PATH 417

Case 2: A New Partner

Bacterial Pathogenesis Questions

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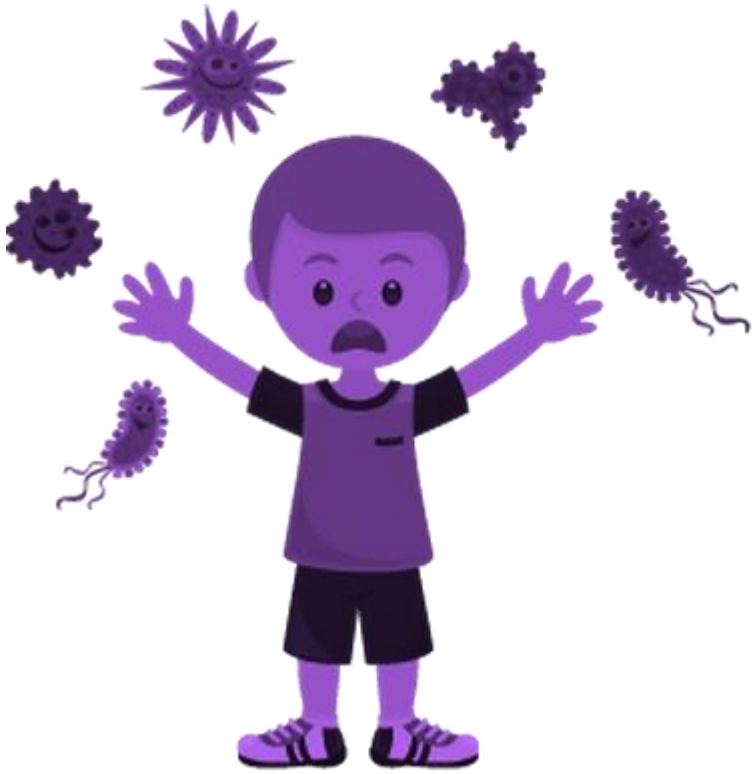


The Case:

21 year old male patient reports burning pain in his penis during urination followed by a greenish discharge.

Recent sexual history includes unprotected sexual intercourse with a new partner about one week ago.

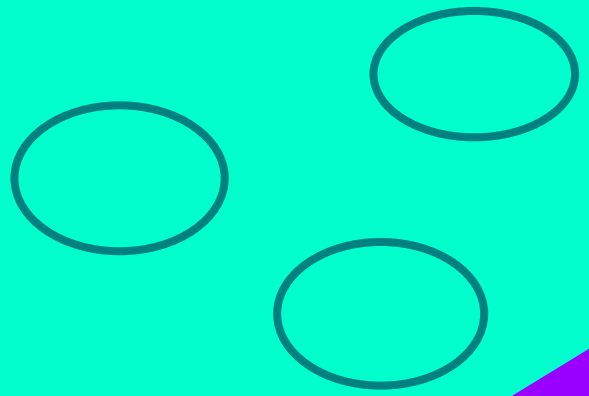
Urine sample has been sent to the Microbiology Laboratory for testing.

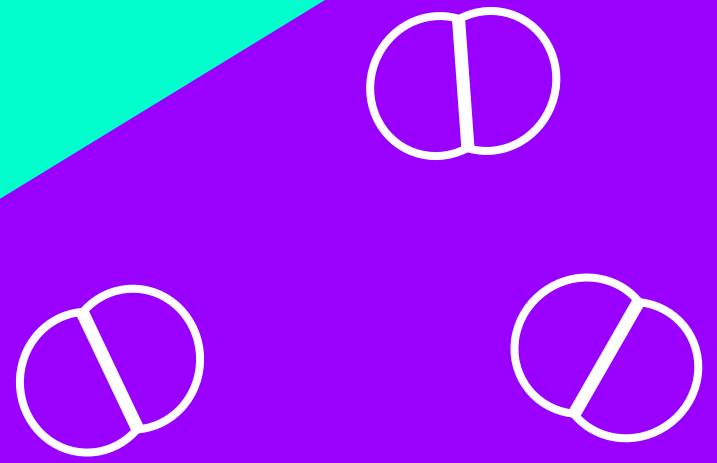
Patient is prescribed antibiotics.

The likely pathogens:

Chlamydia trachomatis

- Gram-negative
- Nonmotile
- Coccoid/rod shaped
- Obligate intracellular parasite





Neisseria gonorrhoeae

- Gram-negative
- Nonmotile
- Diplococci with coffee bean shape

Encounter *Chlamydia trachomatis*

Geographically:
- Very prevalent worldwide
- Especially in South Asia, Africa, Western countries
  - United States – 1.3-5.2%

Flourishes in hot, dry areas with storage of water + standards of hygiene are low

Host Wise:
- Humans – higher prevalence rate in women than men + common in sexually active 15-24 year olds

- Colonizes tissues of eye + urogenital tract
- PV-resident (pathogen containing vacuole)

- Resides/infects columnar epithelial cells or urethra

Bacterial characteristics to allow this:
- Obligate cellular parasite that depends on host for ATP + intermediates
- Two forms:

Reticulate Bodies (RB)
- intracytoplasmic
- replicating form

Elementary Bodies (EB)
- non-replicating
- infectious
- inert until attachment
- transforms to RB form

"bodies" act as a rigid membrane to protect DNA and enzyme from damage by host responses or environmental factors

-> May take on an intermediate persistence state that is less metabolically active until favourable conditions return

- *Shows tropism for these tissue types due to their major outer membrane protein **(MOMP)** mediating attachment*

Encounter *Neisseria gonorrhoeae*

Geographically:

- Prevalent worldwide
- Highest prevalence in the African, Southeast Asian and Western Pacific regions
- Prevalence of 0.75-2.1%

Host Wise:

- Humans – higher prevalence rate in men than women + common in sexually active 15-29 year olds
- Site of infection is site of residence
- Commonly found intracellularly in polymorphonuclear leukocytes (neutrophils) of the gonorrhea pustular exudate

Bacterial characteristics to allow this:

- Only capable of binding and exploiting human transferrin and lactoferrin (iron binding proteins needed for the in vitro growth of the bacteria)
- Pili and Opa proteins – help with adherence

Specific to the microvilli of non-ciliated columnar epithelial cells (present in mucosal cells that line the urethra , cervix, rectum, pharynx, conjunctiva and prepubescent vaginal epithelium)

The patient must have come in contact with one or both of the bacteria through unprotected sexual intercourse (direct genital contact) with his new partner who may have been an asymptomatic carrier.

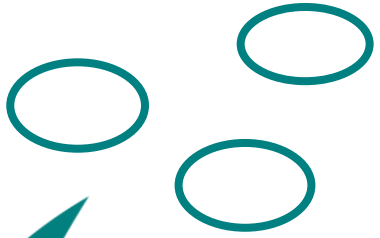
Entry

Chlamydia trachomatis

- Transmitted via sexual contact with infected individuals
- Also transmitted by fomites and flies as well as vertical transmission to newborns

EBs attach to + enter cells

A hemagglutinin may facilitate attachment



Outer membrane protein 2 (**OMP2**)
- Cysteine-rich protein
- Provides stability in the EBs via extensive disulfide cross-links

They then take up residence in the columnar epithelial cells

Host Protein - Protein disulfide isomerase (**PDI**)

2 roles:

1) Structurally required for chlamydial attachment
2) Thiol-mediated redox function required for entry

Intracellularly, they are converted to **RBs**

EB receptors are restricted to nonciliated columnar, cuboidal and transitional epithelial cells – found on the mucous membranes of the urethra, endocervix, endometrium, fallopian tubes, anorectum, respiratory tract and conjunctivae

Initial step of adhesion:
- *OmcB (expressed on bacterial cell surface) binds to host heparin sulfate (**HS**)*

Entry via endocytosis:
- *Translocated actin recruiting protein (**TARP**) is injected into cell via type III secretion system*

-> Leads to rearrangement of actin skeleton to allow endocytosis

Entry

Neisseria gonorrhoeae

- Transmitted via sexual contact with infected individuals

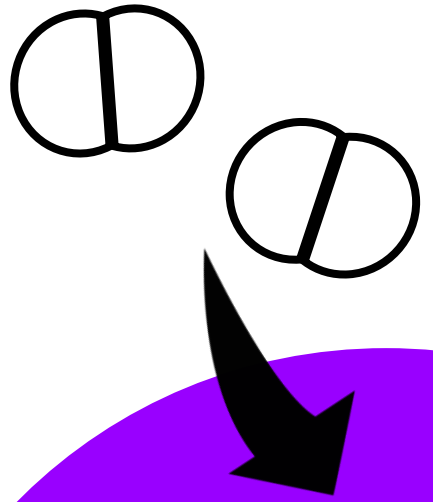
They then take up residence in the columnar epithelial cells

Factors involved in attachment + adherence:

- ***Pili**: Type IV pili binds to CD46 host receptor -> transduces signal to host for Ca2+ mobilization*
- ***Opa** proteins: bind host CD66 + heparin sulfate; also bind carcinoembryonic antigen-related cellular adhesion molecules (CEACAM) -> bacterial internalization + activation of sphingomyelinase -> release ceramides -> combines + acts as membrane platforms for internalization*

Entry via parasite-directed endocytosis:

- *Host syndecan receptors 1-4 required for entry*
- *Uptake mediated by HS chains of syndecan receptors binding to hypervariable regions of OpaA protein*
- *HSPGs binds with Opa proteins (mediated by fibronectin + vitroanectin) -> important for entry + allows persistence in urethra*
- *Por (protein I): possible invasin*
- *IgA protease: cleaves IgA1 + important in colonization*
- *Activation of PC-PLC + ASM is essential of entry into distinct non-phagocytic human cells*



Only attaches to microvilli of non-ciliated columnar epithelial cells

Spread

Chlamydia trachomatis

Exists extracellularly (as EBs) + intracellularly (as RBs)

EBs adsorb to the host cell + utilize glycogen -> will undergo germination + primary differentiation

In the vacuole:
- *DNA genome is transcribed into RNA, proteins synthesized + DNA replicated*

-> done by using host resources

20 hours after entry:
- EBs become RBs
- RBs divide by binary fusion

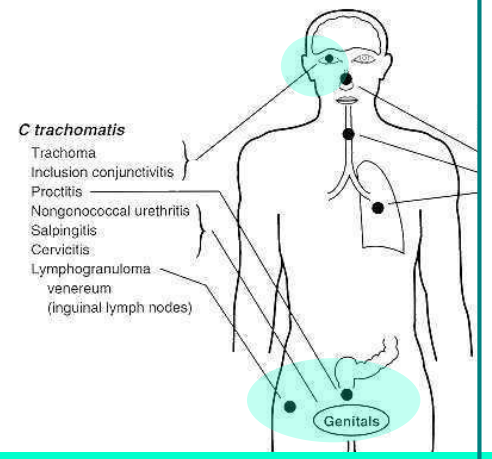
After developing an outer cell wall, they differentiate back into EBs which are exocytosed or lyse the host cell
-> allowing them to spread

EBs can be transferred from the primary urogenital infection site to other tissues (ie. eyes) through contact of contaminated fingers, fomites or towels

- Newborns can be infected by their mother as they pass through the birth canal at birth

(can affect eyes, genitals or rectum)

The patient does not experience symptoms other than of urethritis so it is unlikely that the infection has spread from initial site



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In females: infects cervix -> spread down the endocervical canal (cervitis) -> to endometrium (endometritis) -> to fallopian tubes (salpingitis) -> to pelvic peritoneum (pelvic inflammatory disease PID)

♂

In males: can spread causing non-gonococcal urethritis, epididymitis + procitis

Spread

Neisseria gonorrhoeae

Exists extracellularly + intracellularly

Method of replication:
- *not yet understood*
- *believed to occur within the vacuole after parasite-directed endocytosis*

- Grow primarily in the bloodstream
- Acquire iron from transferrin, lactoferrin + hemoglobin

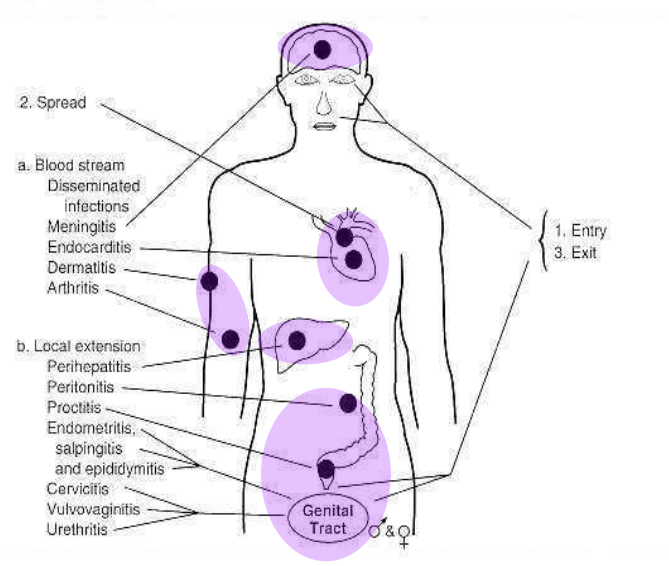
-> expresses Tbp1, Tb2 + Lbp lactoferrin receptors to aid iron acquisition

Localized to the primary site of infection (urethritis, proctitis, pharyngitis, conjunctivitis) -> can disseminate to adjacent organs (PIDs, epididymitis) + bacteremically (septic arthritis, meningitis, endocarditis, tenosynovitis, skin lesions)
-> spread through contractions of pili

♀

In females: can disseminate from endocervical canal -> to endometrium (endometritis) -> to fallopian tubes (salpingitis) -> to pelvic peritoneum (peritonitis)

The patient does not experience symptoms other than of urethritis so it is unlikely that the infection has spread from initial site



Bacterial Damage *Chlamydia trachomatis*

Most harm done to the host is *indirect* through the host's cell-mediated immune response which causes tissue damage

Cytokine upregulation response delayed 20-24 hours after infection

- Host immune response arises and sustained from invaded epithelial cells
- LPS stimulates a relatively weak pro-inflammatory response from macrophages + epithelial cells
- TLR-2 + Myd88 complex plays a large role

Upregulate mRNA expression for: IL-1α, IL-6, IL-8, Growth regulated oncogene + GM-CSF

- Attract macrophages, neutrophils, T-cells
- Upregulate endothelial adhesion molecules
- Increase proinflammatory cytokine secretion of macrophages

Tissue damage occurs by:

- Matrix metalloproteases (MMPs): enzymes released by infected epithelial cells + neutrophils -> tissue damage via Proteolysis
- Neutrophil elastase: protease secreted by neutrophils that destroy both bacteria + host tissue
- Radicals generated through respiratory burst activity of macrophages + neutrophils kill bacteria + damage host tissue
- TNF-α: induces apoptosis in immune cells + can damage tissue

This immune response results in redness, edema, inflammation, destruction of healthy host tissue + mucropulent discharge

The pain that the patient feels is due to the ongoing immune response. The green discharge is due to dead bacterial cells, immune cells + tissues damaged in the immune response.

Bacterial Damage *Neisseria gonorrhoeae*

Most harm done to the host is *indirect* through the host's cell-mediated immune response which causes tissue damage

Two primary components are responsible for the damage:

1. Lipooligosaccharide (LOS)
- an endotoxin on the outer membrane -> activates inflammatory response -> host damage
- Lipid A phospholipid causes toxic effects
- Triggers cytokine up-regulation by cell contact
- LOS binds LPS in serum -> complex binds to CD14 receptors on macrophages -> LOS-LPB-CD14 complex triggers signalling events through TLR-4 -> activates NF-κB transcription
- Can also trigger inflammatory response through epithelial cells with CD14 complex
- Also induced: TNF-α, TGF-β, GM-CSF, IL-1α, IL-1β, IL-6, IL-8, IL-12 + MCP-1

1. Peptidoglycan (PG) Fragments
- Released as part of the growth process -> triggers innate immune response
- CD14 on macrophages is activated + triggers NF-κB transcription
- Epithelial cell Nod1 detects muropeptide of PG -> NF-κB transcription + inflammation

- Cytokine + chemokine upregulation result in inflammation + tissue damage at site of infection
- Bactericidal activity in the area causes collateral damage to tissues + accumulation of dead cells
- Ciliated cells suffer the most

The pain that the patient feels is due to the ongoing immune response. The green discharge is due to dead bacterial cells, immune cells + tissues damaged in the immune response.