Introduction, Bacterial Classification & Immunology Review

Different from parasites and fungi (eukaryotic)

- Prokaryotic organisms
  - Simple (different) unicellular organisms
  - no nuclear membrane
  - no mitochondria
  - no Golgi bodies
  - no endoplastic reticulum
- Complex cell wall
  - Gram-positive
  - Gram-negative

Microbial Disease

- The relationship between many organisms and their diseases is not simple.
- Most organisms do not cause a single, well-defined disease, although some do *e.g., Treponema pallidum*--syphilis.
- More common for infections result in many different manifestations of disease *e.g., S. aureus*--endocarditis, pneumonia, skin infections, bone infections, sepsis, food poisoning.

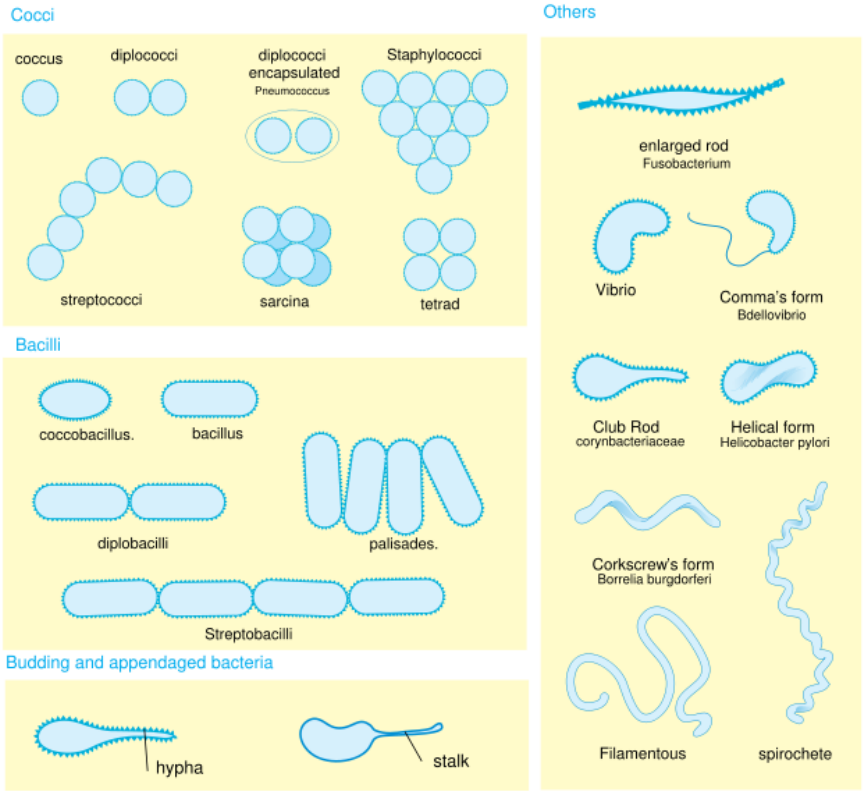
Bacterial Classification

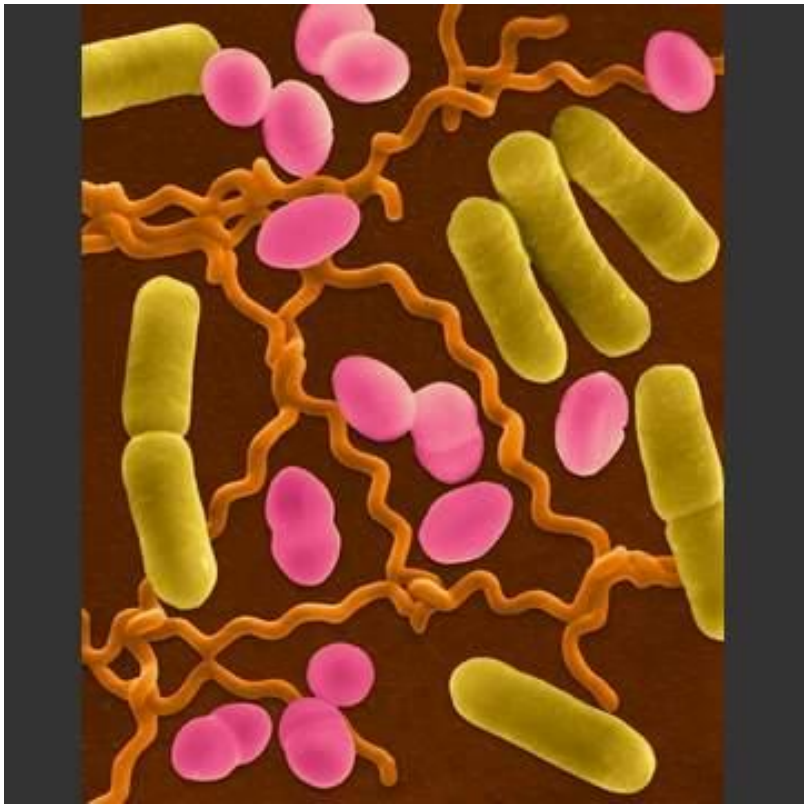
- Phenotypic
- Analytic
- Genotypic

Phenotypic Classification

- Microscopic morphology
  - Gram stain, shape *i.e.*, rods (bacillus), spheres (cocci), curved or spiral, size
- Macroscopic
  - Hemolytic properties on agar containing blood, pigmentation of the colonies, size and shape of colonies, smell and color.
- Serotyping
  - Antibody reactivity to specific antigens
- Antibiogram patterns
  - Susceptibility to antibiotics
- Phage typing
  - Susceptibility to viruses that infect bacteria--bacteriophages

Bacterial Morphologies





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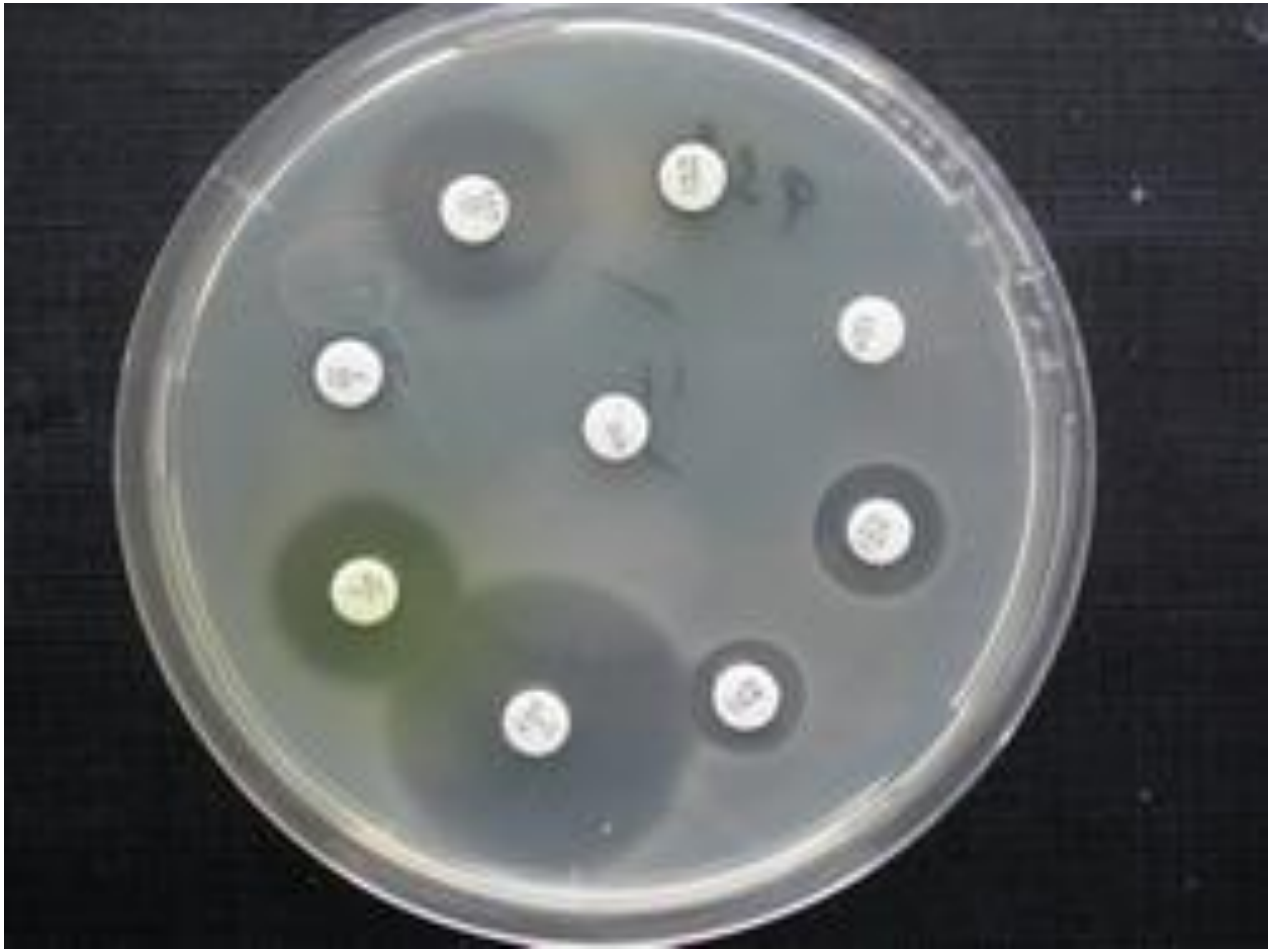
Antibiogram patterns



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Analytic Classification

- Chromatographic pattern of cell wall mycolic acids
- Lipid analysis
- Proteomic analysis

  - These techniques are labor intensive
  - Require expensive equipment
  - Used primarily in reference laboratories

Genotypic Analysis

- Most precise method for bacterial classification.
  - Ratio of guanine to cytosine
  - DNA hybridization
  - Nucleic acid sequence analysis
    - PCR
      - Chromosomal DNA
      - Ribotyping
      - Plasmid analysis

Genotypic Analysis

Parameter — Characteristic for the Genus

	Staphylococcus	Micrococcus	Planococcus	Stomatococcus
GC content of DNA	30-35	70-75	40-51	56-60
Cell wall composition More than 2 mol of glycine per Mol of glutamic acit in Peptidoglycan	+	-	-	-
Type of fructose 1,6-diphsphate Aldolase	I	II	ND	II
Cytochrome C	-	+	ND	+
Lysostaphin Sensitivity	+	-	-	-
Furazolidon Sensitivity	-	+	ND	ND

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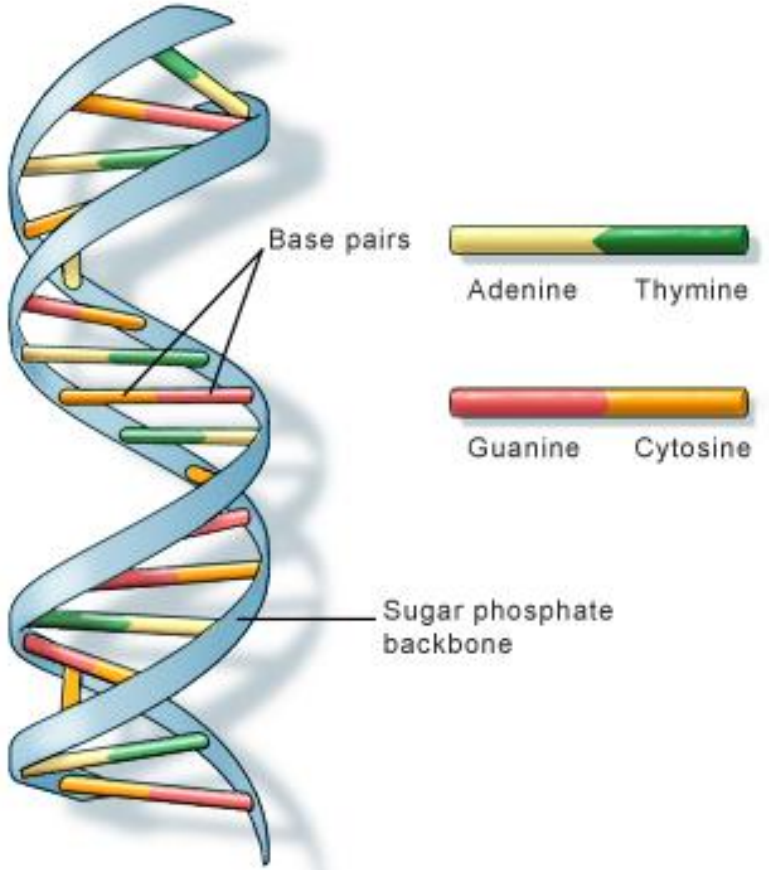
Why is PCR So Sensitive?

4^n

Why is PCR So Sensitive?

4n

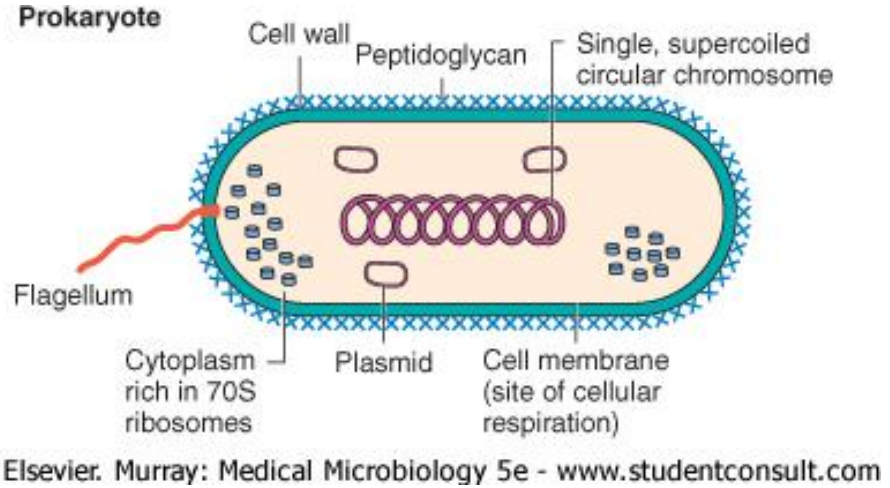
Adenine
Guanine
Cytosine
Thymine

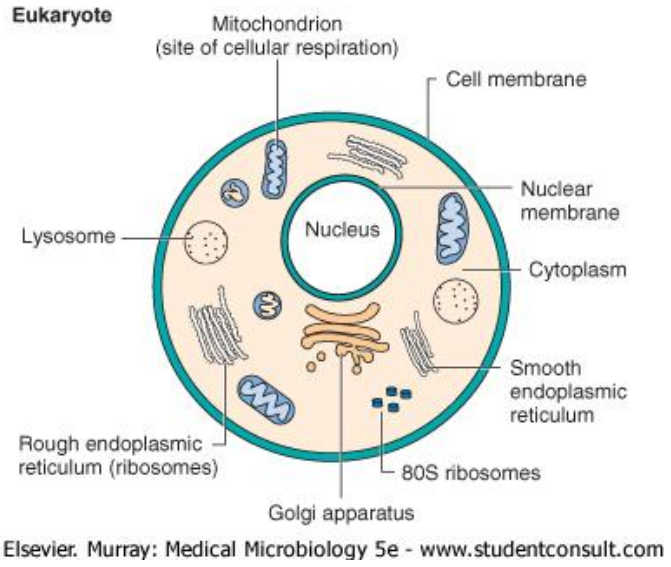


Bacterial Morphology and Cell Wall Structure and Synthesis

Differences between eukaryotes and prokaryotes

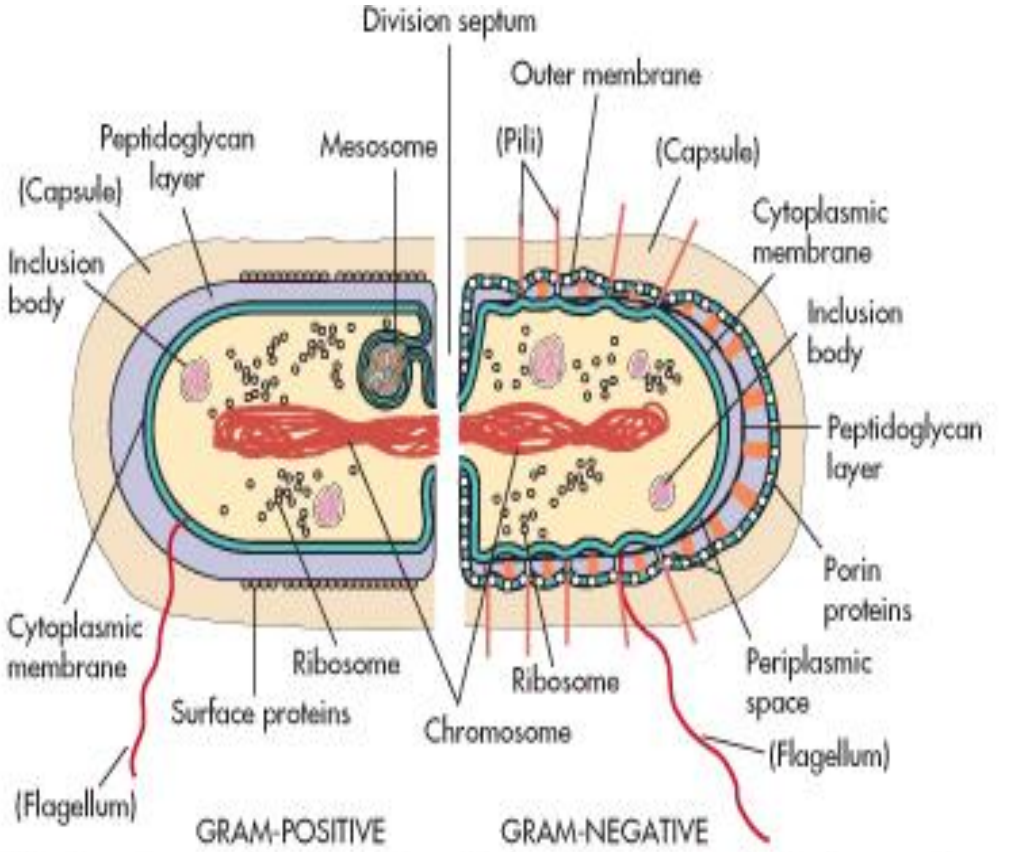
- Eukaryotes-Greek for true nucleus.
  - 80S Ribosome
    - 60S + 40S
- Prokaryotes-Greek for primitive nucleus.
  - 70S Ribosome
    - 50S + 30S (16S + 23S rRNA).
- Peptidoglycan cell wall.





Characteristic	Eukaryote	Prokaryote
Major Groups	Algae, fungi, protozoa, plants, animals	Bacteria
Size (approximate)	<5μm	0.5-3.5 μm
Nuclear structures -Nucleus -Chromosomes	Classic membrane Strands of DNA diploid genes	No nuclear membrane Single, circular DNA haploid gene, plasmids
Cytoplasmic Structures		
Mitochondria	Present	Absent
Golgi bodies and ER	Present	Absent
Ribosomes	80S (60S +40S)	70S (50S+30S)
Cytoplasmic membrane	Contains sterols	No sterols
Cell wall	Present for fungi, otherwise absent	Complex, proteins, lipids, peptidoglycans
Reproduction	Sexual and asexual	Asexual (binary fission)
Movement	Complex flagellum, if present	Simple flagellum, if present
Respiration	Via mitochondria	Via cytoplasmic membrane

Bacterial Ultrastructure-
Cytoplasmic Structures



-Bacterial chromosome is a single, double-stranded circle.

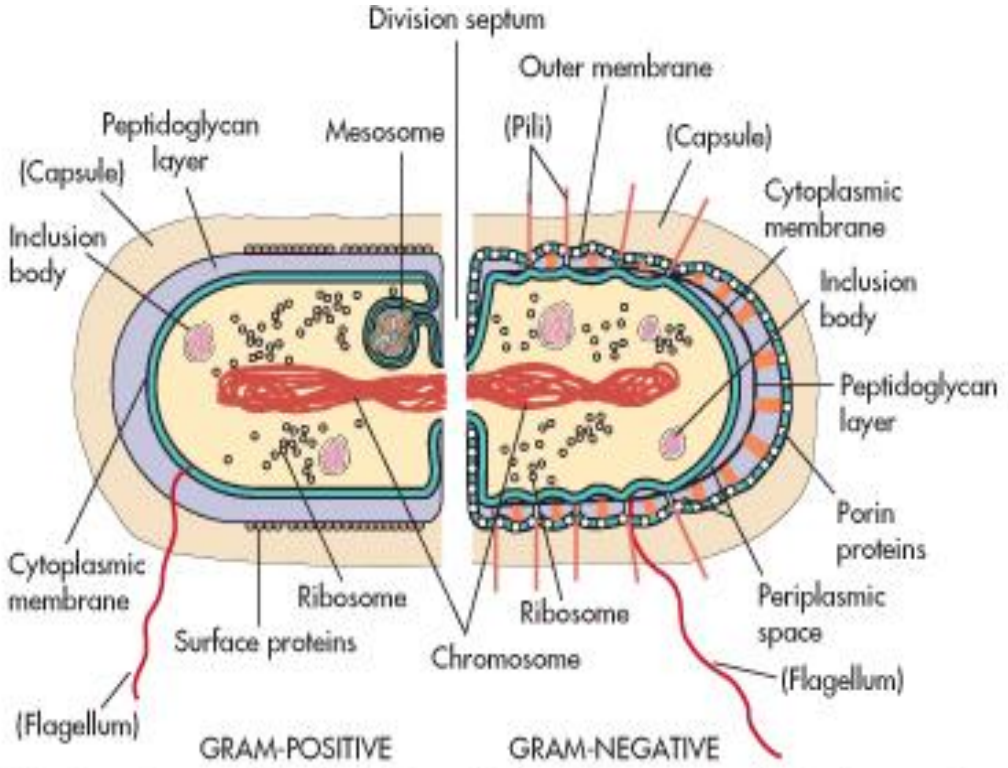
-Ribosomes

-Plasmids present in most bacteria.

- confer virulence
- antibiotic resistance

-Cytoplasmic membrane

Bacterial Ultrastructure-
Cell Wall



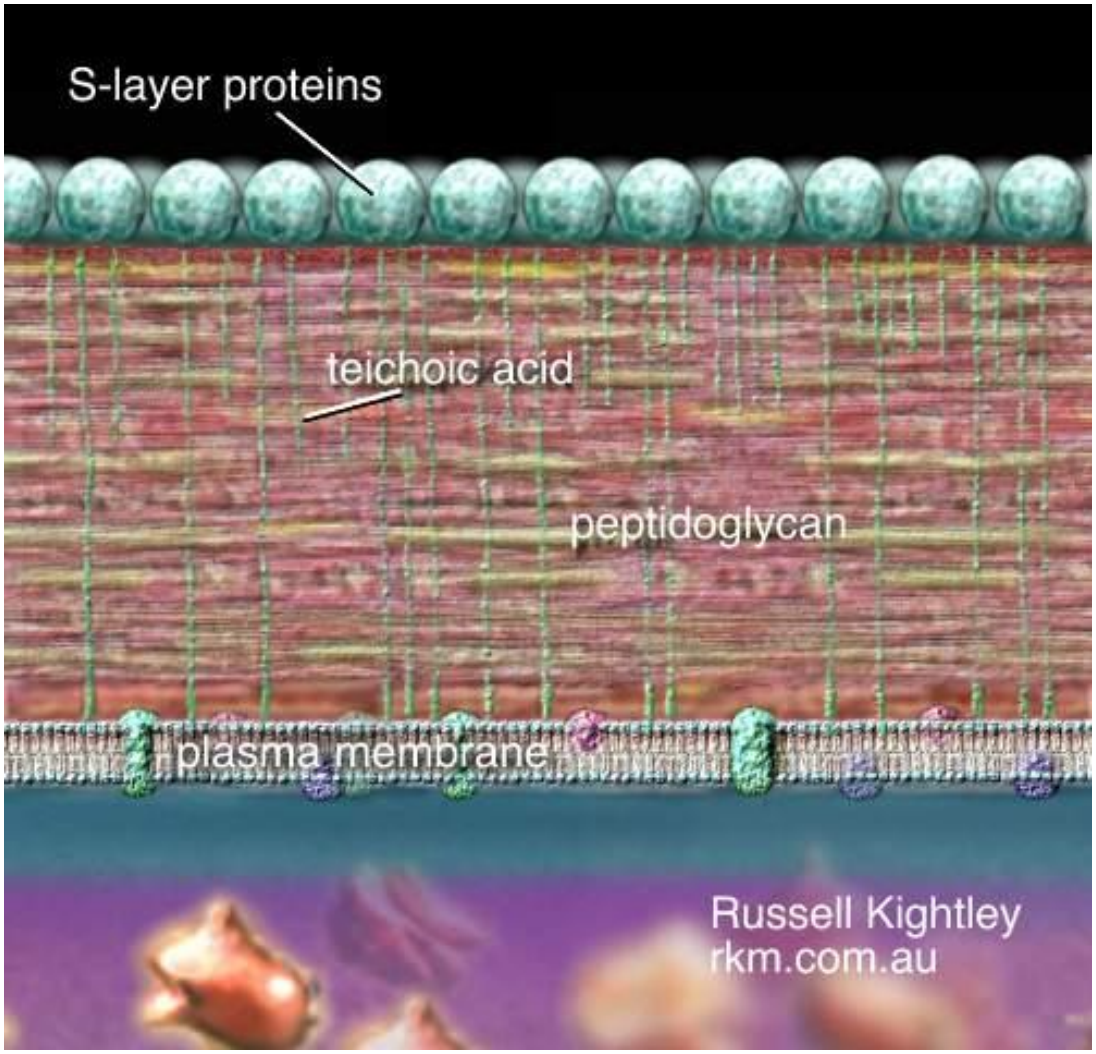
Rigid peptidoglycan layers surround the cytoplasmic membranes of most prokaryotes.

- Both Gram-positive and -negative.

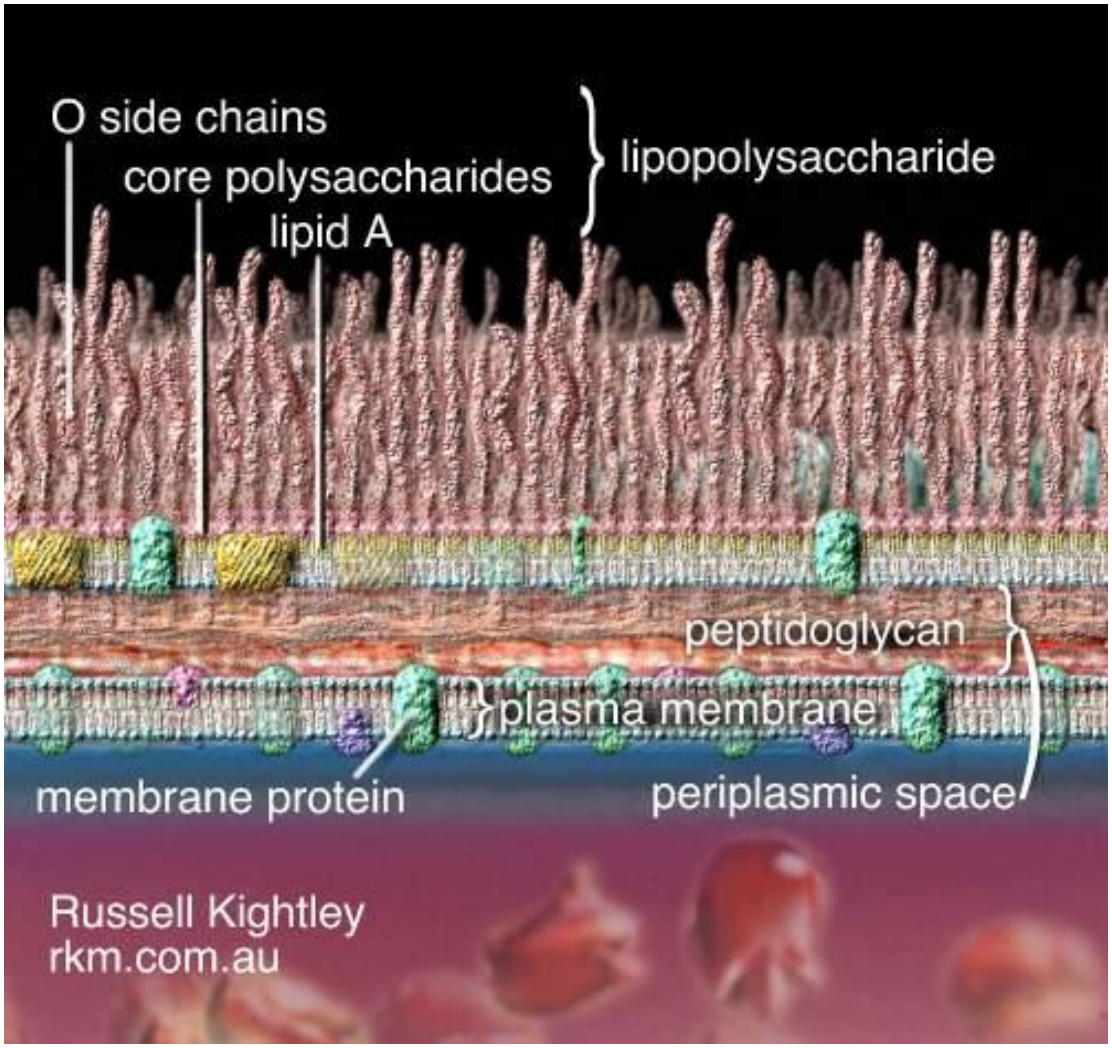
Exceptions are Archaeobacteria organisms and mycoplasmas.

Differences Between Prokaryotes-- The Gram Stain

Gram-Positive Cell wall



Gram-Negative Cell wall



The Gram Stain

In the late 1800's, Christian Gram observed that some genera of bacteria retained an iodine-dye complex when rinsed with alcohol, while other genera were easily decolorized with alcohol and could be then visualized by a contrasting counterstain.

The Gram Stain

This staining procedure defines two bacterial groups: those which retain the primary dyes ("Positive by Gram's Method" or "Gram-Positive") and those which are easily decolorized ("Negative by Gram's Method" or "Gram-Negative").

This is the starting point for bacterial identification procedures.

The Gram Stain

The difference in dye retention is dependent on such physical properties as thickness, density, porosity, and integrity of the bacterial cell wall, as well as, to some extent, the chemical composition.

Gram-Positive bacteria have thick, dense, relatively non-porous walls, while **Gram-Negative** bacteria have thin walls surrounded by lipid-rich membranes.

Some non-bacterial organisms with thick cell walls (*e.g.*, some yeasts) also stain **Gram-Positive**.

Gram-Positive bacteria which have lost wall integrity through aging or physical or chemical damage may stain **Gram-Negative**.

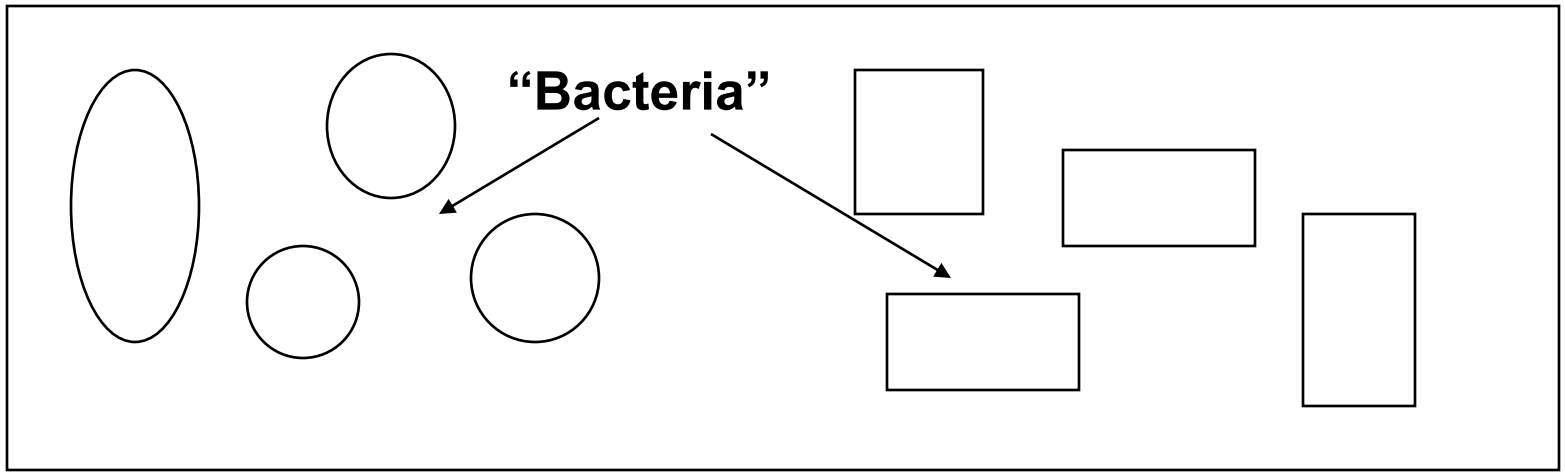
The Gram Stain Procedure

- Step 1-**Prepare a Smear**

  Suspend some of the material to be stained in a drop of water on a microscope slide, spread the drop to about the size of a nickel.

  Allow to air dry. Heat fix by gently warming above a flame or other heat source.

Watch what happens to the “Bacteria” at each step



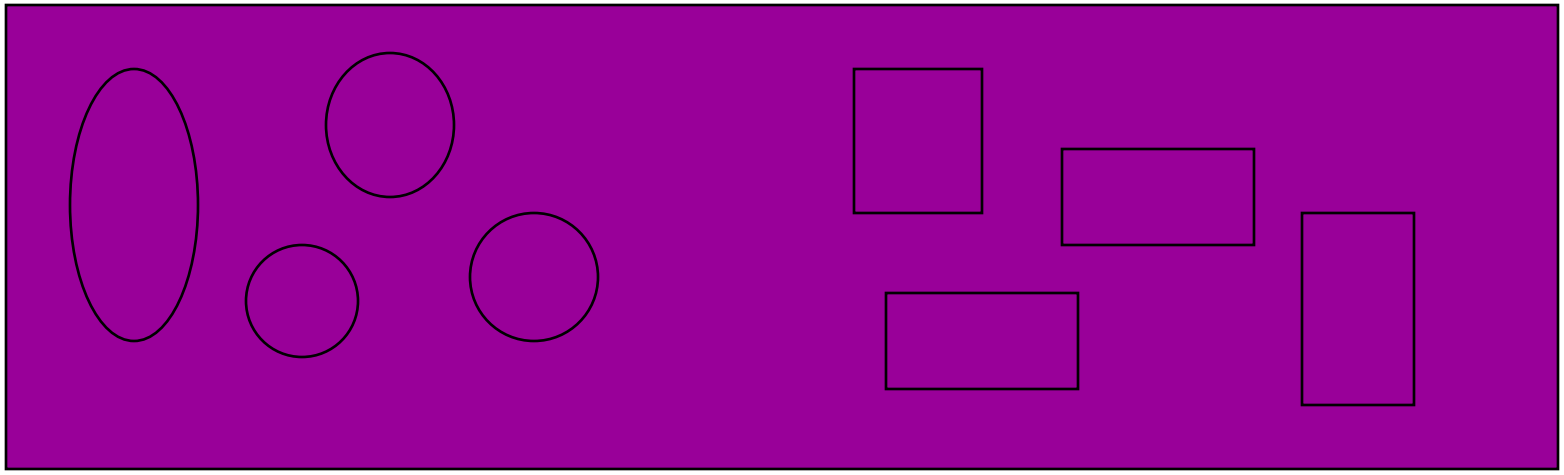
The Gram Stain Procedure

- Step 2-**Apply the Primary Stain**

Flood the Smear with **Crystal Violet**

Allow to stand 30 sec to 1 min

Rinse with water to remove excess stain

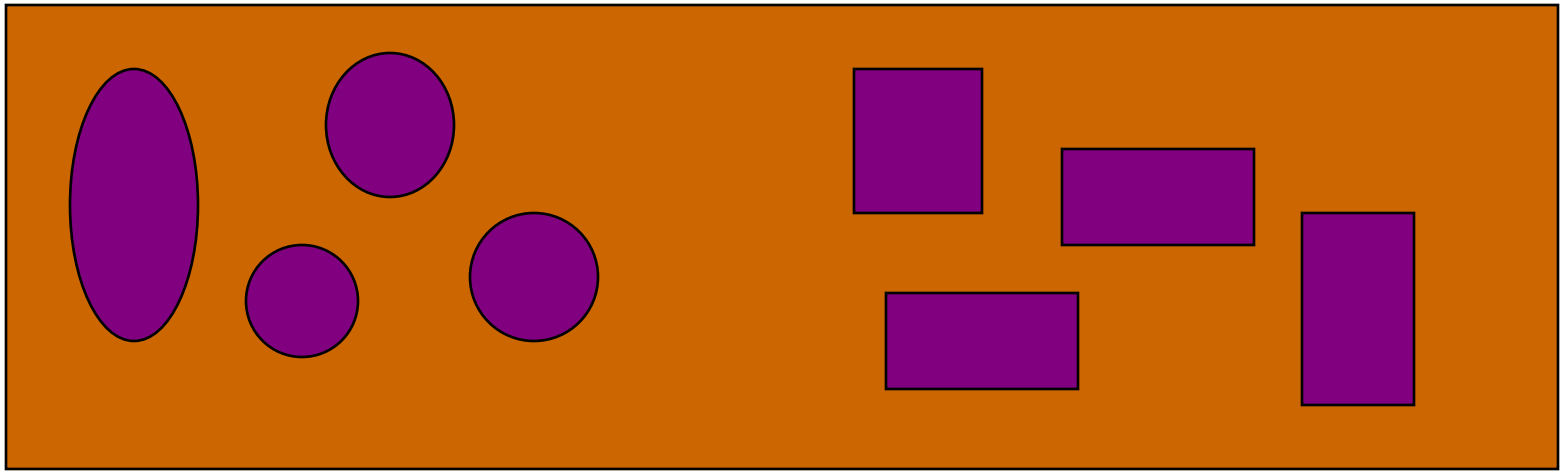


The Gram Stain Procedure

- Step 3-**Apply the Fixing Agent**

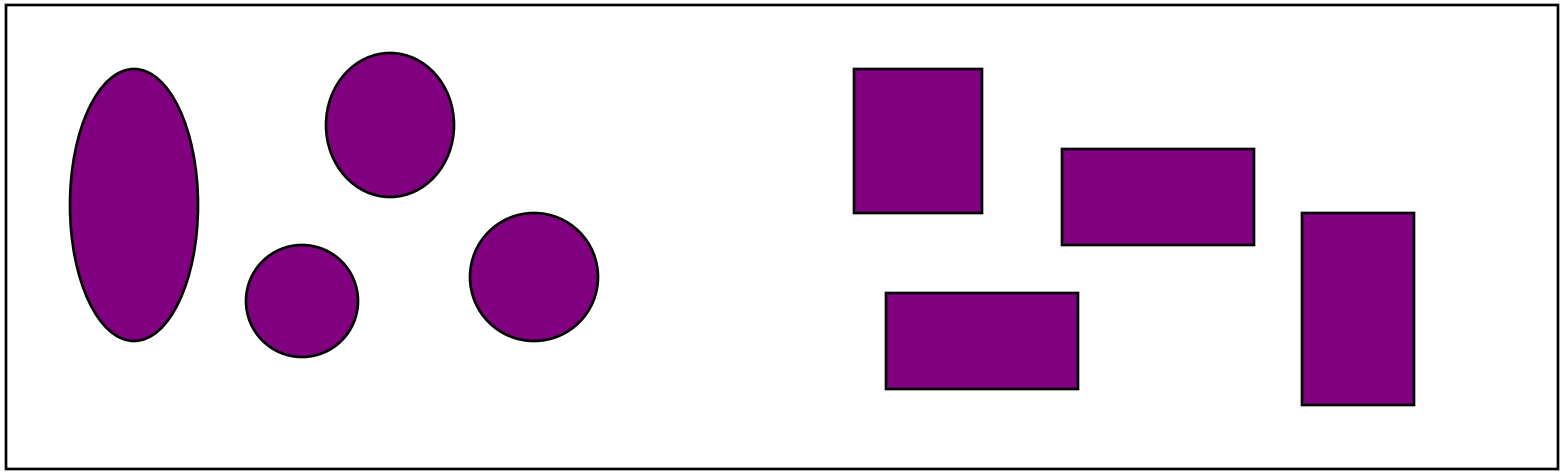
Flood the Smear with **Iodine** solution

Allow to stand 30 sec to 1 min



The Gram Stain Procedure

- Step 4-**Rinse**
- Rinse with water to remove excess Iodine

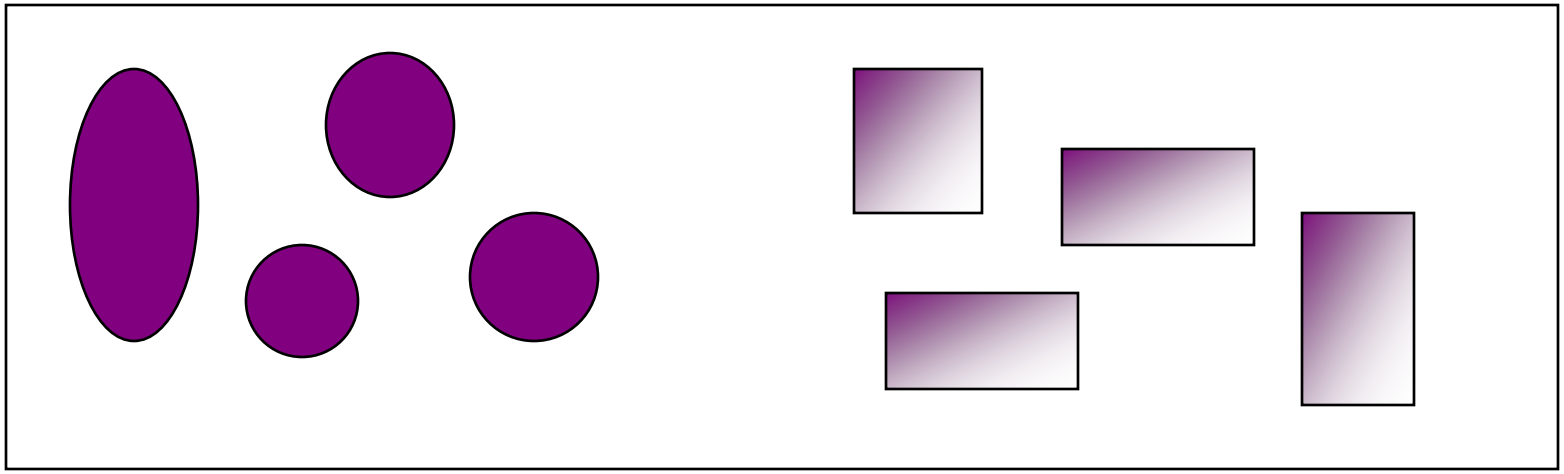


The Gram Stain Procedure

- Step 5-**Decolorize**

  Drip 95% Alcohol across the slide about 5 sec.

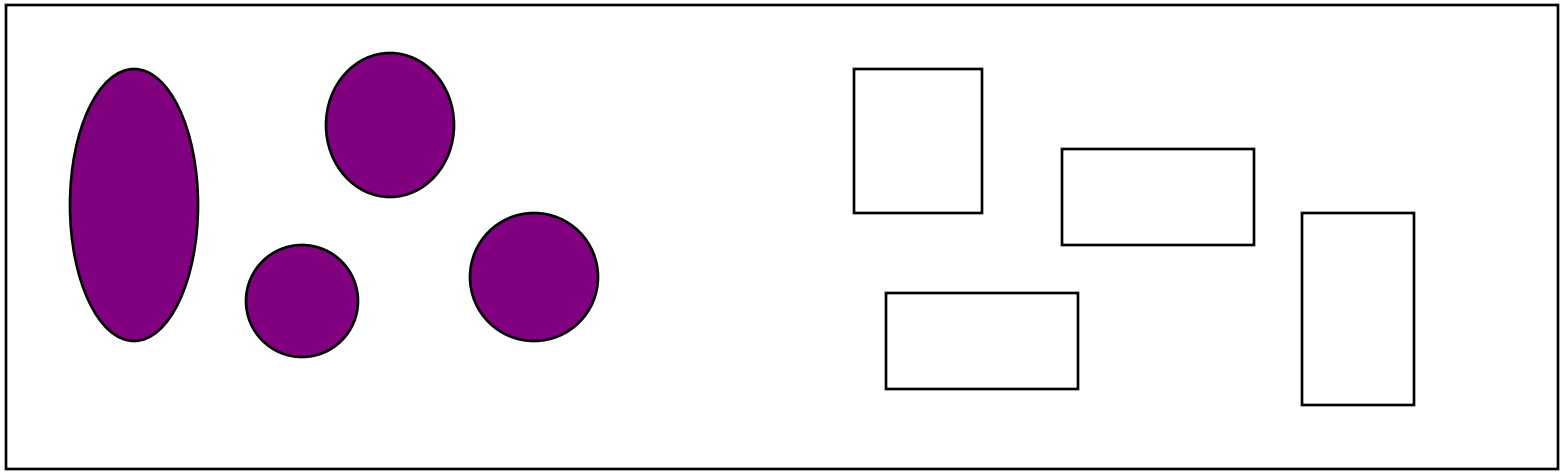
  The effluent should appear pale or clear.



The Gram Stain Procedure

- Step 6-**Rinse**

Rinse with water to remove excess alcohol

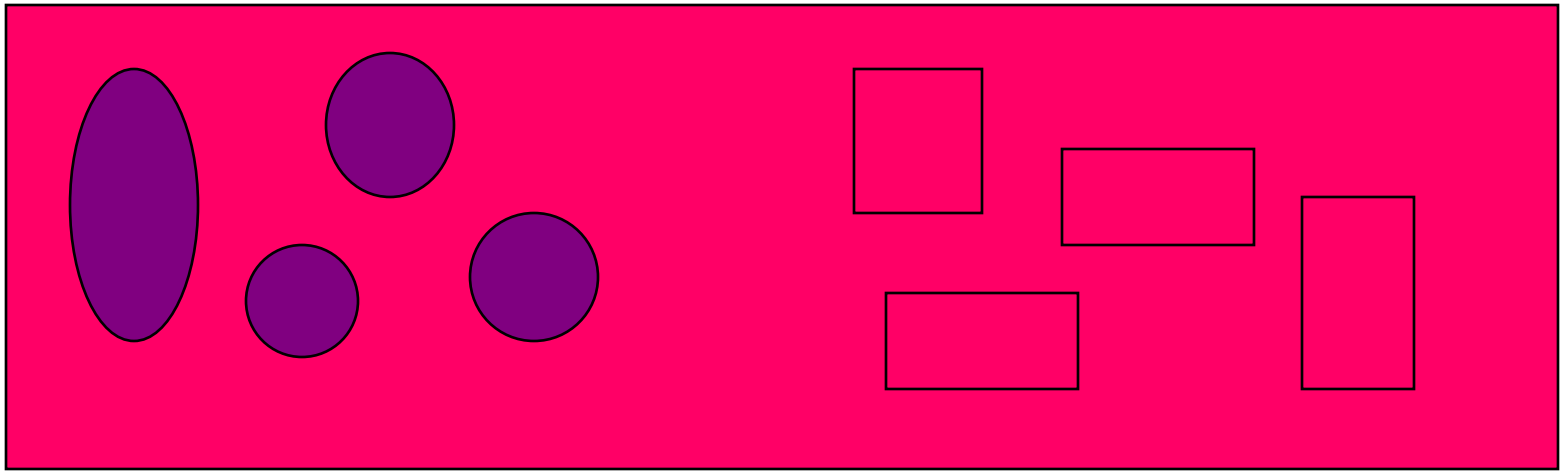


The Gram Stain Procedure

- Step 7-**Counterstain**

  Flood the slide with **Safranin** solution.

  Let stand 30 sec.



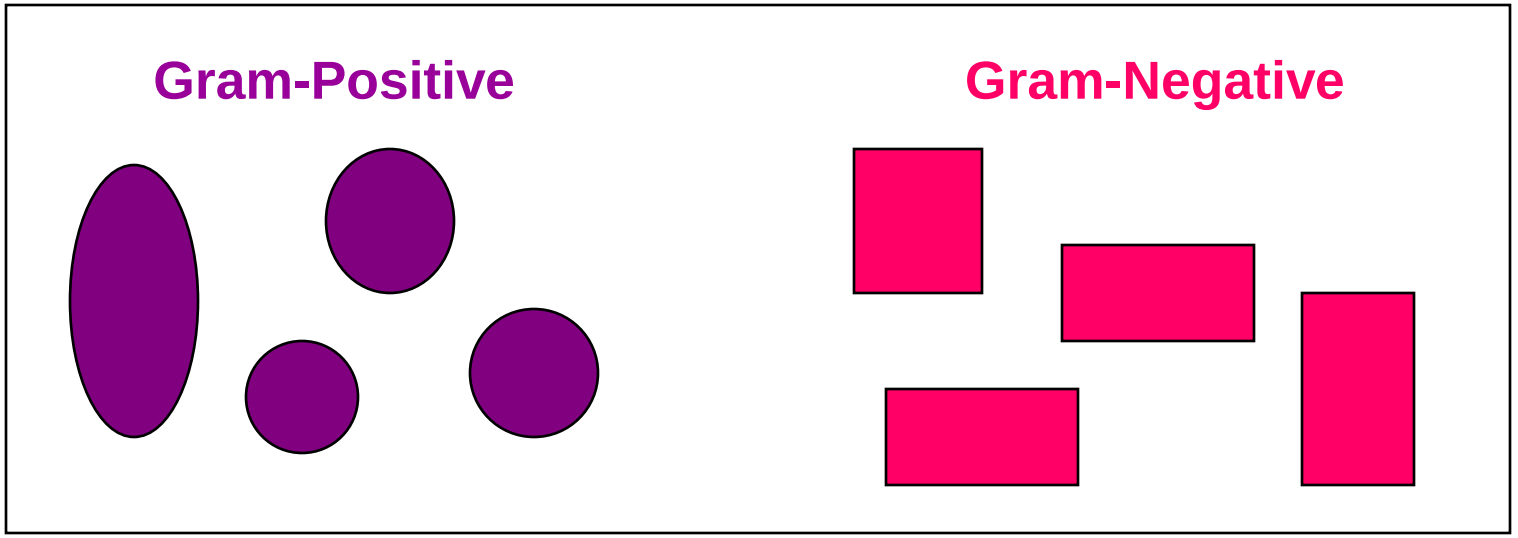
The Gram Stain

- Step 8-**Rinse, Dry and Observe**

  Rinse with water to remove excess stain.

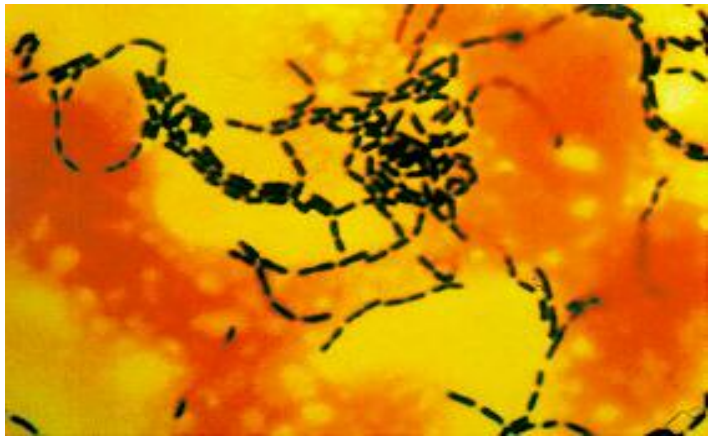
  Blot dry.

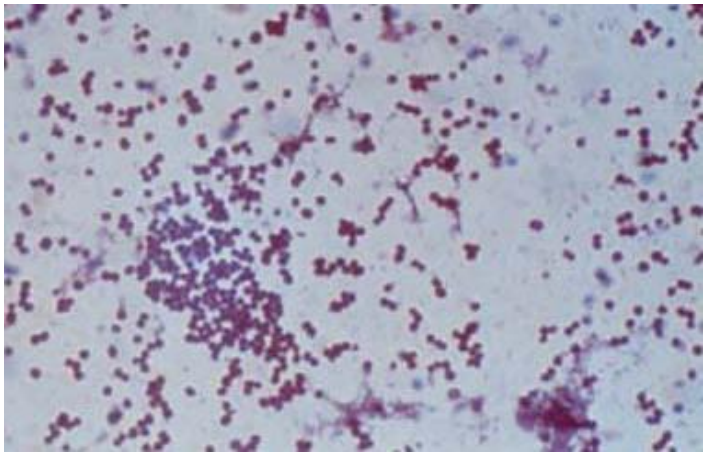
  Observe under oil immersion.



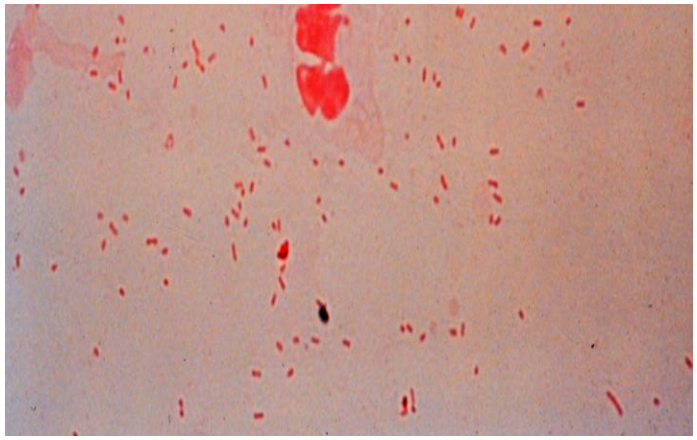
Examples of Gram Stains

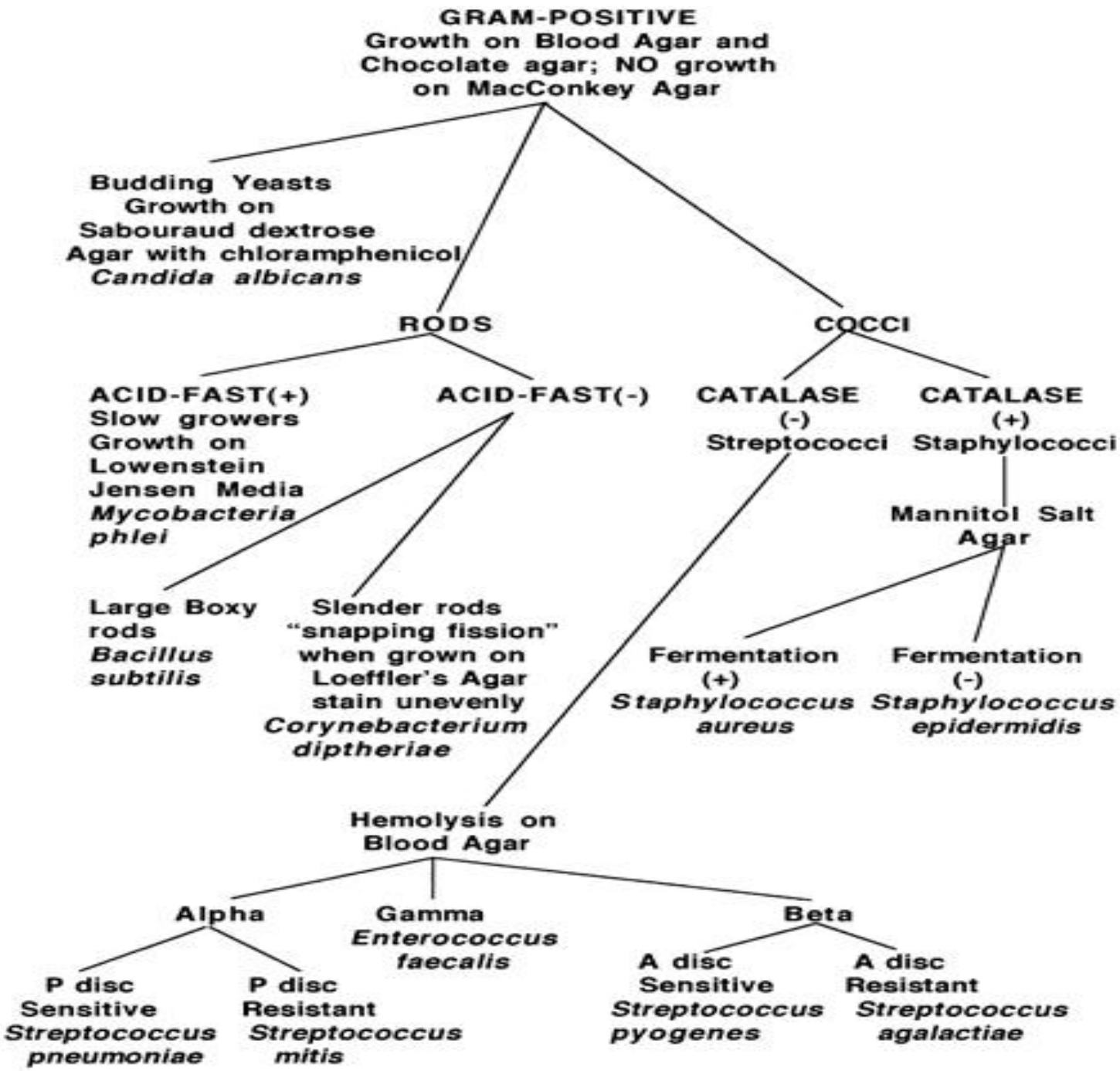
Gram-Positive Rods and Cocci

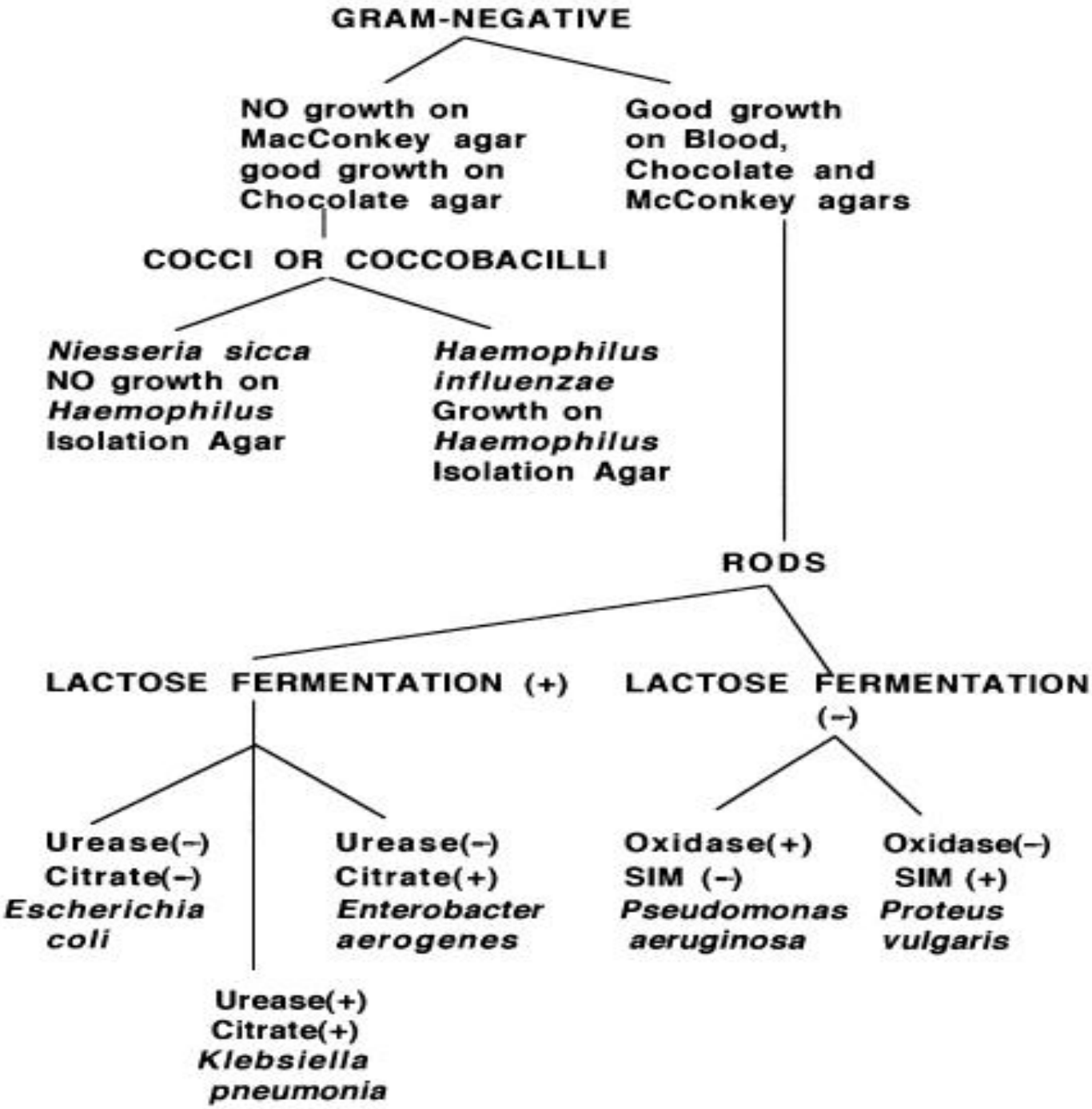




Gram-Negative Rods and Cocci

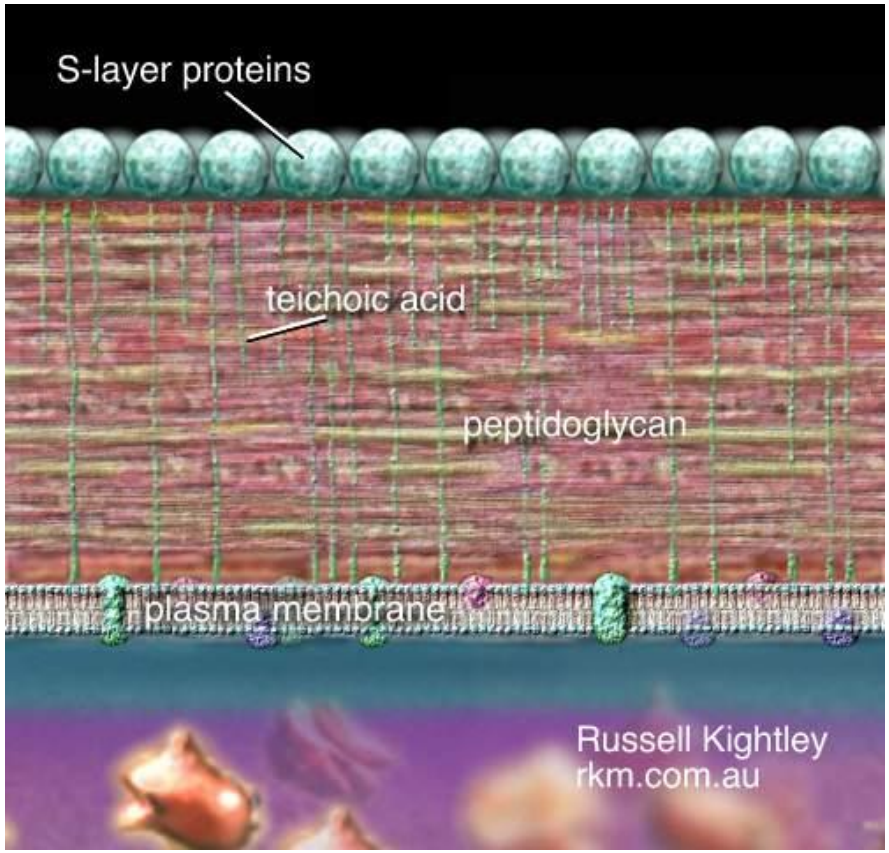



GRAM-POSITIVE
Growth on Blood Agar and Chocolate agar; NO growth on MacConkey Agar
Budding Yeasts
Growth on Sabouraud dextrose Agar with chloramphenicol
Candida albicans
RODS
COCCI
ACID-FAST(+)
Slow growers
Growth on Lowenstein Jensen Media
Mycobacteria phlei
ACID-FAST(-)
CATALASE (-)
Streptococci
CATALASE (+)
Staphylococci
Mannitol Salt Agar
Large Boxy rods
Bacillus subtilis
Slender rods "snapping fission" when grown on Loeffler's Agar stain unevenly
Corynebacterium diptheriae
Fermentation (+)
Staphylococcus aureus
Fermentation (-)
Staphylococcus epidermidis
Hemolysis on Blood Agar
Alpha
Gamma
Enterococcus faecalis
Beta
P disc Sensitive
Streptococcus pneumoniae
P disc Resistant
Streptococcus mitis
A disc Sensitive
Streptococcus pyogenes
A disc Resistant
Streptococcus agalactiae

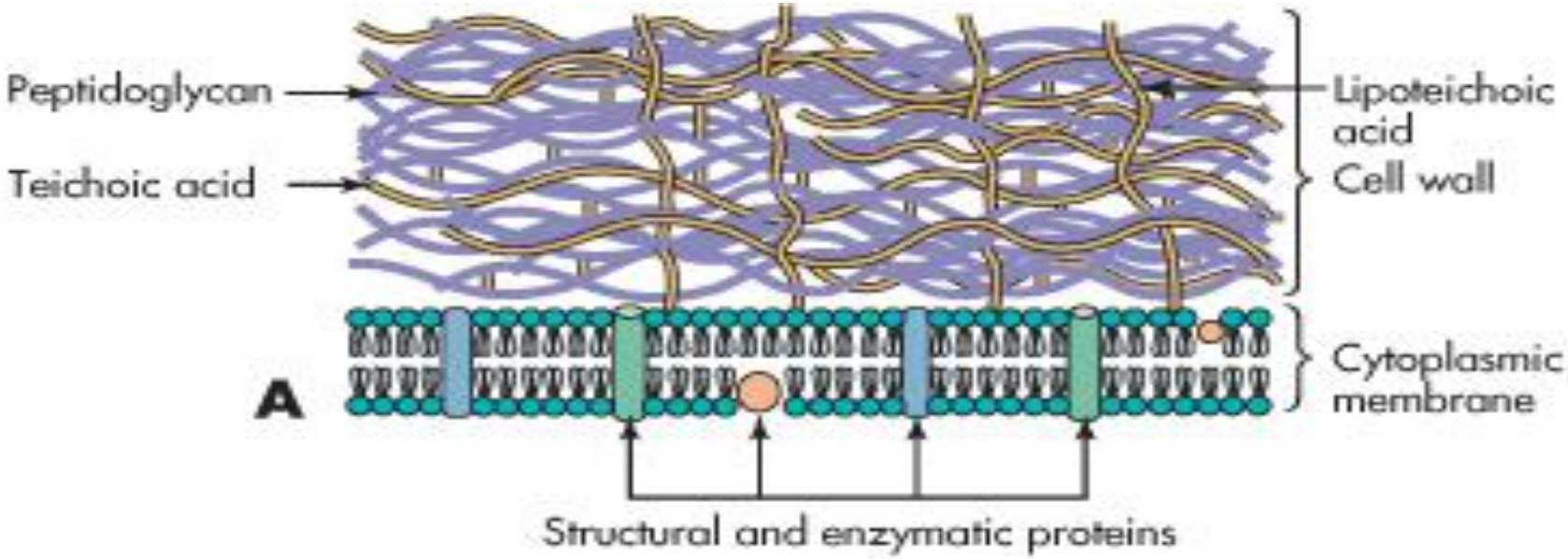

GRAM-NEGATIVE
NO growth on MacConkey agar good growth on Chocolate agar
Good growth on Blood, Chocolate and McConkey agars
COCCI OR COCCOBACILLI
Niesseria sicca NO growth on Haemophilus Isolation Agar
Haemophilus influenzae Growth on Haemophilus Isolation Agar
RODS
LACTOSE FERMENTATION (+)
LACTOSE FERMENTATION (–)
Urease(–) Citrate(–) Escherichia coli
Urease(–) Citrate(+) Enterobacter aerogenes
Urease(+) Citrate(+) Klebsiella pneumonia
Oxidase(+) SIM (–) Pseudomonas aeruginosa
Oxidase(–) SIM (+) Proteus vulgaris

Gram-Positive Cell Wall

- Thick, multilayered cell wall consisting mainly of peptidoglycan (150-500 Å).
- Similar to the exoskeleton of an insect except it is **porous**.



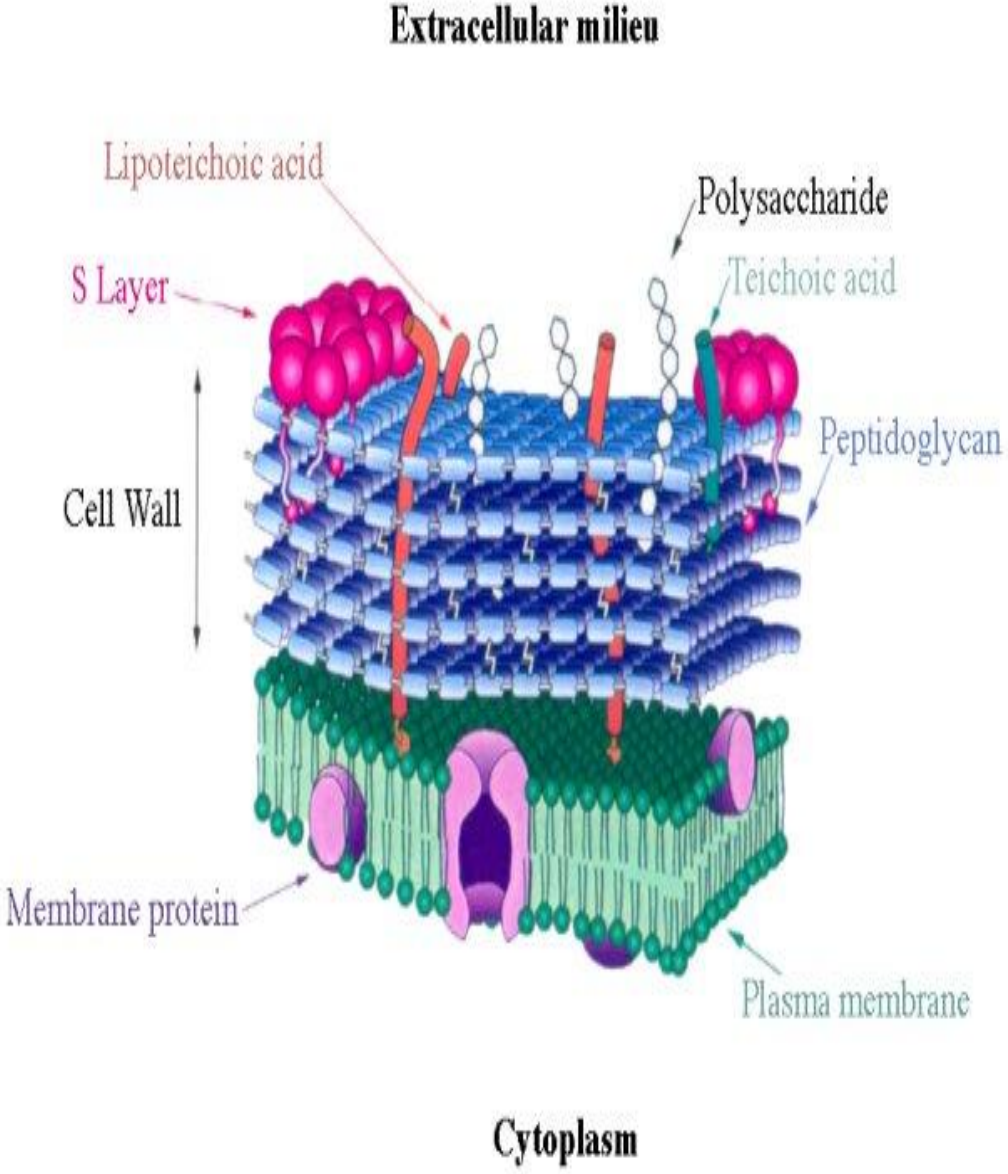
Gram-Positive Cell Wall



- Peptidoglycan essential for structure, replication and survival.
- Can interfere with phagocytosis and stimulate innate immune responses.
- Pyrogenic.

Gram-Positive Cell Wall

- Teichoic acids are water soluble, anionic polymers covalently linked to the peptidoglycan.
- Lipoteichoic acids have a fatty acid modification and are anchored to the cytoplamic membrane.
- Both are common surface antigens that distinguish bacterial serotypes and promote attachment to other bacteria and to specific receptors on mammalian cell surfaces.

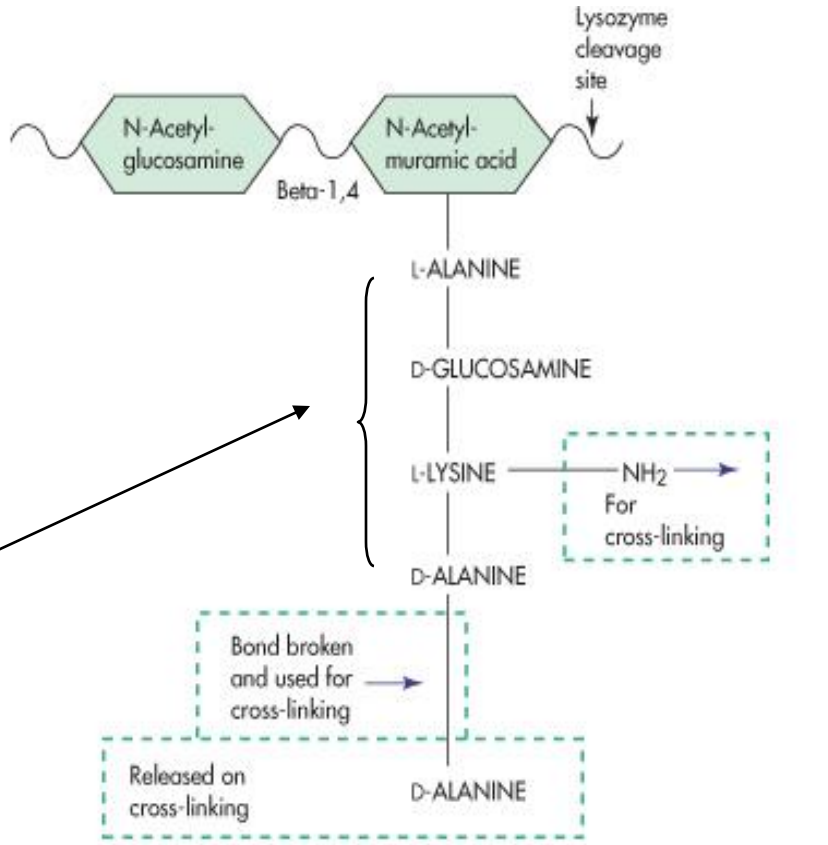


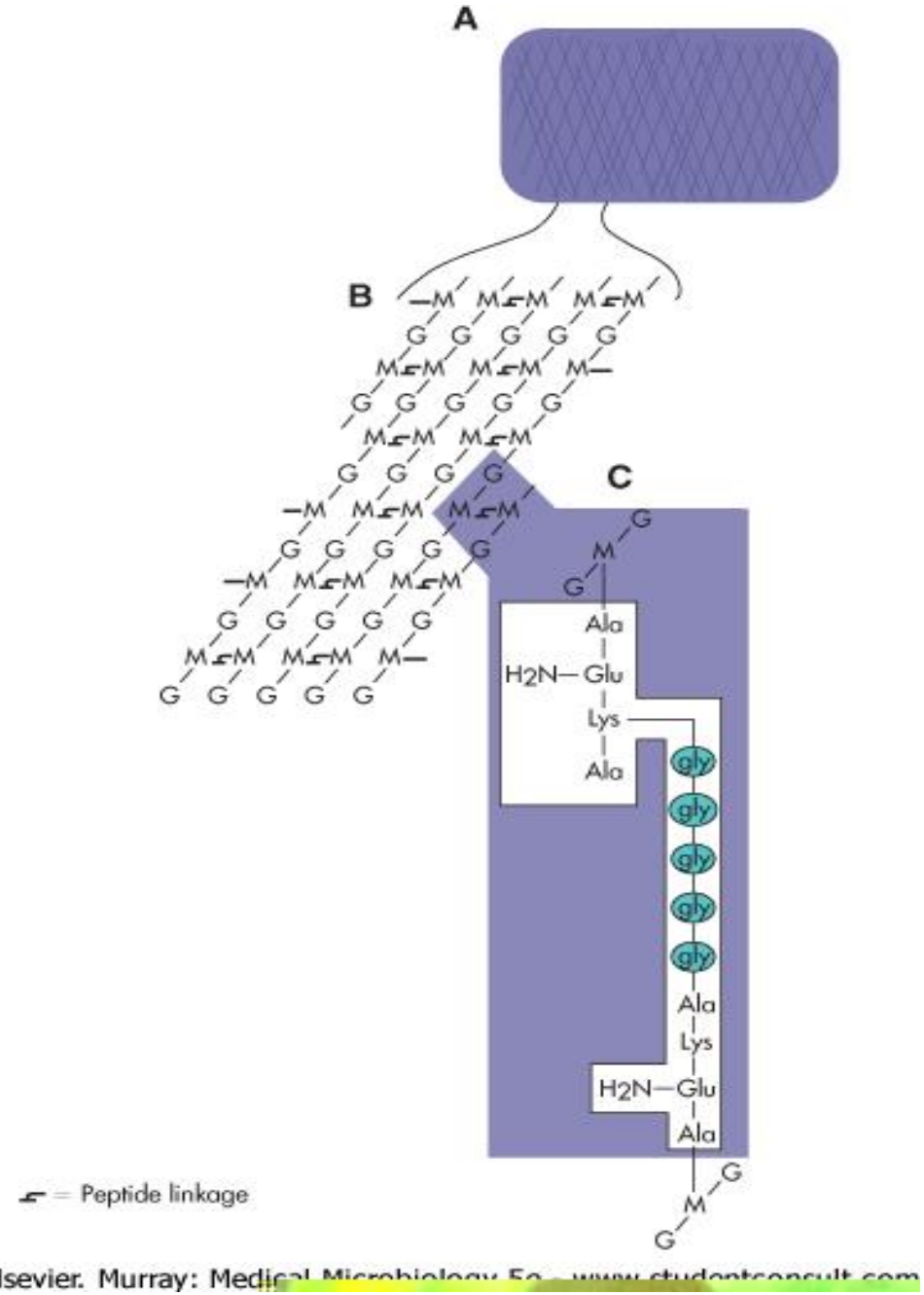
Structure and Biosynthesis of the Major Components of the Bacterial Cell Wall

Cell wall components are prefabricated precursors and subunits of the final structure are assembled on the inside and then brought to the surface.

PEPTIDOGLYCAN

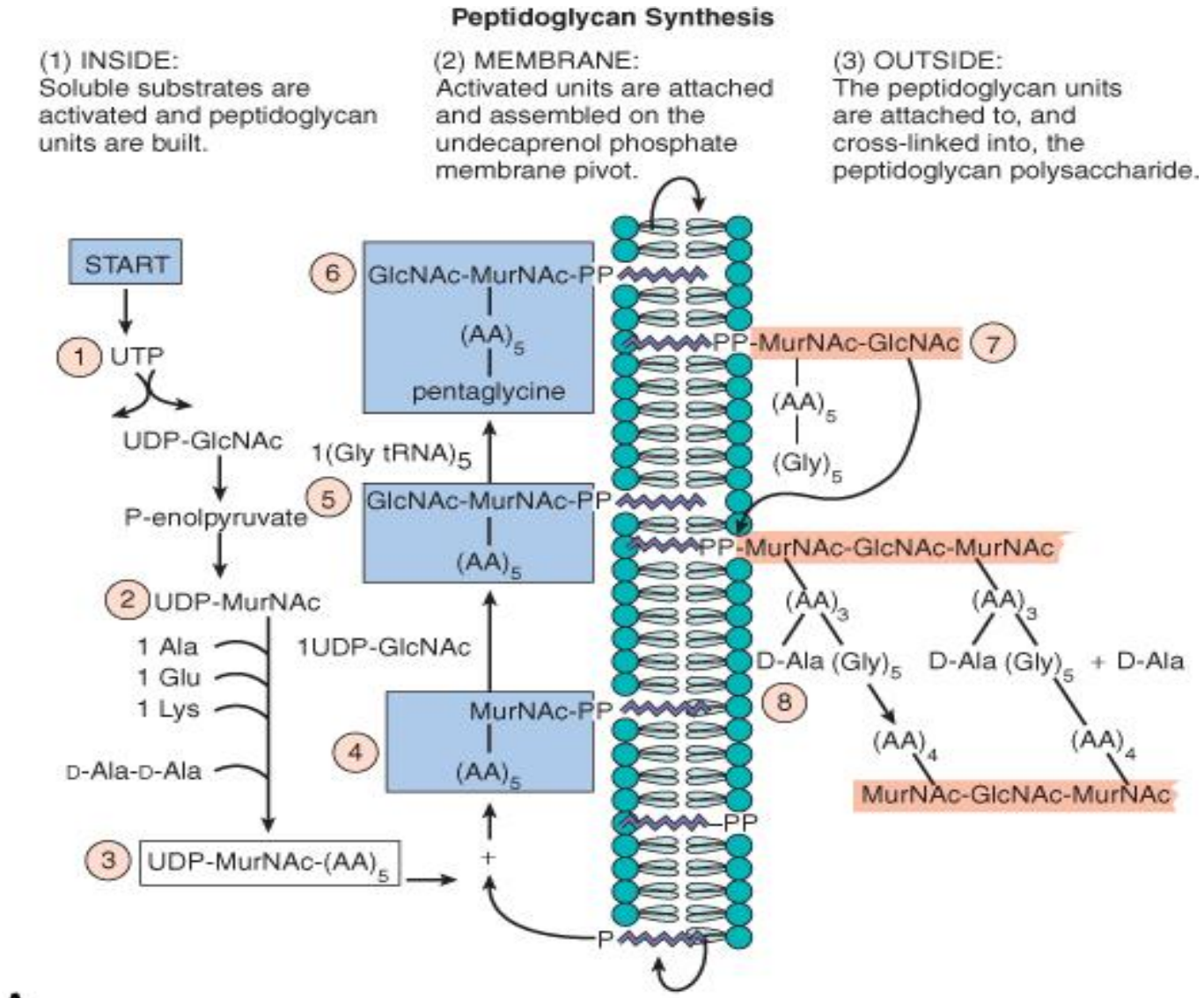
- Peptidoglycan is a rigid mesh made up of ropelike linear polysaccharide chains made up of repeating disaccharides of **N-acetylglucosamine (GlcNAc, NAG, G)** and **N-acetylmuramic acid (MurNAc, NAM, M)**.
- Tetrapeptide attached to MurNAc.



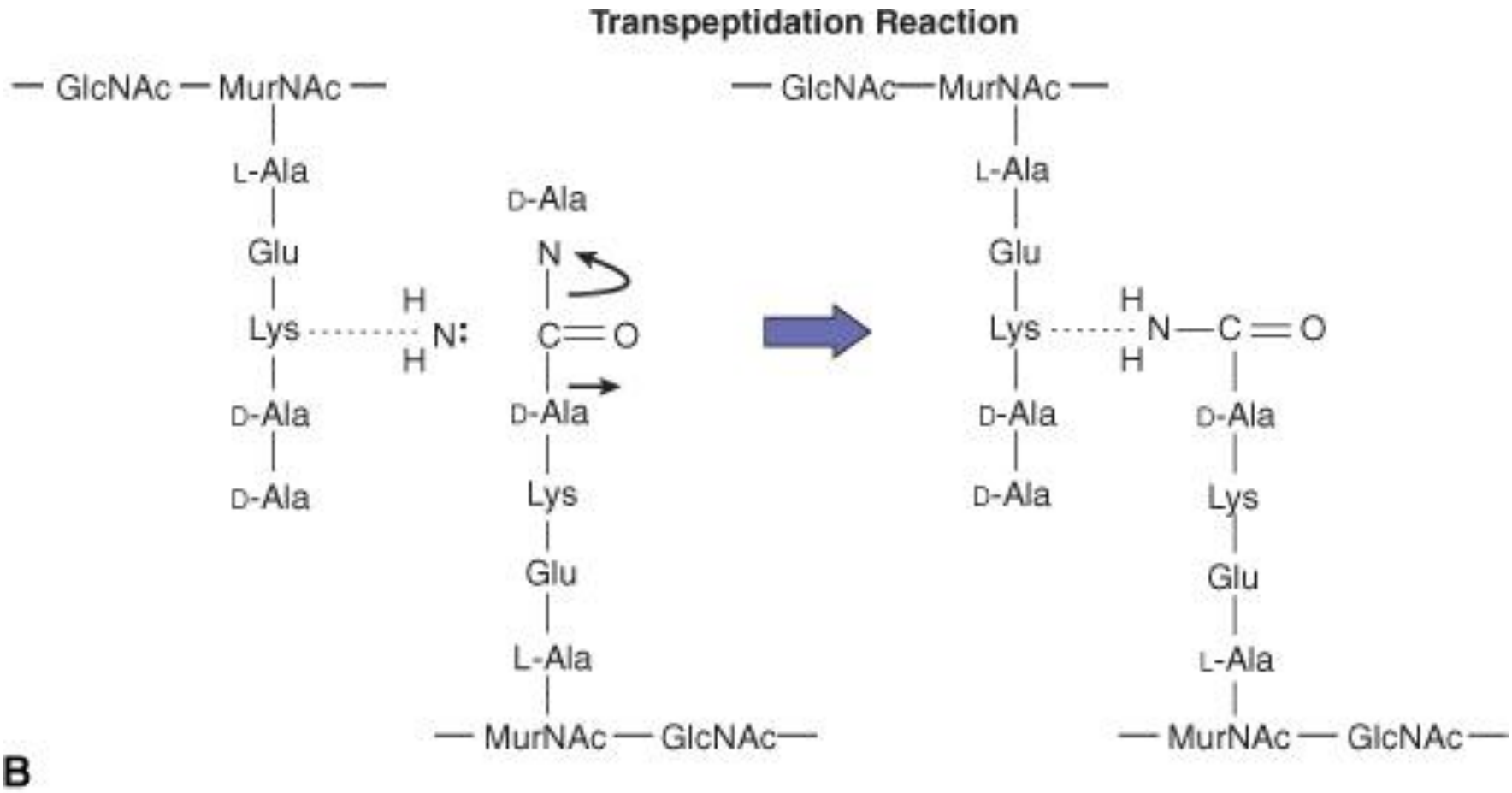


PEPTIDOGLYCAN

PEPTIDOGLYCAN SYNTHESIS



PEPTIDOGLYCAN SYNTHESIS



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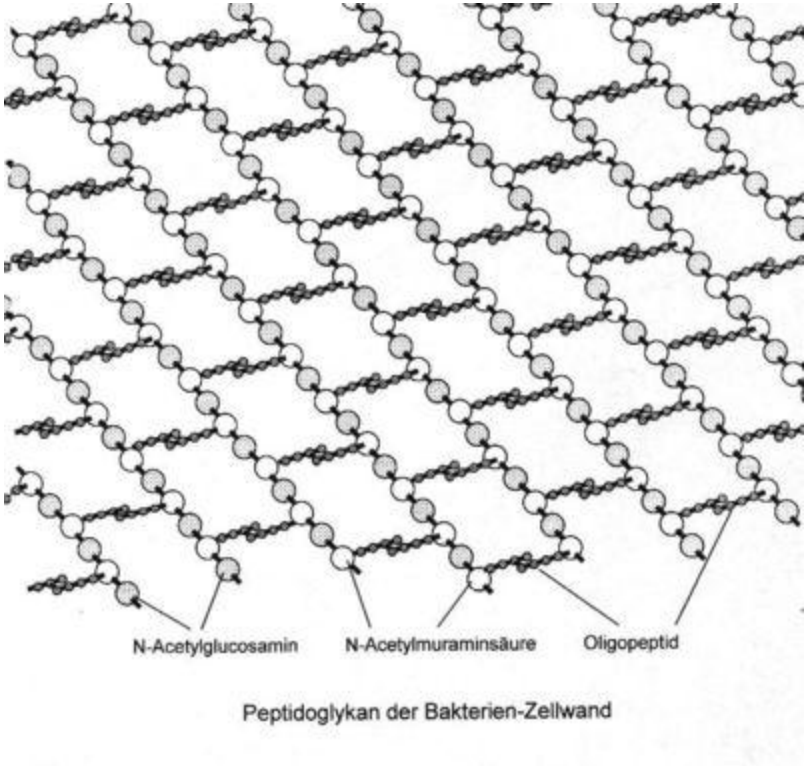
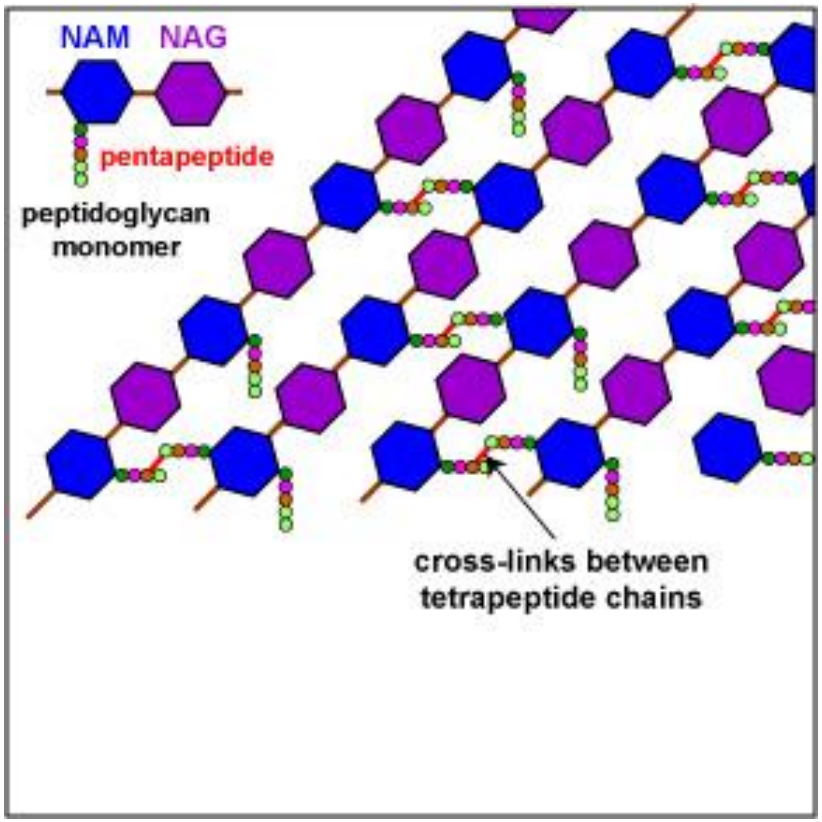


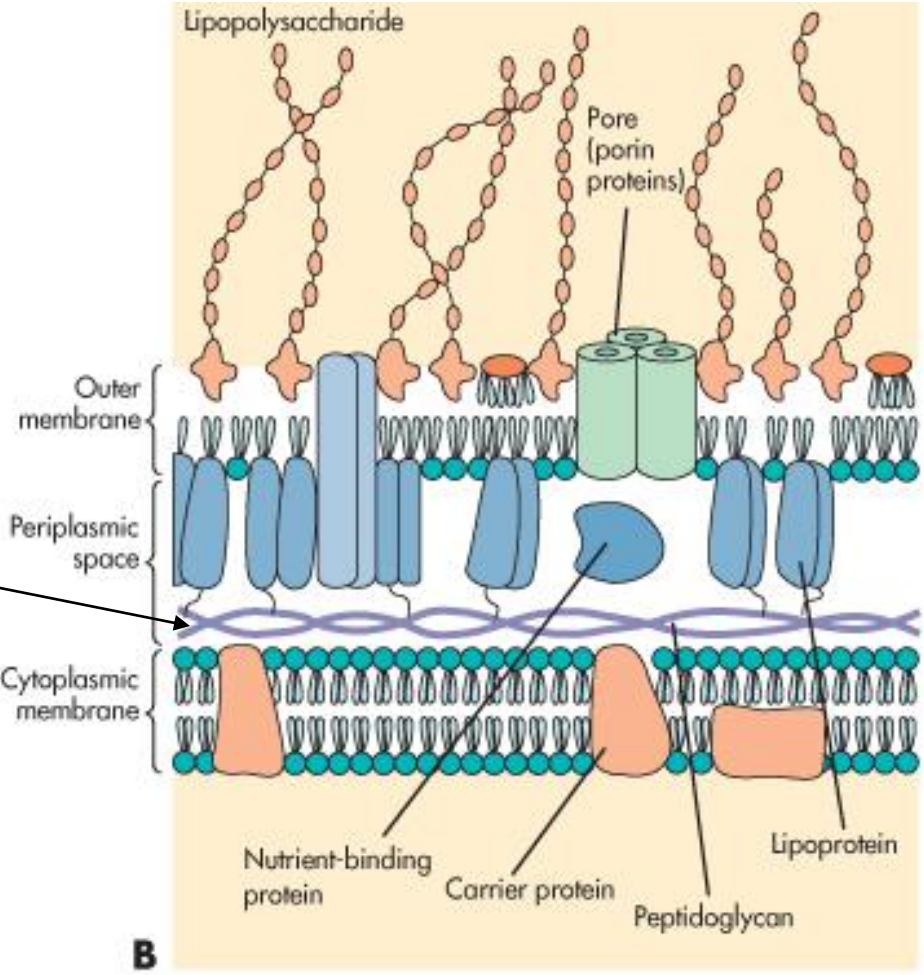
Peptidoglykan der Bakterien-Zellwand

The number of cross-links and the length of the cross-links determine the rigidity of the peptidoglycan mesh.



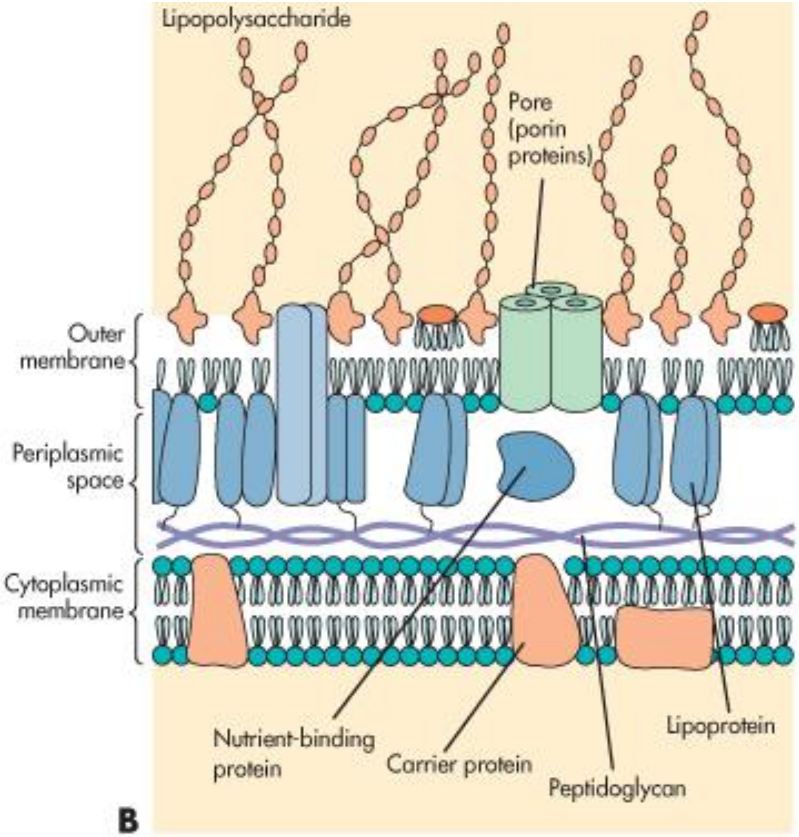
Gram-Negative Cell Wall

- More complex than Gram-positive cell wall.
- 2 layers external to the cytoplasmic membrane.
  - thin peptidoglycan layer (5-10% of the cell wall by weight).
  - external to the peptidoglycan layer is the outer membrane.



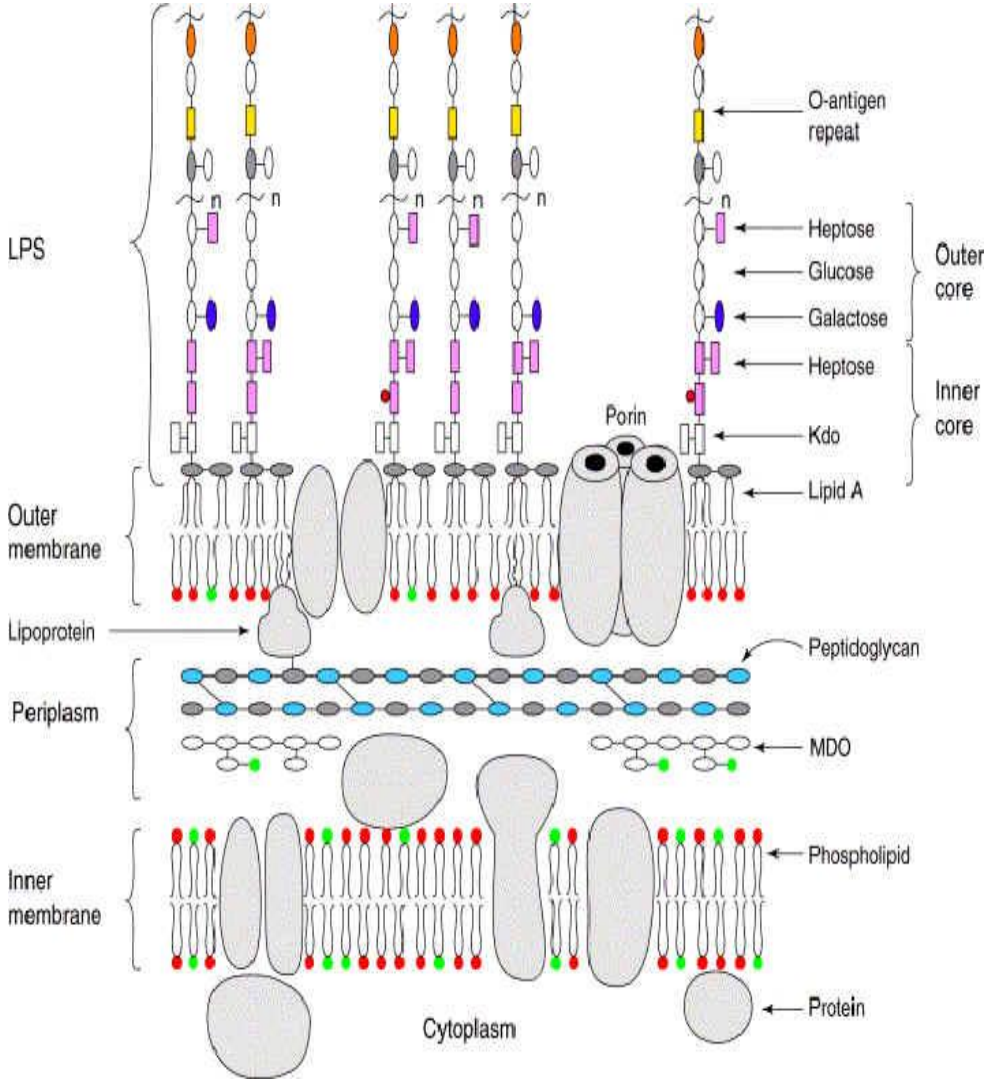
Gram-Negative Cell Wall

- **Periplasmic space-**
  - The area between the external surface of the cytoplasmic membrane and the internal surface of the outer membrane.
  - Contains hydrolytic enzymes important to the cell for breakdown of large macromolecules for metabolism.
  - Also contains enzymes associated with pathology *e.g.*, proteases, hyaluronidase, collagenases and β-lactamase.

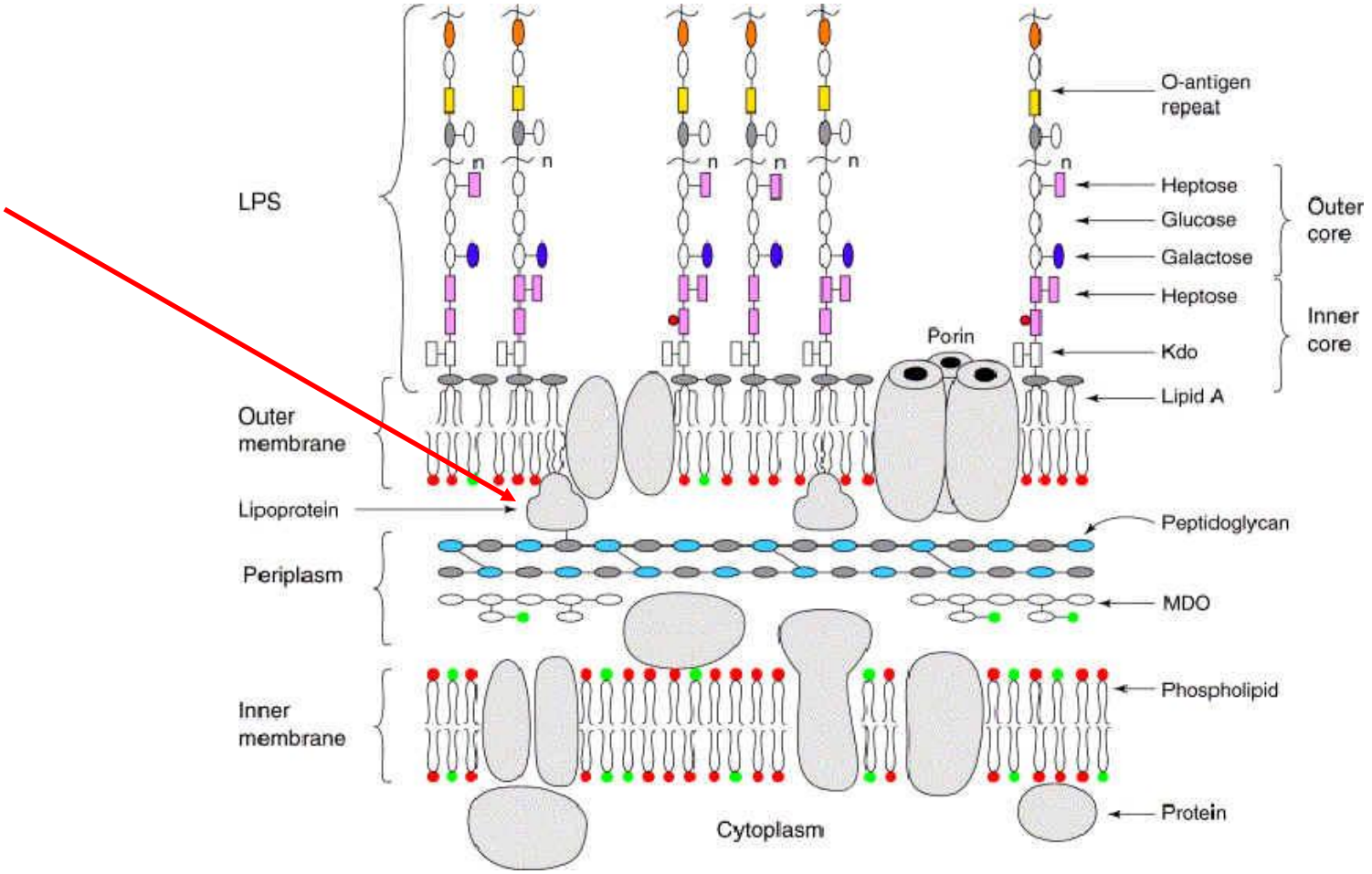


Gram-Negative Cell Wall

- **Outer membrane-**
  - unique to gram negative bacteria.
  - has similar roll as peptidoglycan does in Gram-positive bacteria.
    - *i.e.*, it maintains the bacterial structure and is a permeability barrier to large molecules.
  - Asymmetric.
    - bilayer structure unique among biologic membranes.
      - inner leaflet-phospholipids
      - outer leaflet-LPS which is amphipathic.
      - Only place where LPS is found.
      - LPS=endotoxin

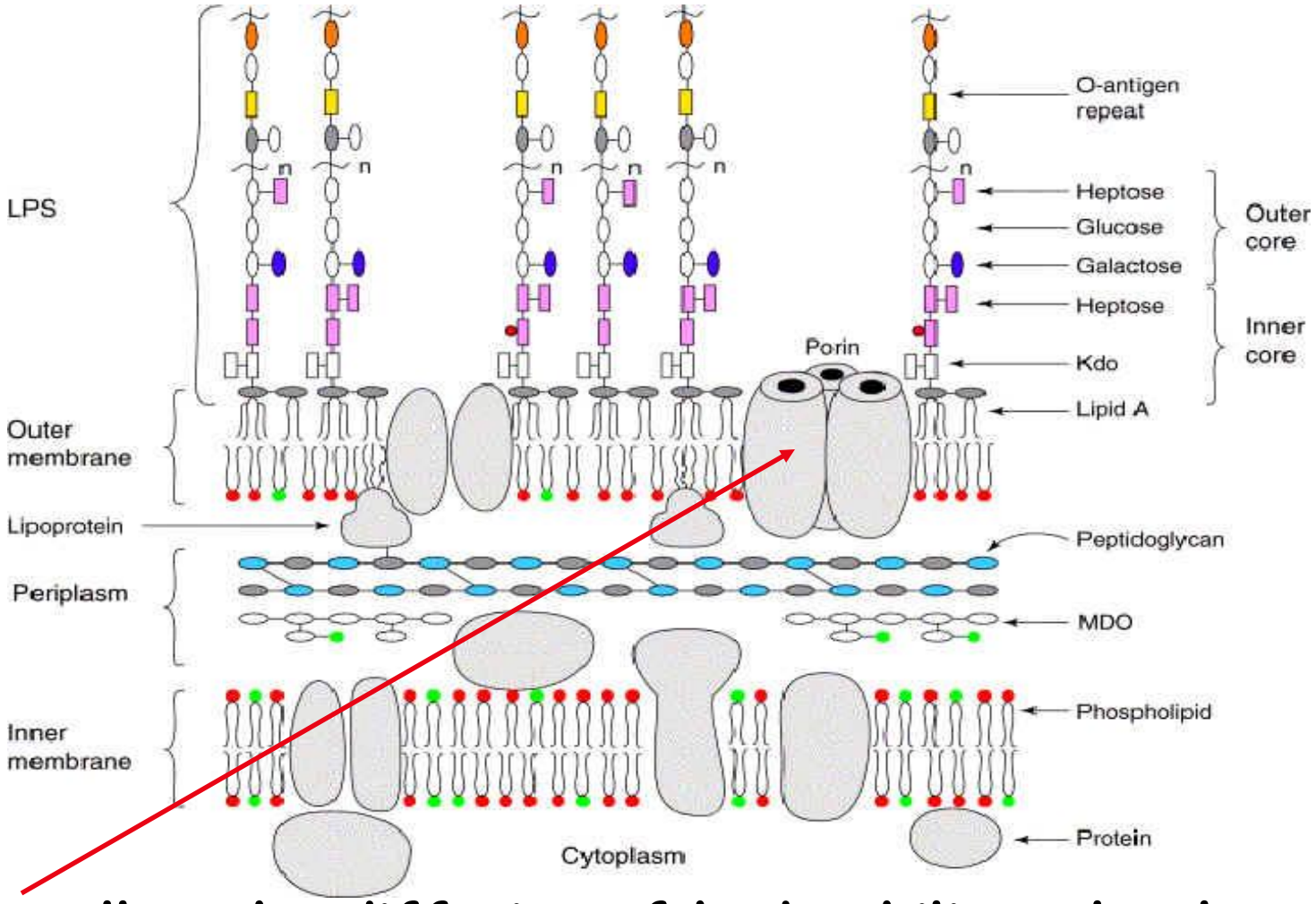


Gram-Negative Cell Wall



The outer membrane is connected to the cytoplasmic membrane at adhesion sites and is tied to the peptidoglycan by lipoprotein.

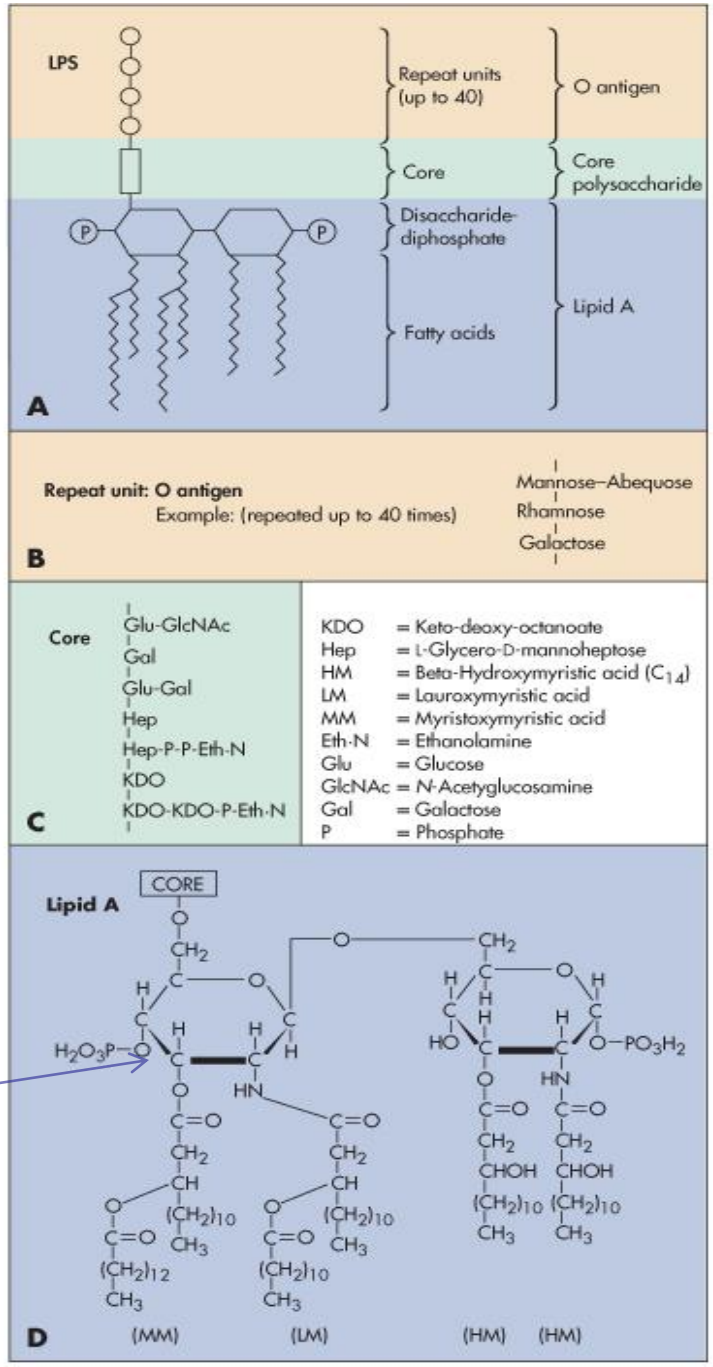
Gram-Negative Cell Wall



Porins allow the diffusion of hydrophilic molecules: metabolites and small hydrophylic antibiotics.

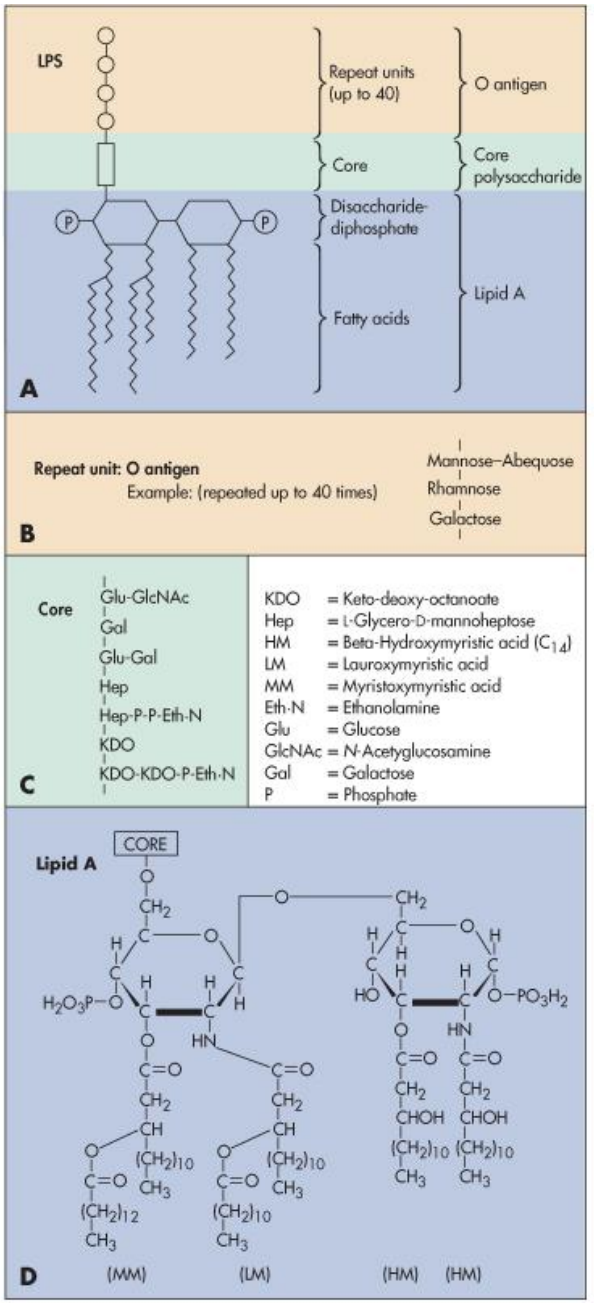
LPS

- Consists of three structural sections:
  - Lipid A
  - Core polysaccharide
  - O-antigen
- Lipid A is responsible for the endotoxin activity of LPS.
  - Phosphorylated glucosamine disaccharide backbone.
  - Phosphates connect LPS molecules into aggregates.



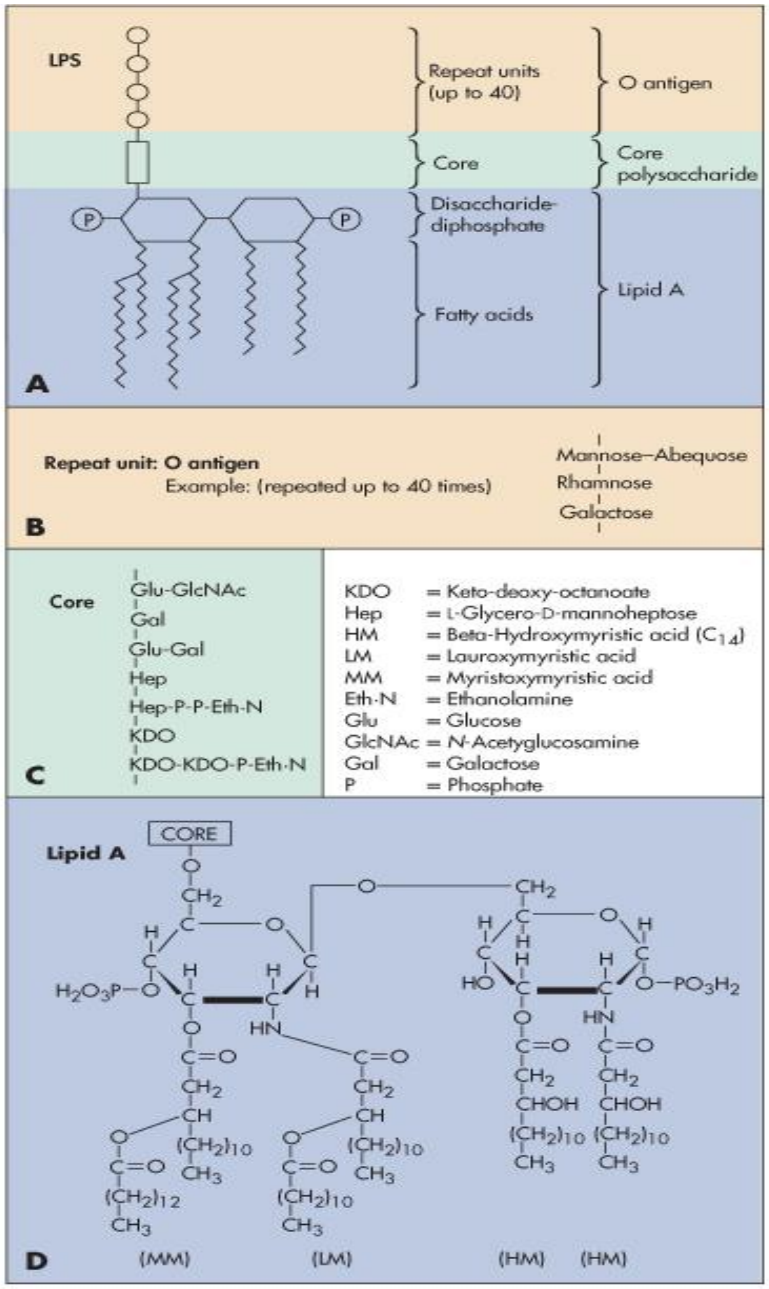
LPS

- Core
  - Polysaccharide is a branched polysaccharide of 9-12 sugars.
  - Essential for LPS structure
- O-Antigen
  - Attached to core
  - Long, linear polysaccharide consisting of 50-100 repeating saccharide units of 4-7 sugars per unit.



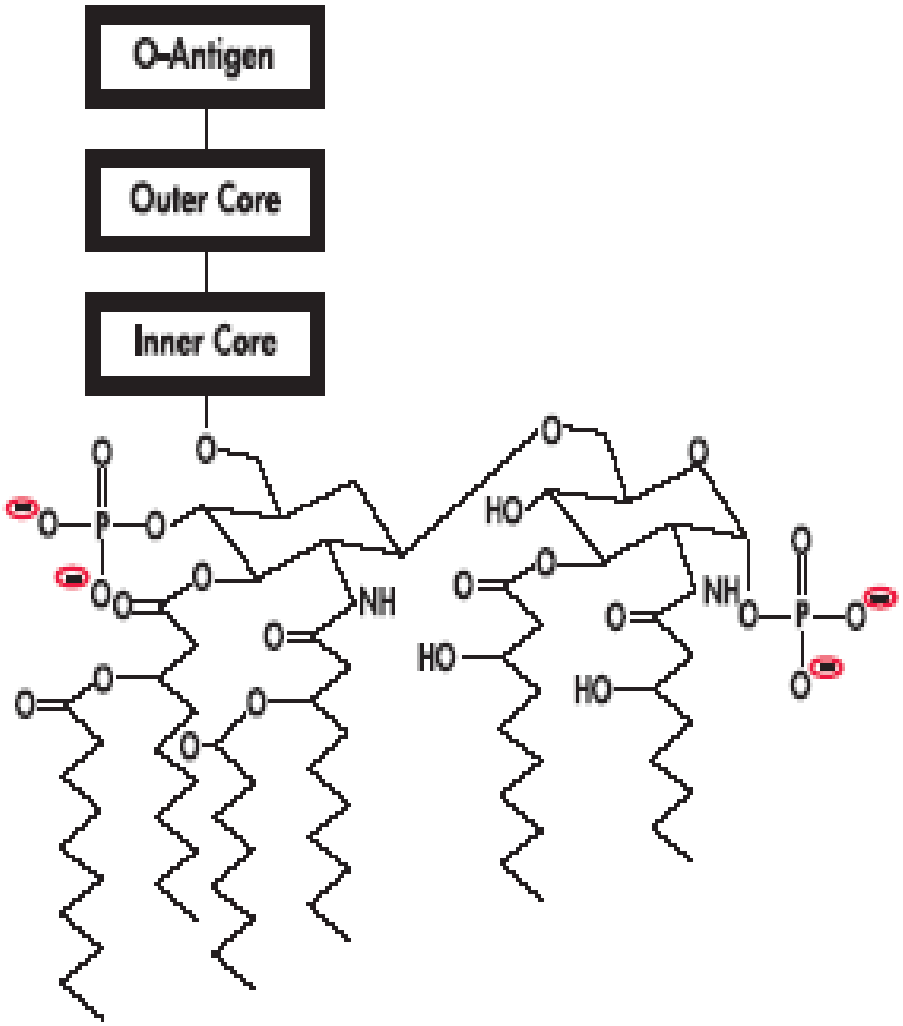
LPS

- LPS structure used to classify bacteria.
  - Lipid A is identical for related bacteria and similar for all Gram-negative Enterobacteriaceae.
- The core region is the same for a species of bacteria.
- The O antigen distinguishes serotypes (stains) of a bacterial species *e.g.*, *E. coli* O157:H7.



LPS

- Powerful nonspecific stimulator of the immune system.
- Activate B cells (non specifically) and induce macrophages, dendritic, and other cells to release IL-1, IL-6, and TNF-α.
- Induce shock if reaches blood stream at elevated levels.
  - Disseminated Intravascular Coagulation.

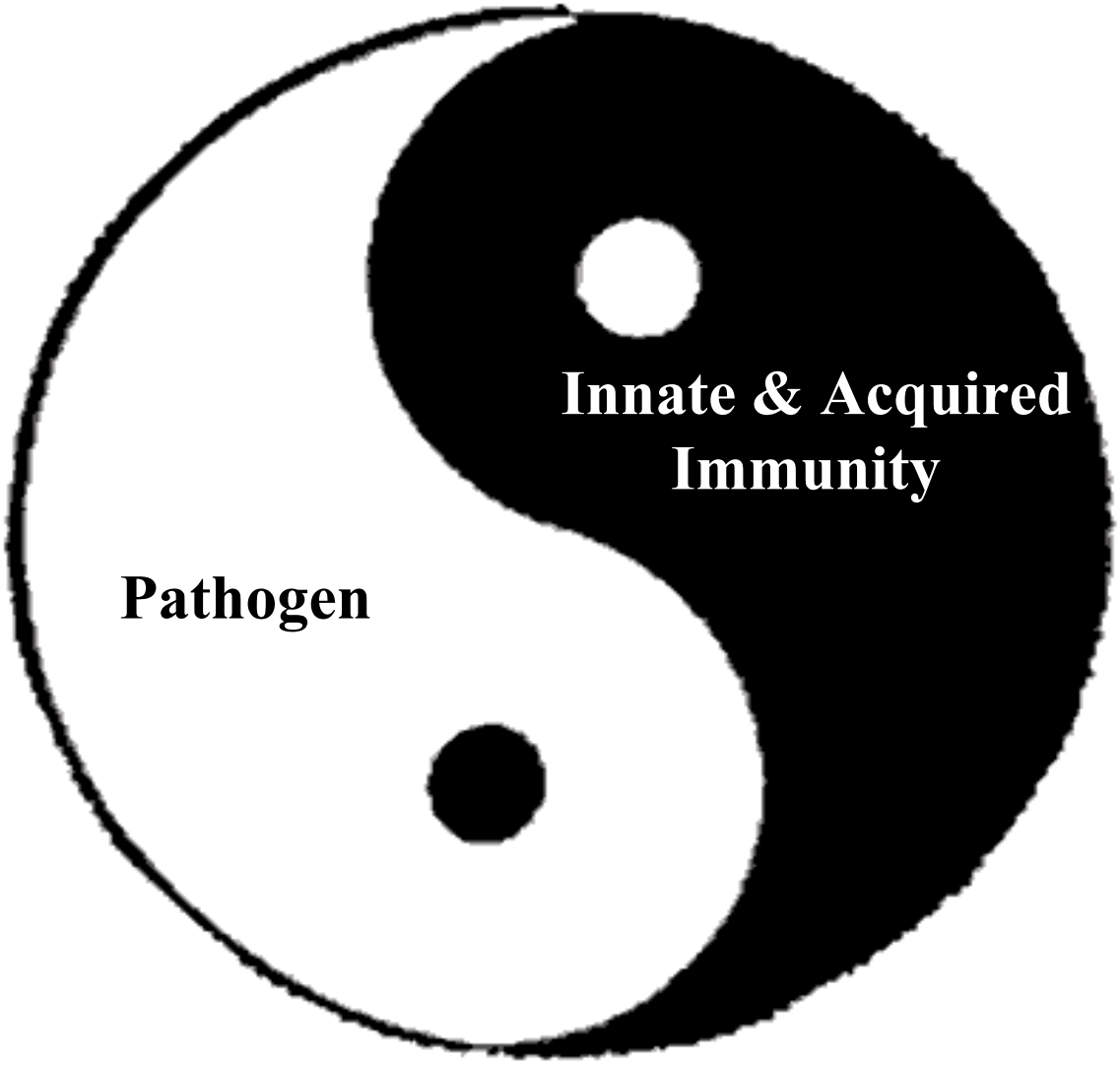


Summary—Gram-positive vs. Gram-negative (membrane characteristics)

Characteristic	Gram-positive	Gram-negative
Outer Membrane	-	+
LPS	-	+
Cell wall	Thicker	Thinner
Teichoic acid	Often present	-
Lysozyme	Sensitive	Resistant
Penicillin susceptibility	More susceptible	More resistant

Immunology Overview/Review

Infection Dynamics



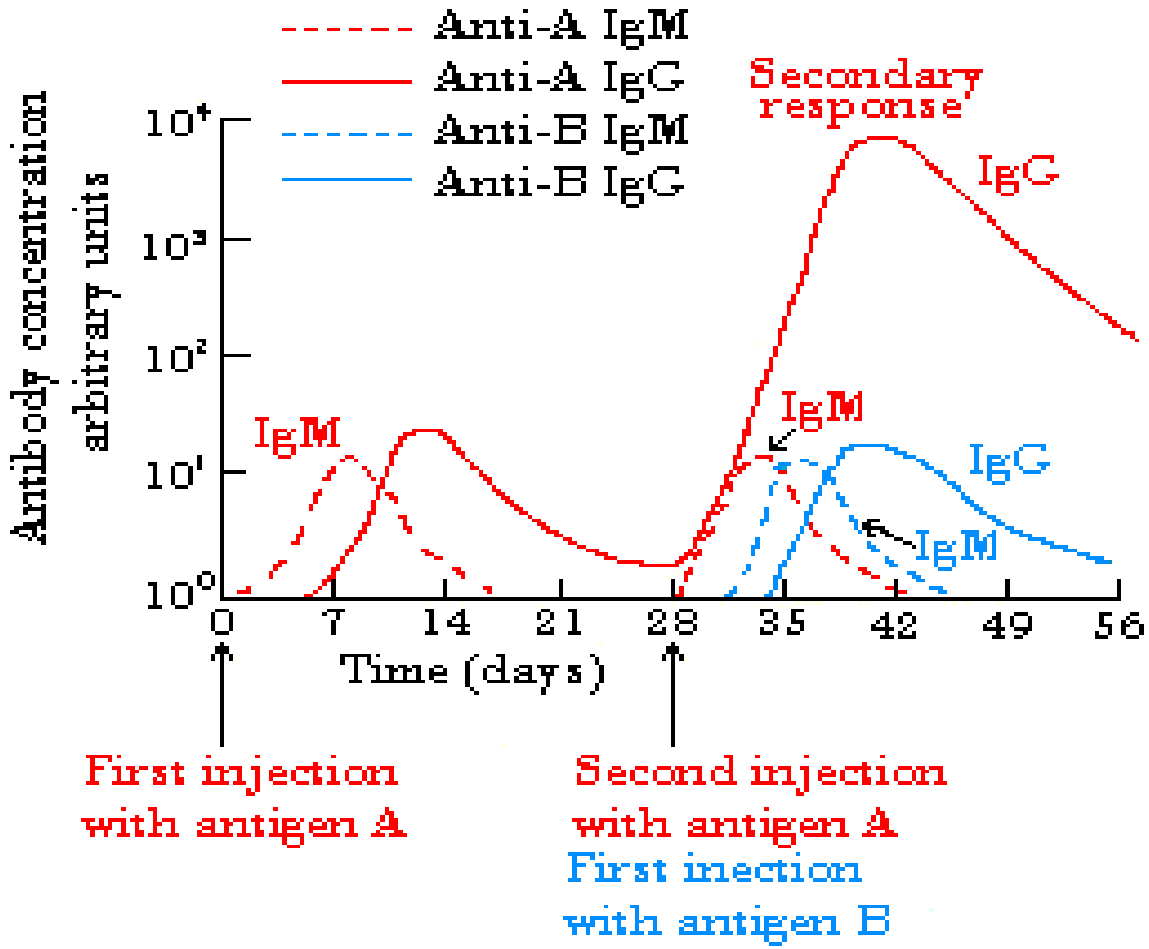
OBJECTIVES

- **1. The general nature of immune responsiveness.**
  - **Memory**
  - **Specificity**
    - **Innate immunity**
    - **Acquired Immunity**
- **2 Infection and Immunity**
- **3. The anatomic basis of immune responsiveness.**

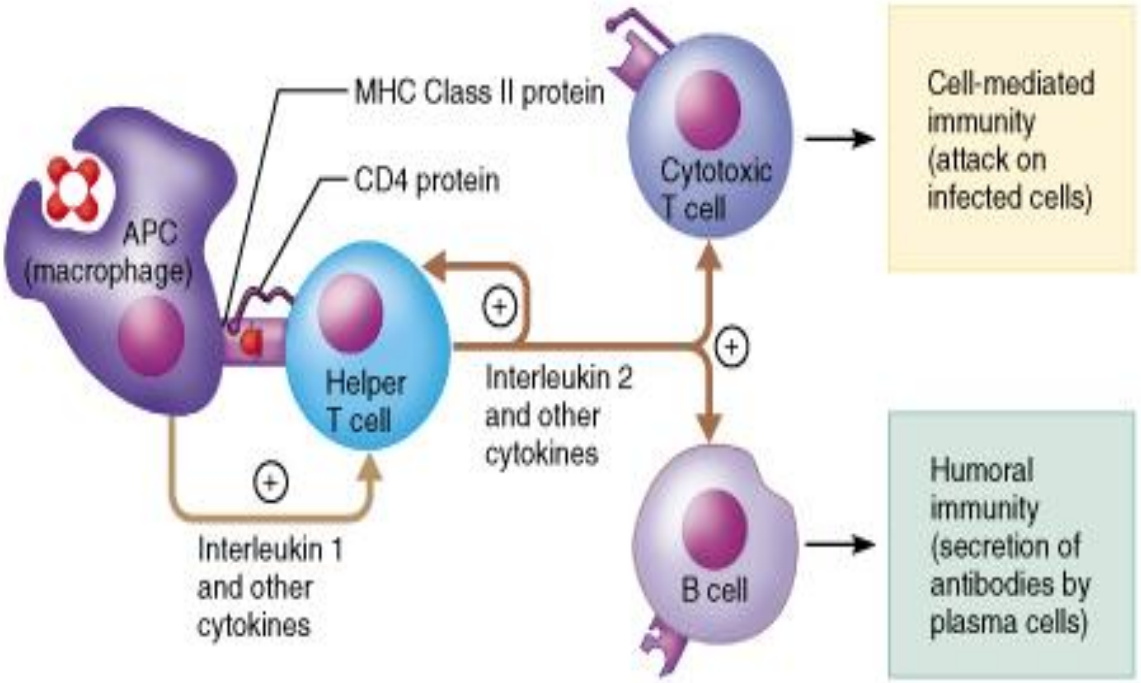
Definitions

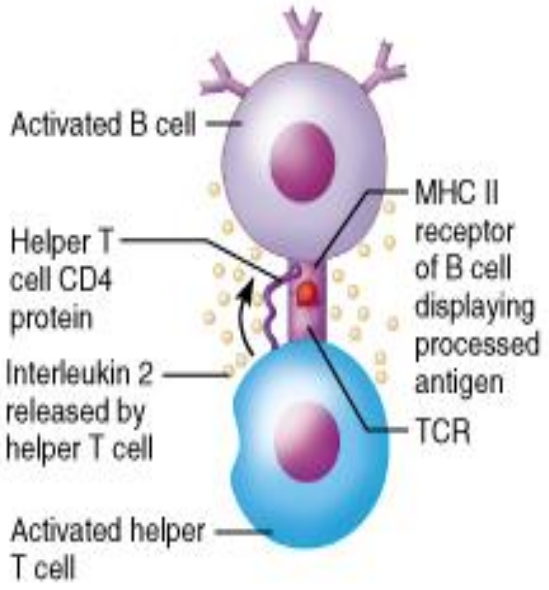
- **Innate**=macrophages, dendritic cells, eosionophils, basophils, neutrophils.
- **Acquired**=T cells; B cells.
- **Humoral**=antibody-mediated
- **Cellular**=dendritic cells, macrophages
- **APC**=antigen presenting cells
- **Antigen**=Any protein, carbohydrate, lipid etc. against which an immune response can be made (Under the right conditions).
- **Cytokines**=proteins (like hormones) used by immune cells to communicate.

Specificity and Memory



Specific & Anamestic Immune Recognition: (Antibodies or Cells other immune components)



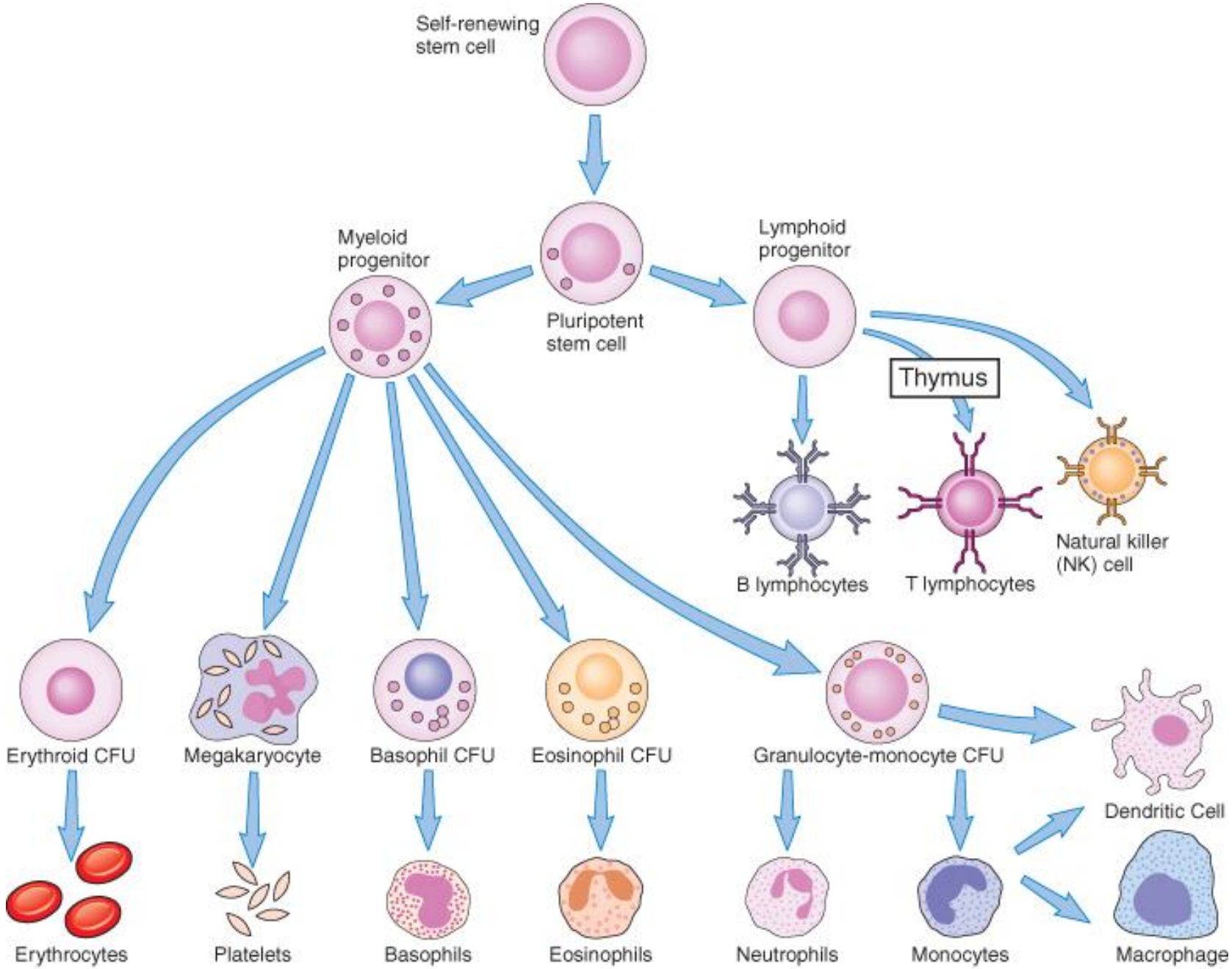


Sensitized lymphocytes

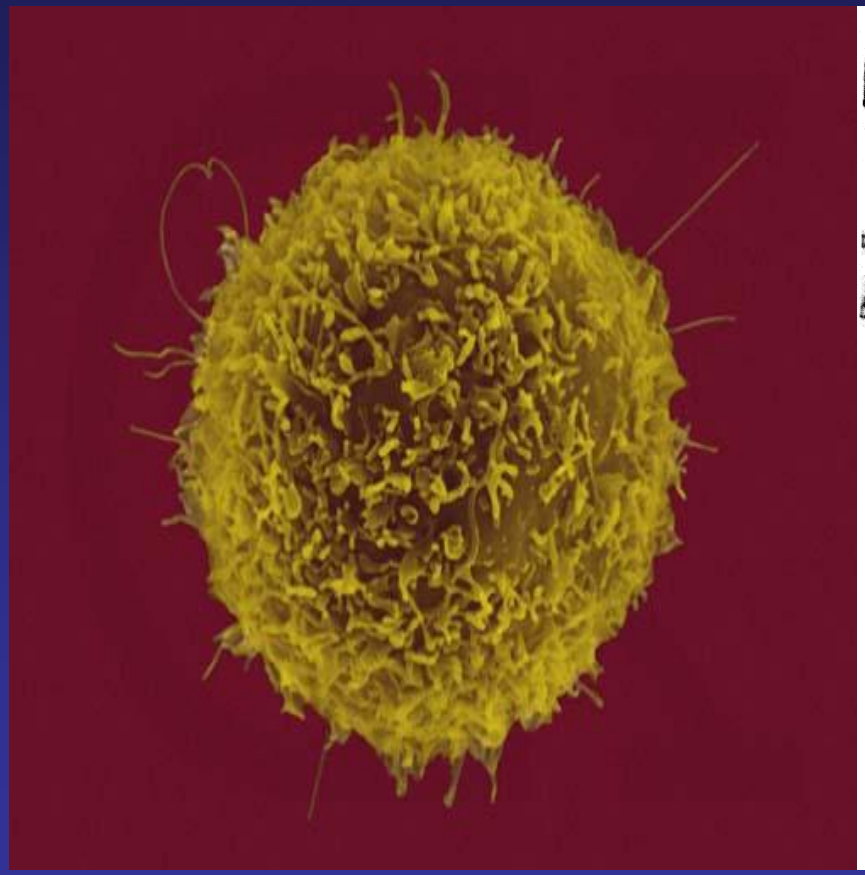
More later…

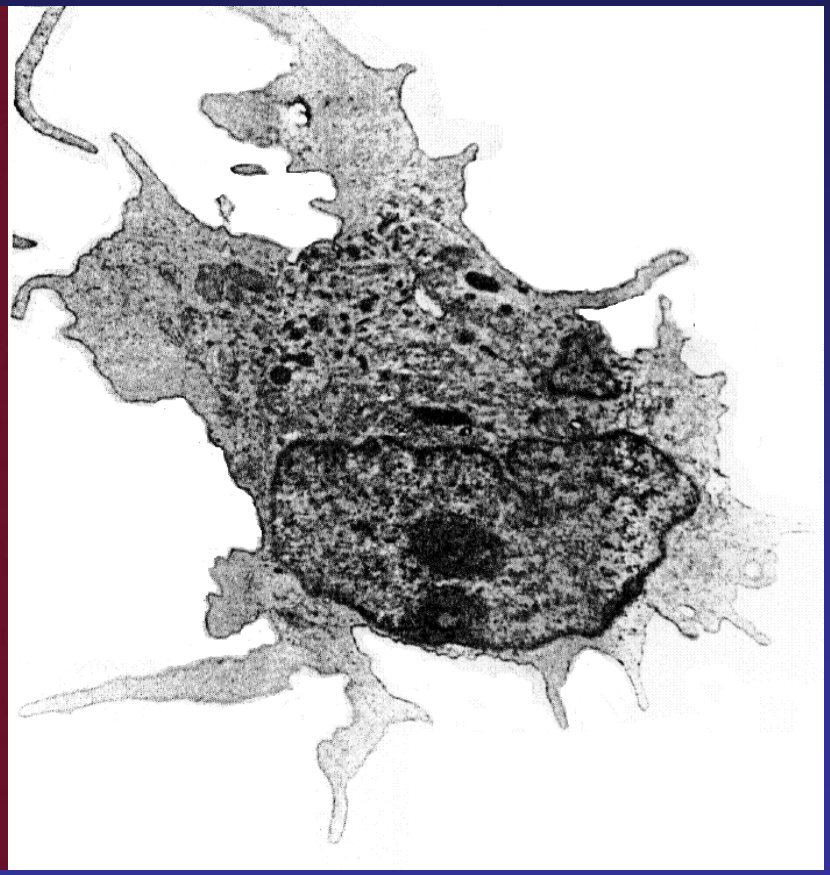
What cells are the main players of the immune system and of an immune response?

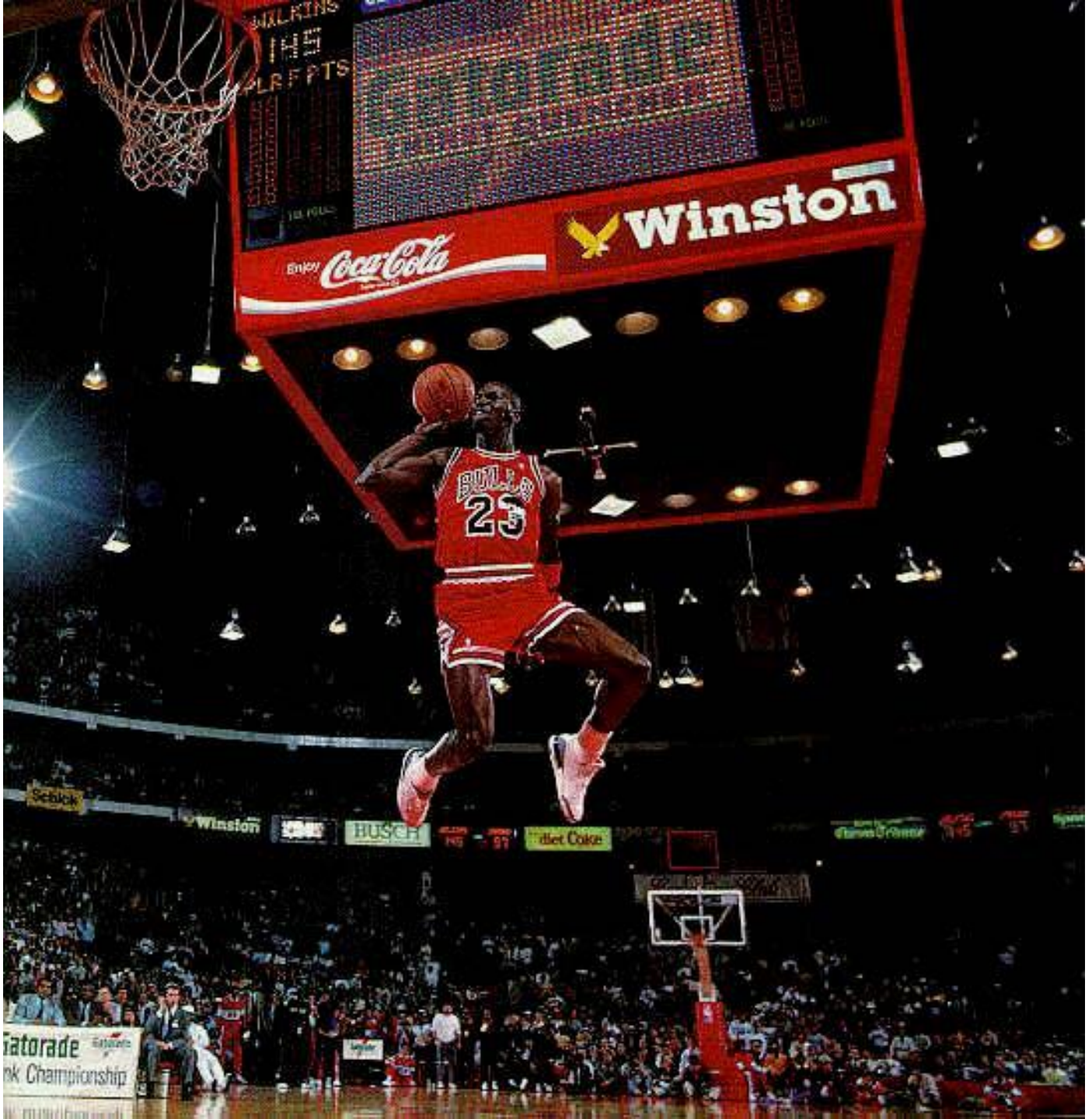
Where to the arise?

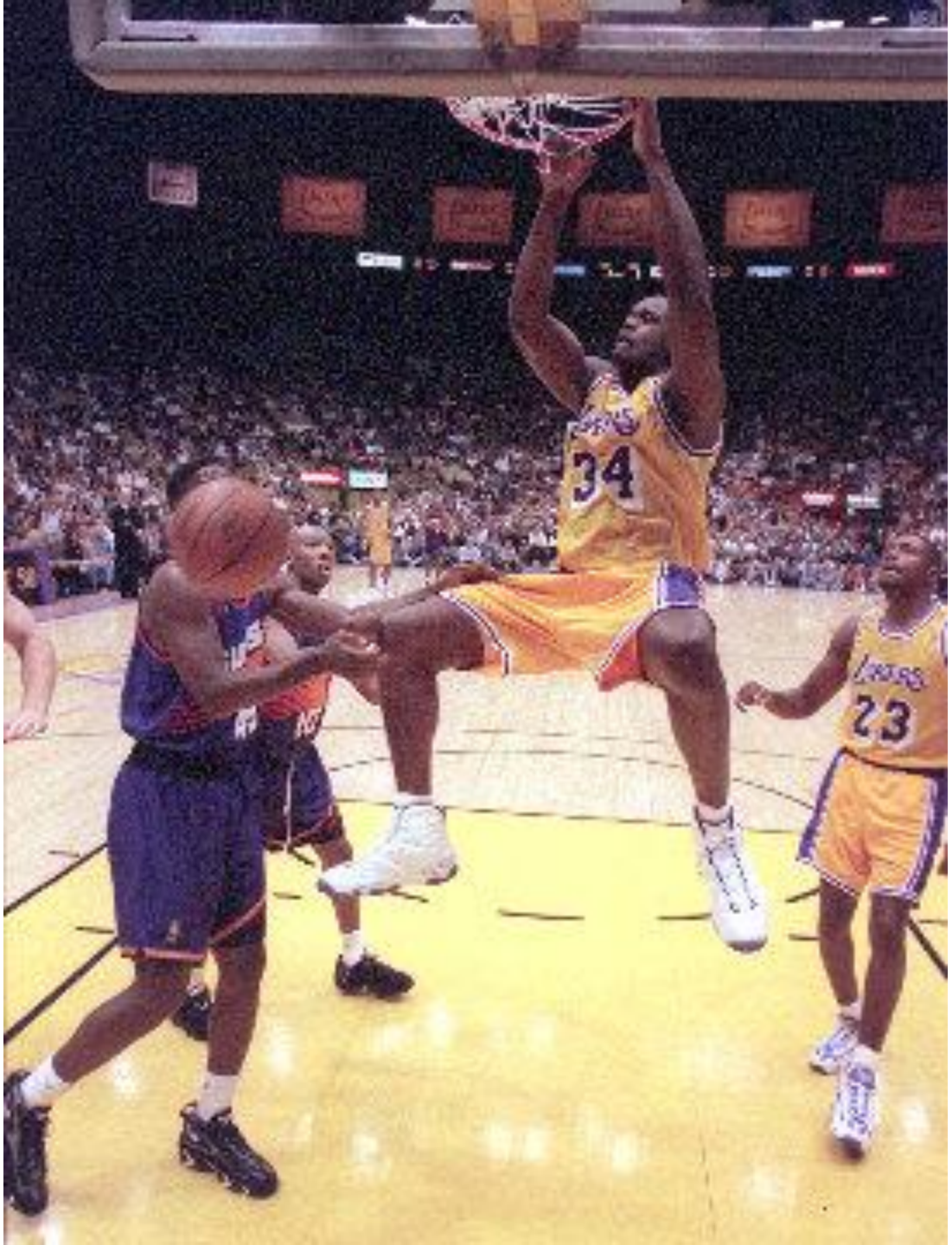


T cell----------MØ

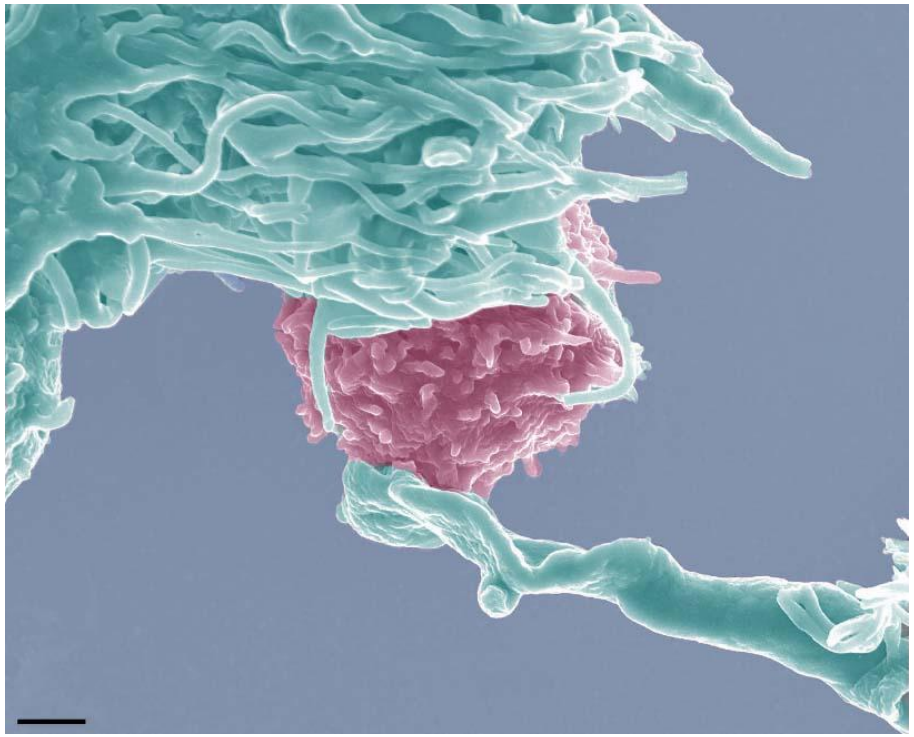


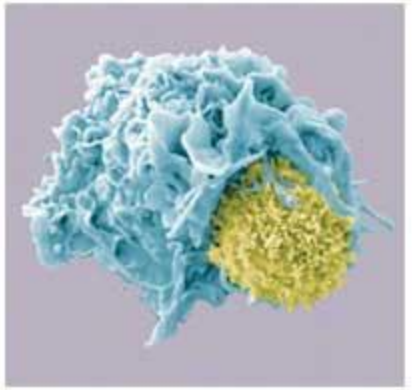


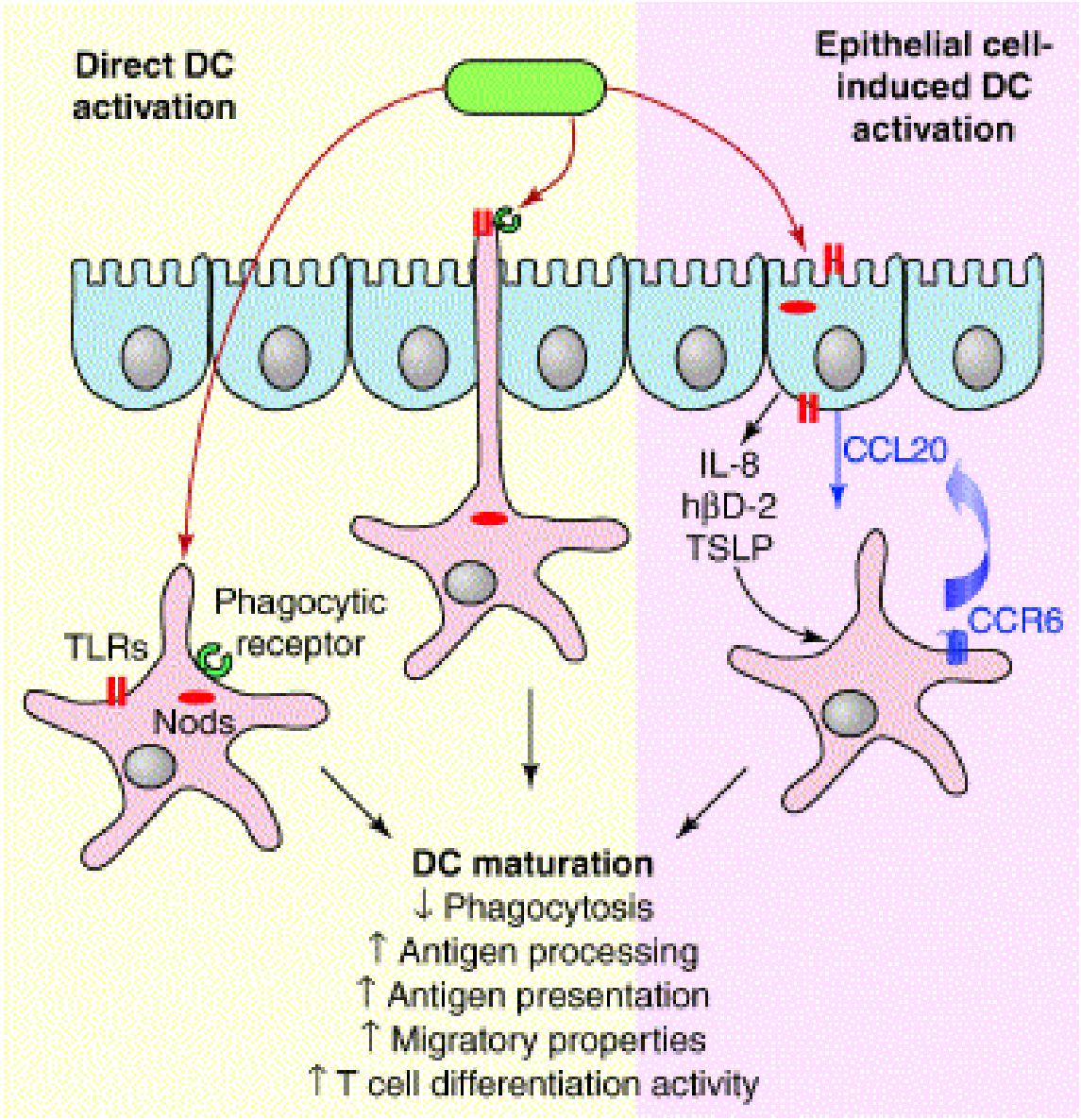

Winston
Coca-Cola
23
Schick
Winston
HUSCH
Diet Coke
Gatorade


34
23

Dendritic-T cell Interaction







TRENDS in Microbiology

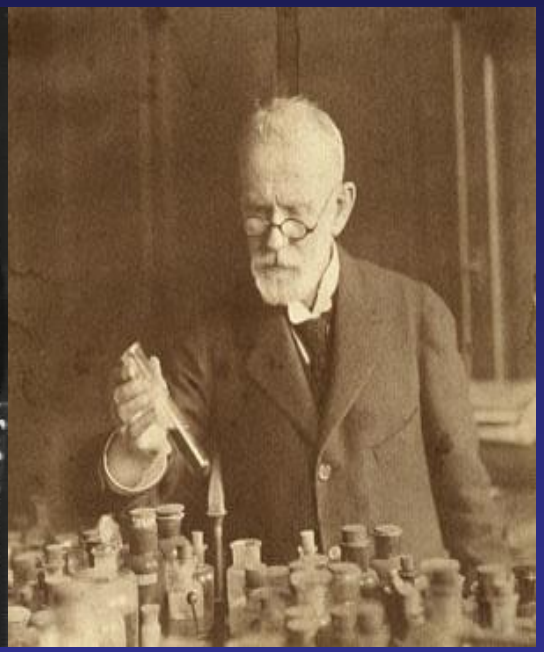
Old vs. New

- Innate-intrinsic *e.g.*, macrophages, neutrophils, DC, NK cells
  - Ancient
  - Recognize general patterns on pathogens (*e.g.*, LPS, carbohydrates).
- Acquired-adaptive, learned *e.g.*, T and B cells
  - Recognize specific protein sequences or structures.

Innate Immune Cells & Defense Mechanisms

Brief History of Complement

Bordet



Ehrlich

Hans Buchner- demonstrated that heating serum inactivated its lytic properties; alexin

Jules Bordet (Nobel 1919)-serum contained heat-stable (Antibodies) and a heat labile component that 'complemented' antibody

- Paul Ehrlich (1899)- coined the name 'complement'

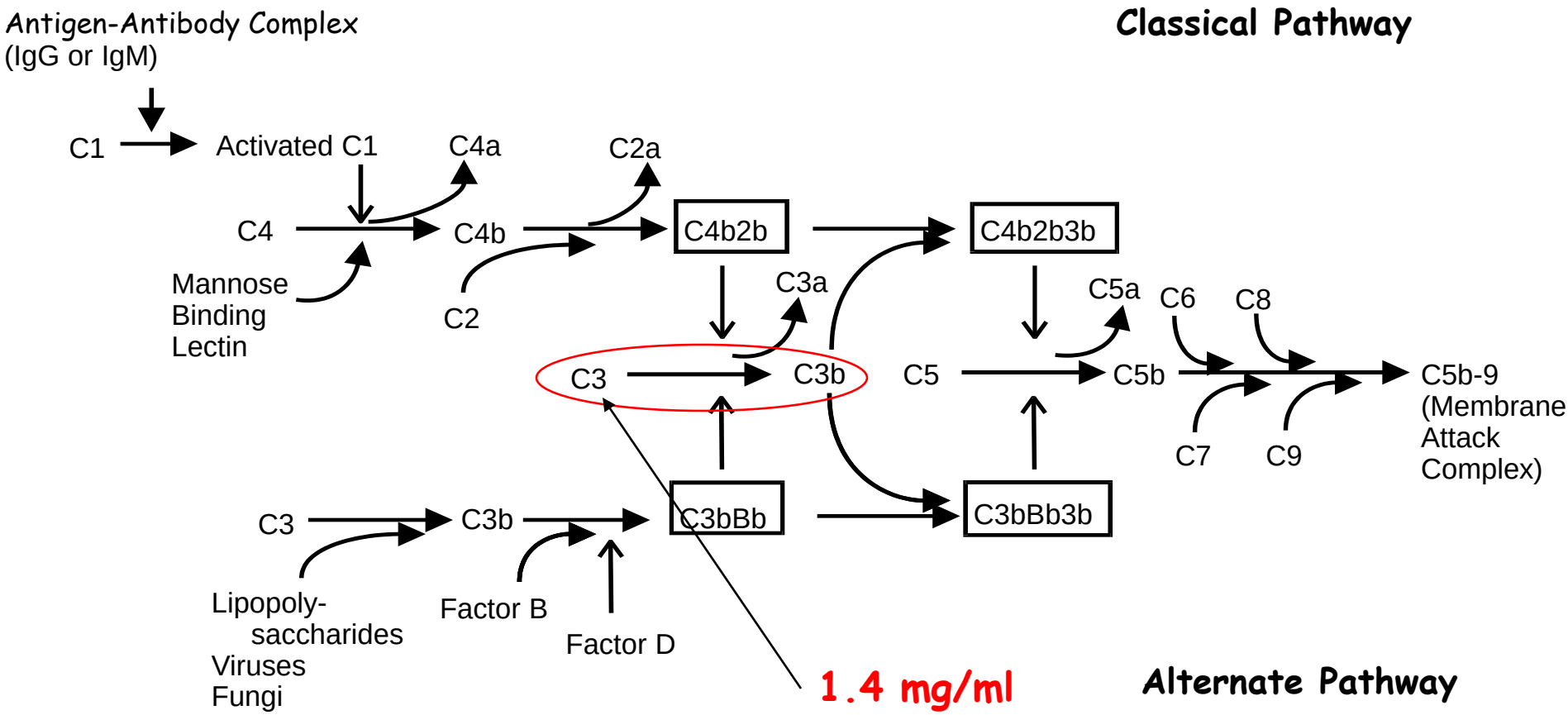
Complement

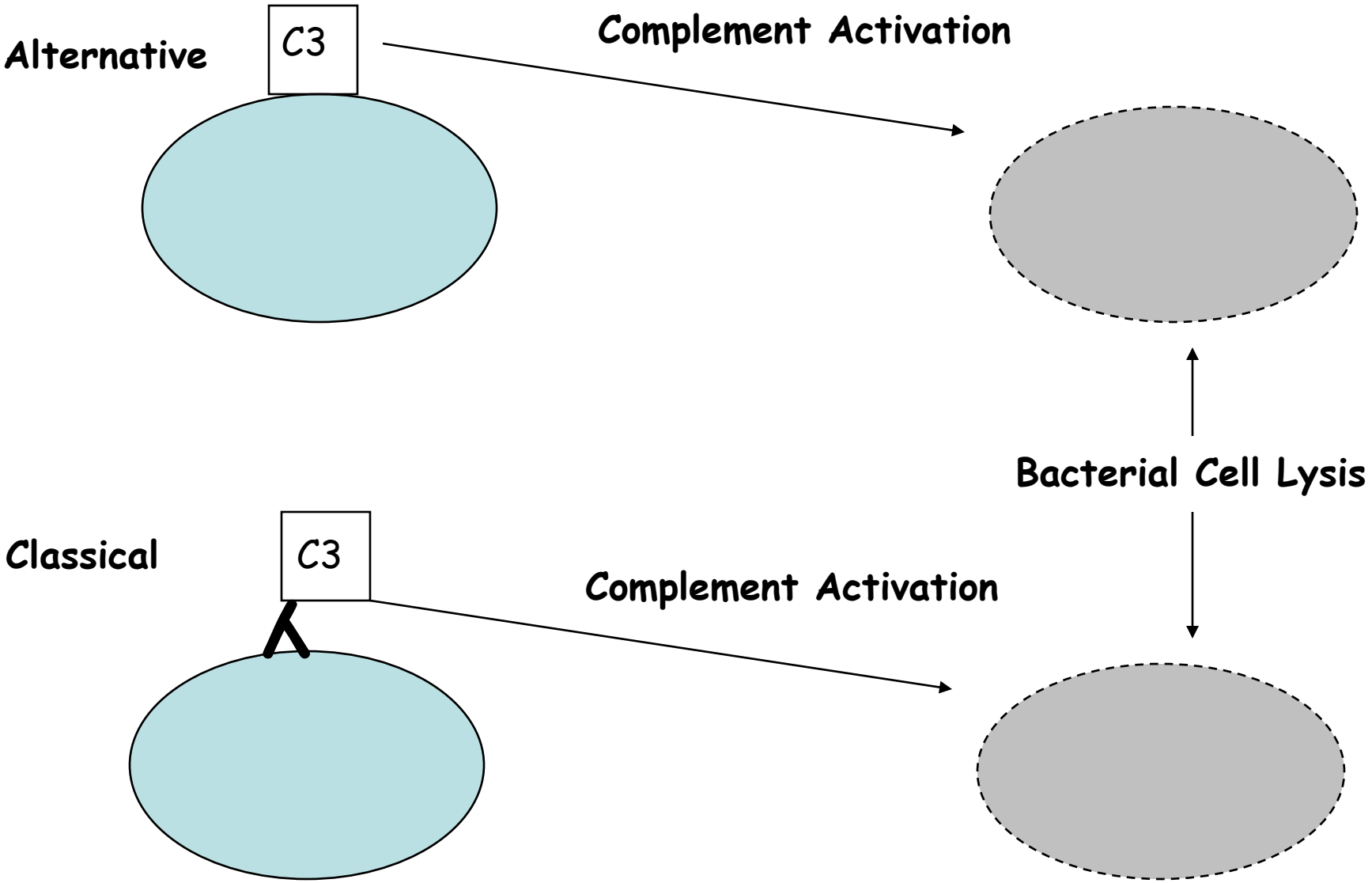
- The complement system comprises more than 30 plasma (1/2 for regulation).
- The liver produces ~90% of the plasma complement components, however...
- Production of virtually all components has been documented in monocytes/macrophages and in astrocytes.

What is complement and why is this important?

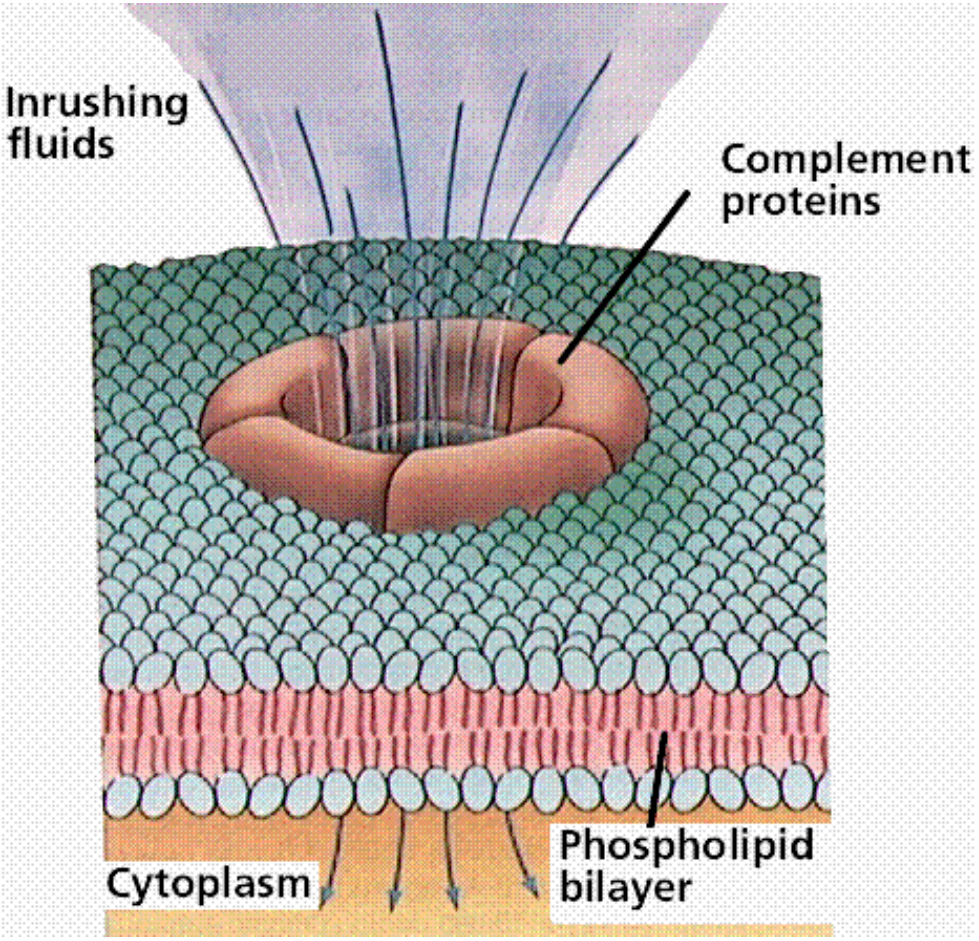
- Complement serves as a primitive surveillance system against microbes.
- Independent from antibodies or T cells.
- During evolution it became intertwined with humoral immunity and now represents a major effector system for antibodies.
- Alternative pathway is 500 million years old. Found in most vertebrates and primitive C3 analogs are present in non vertebrates.

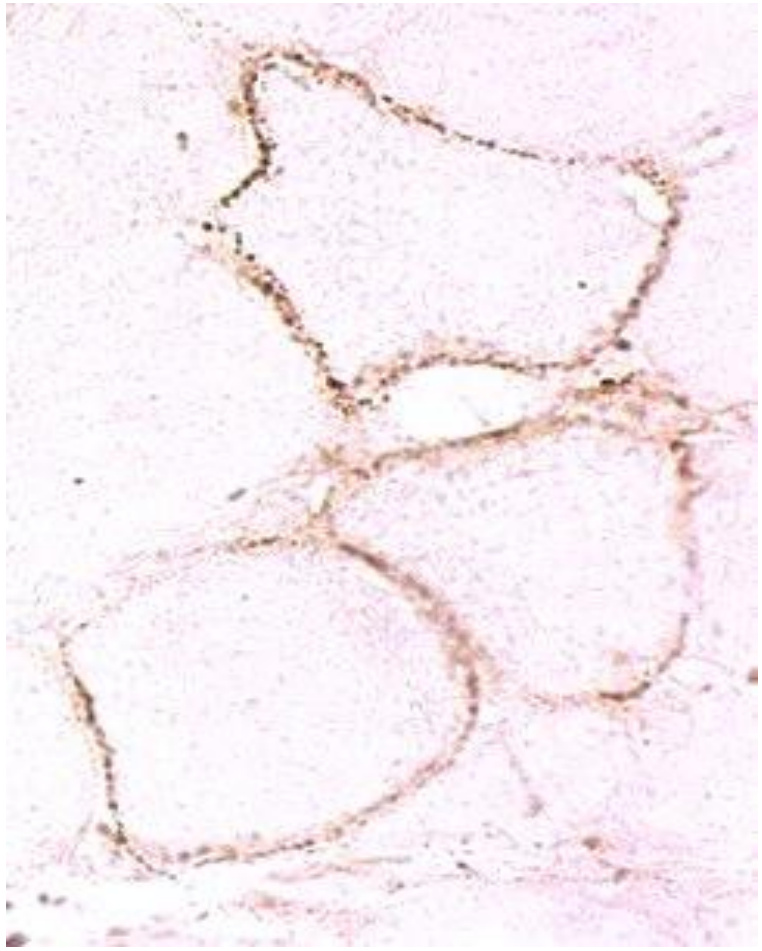
Complement Pathways




Alternative
C3
Complement Activation
Bacterial Cell Lysis
Classical
C3
Complement Activation

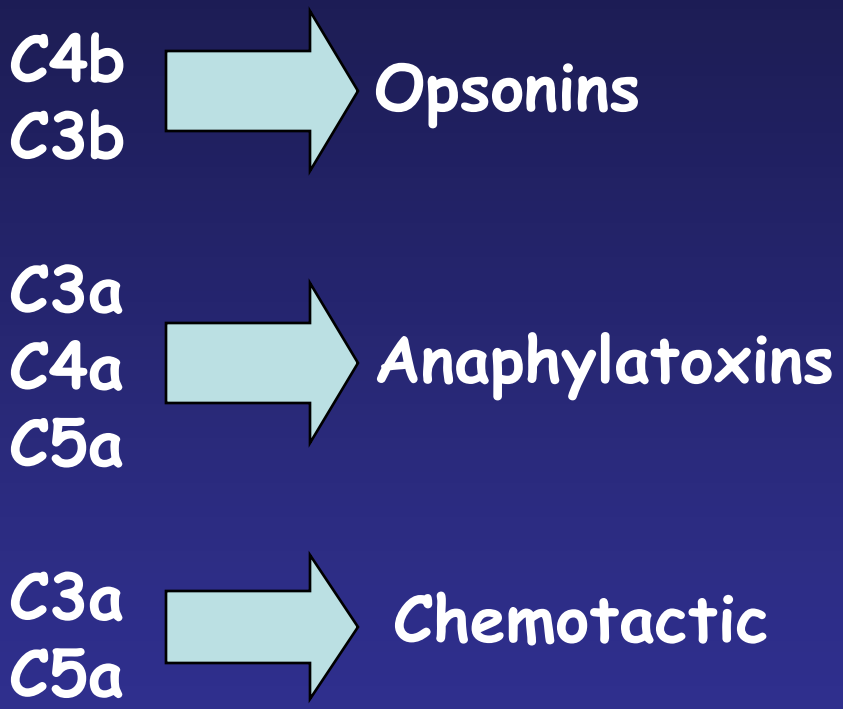
Complement-Mediated Lysis





Biological Functions

- Cytolysis
- Immune complexes
- Opsonization
- Mediate Inflammation
- Chemotaxis



Complement Deficiency States

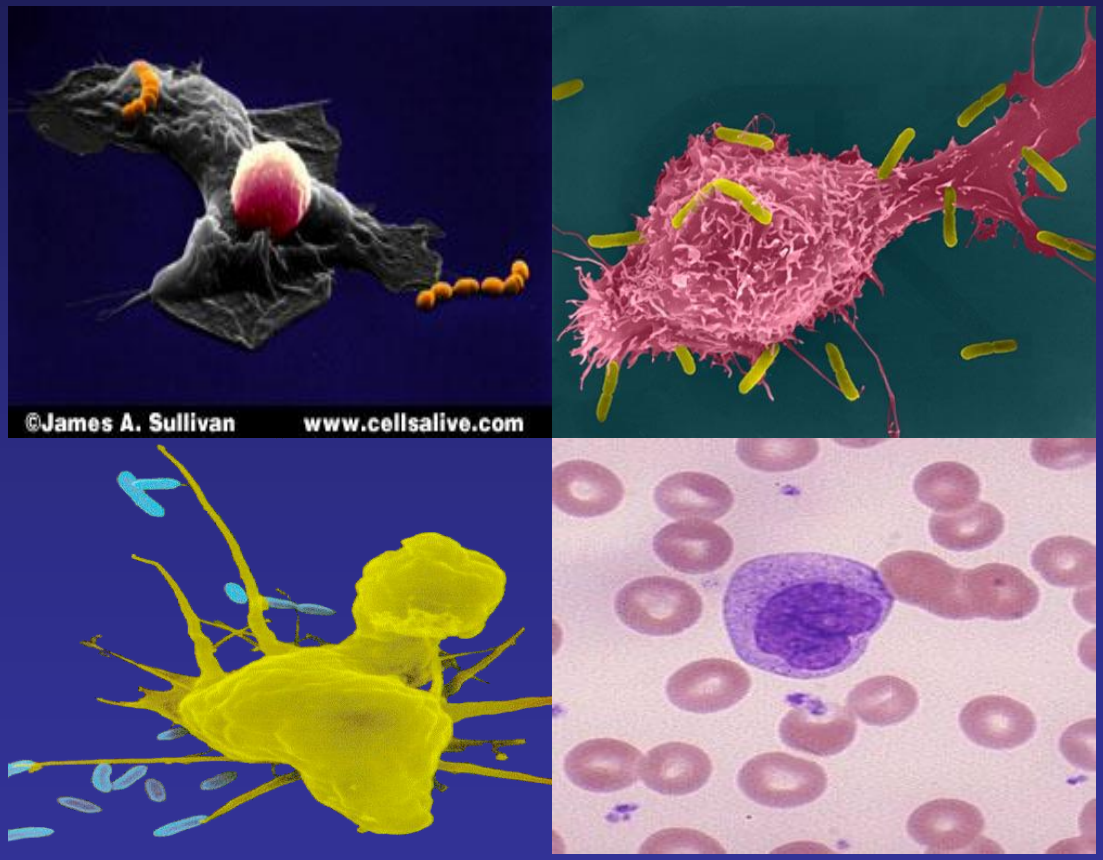
- **Component (Cases)**
  - C1 (50-100)
  - C4 (20-50)
  - C2 (>100)
  - C3 (20-50)
  - B (None)
  - D (3)
  - P (50-100)
  - H (20-50)
  - C5 (20-50)
  - C6 (>100)
  - C7 (>100)
  - C8 (>100)
  - C9 (>100)

- **Disease associations**
  - SLE, bacterial infections
  - SLE, bacterial infections
  - SLE, bacterial infections
  - Bacterial infections
  - Incompatible with life?
  - Bacterial infections?
  - Meningococcal infections
  - “ ” /glomerulonephritis
  - Bacterial infections
  - Meningococcal infections
  - Meningococcal infections
  - Meningococcal infections
  - Meningococcal infections

Because complement is a critical defense against most infectious agents, it is not surprising many pathogens have developed strategies to circumvent the complement cascade.

Cells of the Innate Immune System

Macrophages Doing Their Thing

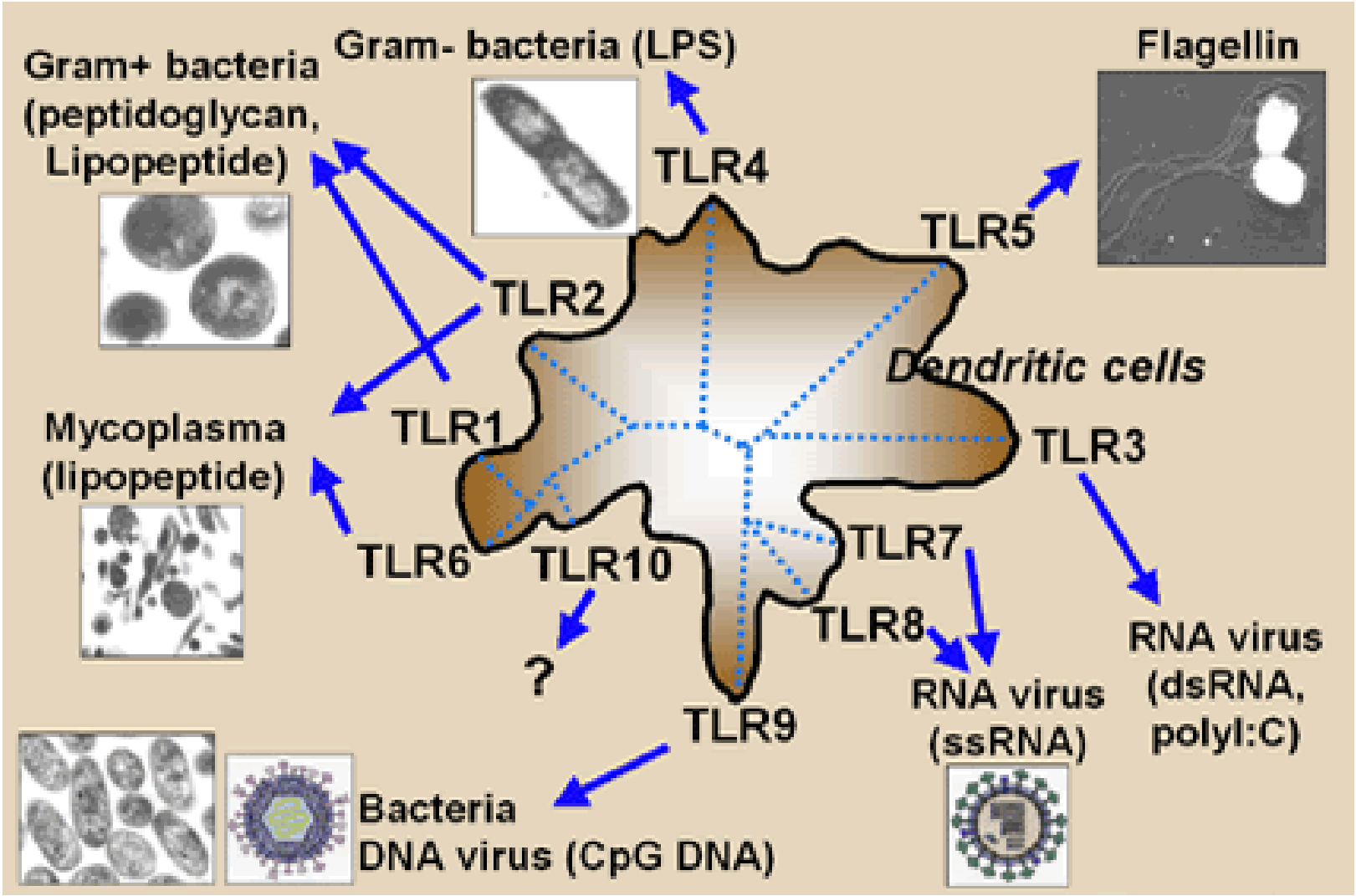


But what makes them 'eat'?

The Activated Macrophage

- Professional APCs posses a myriad of receptors recognizing molecular structures on microbial pathogens.
- Bacterial attachment to macrophages via receptors can lead to survival or death.

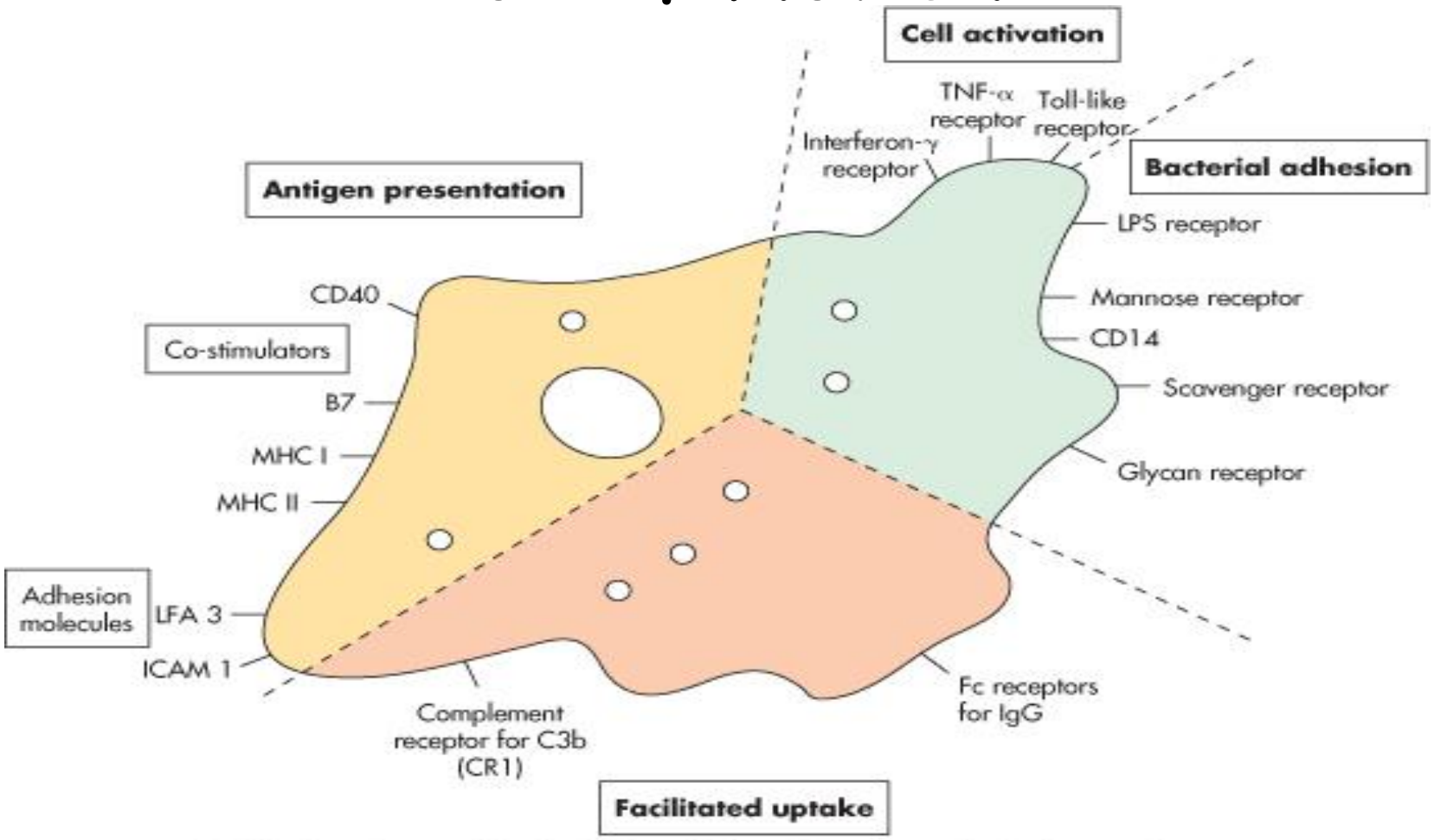
Toll Receptors

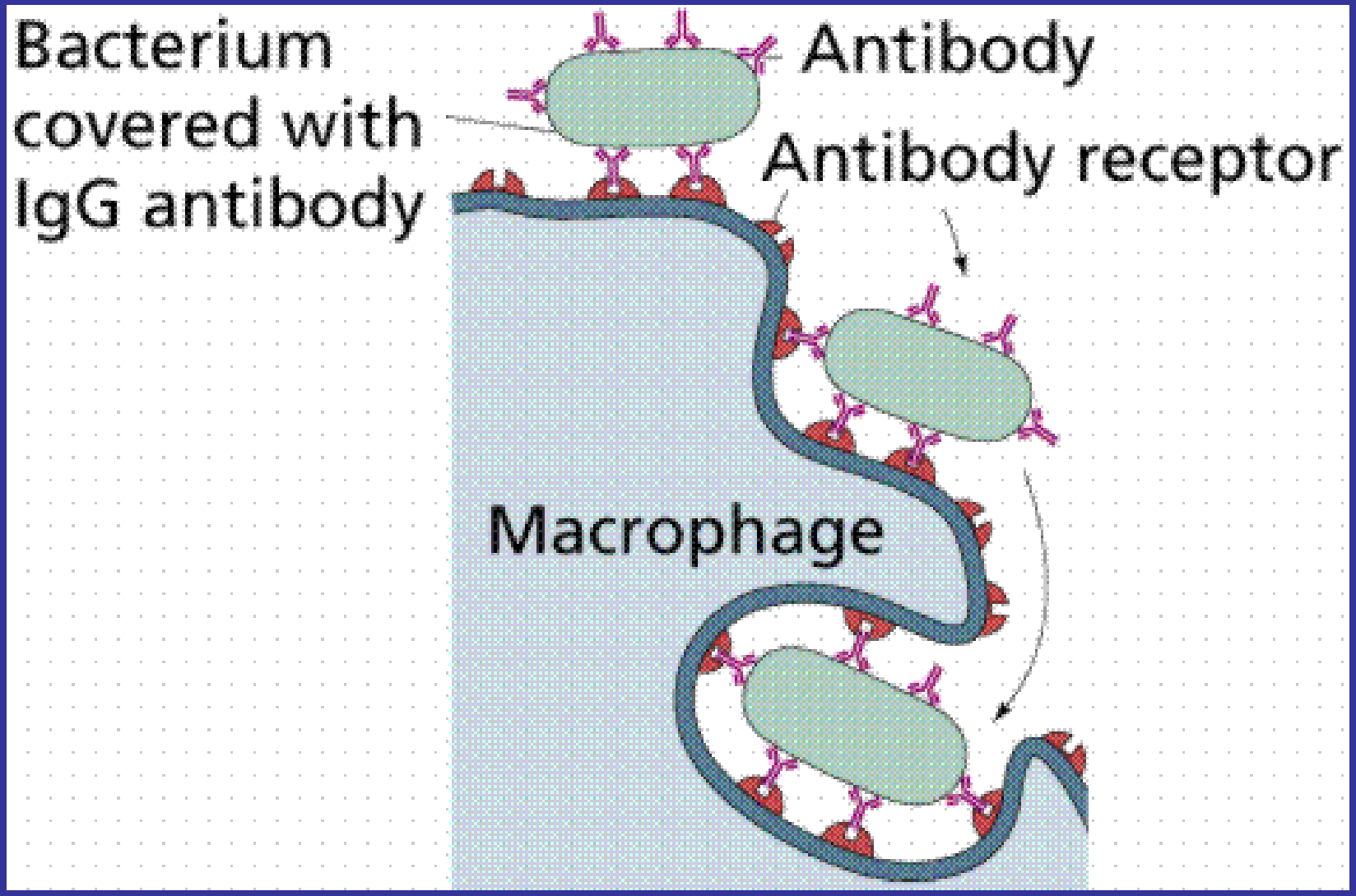


Macrophage Receptors
Pattern Recognition

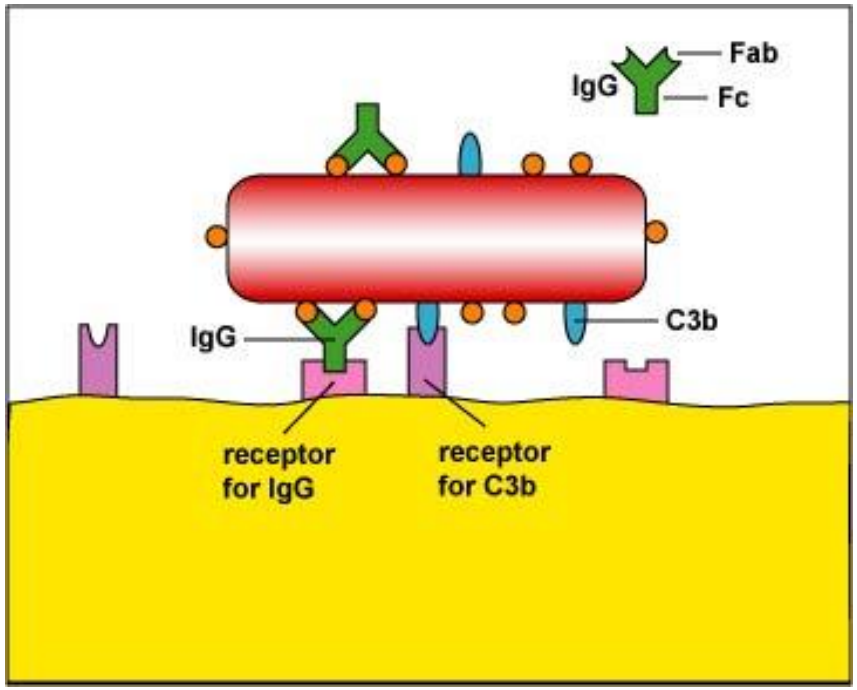
- **Fcγ receptors/ Opsonization**
- **Scavenger receptors**
- **Complement receptors**
- **Cytokine receptors**

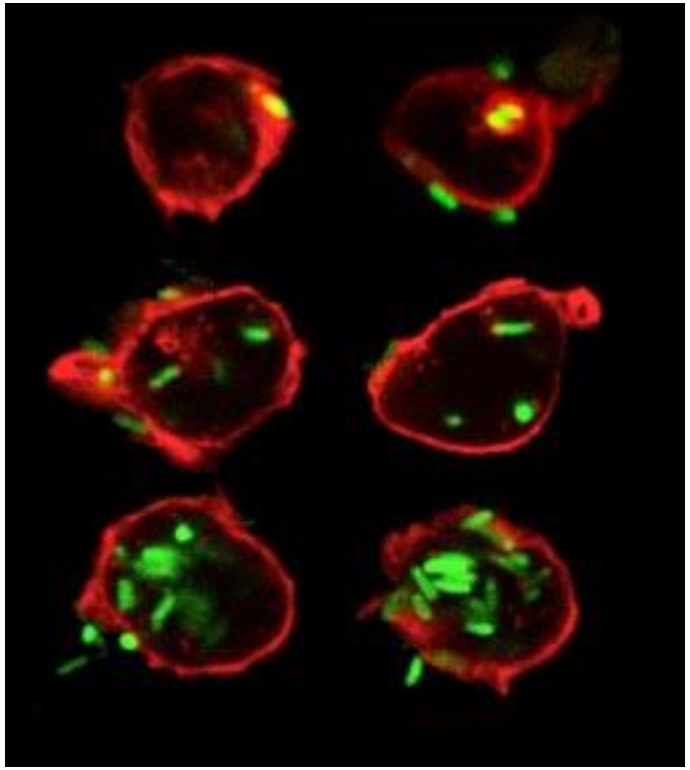
MØ surface structures mediate cell function




Bacterium
covered with
IgG antibody
Antibody
Antibody receptor
Macrophage

Opsonophagocytosis





Antibacterial Capacities of Activated Macrophages

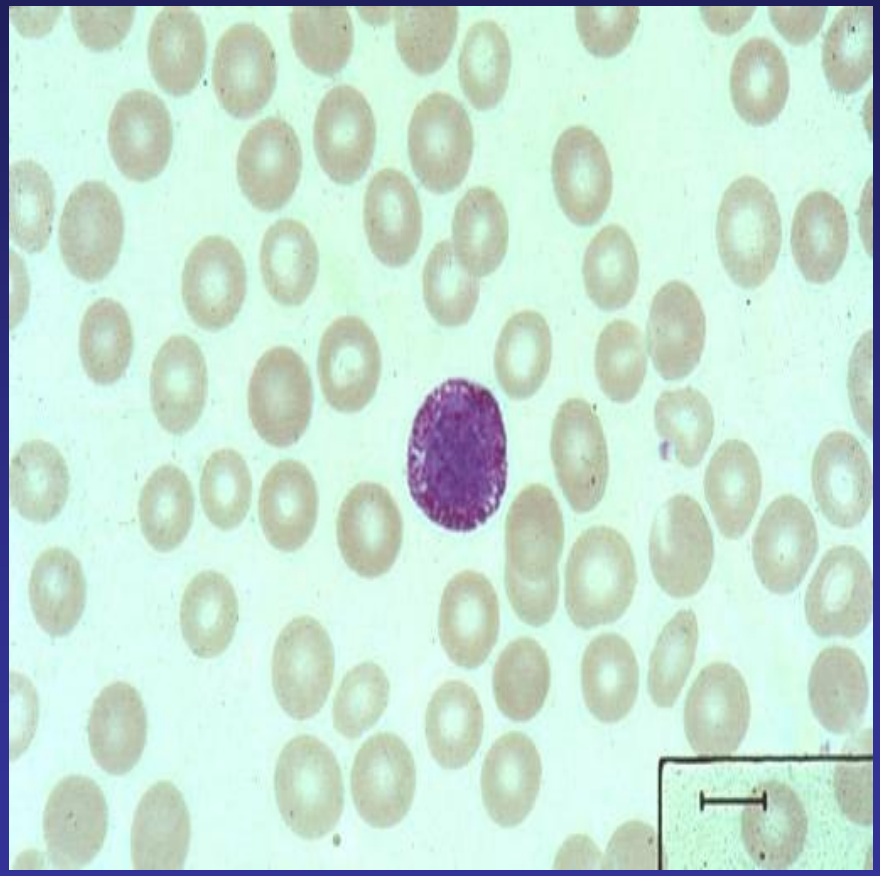
Macrophage effector capacity	Microbial evasion mechanism
Defensins	Unknown
Phagosome acidification	Phagosome neutralization
Phagosome–lysosome fusion	Inhibition of phagosome–lysosome fusion
Lysosomal enzymes	Resistance against enzymes
Intraphagolysosomal killing	Evasion into cytosol Robust cell wall
ROI	CR-mediated uptake, ROI detoxifiers, ROI scavengers
RNI	Unknown (ROI detoxifiers probably interfere with RNI)
Iron starvation	Microbial iron scavengers (e.g., siderophores)
Tryptophan starvation	Unknown

ROI, reactive oxygen intermediates; RNI, reactive nitrogen intermediates.

Other cells of the Innate Immune System (The Large Granular Lymphocytes)

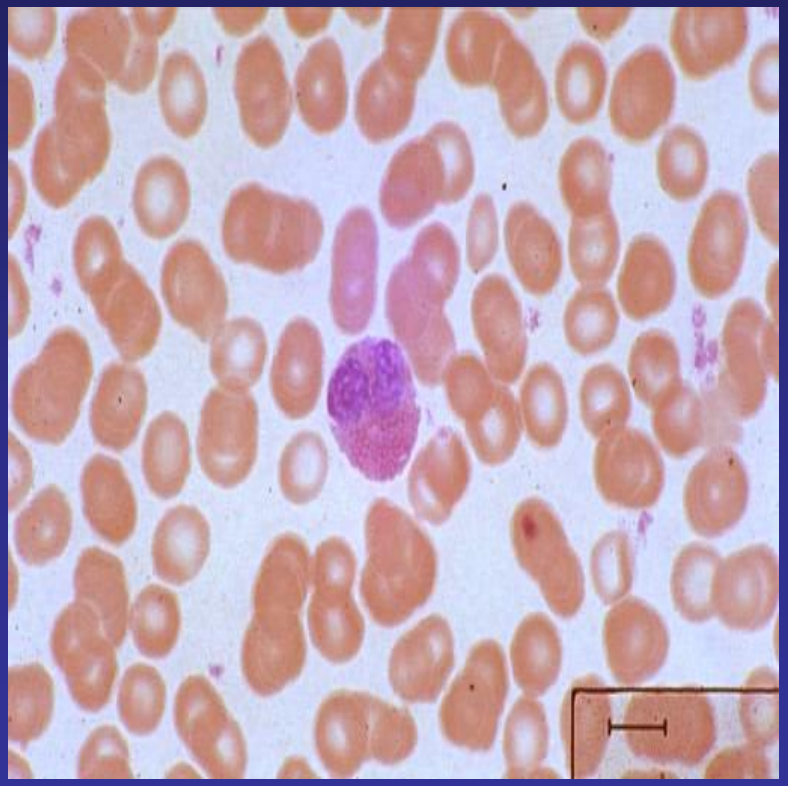
The Polymorphonuclear Monocytes…

- Basophiles
  - Bind IgE and some IgG
  - 1% of leukocyte
  - Release histamine and seratonin
  - Initiate allergy and anaphylactic-type responses



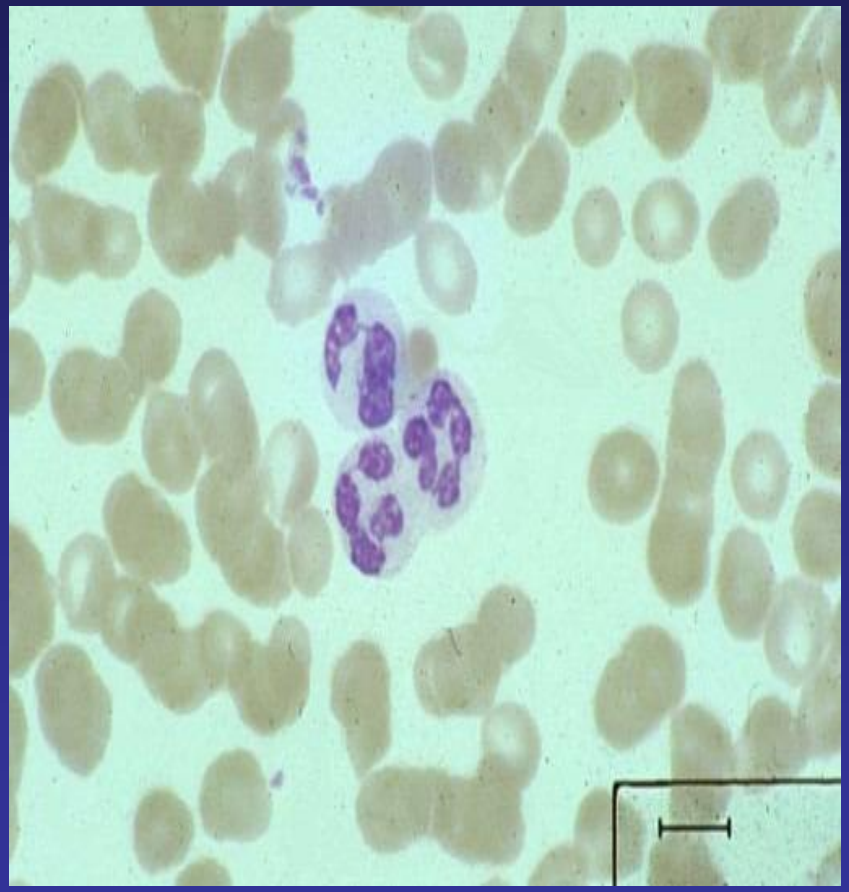
The Polymorphonuclear Monocytes…

- Eosinophils
  - 2-5% leukocytes
  - IL-5-induced
  - Helminth infections
  - Mucosal epithelia



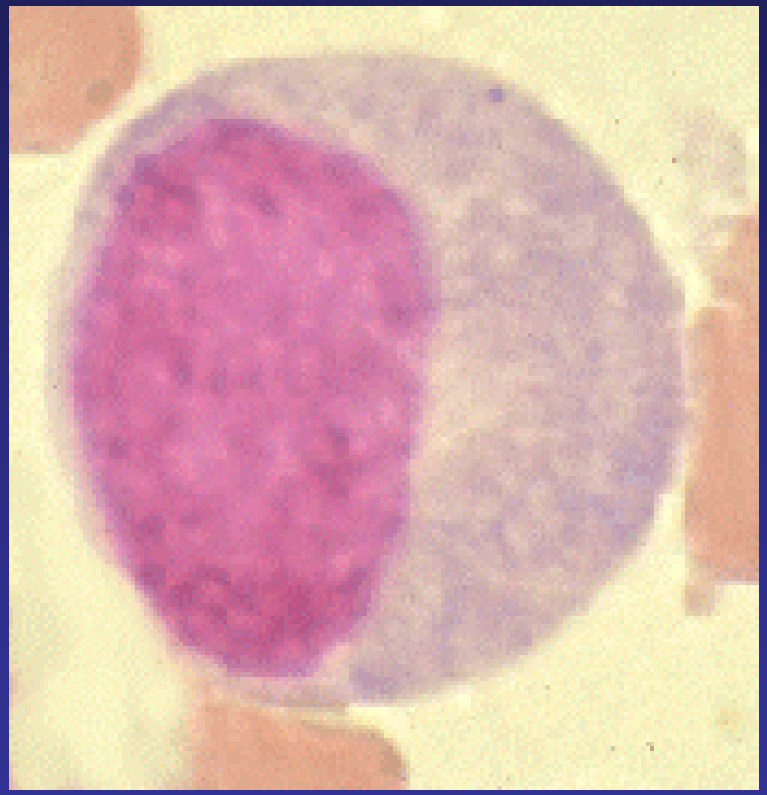
The Polymorphonuclear Monocytes…

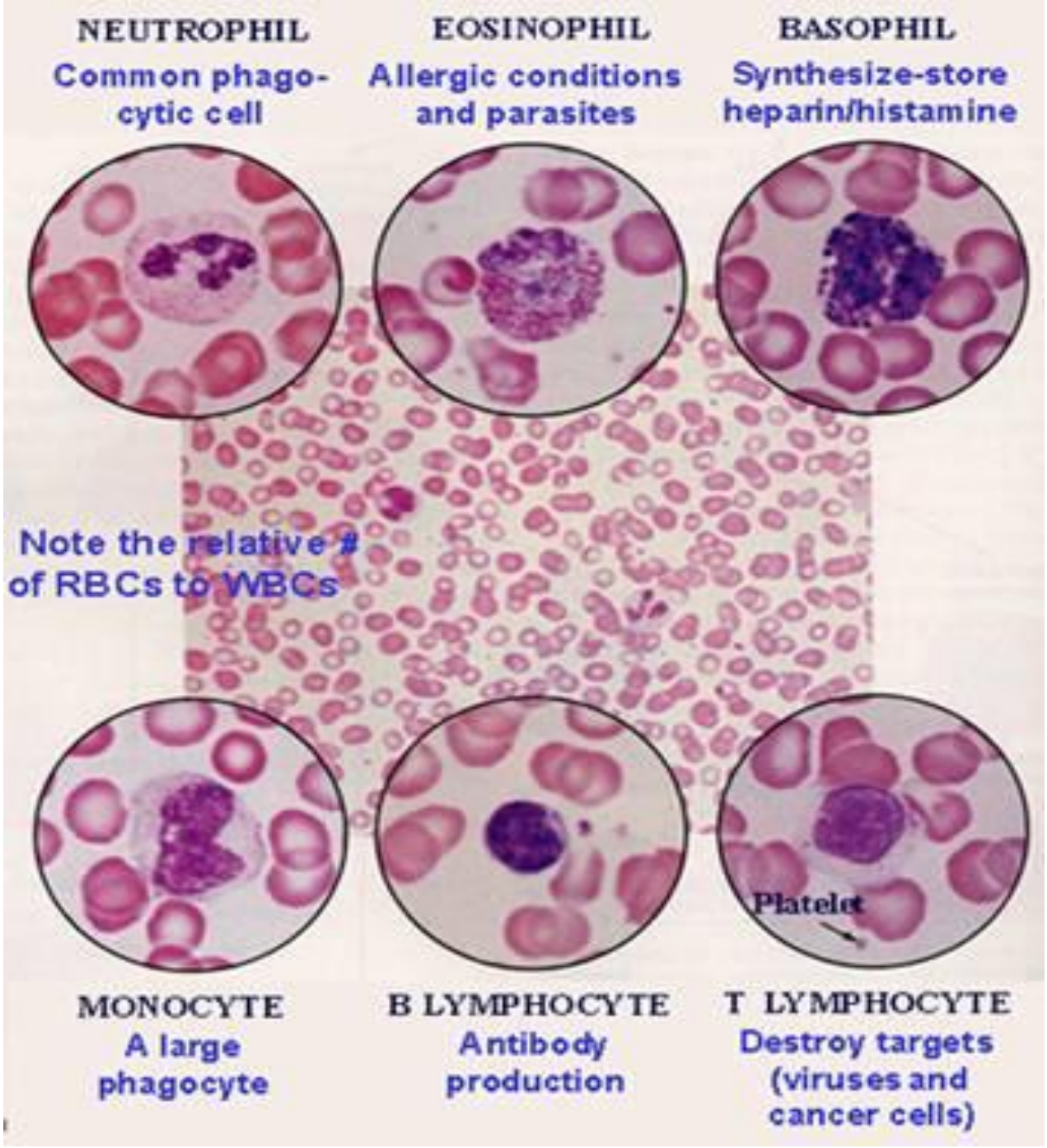
- Neutrophils
  - >40-50% leukocytes
  - $1x10^8$/day
  - Mediate wide range of inflammatory reactions
  - Primary line of defense
  - Extracellular bacteria



NK cells--Lymphocytes

- Natural Killer cells
  - Hybrid between acquired and innate.
  - Acts like CTL (cytotoxic T lymphocyte).
  - Present in unimmunized individuals (opposite of CTLs).
  - These cells 'scan' the MHC I density of other cells…why?

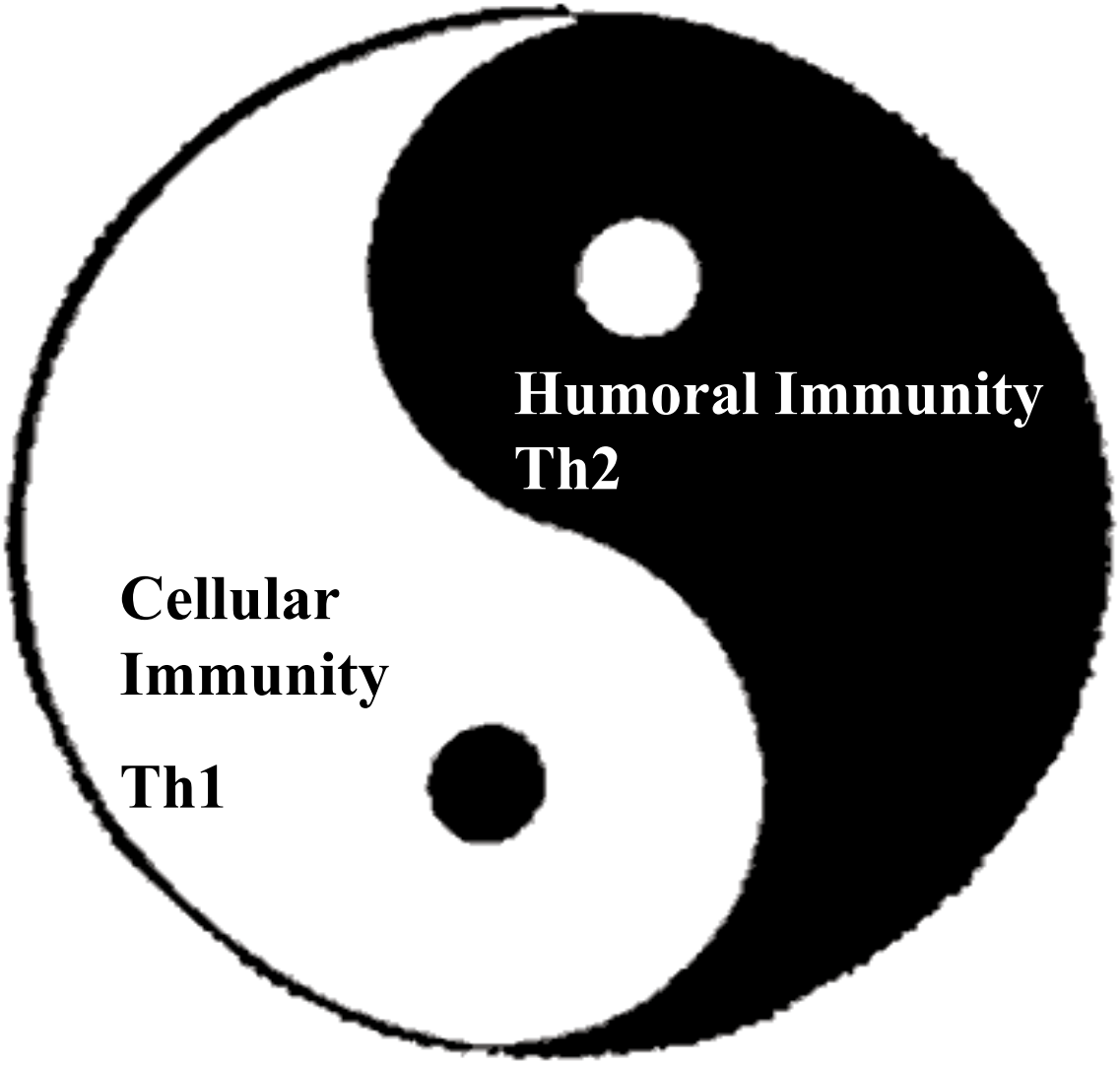



NEUTROPHIL
Common phago-cytic cell
EOSINOPHIL
Allergic conditions and parasites
BASOPHIL
Synthesize-store heparin/histamine
Note the relative # of RBCs to WBCs
Platelet
MONOCYTE
A large phagocyte
B LYMPHOCYTE
Antibody production
T LYMPHOCYTE
Destroy targets (viruses and cancer cells)

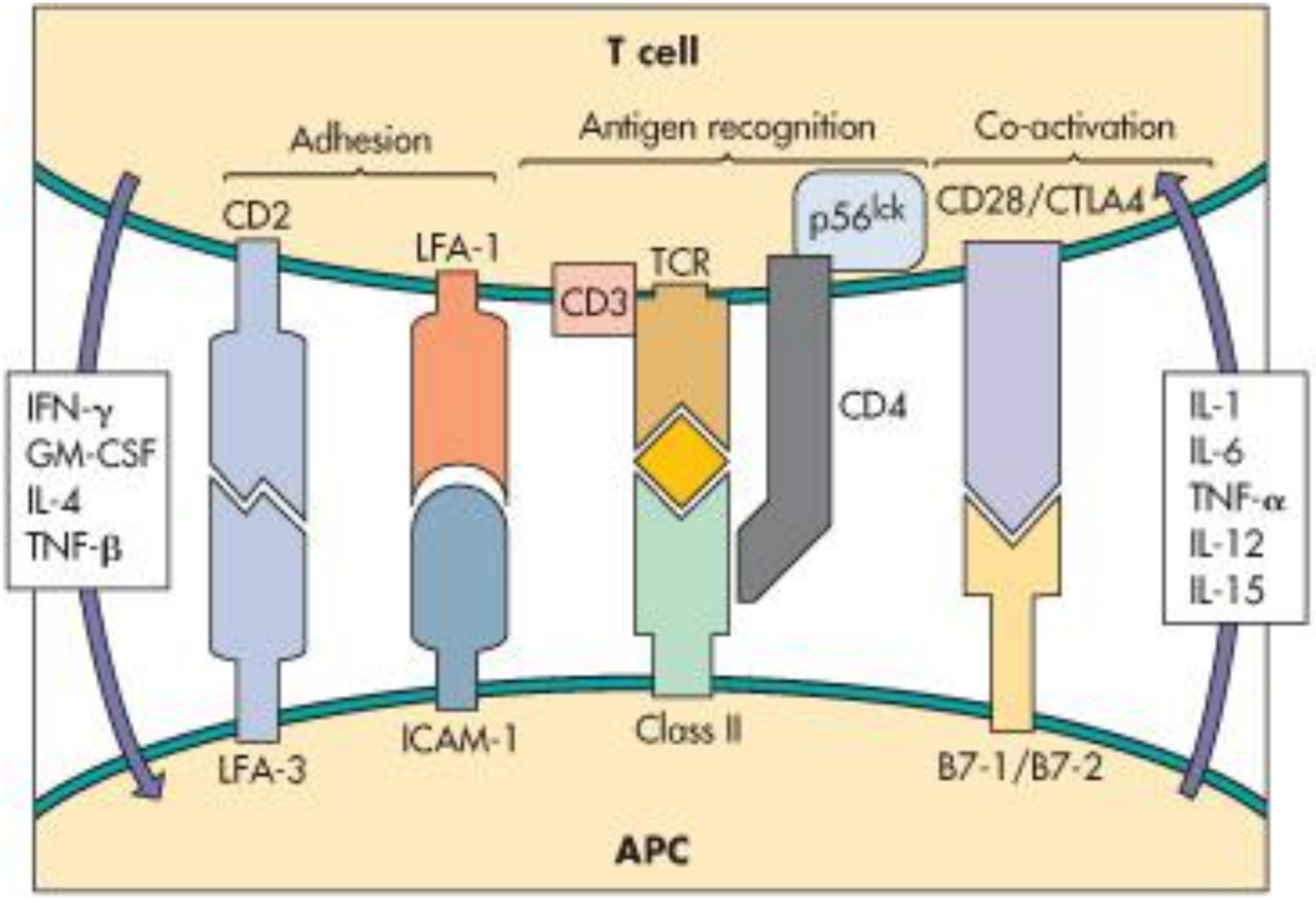
Acquired Immunity

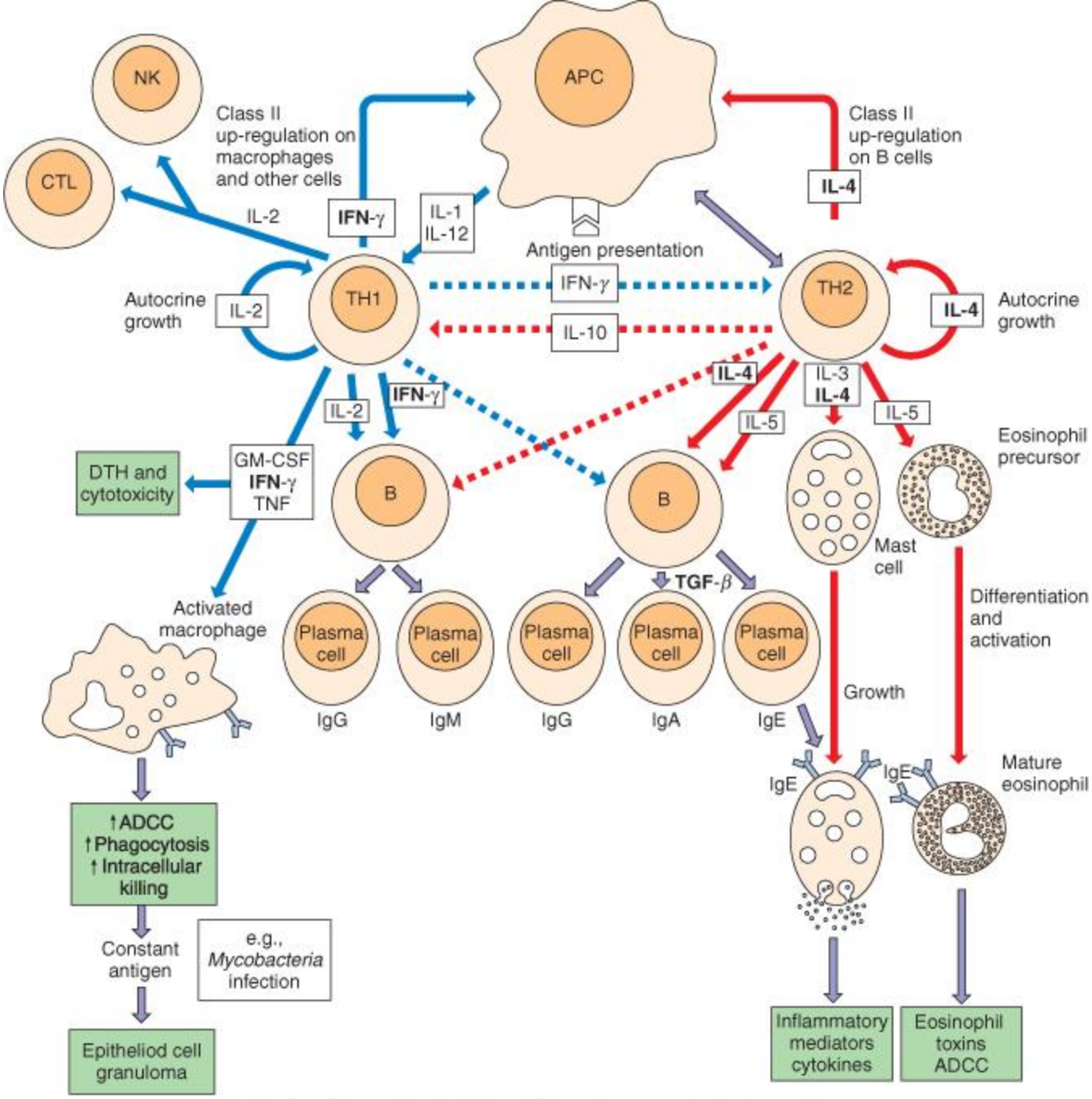
- Learned.
- Responsible for immunologic memory.
- Cells of the Acquired Immune System:
  - T-cells
  - B-cells
  - NK cells

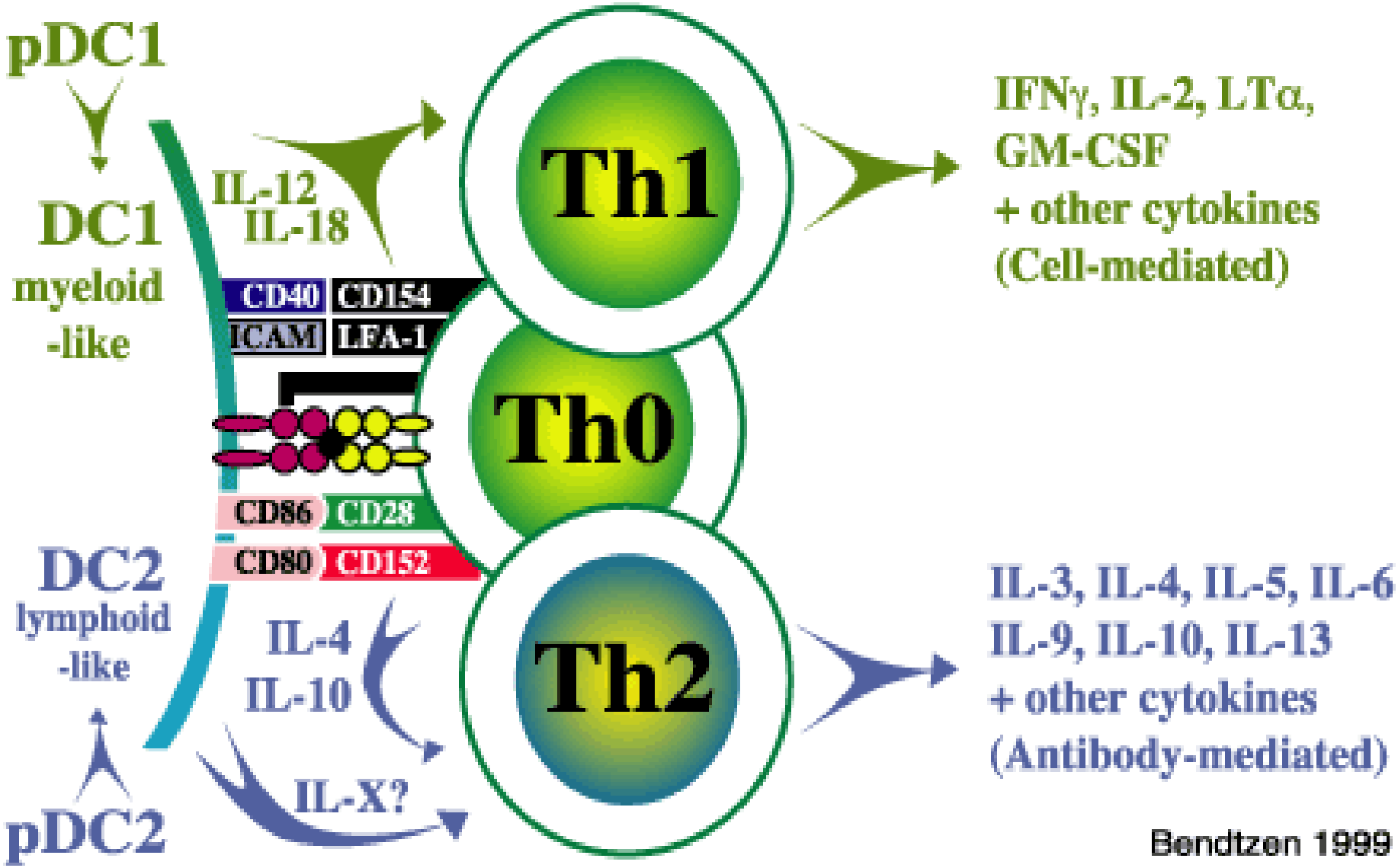
Immune System Dynamics



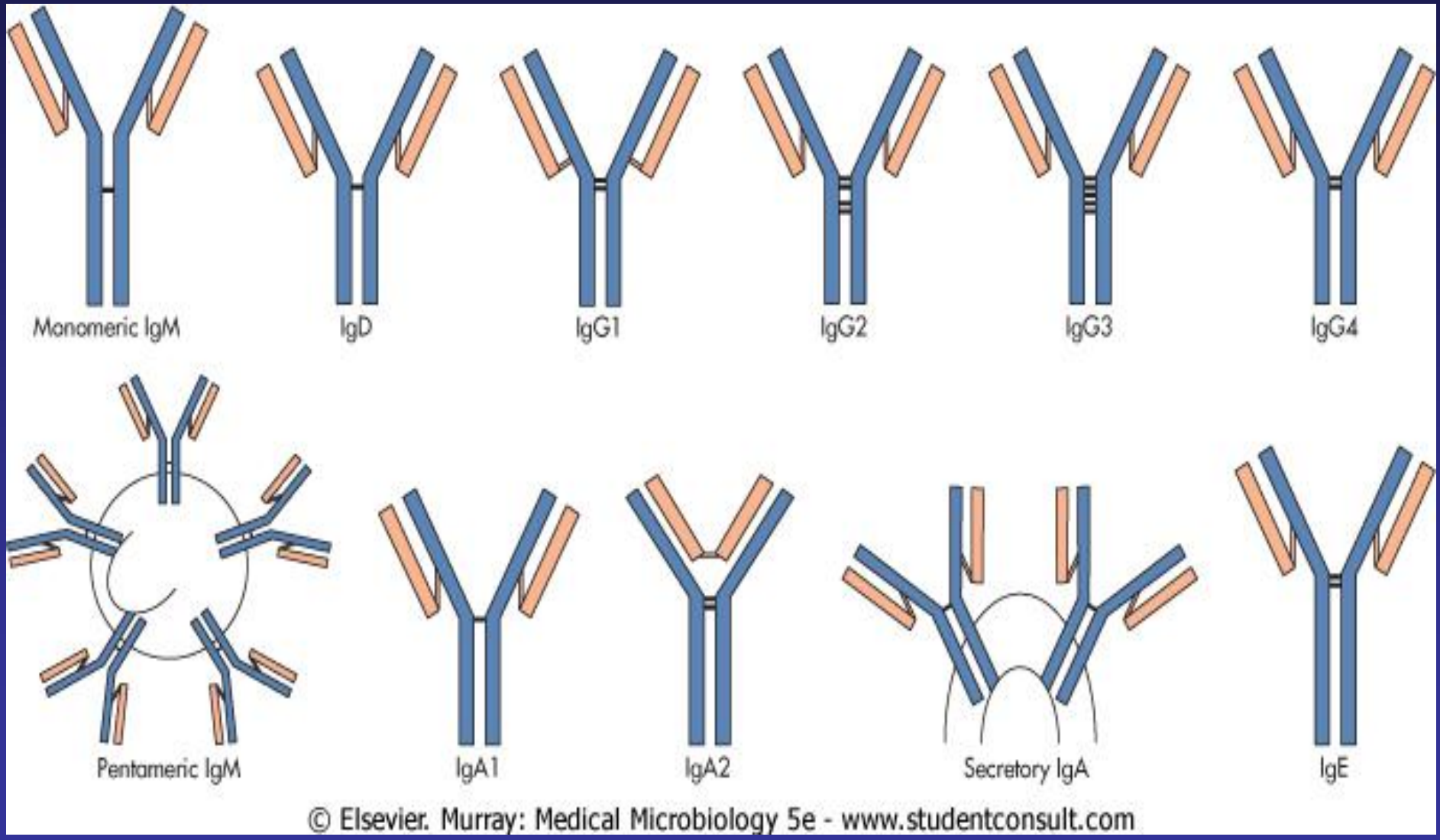
Response Initiation






pDC1
DC1
myeloid
-like
IL-12
IL-18
CD40
CD154
ICAM
LFA-1
CD86
CD28
CD80
CD152
DC2
lymphoid
-like
IL-4
IL-10
IL-X?
pDC2
Th1
Th0
Th2
IFNγ, IL-2, LTα,
GM-CSF
+ other cytokines
(Cell-mediated)
IL-3, IL-4, IL-5, IL-6
IL-9, IL-10, IL-13
+ other cytokines
(Antibody-mediated)
Bendtzen 1999

Antibody Classes



OBJECTIVES

- 1. The general nature of immune responsiveness.
  - Memory
  - Specificity
    - Innate immunity
    - Acquired Immunity
- 2 Infection and Immunity
- 3. The anatomic basis of immune responsiveness.

Nature of Infection

- Plays a critical role in the interactions between Acquired and Adaptive immunity
  - Intracellular pathogens
  - Extracellular pathogens
  - Dose
  - Route

Infection-Immunity-Pathogenicity

- Only rarely is the infectious disease the direct and invariable consequence of an encounter between host and pathogen.

- Rather, it is the eventual outcome of complex interactions between them

Intracellular Bacteria

- Routs of Infection
  - Directly into the blood *e.g., Rickettsia* sp.
  - Mucosa *e.g., M. tuberculosis* and *L. pneumophilia*
  - Intestine *e.g., S. enterica* and *L. monocytogenes*

Fate of Bacteria

- Removed nonspecifically by mucociliary movements and gut peristalsis
- Destroyed by professional phagocytes without SPECIFIC attention of the immune system
- Cells surviving these nonspecific mechanisms colonize deeper and stably infect a suitable niche.

Hallmark 1

Intracellular lifestyle represents the distinguishing feature of intracellular bacteria.

Invasion of host cells is not restricted to these pathogens.

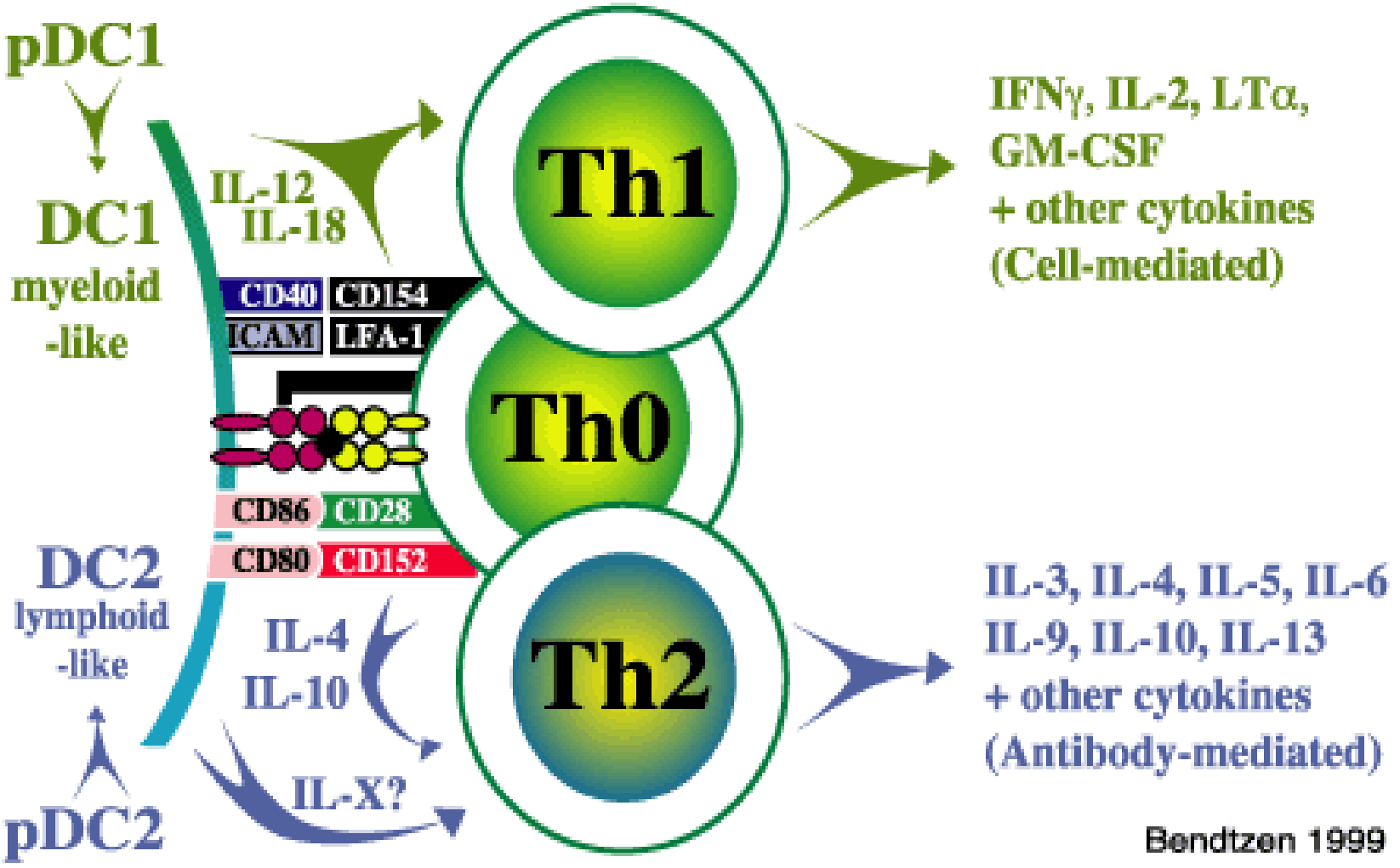
Transient trespassing through epithelial cells is a common invasion mechanism of BOTH intracellular and extracellular pathogens.

Hallmark 2

- T cells are the central mediators of protection
- These T cells do not interact with microbes directly
- Interact with the infected host cell.
- In contrast, antibodies that recognize microbial antigens directly are of exquisite importance for defense against extracellular bacteria.

Hallmark 3

- Infections are accompanied by delayed-type hypersensitivity (DTH).
- DTH expresses itself after local administration of soluble antigens as a delayed tissue reaction
- DTH is mediated by T cells and effected by macrophages.


pDC1
DC1
myeloid
-like
IL-12
IL-18
CD40
CD154
ICAM
LFA-1
CD86
CD28
CD80
CD152
DC2
lymphoid
-like
IL-4
IL-10
IL-X?
pDC2
Th1
Th0
Th2
IFNγ, IL-2, LTα,
GM-CSF
+ other cytokines
(Cell-mediated)
IL-3, IL-4, IL-5, IL-6
IL-9, IL-10, IL-13
+ other cytokines
(Antibody-mediated)
Bendtzen 1999

Tuberculin Test

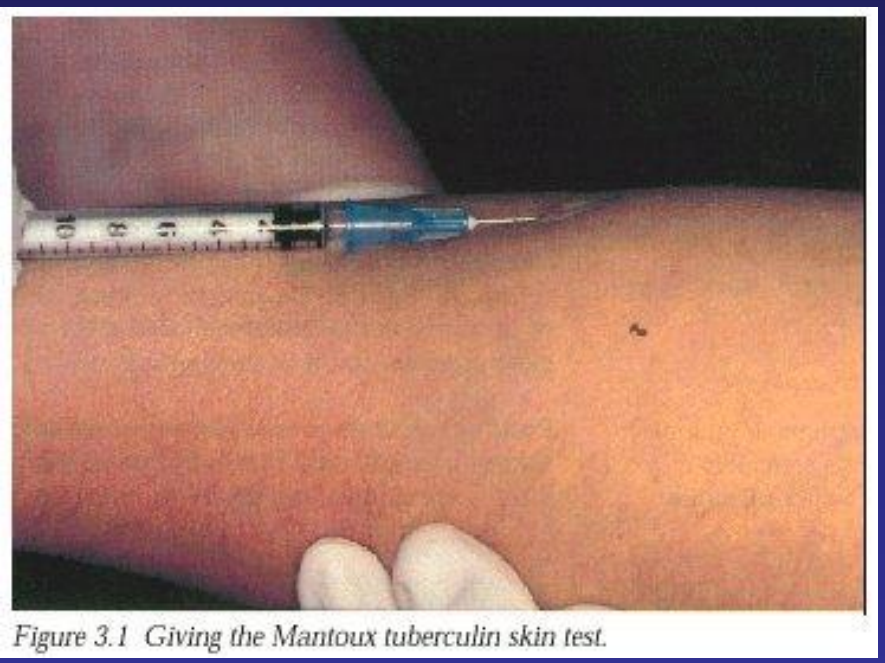
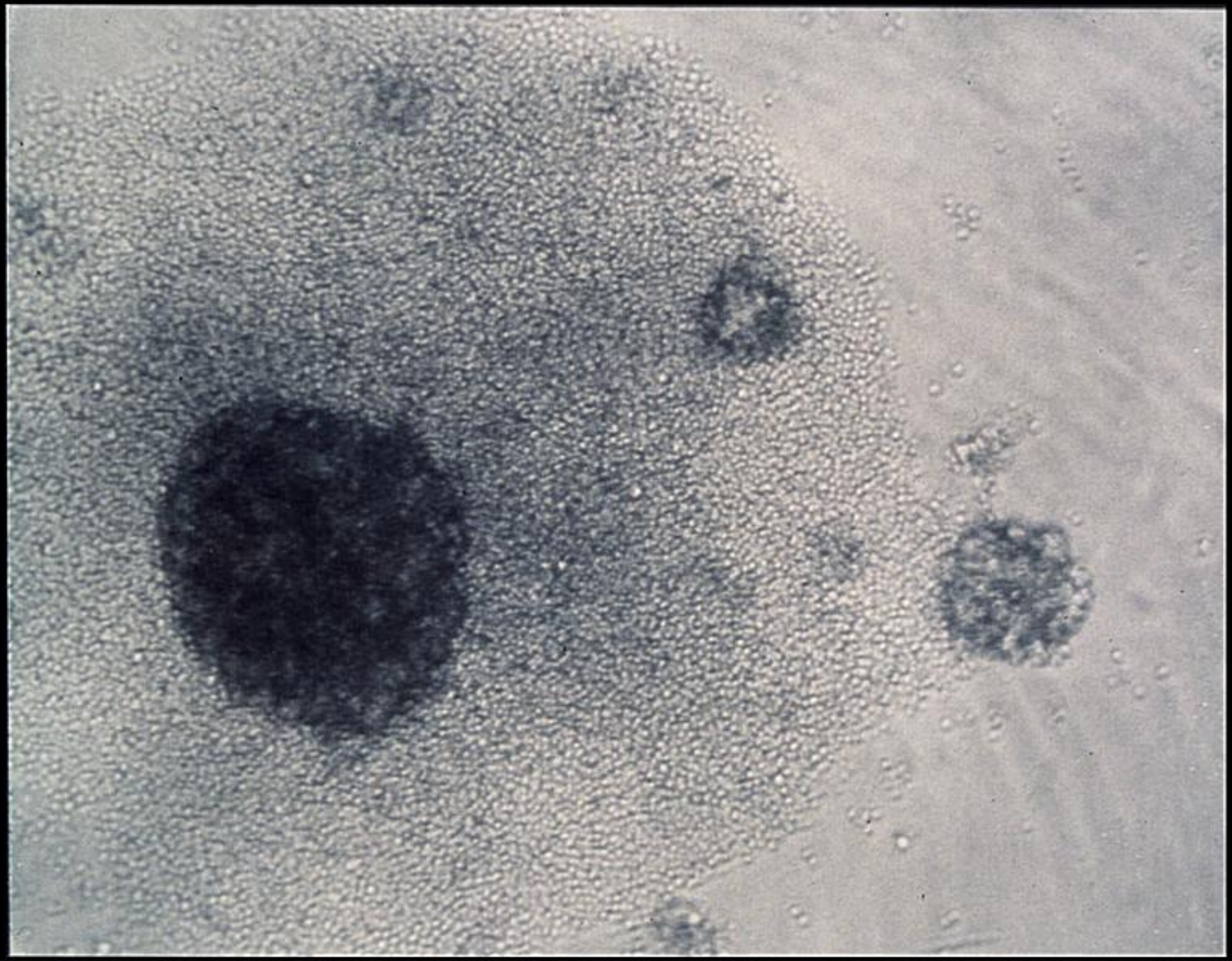


Figure 3.1 Giving the Mantoux tuberculin skin test.



Hallmark 4

- Tissue reactions against intracellular bacteria are granulomatous.

- Rupture of a granuloma promotes bacterial dissemination and formation of additional lesions.

- In contrast, tissue reactions against extracellular bacteria are purulent and lead to abscess formation or systemic reactions.

Hallmark 5

- Intracellular bacteria express little or no toxicity for host cells by themselves
- Pathology is primarily a result of immune reactions, particularly those mediated by T-lymphocytes.
- In contrast, extracellular bacteria produce various toxins, which are directly responsible for tissue damage.

Hallmark 6

- Intracellular bacteria coexist with their cellular habitat for long periods.
- A balance develops between persistent infection and protective immunity, resulting in long incubation time and in chronic disease.
- Infection can be dissociated from disease.
- In contrast, extracellular bacteria typically cause acute diseases, which develop soon after their entry into the host and are terminated once the immune response has developed.

Two Types of Intracellular Bacteria

- Facultative
- Obligate

Major infections of humans caused by facultative intracellular bacteria

Pathogen	Disease	Preferred target cell
• *Mycobacterium tuberculosis/M. bovis*	Tuberculosis	Macrophages
• *Myocabacterium leprae*	Leprosy	Macrophages
• *Salmonella enterica*	Typhoid fever	Macrophages
• *Brucella sp.*	Brucellosis	Macrophages
• *Legionella pneumophila*	Legionnaire's disease	Macrophages
• *Listeria monocytogene*	Listeriosis	Macrophages
• *Francisella tularensis*	Tularaemia	Macrophages

Major infections of humans caused by obligate intracellular bacteria

Pathogen	Disease	Preferred target cell
Rickettsia rickettsii	Rocky Mountain spotted fever	Endothelial cells, smooth muscle cell
Rickettsia prowazekii	Endemic typhus	Endothelial cells
Rickettsia typhi	Typhus	Endothelial cells
Rickettsia tsutsugamushi	Scrub typhus	Endothelial cells
Coxiella burnetii	Q-fever	Macrophages, lung parenchyma cells
Chlamydia trachomatis	Urogenital infection, conjunctivitis, trachoma, lymphogranuloma venerum	Epithelial cells
Chlamydia psittaci	Psittacosis	Macrophages, lung parenchyma cell
Chlamydia pneumoniae	Pneumonia, coronaryheart disease (?)	Lung parenchyma cells

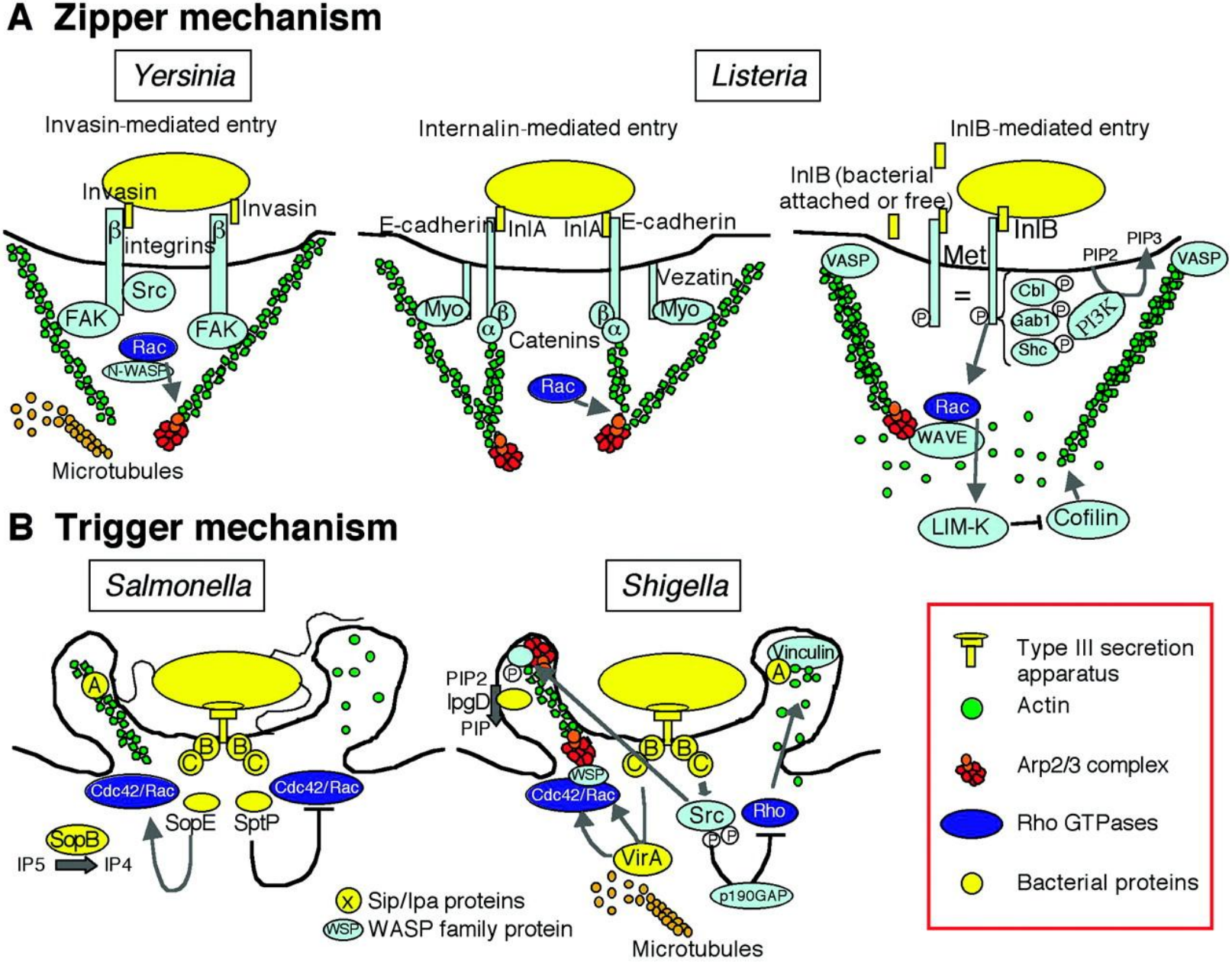
Mechanisms of Immune Evasion

- Easy way—avoid the immune system entirely…how?
- MIMs (Microbial Immunomodulatory Molecules)


He taught him the secret to Karate lies in the mind and heart. Not in the hands.
The Karate Kid
COLUMBIA PICTURES Presents
A JERRY WEINTRAUB Production of A JOHN G. AVILDSEN Film
"THE KARATE KID"
Starring RALPH MACCHIO · NORIYUKI "PAT" MORITA · ELISABETH SHUE
Music by BILL CONTI
Music Supervised by BROOKS ARTHUR
Executive Producer R.J. LOUIS
Written by ROBERT MARK KAMEN
Produced by JERRY WEINTRAUB
Directed by JOHN G. AVILDSEN

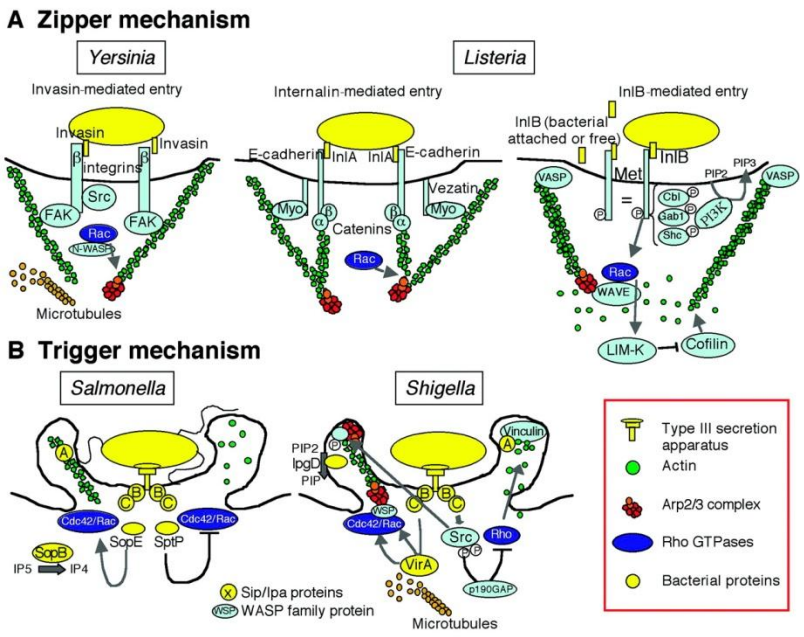
Bacterial Invasion

- Invasive bacteria actively induce their own uptake by phagocytosis in normally nonphagocytic cells.
  - Establish a protective niche.
  - Avoid immunity.
  - Multiply.
  - Active process.
    - Opposite to phagocytosis by phagocytes which is active.


A Zipper mechanism
Yersinia
Invasin-mediated entry
Invasin
Invasin
β integrins β
Src
FAK
FAK
Rac
N-WASP
Microtubules
Listeria
Internalin-mediated entry
E-cadherin
InlA
InlA
E-cadherin
Vezatin
Myo
Myo
β α
β α
Catenins
Rac
InlB-mediated entry
InlB (bacterial attached or free)
InlB
Met
PIP2
PIP3
VASP
VASP
Cbl
Gab1
Shc
PI3K
P
Rac
WAVE
LIM-K
Cofilin
B Trigger mechanism
Salmonella
A
B B
C C
Cdc42/Rac
Cdc42/Rac
SopE
SptP
SopB
IP5 IP4
Shigella
PIP2
IpgD
PIP
Vinculin
A
B B
C C
WSP
Cdc42/Rac
Src
Rho
VirA
p190GAP
Microtubules
X Sip/Ipa proteins
WSP WASP family protein
Type III secretion apparatus
Actin
Arp2/3 complex
Rho GTPases
Bacterial proteins

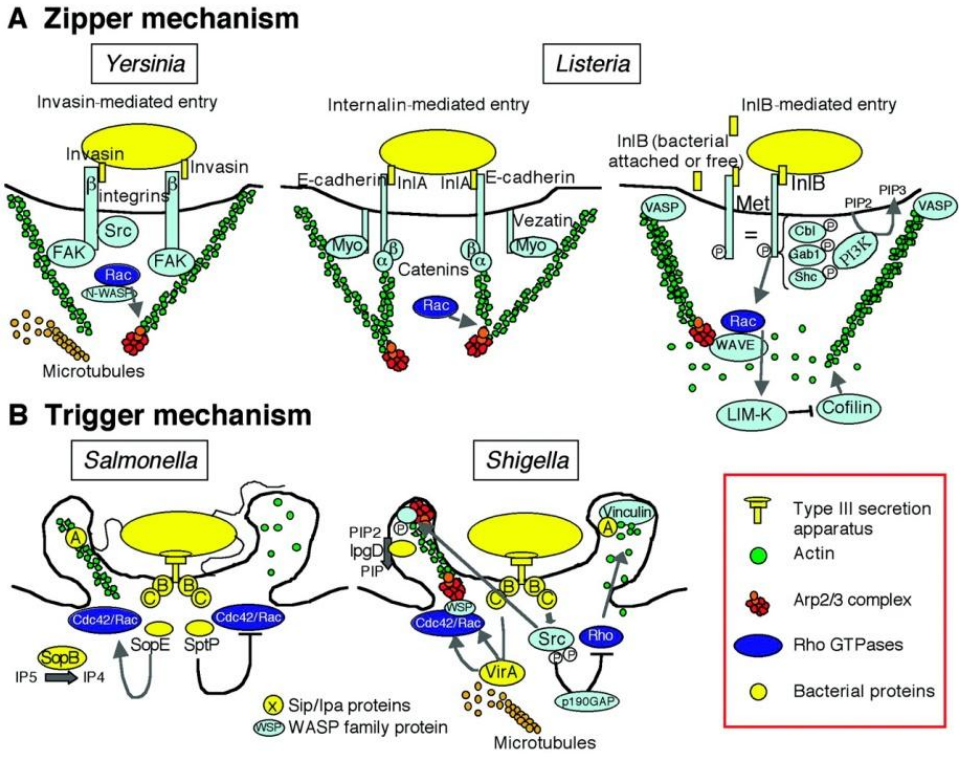
Zipper Mechanism

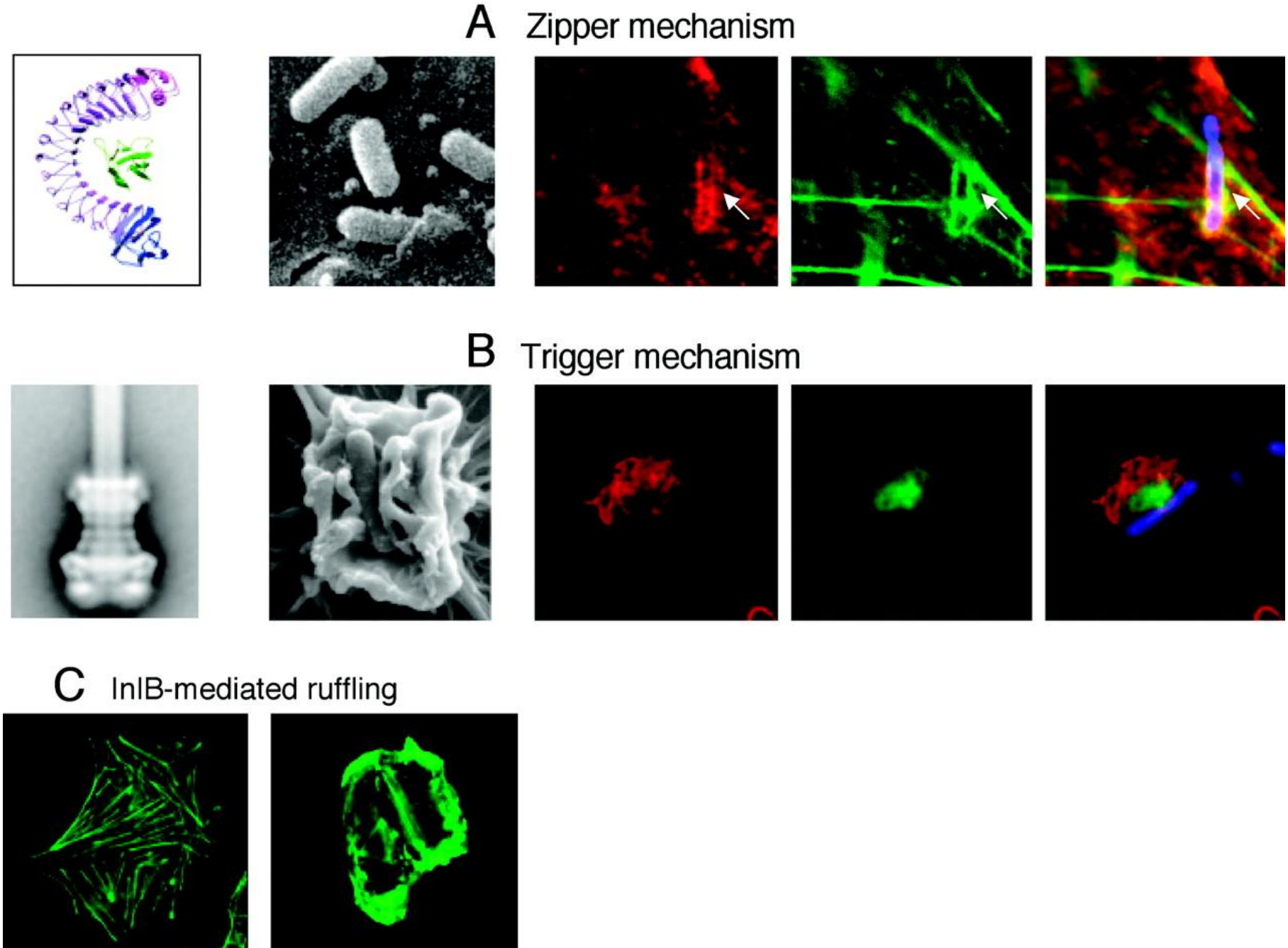
- 1-Contact and adherence
- 2-Phagocytic cup formation
- 3-Phagocytic cup closure and retraction, and actin depolymerization.



Trigger Mechanism—Requires a Type III Secretory System (TTSS)

- 1-Pre interaction stage.
  - TTSS assembled
- 2-Interaction stage.
  - Injection of material via needle.
- 3-Formation of the macropinocytic pocket.
- 4-Actin depolymerization and closing of the macropinocytic pocket.



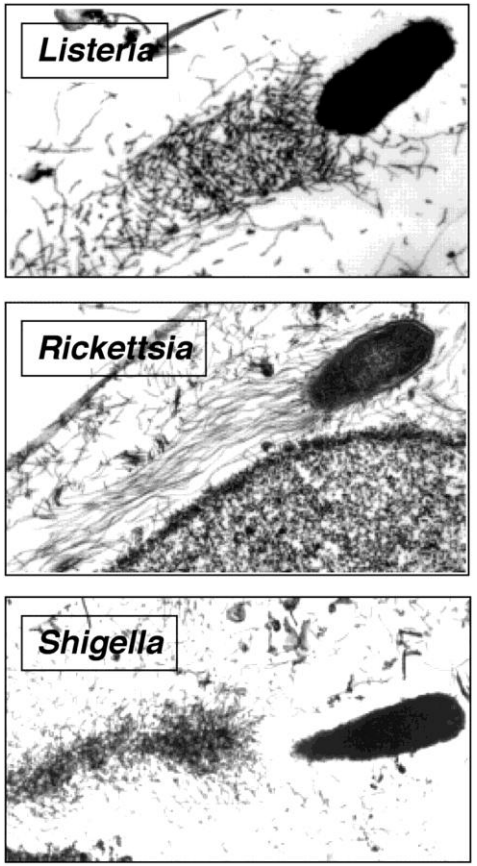

A Zipper mechanism
B Trigger mechanism
C InlB-mediated ruffling

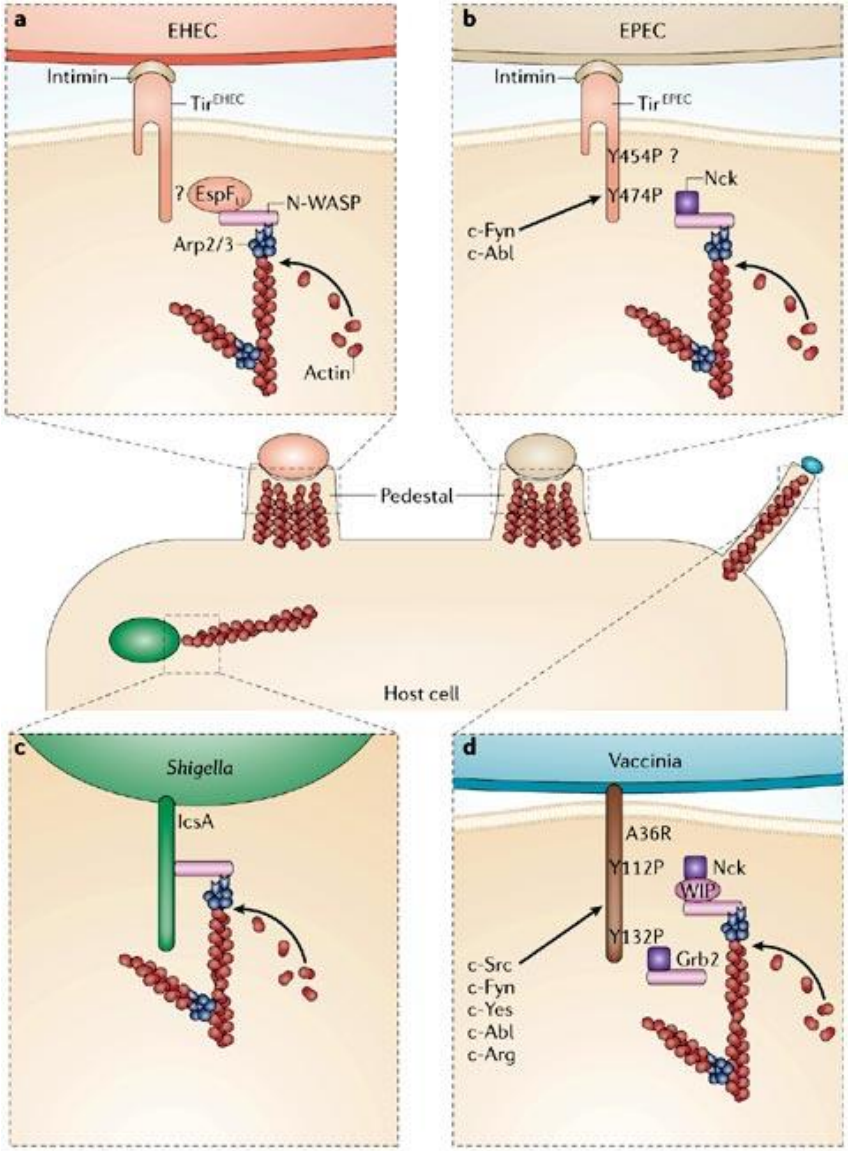
Following Internalization…

- Bacteria that replicate inside the internalization vacuole have developed an impressive array of survival strategies.
  - Adapt to and eventually resist the hostile conditions.
  - Alter the dynamics of the vacuolar compartment.
  - Combinations of the two *e.g.*, Salmonella

Following Internalization…

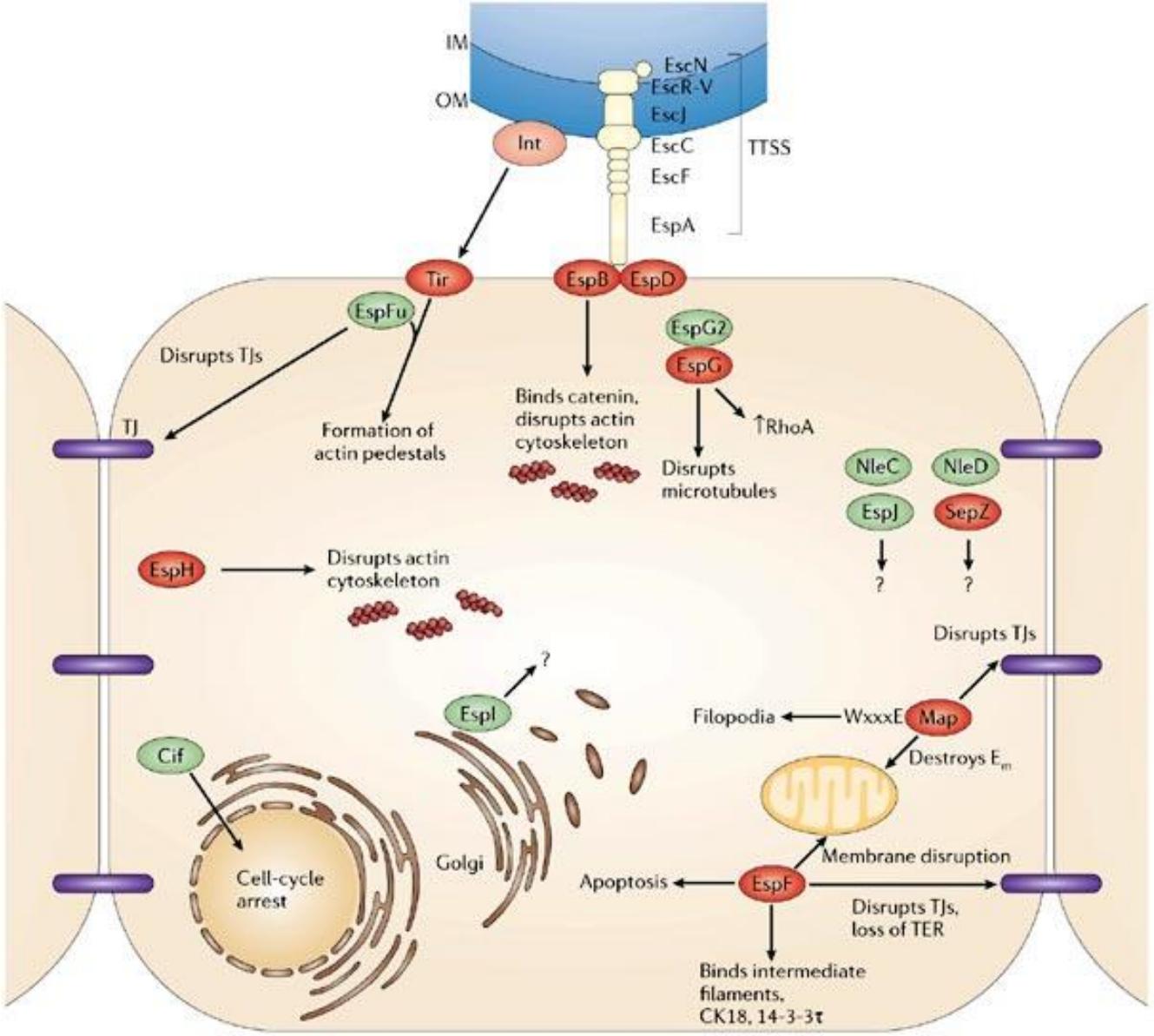
- Some bacteria later 'escape' the vacuole, replicate in the cytosol, and move by recruiting and polymerizing actin (actin tails).
- Facilitates transmission to other cells.





Hayward *et al. Nature Reviews Microbiology*;
published online 03 April 2006 | doi:10.1038/nrmicro1391

nature REVIEWS MICROBIOLOGY



Hayward *et al. Nature Reviews Microbiology*;
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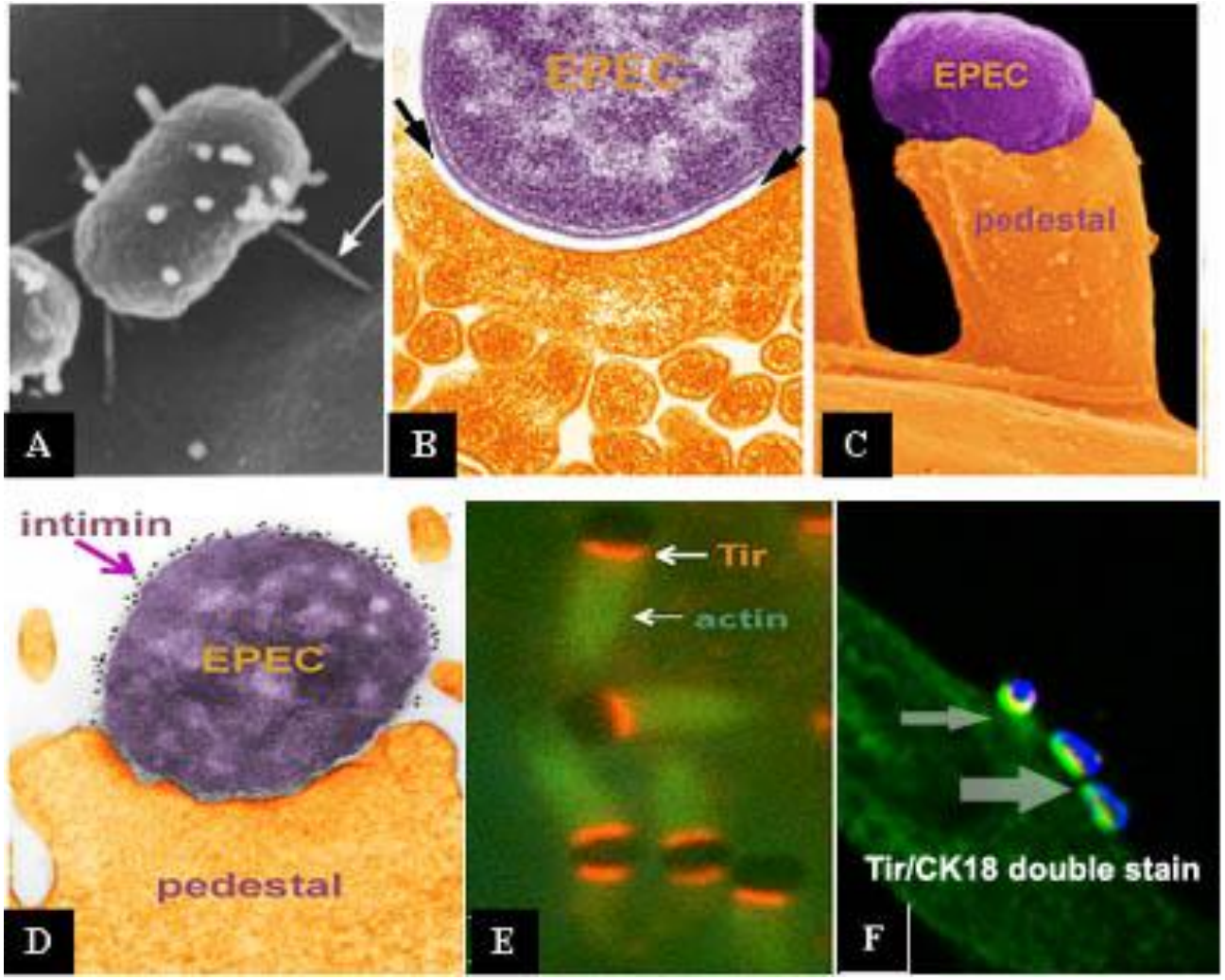
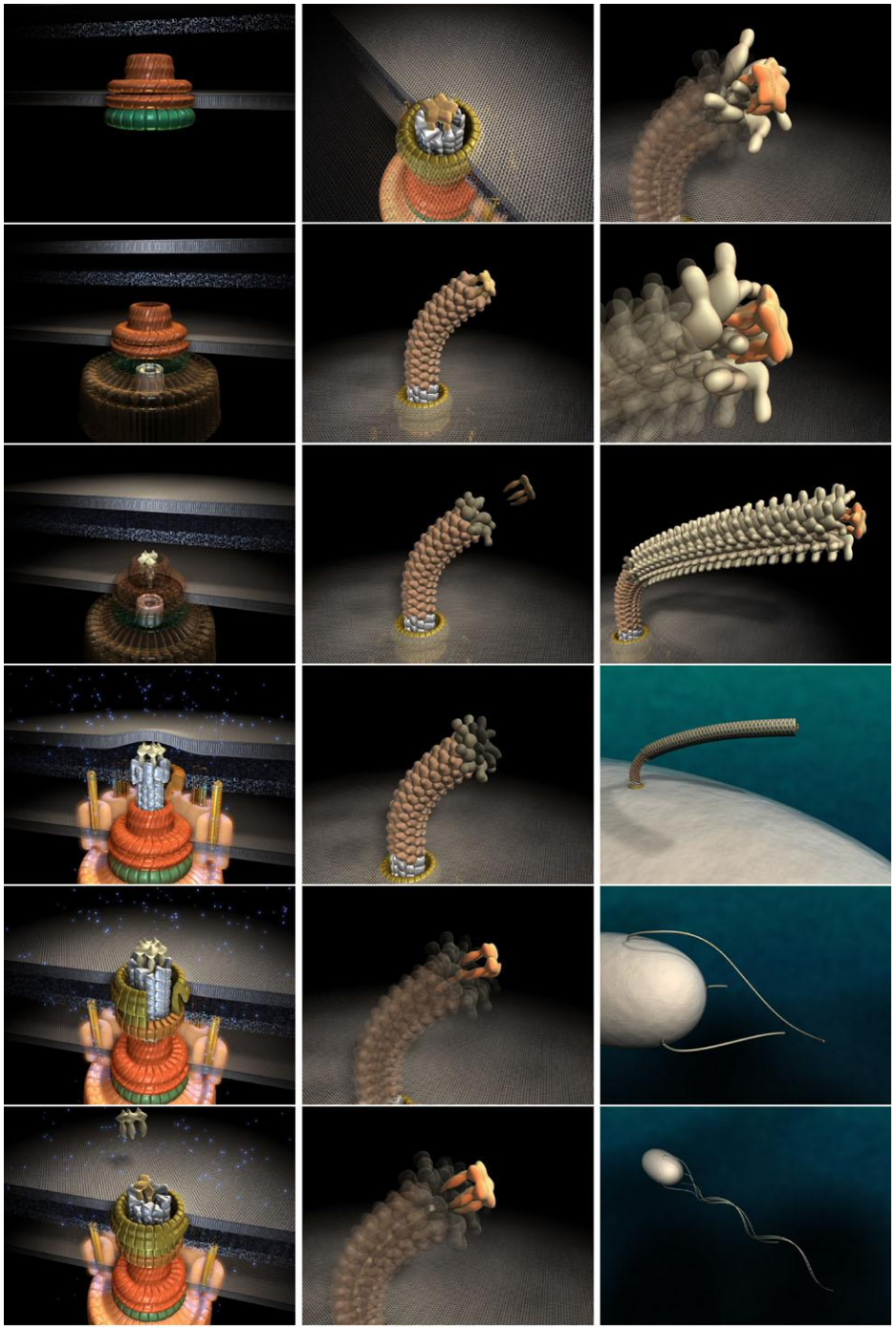
nature REVIEWS MICROBIOLOGY

Pedestal Formation

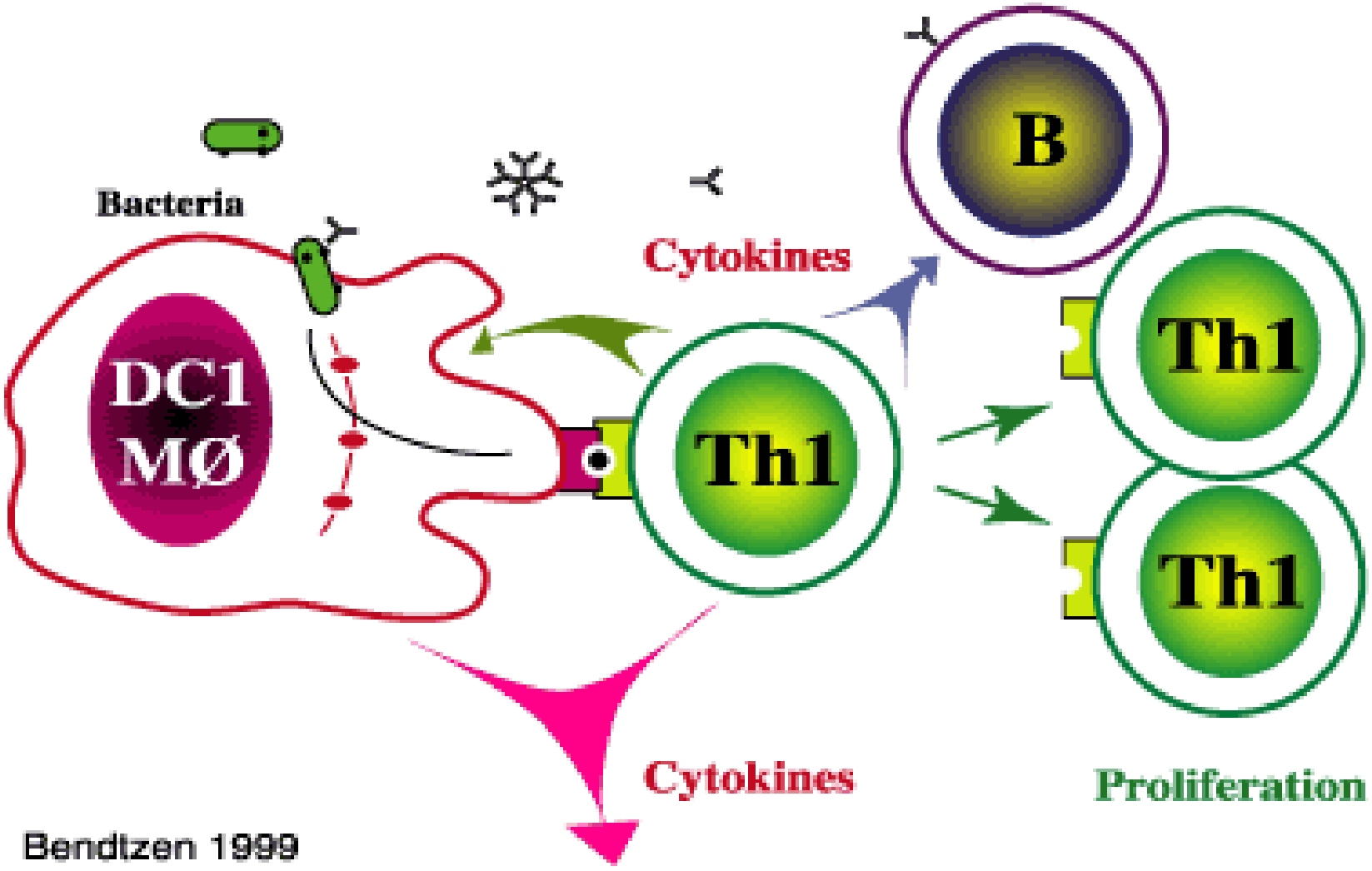




Flagella and T3SS

Extracellular bacteria

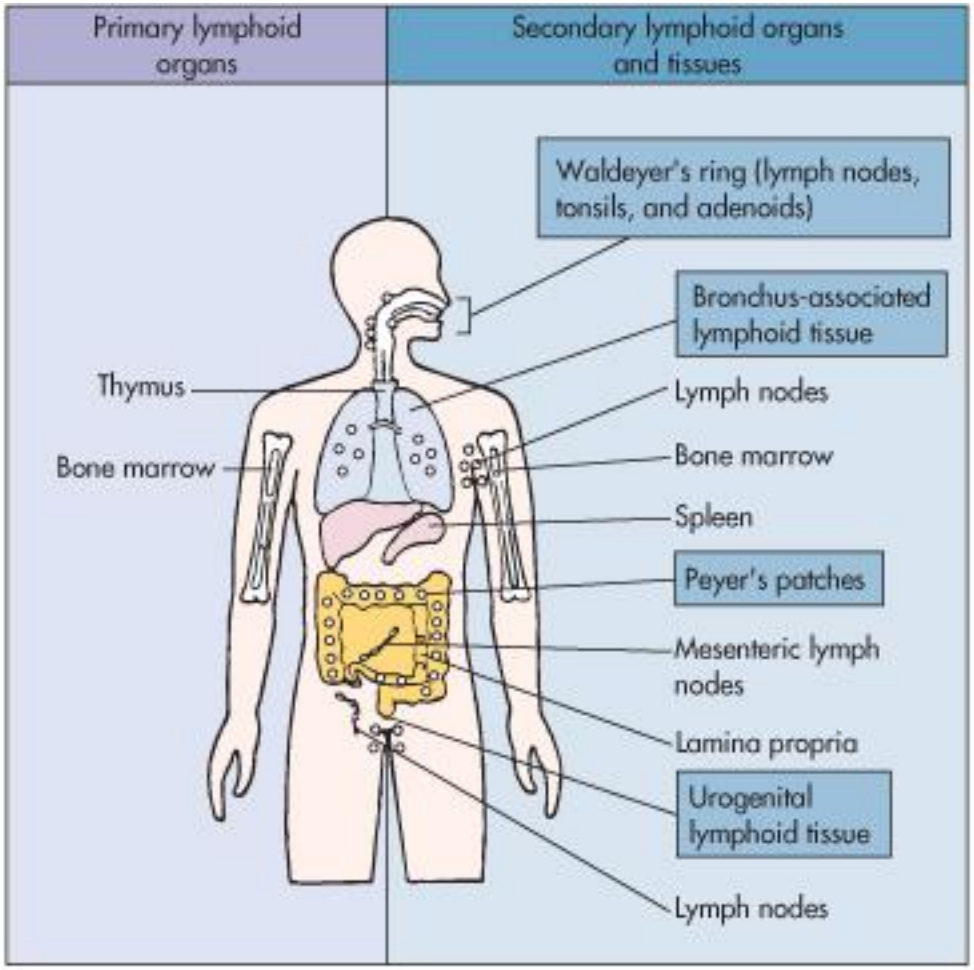
Species	Diseases
N. gonorrhoeae	urethritis, cervicitis salpingitis
N. meningitidis	meningitis, arthritis, pneumonia
H. influenzae	meningitis, sepsis, arthritis
H. ducreyi	genital ulcer disease
B. pertusis	whooping cough
P. aeruginosa	pneumonia, sepsis
E. coli	UTI, sepsis, diarrhea, meningitis
V. cholera	diarrhea
H. pylori	peptic ulcer disease
T. pallidum	syphilis
S. pneumoniae	pneumonia, otitis media, meningitis
S. aureus	impetigo, foliculitis, boils, toxic shock
	osteomylitis, enocarditis, bacteremia
S. pyogenes	scarlet fever, necrotizing fasciitis


Bacteria
B
Cytokines
DC1
MØ
Th1
Th1
Th1
Cytokines
Proliferation
Bendtzen 1999

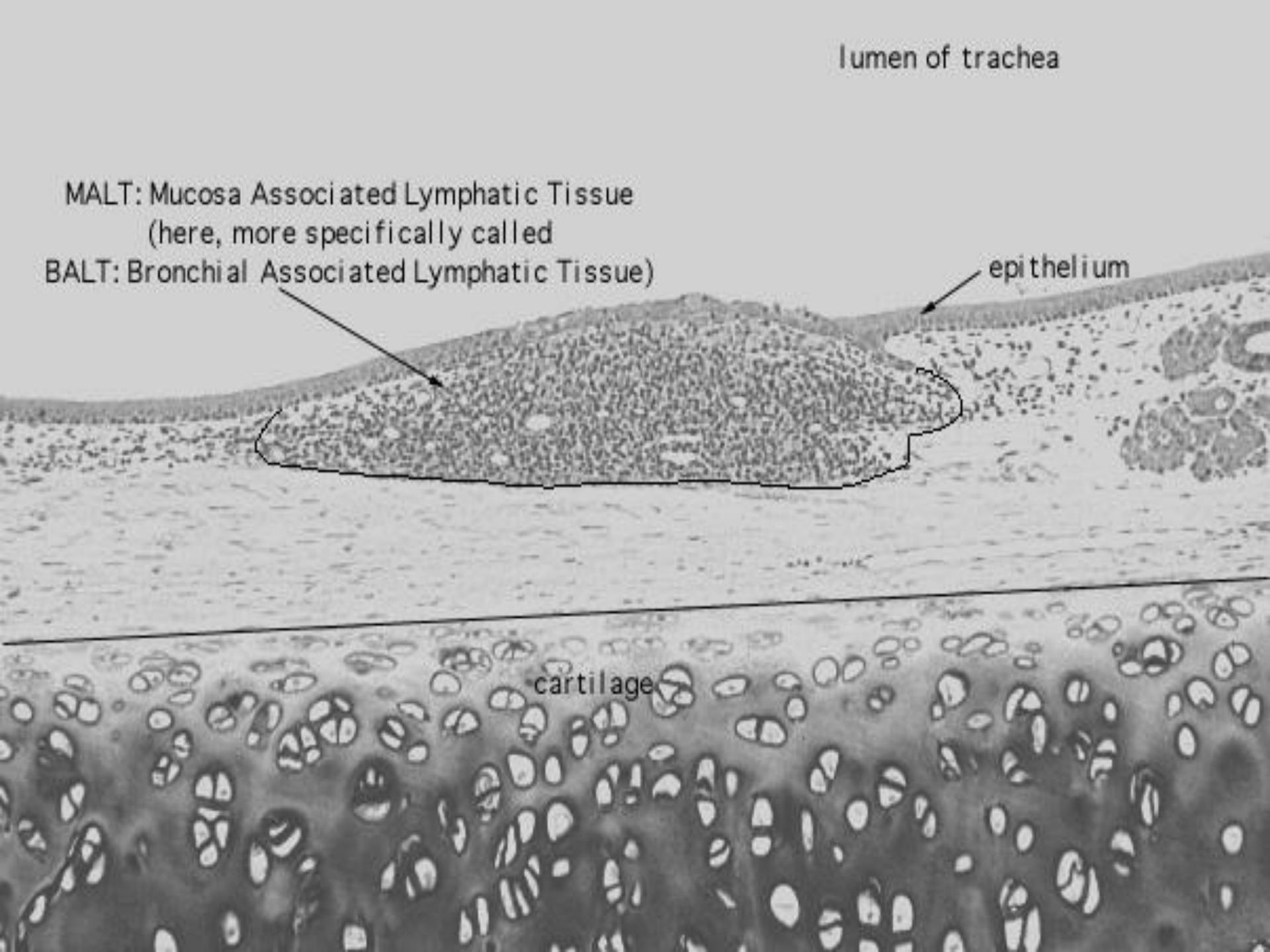
OBJECTIVES

- **1. The general nature of immune responsiveness.**
  - **Memory**
  - **Specificity**
    - **Innate immunity**
    - **Acquired Immunity**
- **2 Infection and Immunity**
- **3. The anatomic basis of immune responsiveness.**

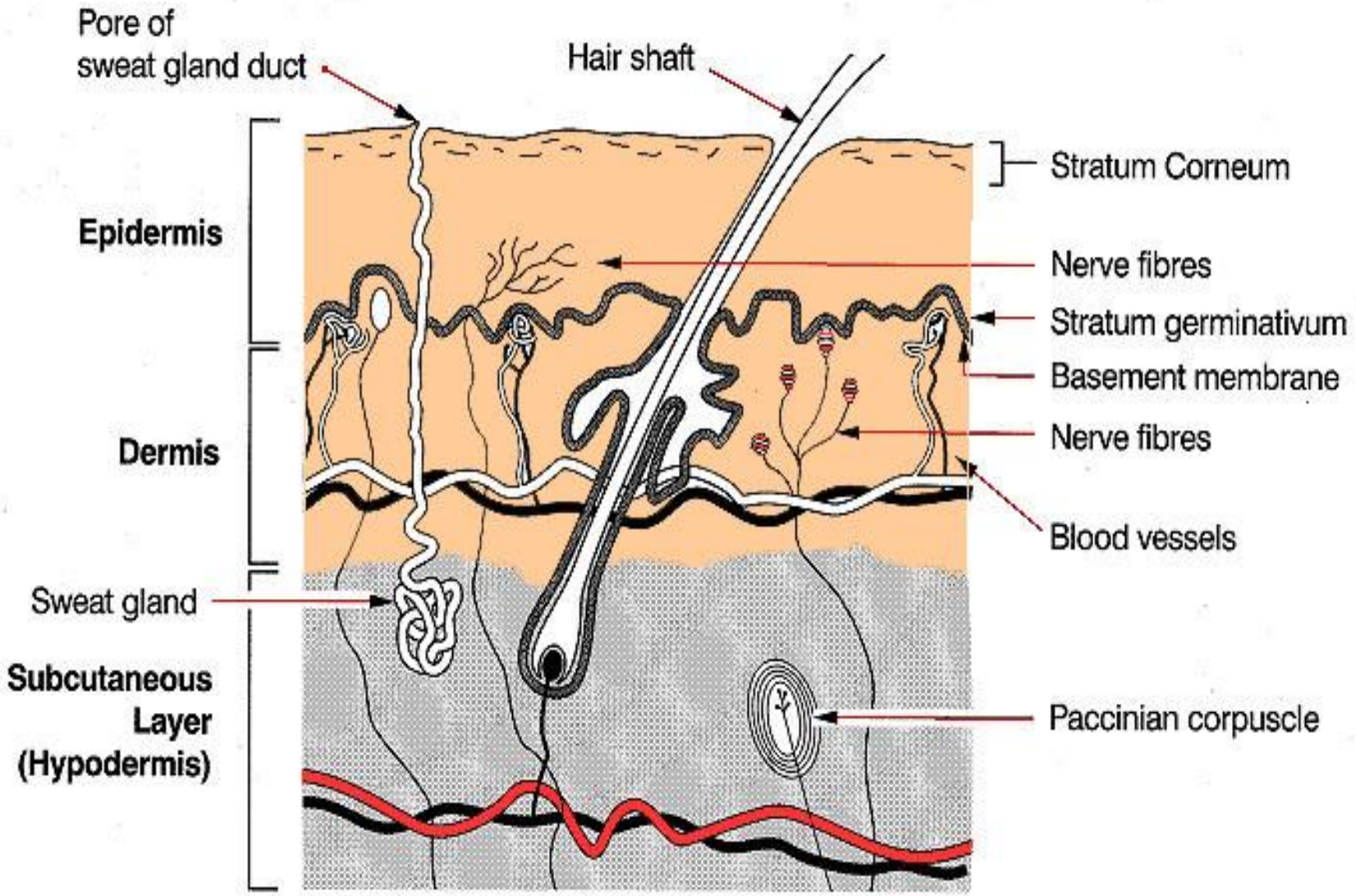
Where things happen



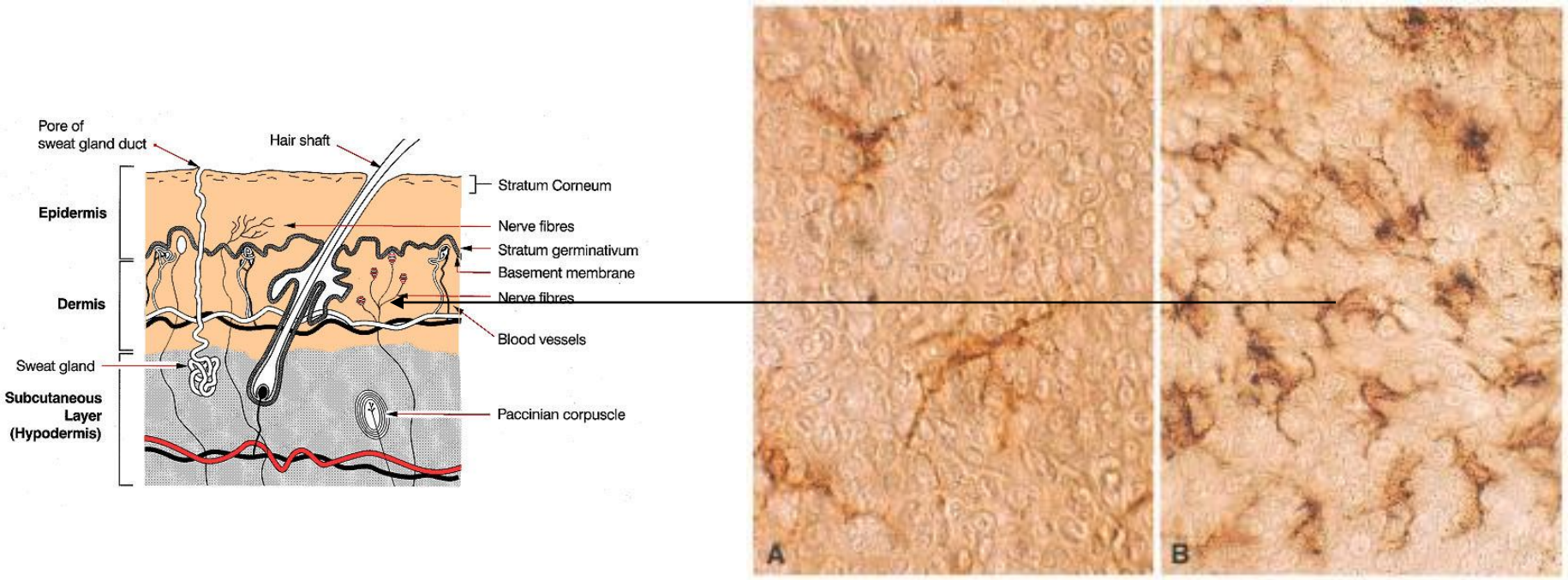
But…

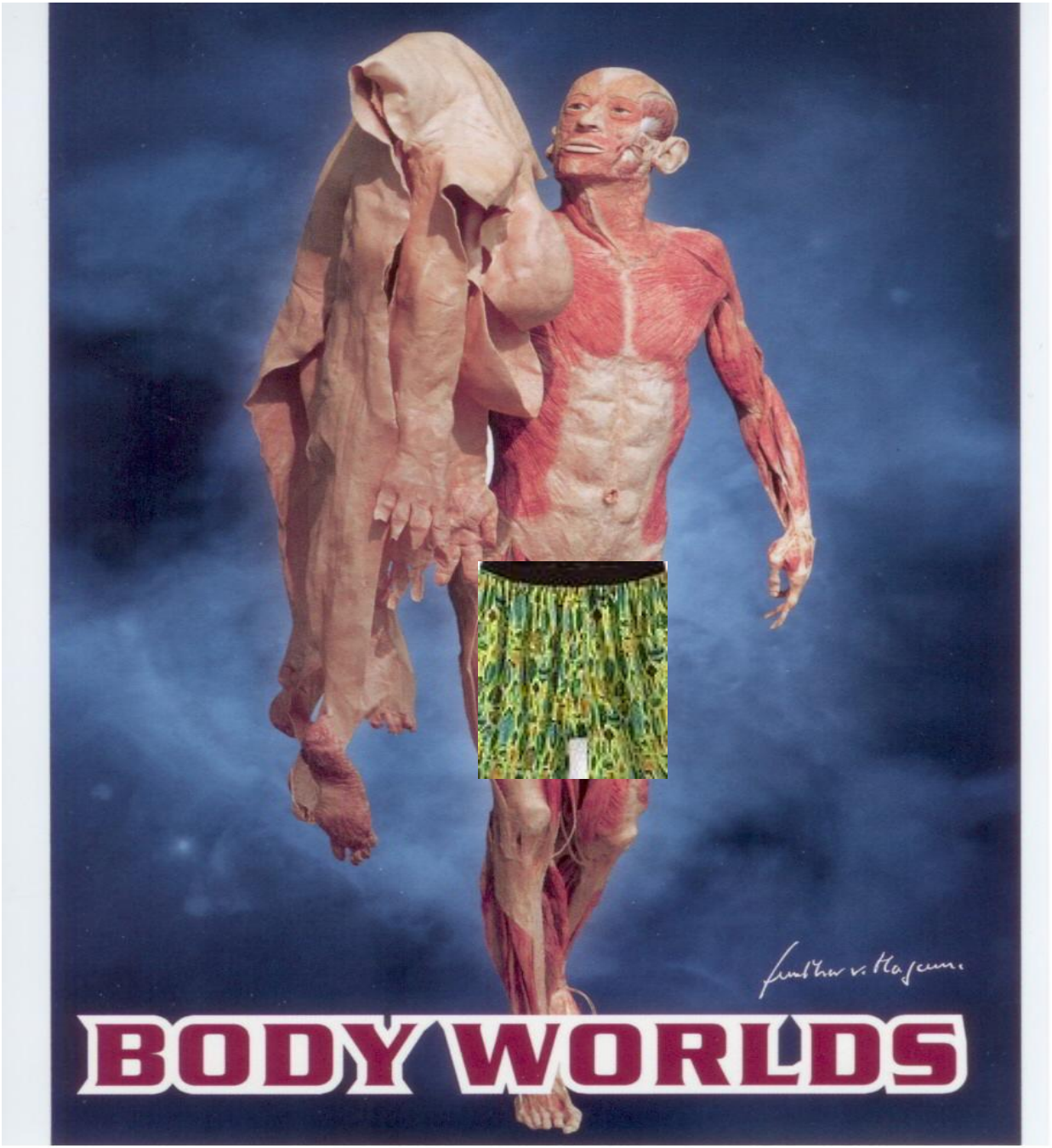

lumen of trachea
MALT: Mucosa Associated Lymphatic Tissue
(here, more specifically called
BALT: Bronchial Associated Lymphatic Tissue)
epithelium
cartilage

Mounting a Response



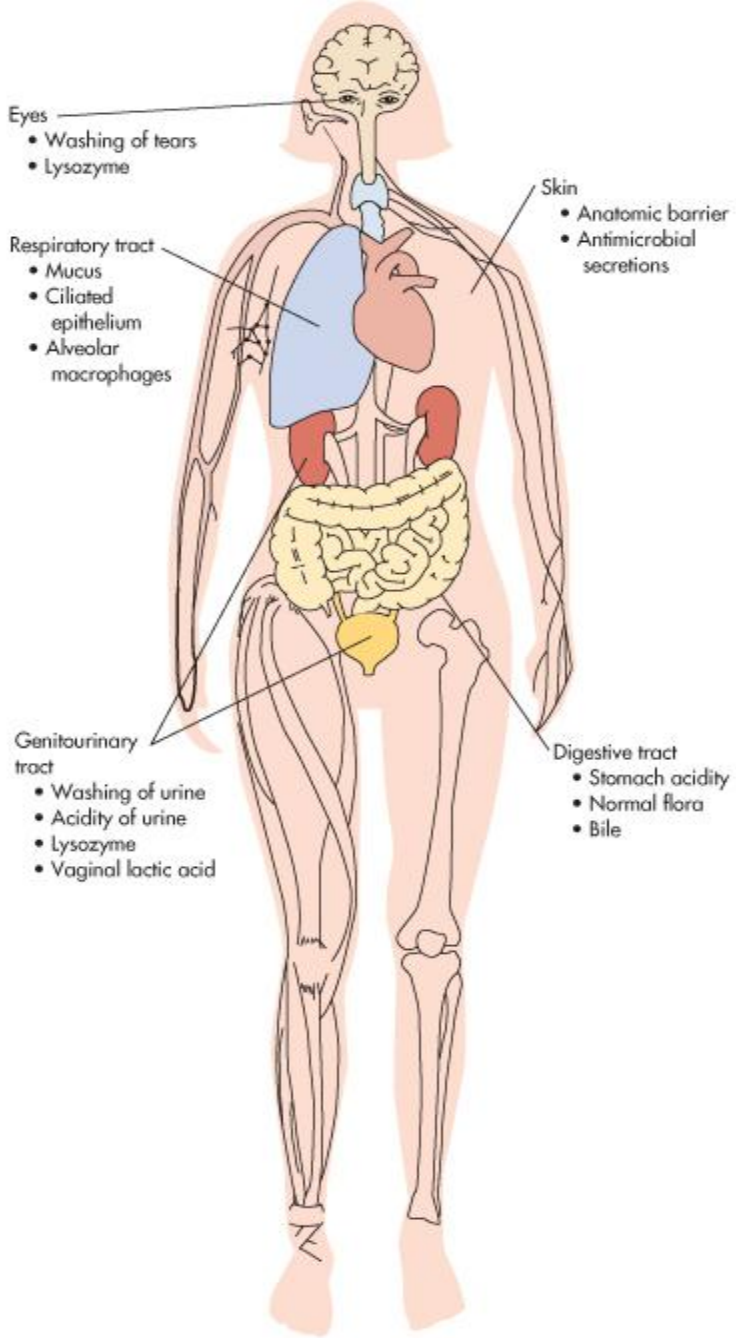
Mounting a Response



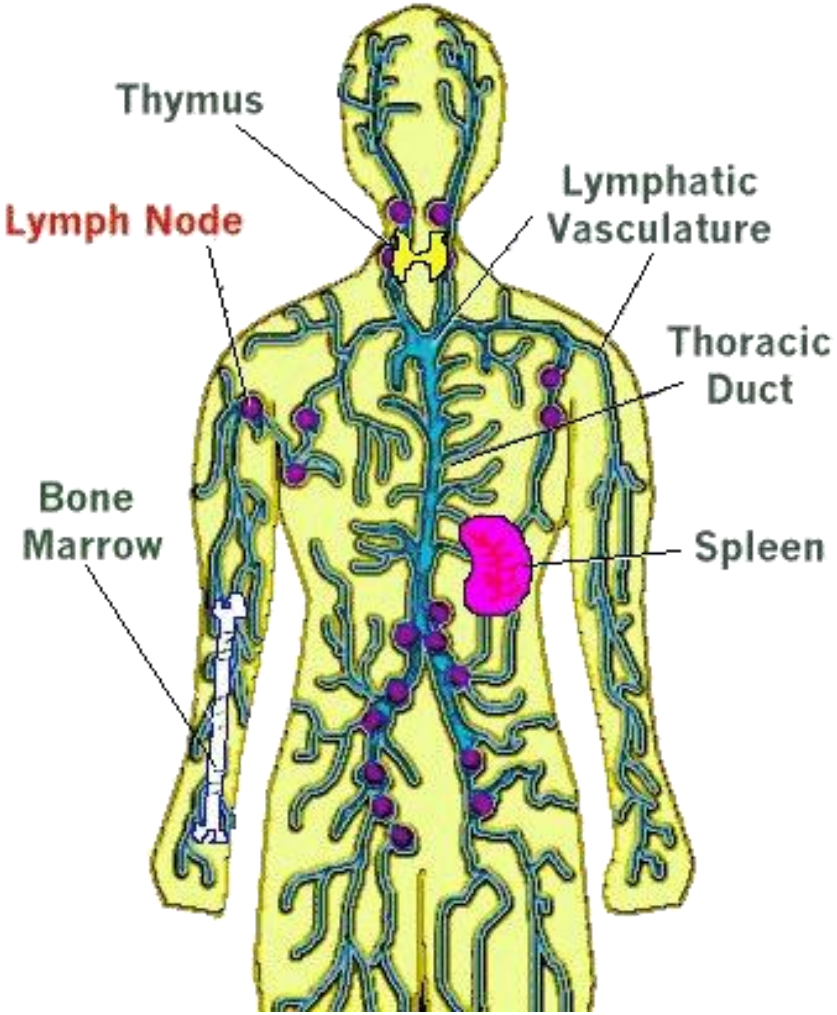


The Largest Immune Organ

Additional Barriers



Mounting a Response



Mounting a Response

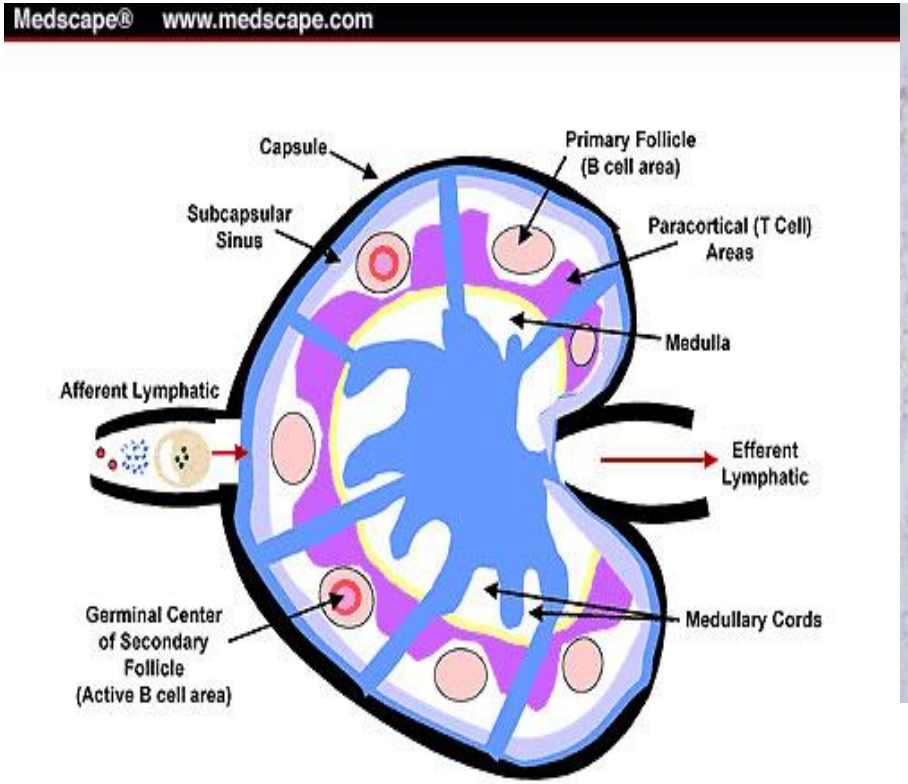
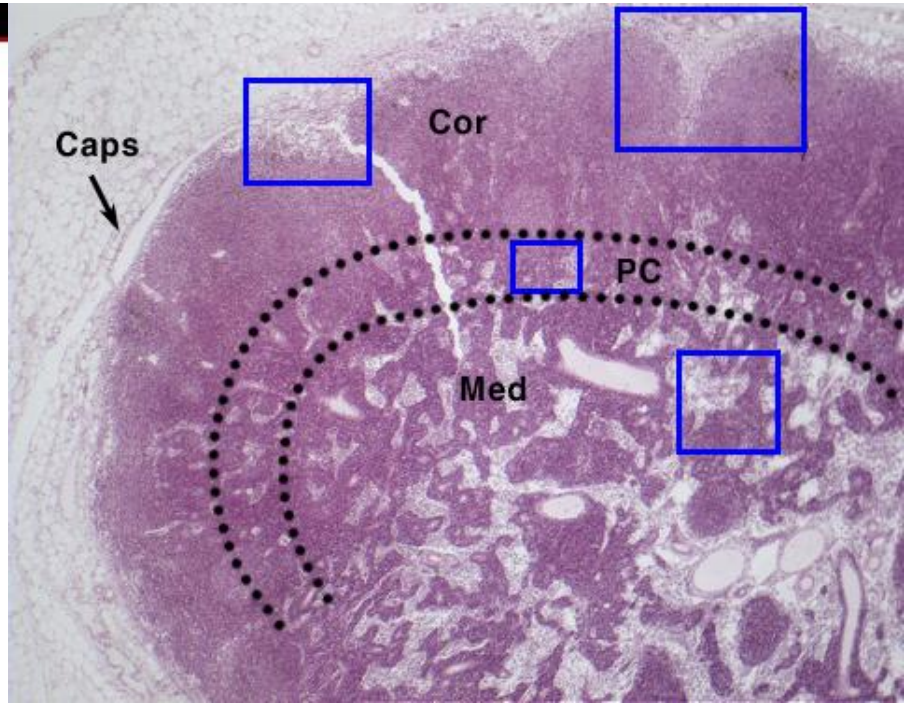
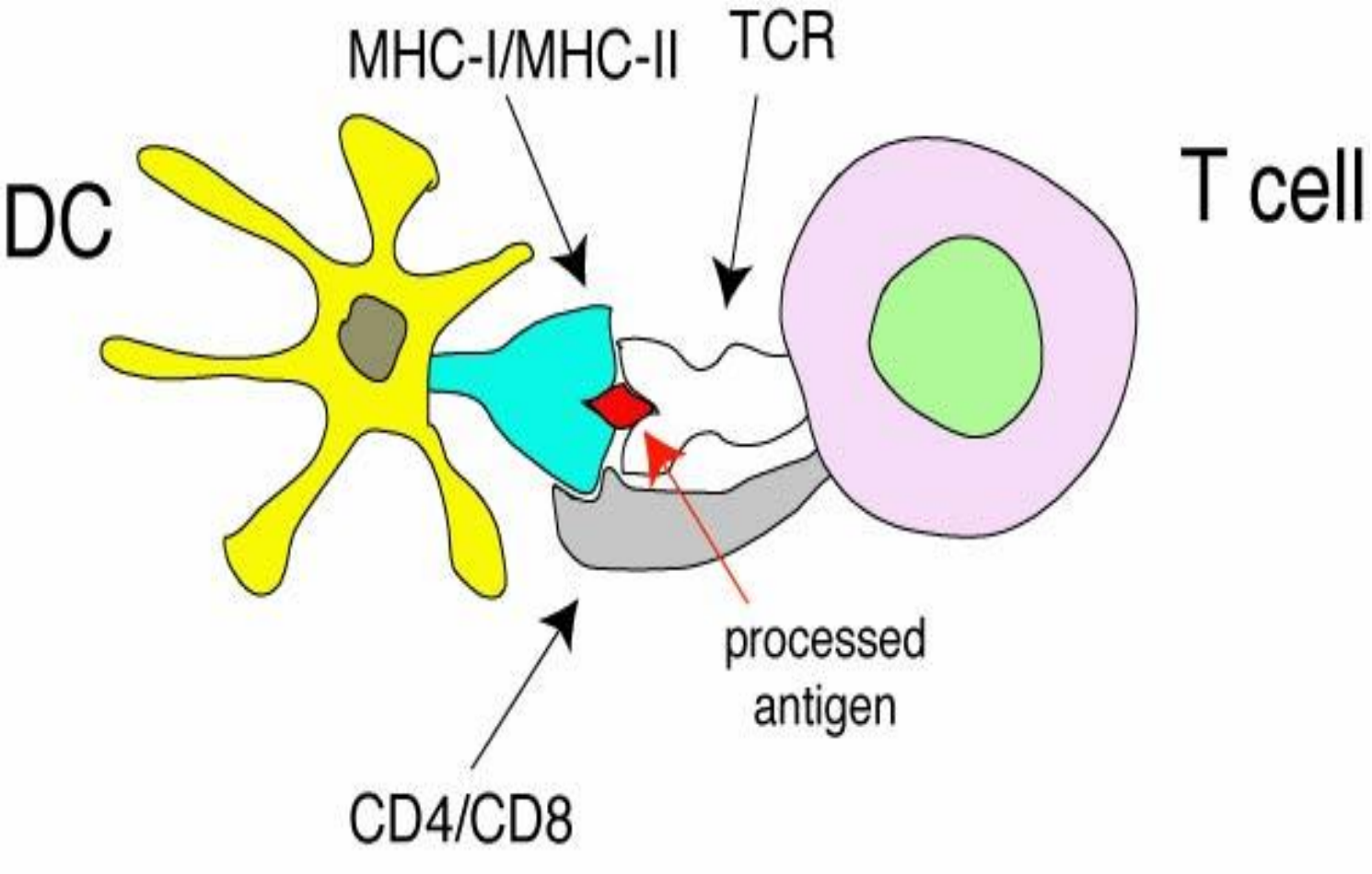


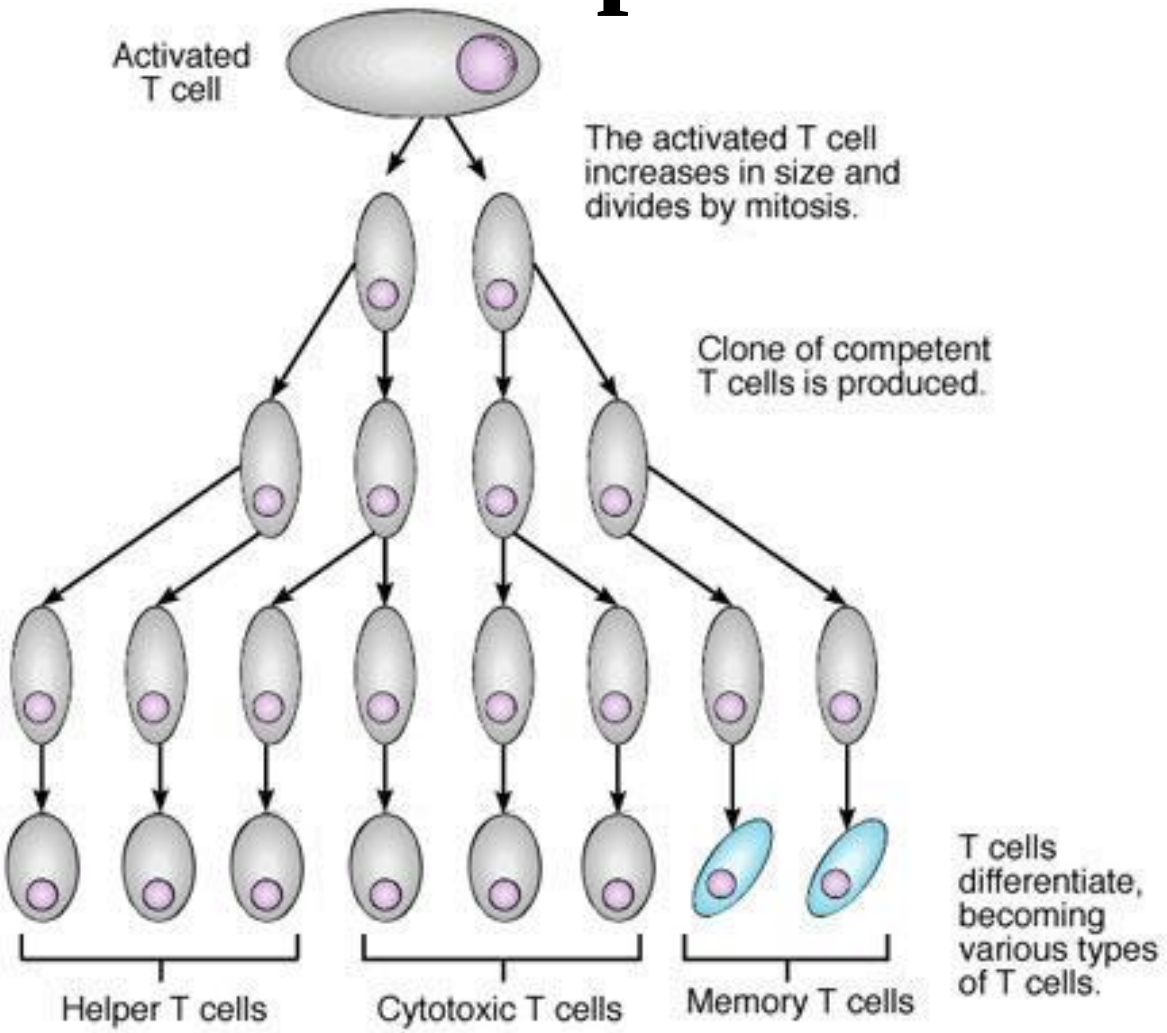
Figure 2. The lymph node.



Mounting a Response



Clonal Expansion



Distribution of Activated/Primed Lymphocytes