774.

Phenotypes associated with AEEC infection

In vivo intestinal Epithelium





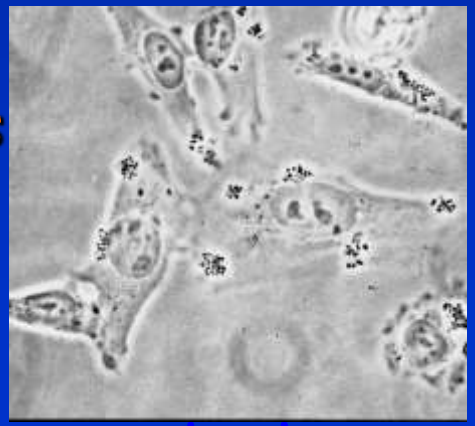
Transmission EM

Attaching-Effacing lesion(A/E)

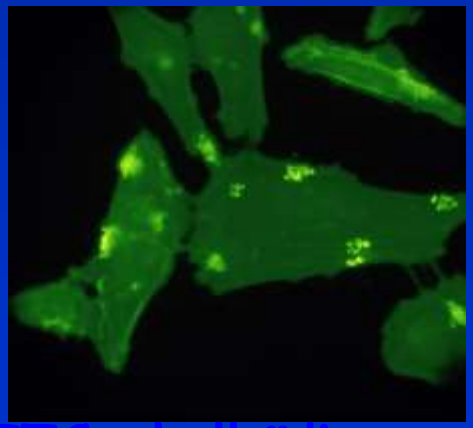
Finlay *et al.*

Fluorescent Actin Staining test (FAS)

In vitro Hela cells



Phase contrast



FITC-phalloïdine

Life-threathening diarrhoea “production” of a single patient in a day