Case 2 – Bacterial Pathogenesis

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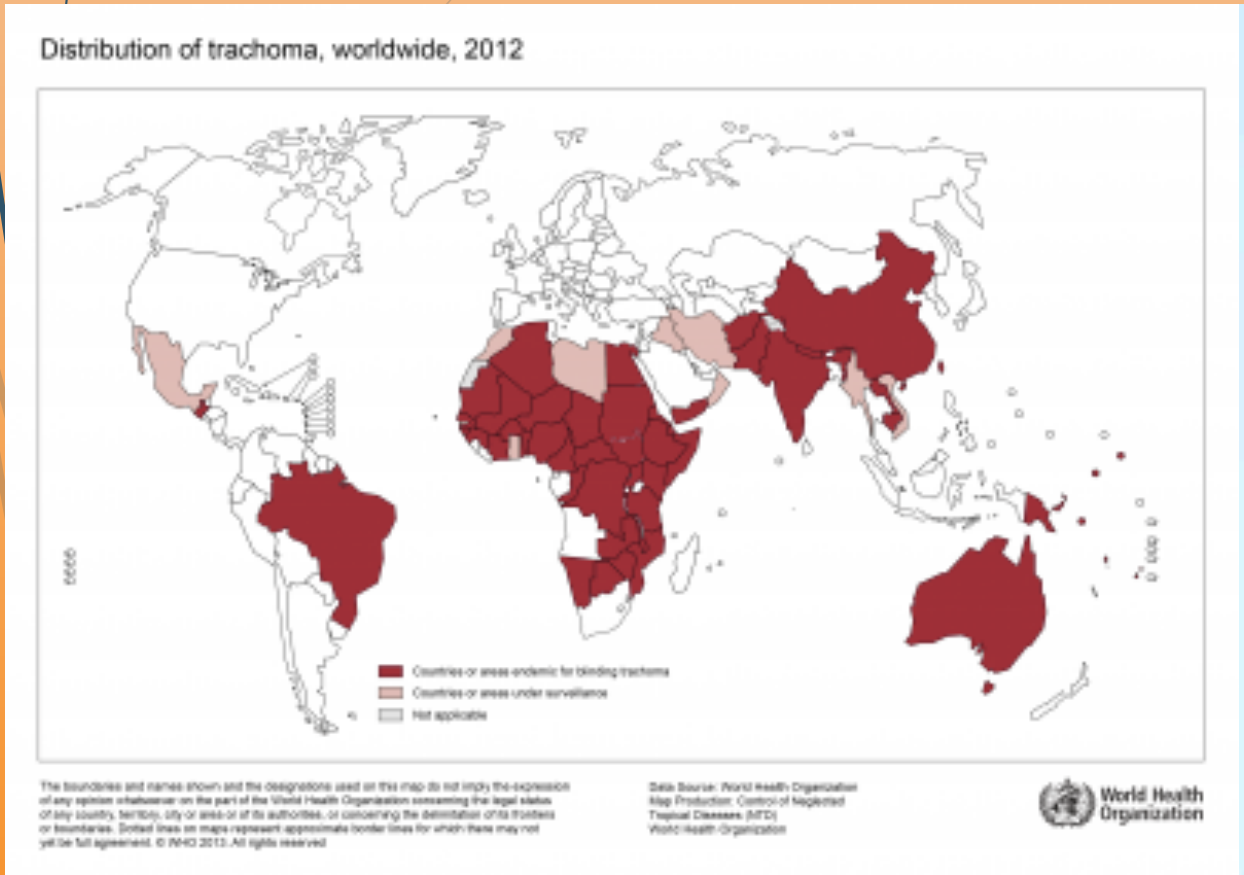
Case 2 – A New Partner

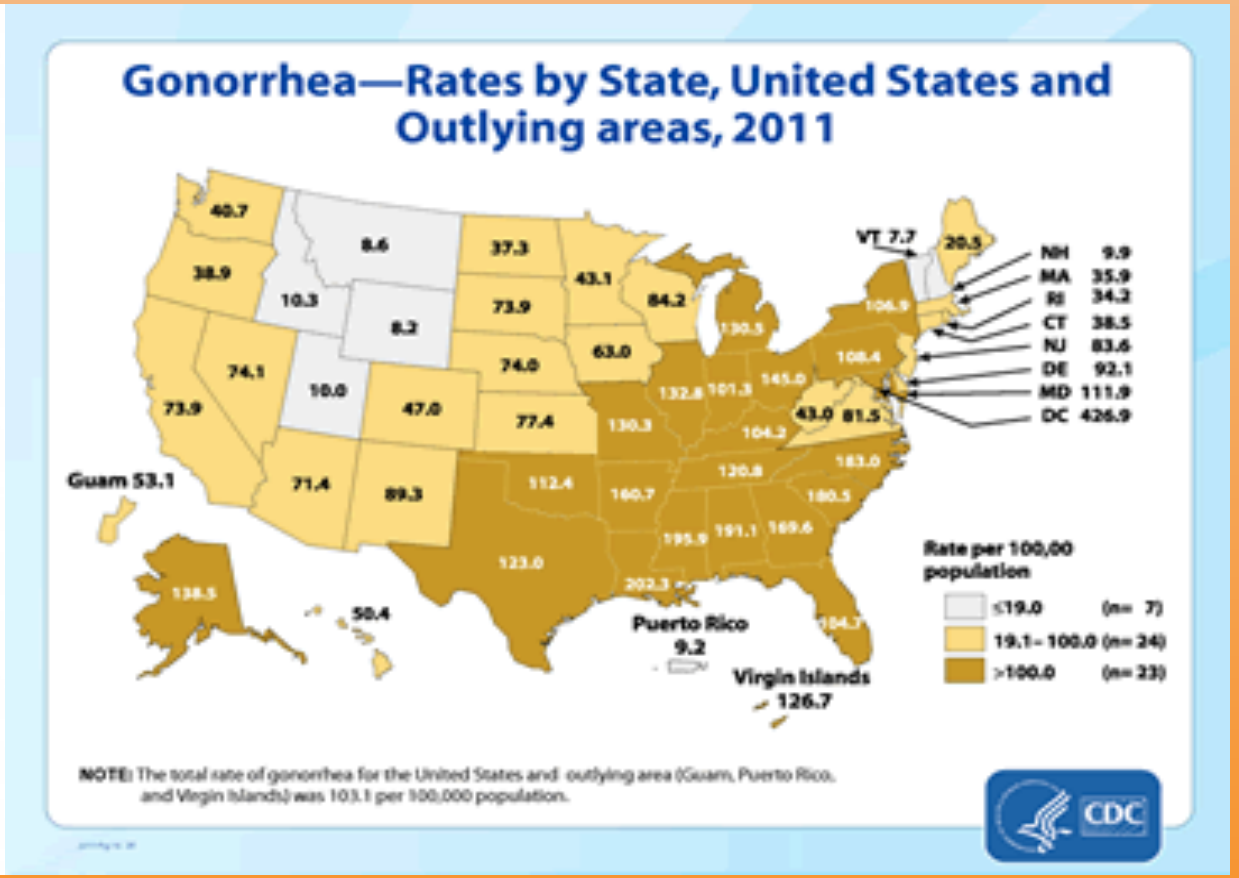
- 21-year-old Naser G. recently hooked up with a new sexual partner. This morning he noticed a burning pain in his penis during urination followed by a greenish discharge. He immediately goes to the student health clinic. The clinic doctor asks Naser about his recent sexual history and he recounts how he had unprotected sexual intercourse with a new partner about one week ago. The new partner claimed that she did not have any sexually transmitted infections. The doctor asks Naser to provide a urine sample to send to the Microbiology Laboratory. The doctor prescribes antibiotics for him and counsels him on safe sex practices and on the importance of encouraging his new partner to come in for testing too.

Question 1

Encounter: where do these organisms normally reside, geographically and host wise, and what are the bacterial characteristics that leave them suited to these places of residence. How would our patient have come in contact with these bacteria.

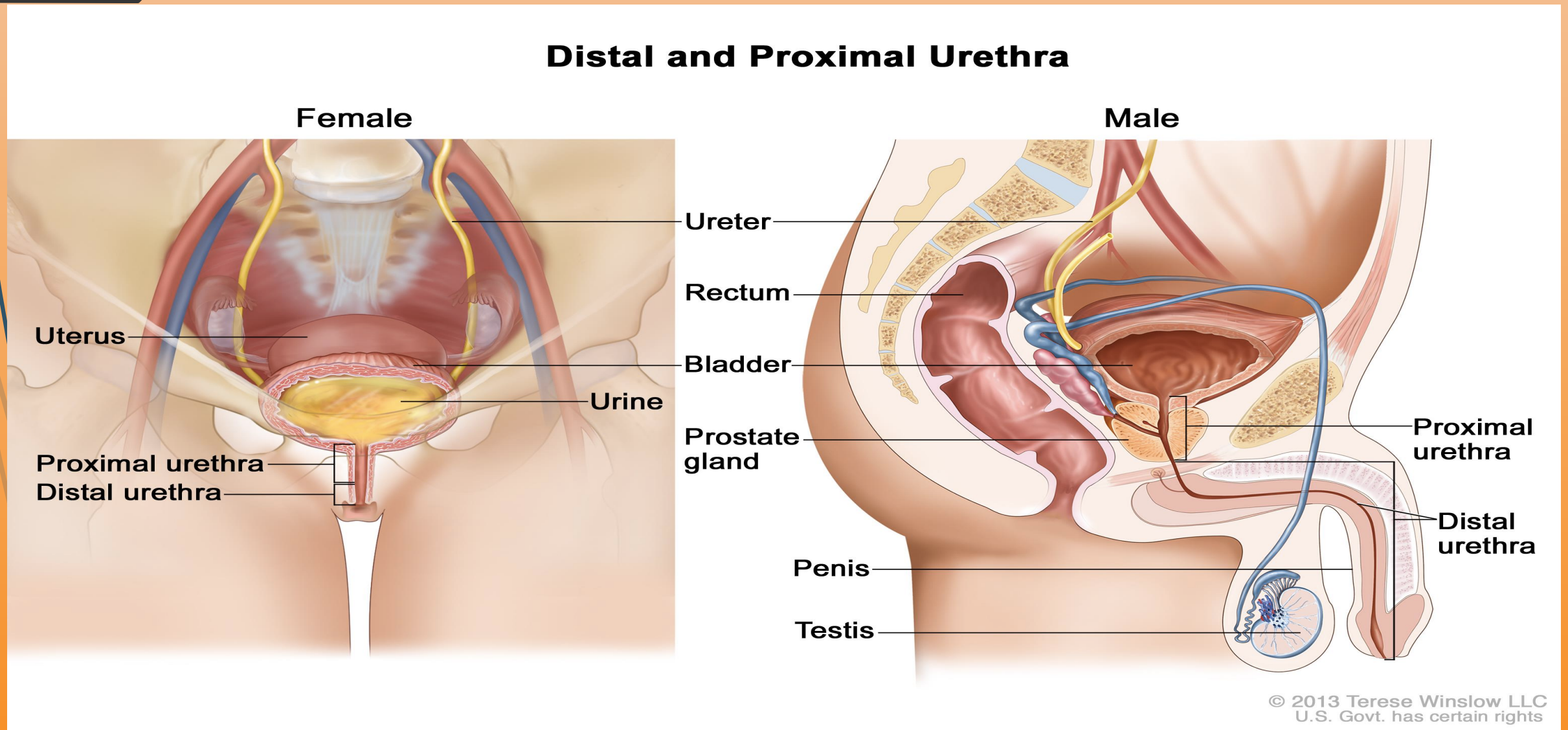
Distribution of *C. trachomatis* and gonorrhea infection





Bacterial Pathogen	*Chlamydia trachomatis*	*Neisseria gonorrhoeae*
Geography	- In South Asia, Africa, Western countries (eg. US) - Hot, dry areas with shortage of water and low hygiene standard	- Prevalent world-wide with the highest prevalence in the African, Southeast Asian, and Western Pacific regions (eg. US)
Host	- Higher prevalence in young females (15-24 years) especially those who are sexually active - As an obligate intracellular parasite, they infect columnar epithelial cells or in urethra of humans because they lack the ability to produce their own ATP	- Higher prevalence in young males (15-29 years), but many infections are asymptomatic - Found intracellularly in polymorphonuclear leukocytes (neutrophills) - Exclusively human pathogens as they are only capable of binding to exploit human transferrin and lactoferrin
Bacterial characteristics	- Major outer membrane protein (MOMP) mediates attachment to columnar epithelial cells - In an inert elementary body (EB) form, they are non-replicating and infectious - Once they attach to the host cell, EB transforms into the reticular body (RB) form, where they replicate and grow - In adverse conditions, they may take an intermediate persistence state where they only become active when favorable conditions return	- Aerobic bacteria but the growth is stimulated by CO2 - Temeprature range for growth is 22-40C (optimally, 35-38C) - pH range for growth is 6.0-8.0 (optimally, 7.4-7.6) - Adhere to the host cells using their pili and Opa proteins specifically to the microvilli of non-ciliated columnar epithelial cells (most commonly in urethra, cervix, rectum, pharynx, conjunctiva, and prepubescent vaginal epithelium)
How they come into contact with hosts	- Transmitted via genital, anal, and oralgenital sex	- Direct genital contact allows it to attach to the microvilli of non-ciliated columnar epithelial cells

Target sites of bacterial infection



Question 2

Entry: how do these bacteria enter into the human host and where do they take up residence. What are the molecular, cellular and/or physiological factors at play in this site specificity and in the initial adherence step.

Chlamydia trachomatis

Entry

- Via sexual contact or contact with infected individuals, fomites, flies
- Vertical transmission to newborns is also possible, during passage through the birth canal
- Upon entering the host, they reside in the columnar epithelial cells

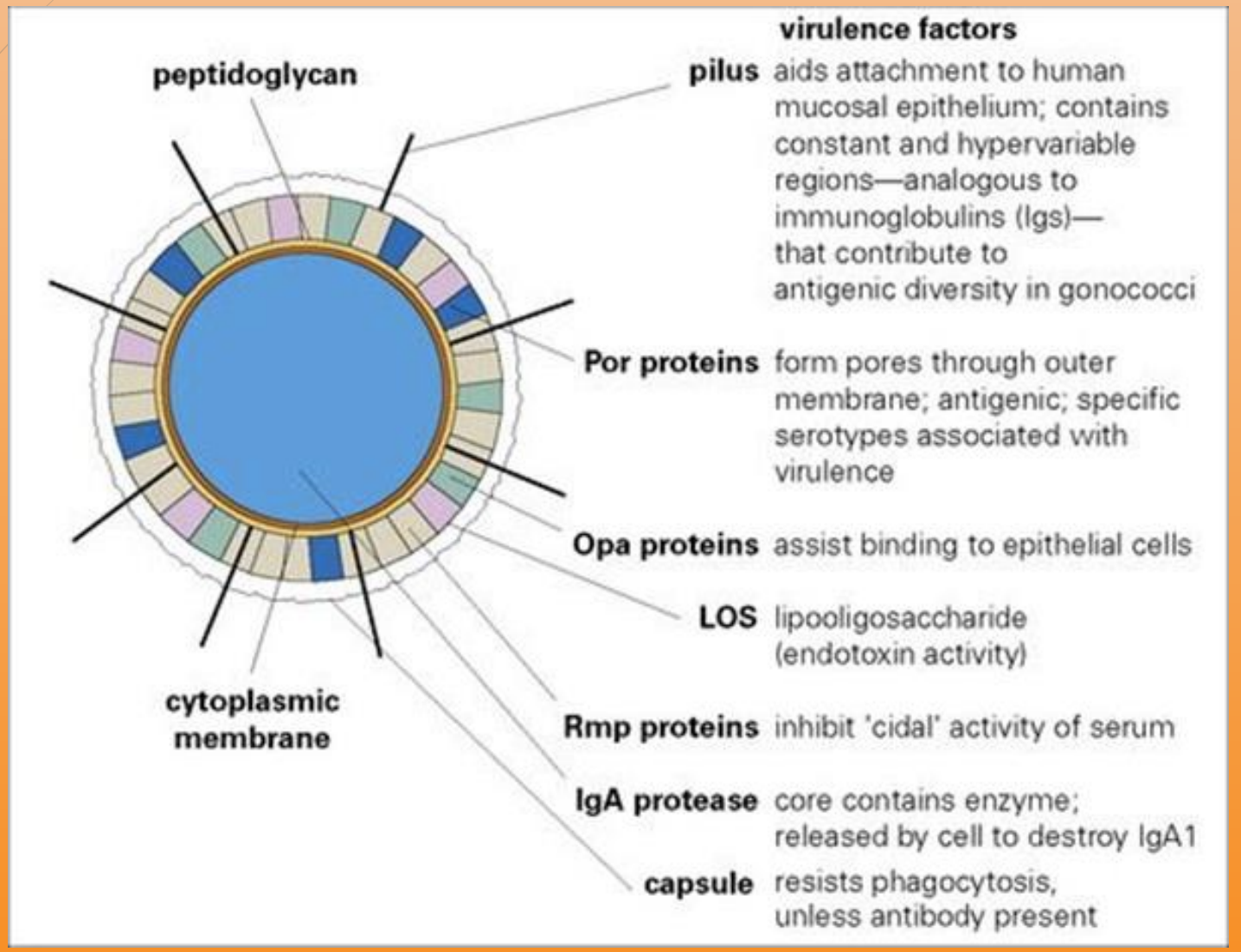
Adherence

- In the infectious EB form, the pathogen attaches to nonciliated columnar, cuboidal, and transitional epithelial cells on the mucous membranes of the urethra, endocervix, endometrium, fallopian tubes, anorectum, respiratory tract, and conjunctivae.
- EB has MOMP2 outer membrane protein that is cysteine-rich and this is responsible for the extensive disulfide cross-links that provide the stability.
- The pathogen's hemagglutinin may facilitate attachment to cells.
- OmcB protein expressed on EB's surface binds to the host cell's heparan sulfate and functions as a chlamydial adhesin.
- Protein disulfide isomerase (PDI), a host protein, is structurally required for chlamydial attachment.

Endocytosis

- PDI is also necessary for entry; PDI-mediated disulfide reduction is necessary for Chlamydia entry into cells.
- EBs induce endocytosis as they translocate actin recruiting protein (Tarp) into the host cell via a type III secretion system; this makes the cell to rearrange its actin skeleton, allowing the endocytosis of EB.
- Once in the cell, Ebs convert into RBs, the metabolically active replicating chlamydial form.

Neisseria gonorrhoeae



Neisseria gonorrhoeae

Entry: Sexual contact

- Resides inside the non-ciliated columnar epithelial cells
- Ex. Urethra, cervic, rectum, pharynx, conjunctiva, and prepubescent vaginal epithelium

Adherence

- Mediated by Type IV pili (contains PilE and PilC subcomponents) and Opa proteins (protein II)
  - Pili bind to CD46 receptor on host cells to transduce signals inside the host cell to mobilize Ca2+
  - Opa proteins bind to CD66 and heparan sulfate proteoglycan receptors to make connections tight; later this interaction mediates the uptake of the pathogen
- Some nonspecific factors such as surface charge and hydrophobicity may be important

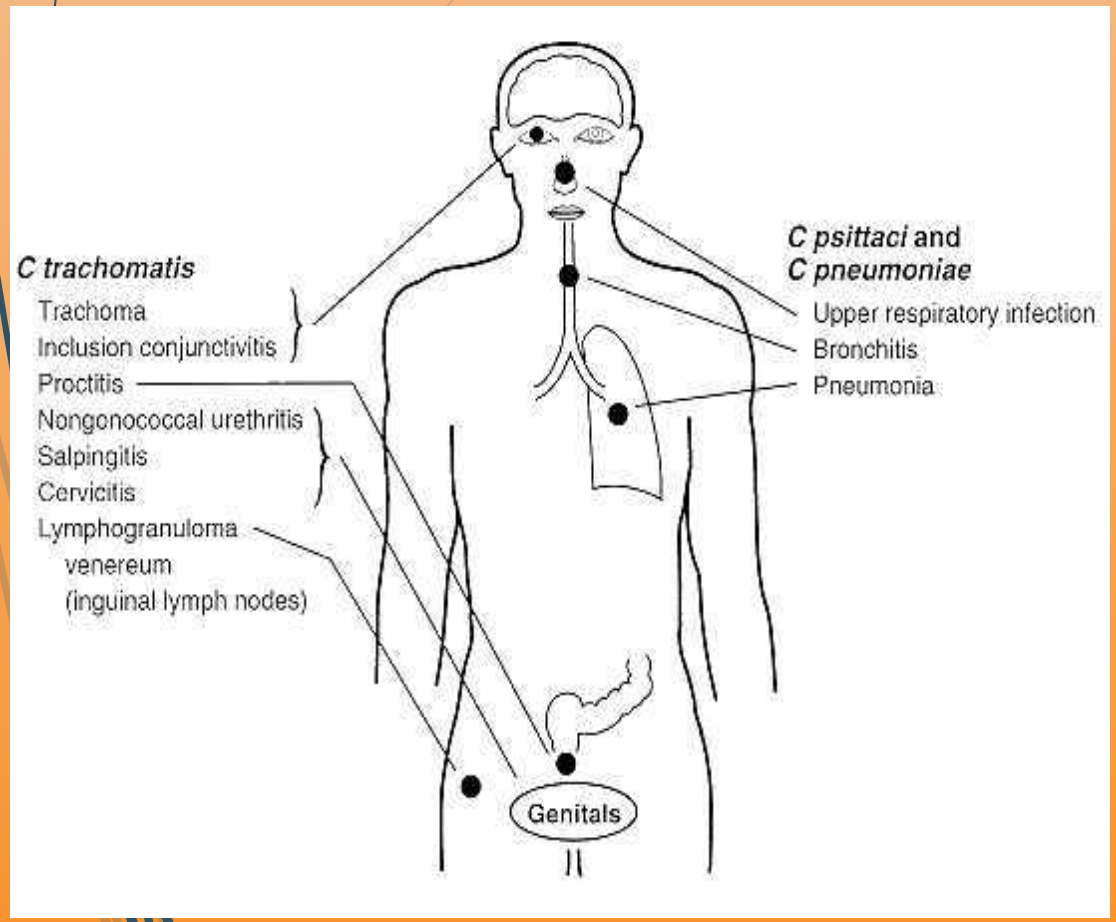
Endocytosis

- Parasite-directed endocytosis of non-phagocytic host cells, initiated by microbial factors
- Opa proteins bind to carcinoembryonic antigen-related cellular adhesion molecultes to activate acid sphingomyelinase, which causes release of ceramides; this coalesces to ultimately initiate internalization of bacteria;
- Host heparin sulphate chains of the syndecan receptors 1-4 interact with OpaA protein, which is also required for bacteria entry; this is mediated by fibronectin and vitronectin
- Proin protein (Por or protein I) has also been proposed as a candidate invasin
- Pathogen enters the cervical non-ciliated epithelial cells in females by interacting with CR3 on host cell surfaces, which results in membrane ruffling and internalization
- After endocytosis, vacuole containing the bacteria is released by exocytosis into the subepithelial tissue

Question 3

Multiplication and Spread: do these organisms remain at the entry site and/or do they spread beyond the initial site; are there, for instance, secondary sites of infection. Do they remain extracellular and/or do they enter into cells and what are the molecular and cellular determinants of these events.

Chlamydia trachomatis



Primary site of infection

- C. trachomatis exist as EB extracellularly and RB intracellularly. EB adsorbs and internalizes into the host cell and differentiate into RB. Proteins are synthesized and DNA gets replicated using many host resources (mitochondria, nucleotides, enzymes, etc.)
- RB undergoes binary fusion and after developing an outer cell wall, differentiate back into EB.
- New EBs are exocytosed or cause the host cell to lyse to infect other host cells or hosts.
- Primary infection sites include cervix and urethra

Secondary site of infection

- EBs can spread to other tissues to cause conjunctivitis, cervicitis, endometritis, salpingitis, PID, urethritis, epididymitis, and procitis
- Mode of transmission include sexual contact or direct contact of contaminated fingers, fomites, or towels
- In the eyes, C. trachomatis can cause ocular tissue inflammation, scarring and blindness
- Newborns can become infected while passing through the birth canal if the mother is infected, possibily leading to acute conjunctivitis

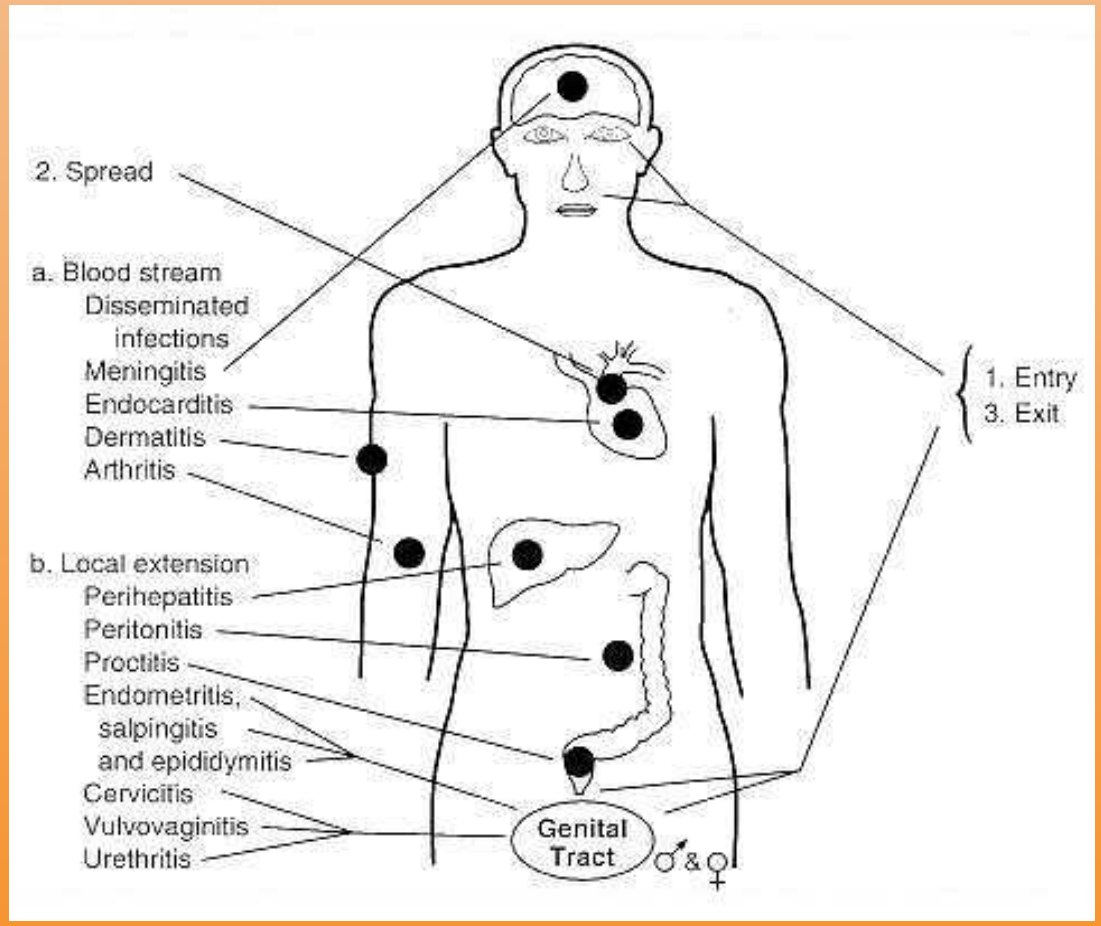
Neisseria gonorrhoeae

Primary site of infection

- Exist Intracellularly following the parasite-directed endocytosis
- Extracellularly, they grow primarily in the bloodstream, acquiring iron from transferrin, lactoferrin, and hemoglobin (express Tbp1, Tb2 and Lbp lactoferrin receptors on their surface to acquire iron)
- Usually localized to the primary site but can disseminate to other areas
- Local infections: urethritis, procitis, pharyngitis, conjunctivitis, and urethritis

Secondary sites of infection

- Upon dissemination, other complications can occur: PID, epididymitis, septic arthritis, meningitis, endocarditis, tenosynovitis, skin lesions
- Untreated, asymptomatic infections (especially for females) can lead to disseminated infections
- Most common form: dermatitis-arthritis syndrome

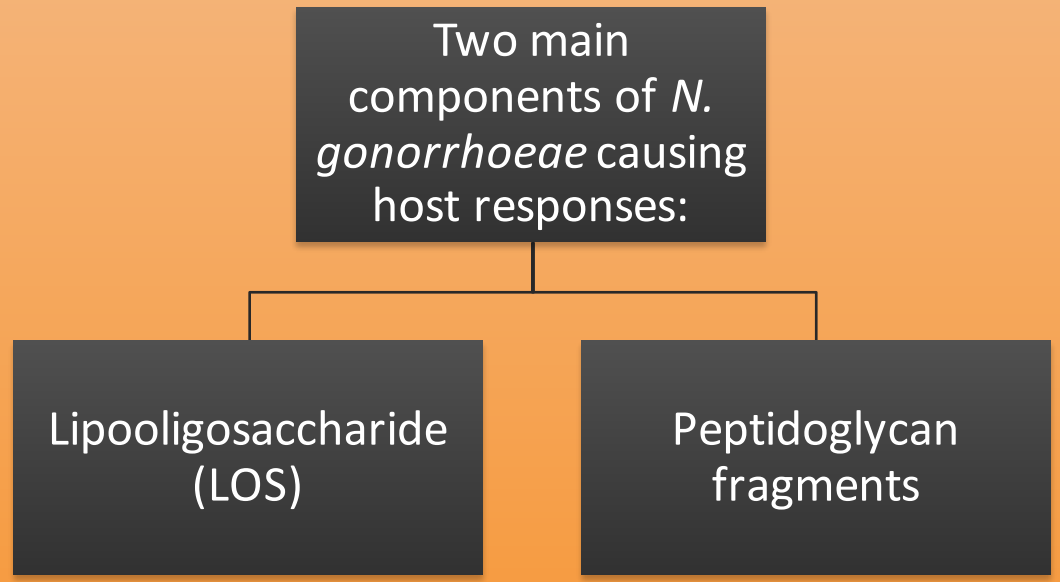


Question 4

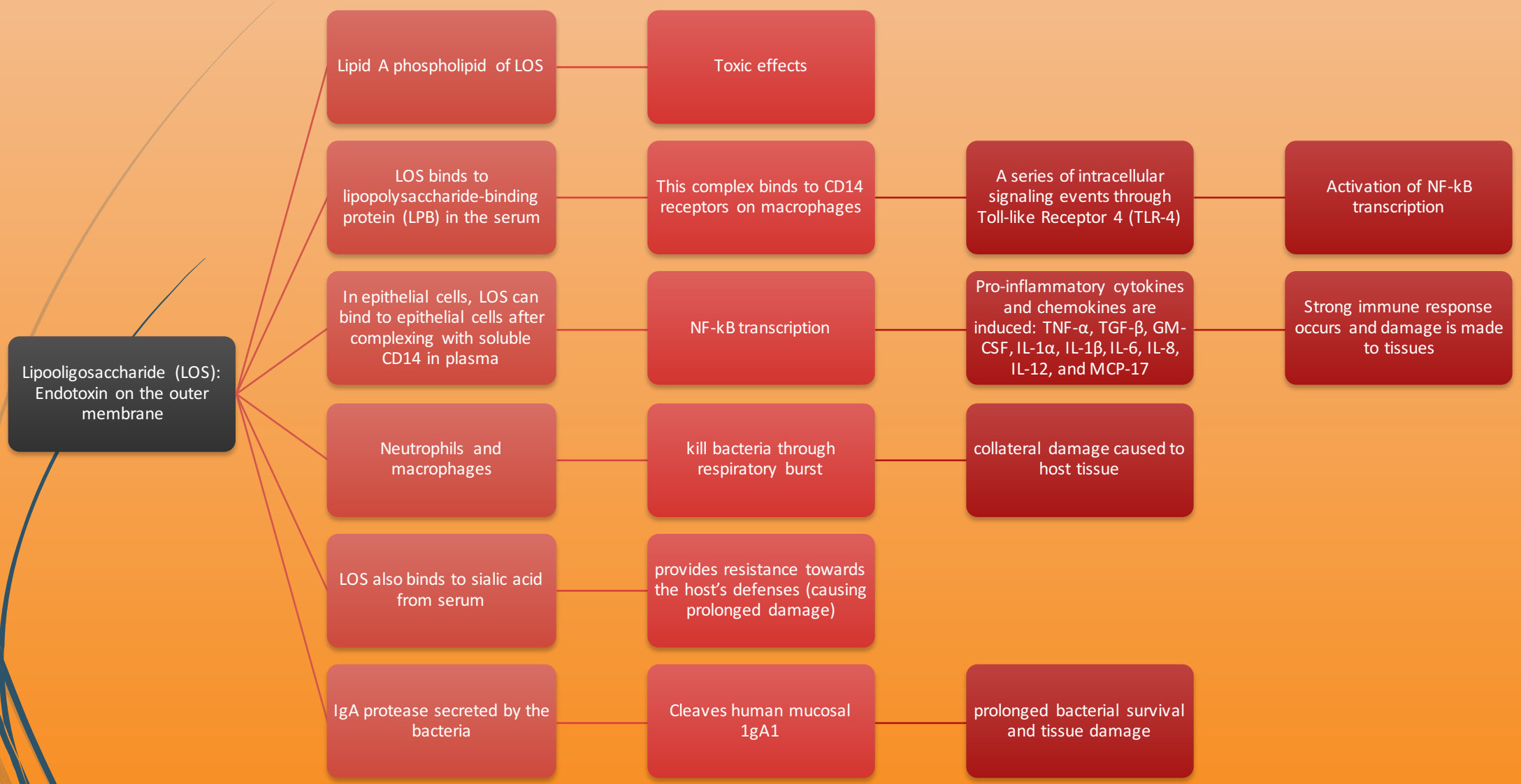
Bacterial Damage: do the bacteria cause any direct damage to the host (or is the damage fully attributable to the host response, as indicated below) and, if so, what is the nature of the bacterial damage. Can it be linked to any of the signs and symptoms in this case?

Neisseria gonorrhoeae

- Mostly indirect damage from the inflammatory immune response of the host
- Invasion into cells is not necessary and often cell contact is enough to trigger cytokine up-regulation



Neisseria gonorrhoeae



Neisseria gonorrhoeae

- Peptidoglycan fragments
  - Constantly released as part of the bacterial growth
  - It binds to CD14 receptor of macrophage to trigger NF-kB upregulation, causing inflammatory response
  - Inside the epithelial cells, Nod1 detects peptidoglycan fragments also leading to NF-kB upregulation and inflammation

Chlamydia trachomatis

- Like that of *N. gonorrhoeae*, the damage from *C. trachomatis* is mostly caused indirectly by host's immune response to the pathogen.
- Response could be delayed by 20-24 hours upon infection as intracellular growth is required for cytokine upregulation
- Studies have shown that Chlamydial lipopolysaccharide (LPS) can stimulate weak proinflammatory response
- Intracellular TLR-2 and Myd88 form complex with chlamydial inclusion to express cytokines and chemoattractants: IL-1α, IL-6, IL-8, Growth regulated oncogene (GROα) and GM-CSF
- Macrophages, neutrophils, and T-cells migrate towards the tissue to increase inflammatory response and cytokine secretion
- Epithelial cells release matrix metalloproteases (MMPs) for proteolysis
- Neutrophil elastase (protease) destroy both host cells and bacteria
- TNF-α induce apoptosis (negative consequences if the infection is chronic)

Relation to signs and symptoms

- Naser would have felt the burning sensation and observed green discharge due to the ongoing inflammatory immune response against the bacteria and from an exudate of dead bacterial cells, immune cells, and tissues resulted from the immune responses.