Hilury Ha
PATH 417A
Case 2 – Q3

Introduction

Patient Naser experiences dysuria and green discharge from his penis after having unprotected sexual intercourse with a new partner. These symptoms are similar to those characterized in many sexually transmitted diseases and genitourinary infections. Based on his symptoms of painful urination and green discharge, it is likely that they are caused by *Neisseria gonorrhoeae* and/or *Chlamydia trachomatis*.

I. Encounter

A) *Chlamydia Trachomatis*

Chlamydia trachomatis are bacteria that infect both birds and mammals, but are mostly limited to humans, particularly in the tissues of the eye and urogenital tract (Todar, n.d.). These bacteria commonly cause chlamydia, one of the most prevalent sexually transmitted diseases in Western countries (Lee et al., 1995). C. *trachomatis* infection is more common in younger age groups when compared to other sexually transmitted diseases such as gonorrhoea (Zimmerman et al., 1990). *C. trachomatis* is widely distributed around the world, but recent studies indicate the emergence of new genotypes in regions of Europe, Sweden, and Spain (Bebear & de Barbeyrac, 2009). In particular, geographic distribution of different genotypes of *C. trachomatis* appears to be dependent on sexual behaviour (Piñeiro et al., 2014). For example in Spain, genotype L2b is more common in men who have sex with men, while genotypes E and F are more frequent in women and heterosexual men (Piñeiro et al., 2014). *C. trachomatis* can also cause non-gonococcal urethritis, characterized by clear discharge and dysuria (Ronald & Alfa, 1996).

As obligate intracellular parasites of eukaryotic cells, Chlamydia bacteria must remain inside the host in order to sustain metabolism due to their lack of ability to produce adequate ATP independently (Todar, n.d.). Within the human body, *C. trachomatis* resides and replicates within the epithelial monolayer lining the reproductive tract (Johnson, 2013). Attachment to these cells is mediated by hemagglutinin (Todar, n.d). *C. trachomatis* load in the human genital tract also varies based on gender. In females, *C. trachomatis* colony concentration was found to be highest in cervical swabs, and lowest in urine specimens (Vodstrcil et al., 2014). On the other hand, *C. trachmatis* load in men were found to be highest in rectal swabs, and similar for urethral swabs and urine specimens (Vodstrcil et al., 2014). Chlamydiae exist in two forms: as non-replicating and infectious elementary bodies (EB), or as intracytoplasmic reticulate bodies (RB) (Figure 1) (Becker 1996). In elementary bodies, the presence of a rigid cell wall protecting the DNA genome and RNA polymerase needed for transcription during transmission between individuals allows Chlamydiae to persist (Becker 1996). During bacterial development stages, the DNA genome, proteins, and ribosomes needed are protected by the membrane bound reticulate bodies (Becker 1996). Therefore it is evident that there are suitable protective mechanisms in place for the pathogen to ensure survival throughout the human environment.

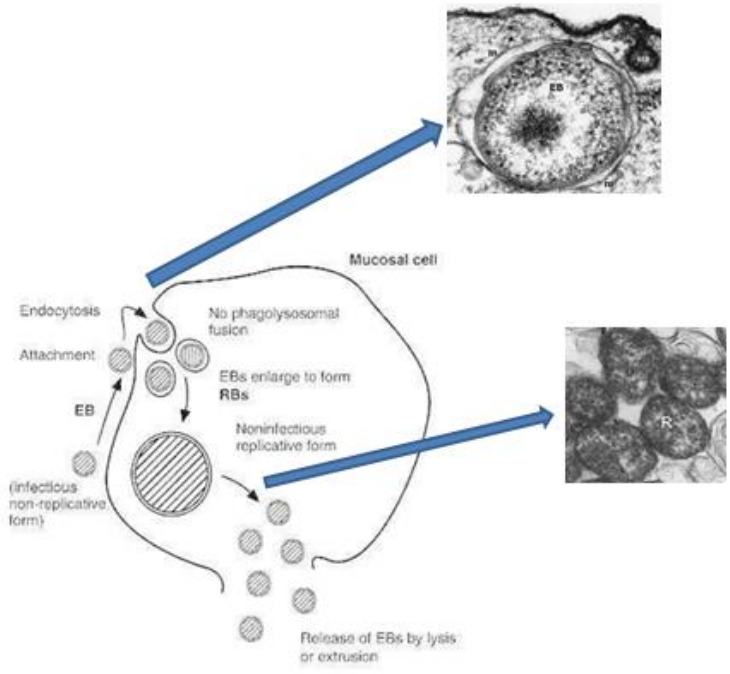

Figure 1. Replication cycle of *Chlamydia*. Image adapted from Ronald & Alfa (1996). Elementary body (EB) after entry into cell, indicating membrane (m) as well as size comparison to clathrin-coated pit, and reticulate bodies (R). Images adapted from *Chlamydial Cell Biology* by M. Ward (2007).

B) *Neisseria gonorrhoeae*

Geographically, *Neisseria gonorrhoeae* is present worldwide and most commonly cause gonorrhoea infections in people between age 15 and 29, with higher infection rates in males (Morse, 1996). *N. gonorrhoeae* are exclusively human pathogens because they are only capable of binding human transferrin and lactoferrin, which are required for bacterial growth (Morse, 1996). Distribution in hosts depends on the number of sexual partners, sexual preference, contraceptive sex practices, and population mobility (Morse, 1996). *N. gonorrhoeae* are Gram-negative aerobic bacteria that are not part of the human normal flora; rather, they are only acquired upon sexual or direct contact with an infected individual (Todar, 2008). *N. gonorrhoeae* commonly reside in the mucus membranes of the urethra in males, and the endocervix and urethra in females (Todar, 2008), although infection in the pharynx and rectal sites have been reported (Li & Wong, 2009).

Similar to *C. trachomatis*, *N. gonorrhoeae* also possesses molecular characteristics that render them well suited for survival in the human environment. The bacteria persist by attaching to non-ciliated epithelial cells through the use of pili (fimbriae) and Opa proteins, and through the production of lipopolysaccharide endotoxin (Todar, 2008). In addition, the production of IgA proteases promotes virulence (Todar, 2008). After attachment the bacteria enter epithelial cells

through endocytosis in a membrane bound vacuole, therefore protecting the bacteria throughout the process (Todar, 2008). Globally, *N. gonorrhoeae* strains have also become increasingly resistant to several antimicrobial agents such as penicillin, tetracycline, quinolones, and third-generation cephalosporins (Li & Wong, 2009), thereby allowing them to persist in hosts and limiting therapeutic choices available for treatment.

Likely Method of Contact:

The primary risk factors for genital infection with *C. trachomatis* and *N. gonorrhoeae* infection in young, sexually active persons such as Naser, is engagement in unprotected sexual intercourse. This is the most likely method of contact between Naser and these bacteria based on his recent sexual history. It has been found that about 1/3 of heterosexual men infected with *N. gonorrhoeae* are also co-infected with *C. trachomatis* (Ronald & Alfa, 1996) therefore it is likely that there may be more than one pathogen responsible for Naser's symptoms; however microbiology laboratory testing would be required to determine the pathogens responsible.

Although Naser's partner claims that she does not have any sexually transmitted diseases, given that she had previously been sexually active, it is likely that she is a carrier of those bacteria, but does not recognize this because she is asymptomatic. For example, many *C. trachomatis*-positive women are asymptomatic, without any correlation with demographic and sexual characteristics (Morre et al., 2002). Furthermore, about 50% of women with cervical *N. gonorrhoeae* infection are asymptomatic (Todar, 2008) which contributes to the likelihood that Naser's new partner is a carrier of sexually transmitted disease. Therefore engaging in unprotected sexual intercourse with Naser is a very likely reason for his contact with these bacteria. However, it would be necessary to bring her in for testing as well to confirm the identity/identities of bacteria present (if any).

II. Entry

A) *C. trachomatis*

Upon entering the host, *C. trachomatis* commonly reside in the tissues of the eye and mucosal surfaces of the urogenital tract (Todar, 2008). Genital infections with *C. trachomatis* occur through sexual transmission, whereas transmission of the bacteria from the urogenital tract to the eyes and vice versa can occur through direct contact with contaminated fingers, towels, or fomites (Becker, 1996). As obligate intracellular parasites of eukaryotic cells, *C. trachomatis* are dependent on their host for energy metabolism and biosynthetic pathways (Becker, 1996). As discussed in Section I, Chlamydiae exist as elementary bodies (EB) and as reticulate bodies (RB). Elementary bodies are small particles about 0.25 to 0.3μm in diameter that are released from ruptured infected cells during transmission from one individual to another (Figure 1) (Becker 1996). On the other hand, reticulate bodies are slightly larger (0.5-0.6μm) than EB's and are prevalent during the replication and growth phases of the bacteria (Becker 1996). The rigid cell walls of EB's protect the DNA genome, RNA polymerase, ribosomes, and ribosomal

subunits needed to initiate growth after entry into host cells (Becker, 1996). Similarly in RB's, the DNA genome and necessary proteins and ribosomes are retained and protected within the membrane bound cell (Becker, 1996).

A common method that pathogens utilize to attach to host cells is by expressing adhesins that are capable of binding sulphated glycosaminoglycans (GAGs) which are conveniently widely distributed on the outermost surface of host tissues (Menozzi et al., 2002). In order to attach to host cells, *C. trachomatis* produces a heparin sulphate-like ligand that enables it to bind to a complementary glysosaminoglycans-binding receptor (Menozzi et al., 2002). To further mediate adherence to host epithelial cells, a major outer membrane protein of *C. trachomatis* is a cytoadhesin with lectin affinity for heparin sulphate-containing receptors on epithelial cell surfaces (Menozzi et al., 2002). Elementary bodies also possess a cysteine-rich outer membrane complex protein called OmcB that reinforces binding with GAGs through a heparin-dependent mechanism (Moelleken & Hegemann, 2008). Overall, studies indicate that OmcB proteins are essential for EB's to bind to target cells and thus remains heavily conserved across many strains of Chlyamidiae (Moelleken & Hegemann, 2008).

B) *N. gonorrhoeae*

Transmission of *N. gonorrhoea* is almost exclusively through sexual intercourse. Within the host, the bacteria commonly reside within the mucus membranes of the urogenital tract, the rectum, pharynx, and conjunctiva (Todar, 2008). Intracellularly, *N. gonorrhoeaa* are found in polymorphonuclear leukocytes of gonorrhoea pustular exudate (Todar, 2008). *N. gonorrhoeae* are highly adapted to attachment to human host tissues such as the urogenital mucosa through multiple mechanisms (Figure 2). In particular, *N. gonorrhoeae* attach only to non-ciliated columnar epithelial cells, and after entry through endocytosis begin multiplication in the basement membrane (Figure 3) (Todar, 2008). For initial attachment, *N. gonorrhoeae* rely on the use of pili (such as PilE protein), whereas subsequent adherence steps leading to stronger attachment are mediated by other membrane opacity-associated proteins (Opa proteins) (Figure 2) (Todar, 2008). The presence of these Opa proteins which utilize protein-protein interactions to adhere to carcinoembryonic antigen-related cellular adhesion molecules (CEACAM) render *N. gonorrhoeae* to be highly specified to epithelial cells and neutrophils (Menozzi et al., 2002). Por proteins also play a role in pathogenesis by mediating the passage of solutes through the outer membrane and allowing for survival of the bacteria inside phagocytes (Todar, 2008).

In addition, syndecan receptors 1-4 have been found to be required for *N. gonorrhoeae* entry into human mucosal and epithelial cells (Menozzi et al., 2002). Uptake is mediated by interactions between the heparin sulphate chains of the syndecan receptors and the hypervariable region of OpaA protein. Syndecan receptors' C-termini contain an intracellular EFYA amino acid motif which allows it to interact with the PDZ domain of syntenin that is responsible for the transmission of extracellular signals to the cytoskeleton and the activation of signal pathways (Menozzi et al., 2002). Therefore OpaA proteins in *N.gonorrhoeae* influence host cytoskeleton-

membranes through syndecans-syntenin interactions, ultimately leading to enhanced bacterial infections (Menozzi et al., 2002). Binding of heparin sulphate by Opa proteins is also important to protect the bacteria from elimination in human serum. By binding to sulphated glycosaminoglycans, *N. gonorrhoeae* is able to persist in the urethra. Entry of OpaA gonococci into cells also relies on the action of fibronectin and integrin receptors (Menozzi et al., 2002).

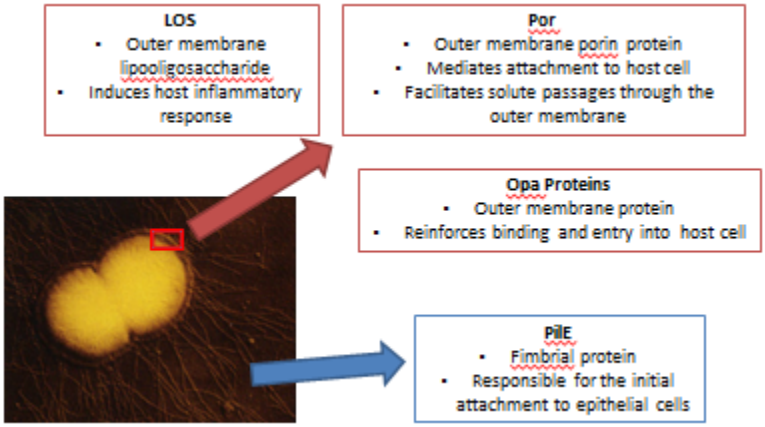

Figure 2. Surface proteins of *N. gonorrhoeae* involved in attachment to host cells. Image adapted from Todar (2008).

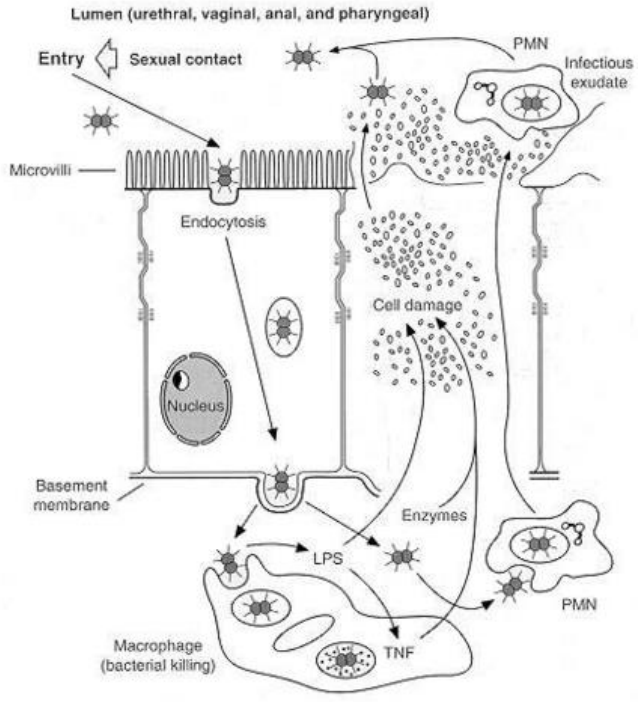

Figure 3. Attachment, entry and spread mechanism of *N. gonorrhoeae* in host cells. Image reproduced from Todar (2008).

III. Multiplication and Spread

A) *C. trachomatis*

C. trachomatis are intracytoplasmic obligate parasites that transmit from infected tissues to other uninfected tissues in the form of elementary bodies (Becker, 1996). After attachment and entry into the cells of the new site, the elementary bodies develop and mature into intracytoplasmic reticular bodies (Figure 1) thus augmenting the infection process in the new tissue (Becker, 1996). *C. trachomatis* also remains intracellular due to the bacteria's reliance on host cells for metabolic pathways and ATP (Becker 1996). However, while disseminating to new sites, EB's protect the DNA genome and vital proteins required for development using its rigid cell wall as discussed in section II.

An additional molecular determinant of intracellular survival of *C. trachomatis* is its Type III secretion system (TTSS). During intracellular chlamydial growth, TTSS promotes the delivery of pathogen effector proteins as well as the secretion of virulence factors that modulate host cell function, promote invasion, and intracellular survival (Byrne, 2010). An example of a secreted effector protein that promotes intracellular survival is IncA, which mediates *C. trachomatis* vesicle fusion with host cell membranes (Hackstadt, Scidmore-Carlson, Shaw, & Fischer, 1999). In addition, phosphorylation of *C. trachomatis* translocated actin recruiting phosphoprotein (TARP) promotes the internalization of the bacteria through an actin-mediated mechanism (Bryne, 2010), thus limiting the amount of time that the bacteria spend in the extracellular environment, where they may be potentially eliminated by the host immune response.

Modifications at the genetic level are also crucial to the survival of *C. trachomatis* as the pathogen adapts to changes in the environment. Like many organisms, this is achieved by coupling an environmental sensor to a transcriptional regulator. For example, in environments where nutrients are limited, *C. trachomatis* will activate tryptophan biosynthetic genes, and utilize indole produced by bacterial flora as a substrate for synthesis (Bryne, 2010).

In particular, genital tract infections are spread through sexual contact and act as a reservoir for elementary bodies that can be spread to other tissues (Figure 5). In the urogenital tract, *C. trachomatis* can spread into different regions and cause non-gonoccocal urethritis, epididymitis, and proctitis in males (Figure 4) (Becker, 1996). In females *C. trachomatis* infection can cause salpingitis and cervicitis (Becker, 1996). Genital infections with *C. trachomatis* have often been found to occur alongside gonorrhoea, and can even ascend the cervix and spread to other locations such as the fallopian tubes (Becker, 1996). Neonates can also be infected with *C. trachomatis* during birth if they pass through an infected birth canal, leading to acute conjunctivitis (Becker, 1996). Direct contact through contaminated fingers, towels or fomites can transmit the elementary bodies from the primary urogenital infection site to other tissues such as the eye. In the eye *C. trachomatis* can cause trachoma and inclusion conjunctivitis by causing inflammation in the ocular tissue, leading to follicle development, scarring and ultimately

blindness once the cornea becomes cloudy and vascularized (Hu, Holland, & Burton, 2013). However, Naser does not experience any of these other symptoms which suggests that the bacteria have not spread from their initial site.

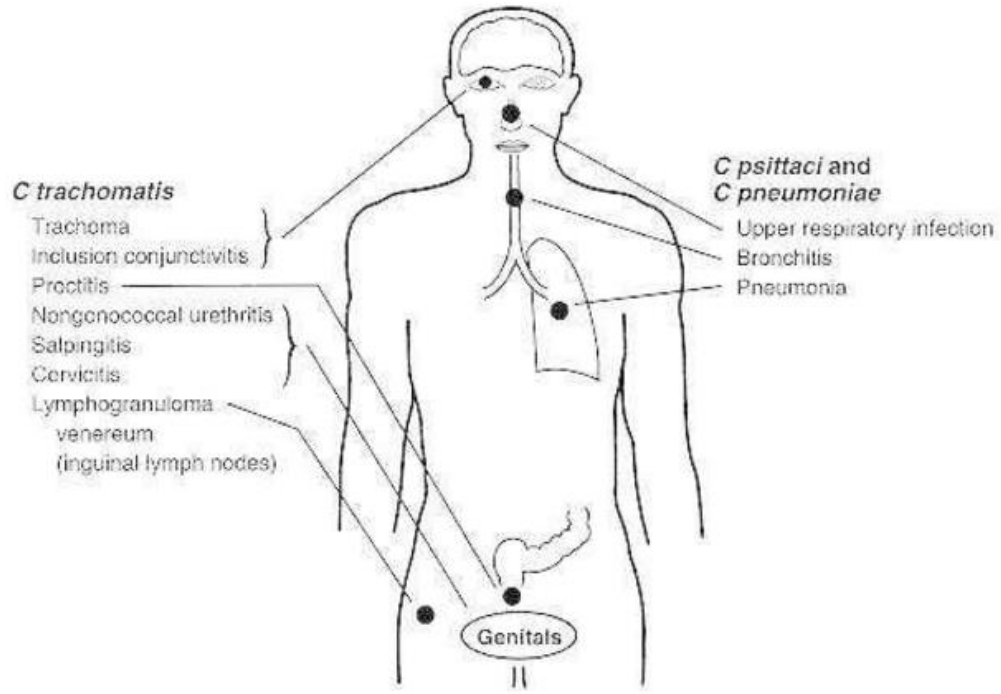

Figure 4. Clinical Manifestations of Chlamydia infections. Image reproduced from Becker (1996).

B) *N. gonorrhoeae*

N. gonorrhoeae are obligate human pathogens capable of entering the blood stream and spreading to other sites in the body (Todar, 2008). However, after attachment to the new infection site, they remain intracellular due to their reliance on human transferrin and lactoferrin for growth (Morse, 1996). A method that the bacteria use to spread to other sites (Figure 5) without being eliminated by the host immune response is through modifications of its lipooligosaccharides (LOS). The bacteria are able to persist in the human body despite the bactericidal serum present due to its ability to utilize host-derived cytidine monophospho-N-acetylneuraminic acid to convert its serum-sensitive LOS into a serum-resistant form through sialylation (Morse, 1996). *N. gonorrhoeae* also possess the ability to alter the antigenic types of LOS expressed, and it has been found that the antigenic similarity between LOS antigens and those of human erythrocytes contribute to the immunotolerance exhibited by the host (Todar, 2008), making this pathogen difficult to eliminate.

Dissemination through the bloodstream can cause dermatitis-arthritis syndrome in which patient's exhibit fever, chills, skin lesions, and arthralgias due to inflammation of the tendon

sheaths (Morse, 1996). Bacteremic spread of *N. gonorrhoeae* can also cause skin lesions, tenosynovitis, septic arthritis, endocarditis, and meningitis (Morse, 1996). In females, *N. gonorrhoeae* can cause cervicitis, urethritis, pelvic inflammatory disease, and lead to long-term complications such as infertility, ectopic pregnancy, chronic pelvic pain, and increased susceptibility to HIV infection (Li & Wong, 2009). Furthermore, *N. gonorrhoeae* can spread through the endometrium into the fallopian tubes and pelvic peritoneum, leading to endometritis, salpingitis, and peritonitis (Morse, 1996).

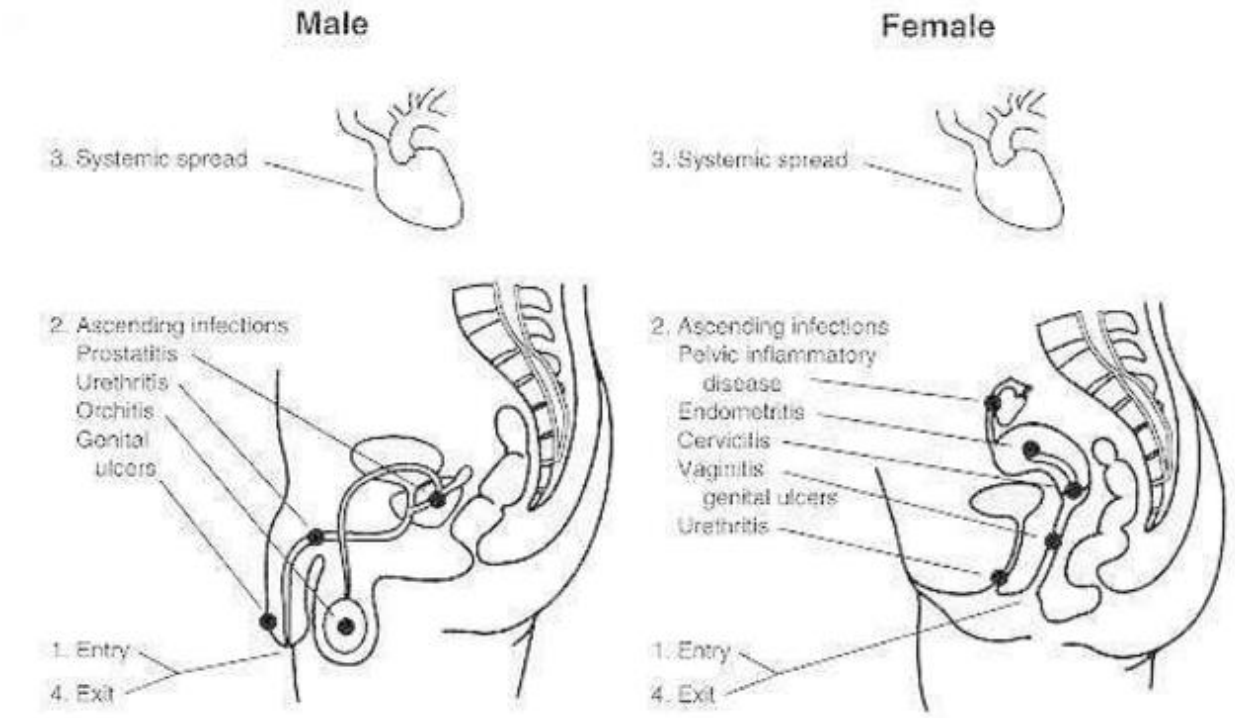

Figure 5. Pathogenesis of genital tract infection. Image reproduced from Ronald & Alfa (1996).

IV: Bacterial Damage

A) *C. trachomatis*

C. trachomatis predominantly cause tissue damage to the host through cell-mediated immune response as well as endotoxin-like toxin release (Becker, 1996). Upon infection, *C. trachomatis* initiate the inflammatory response by binding to, and activating the innate Toll-like immune receptor (Darville & Hiltke, 2010). Immune receptor activation induces the release of interleukin-1α, interleukin-1β, and tumor necrosis factor (TNF) alpha cytokines leading to chronic inflammation, scarring, and tissue damage (Becker, 1996). Further interleukin (IL)-1α and interleukin-1β release by monocytes is induced by *C. trachomatis* lipopolysaccharide (Becker, 1996). Naturally, infection will prompt the activation of the host immune system to protect the body from infection and disease. However it has been found that the T cell response

may actually contribute to the tissue damage seen in *C. trachomatis* infections (Darville & Hiltke, 2010).

In addition to the initial release of cytokines and chemokines from resident tissue macrophages, Chlamydiae infection in epithelial cells causes the release of IL-1, TNF, IL-8, growth-related oncogene (GRO) alpha, IL-6, and granulocyte-macrophage colony stimulating factor (GM-CSF), which attracts host immune cells (Figure 6) (Darville & Hiltke, 2010). Furthermore, the release of matrix metalloproteases (MMP's) from infected epithelial cells causes proteolysis and subsequently reinforces the host immune response by attracting neutrophils, natural killer cells, and monocytes (Figure 6). The attraction of neutrophils is important in eliciting the tissue damage characteristic of Chlamydiae infections. Once recruited to the infection site, neutrophils will further release matrix metalloproteases and elastase which contributes to the already existing tissue damage.

Infection of epithelial cells by *C. trachomatis* induces the host immune response, causing up-regulated release of cytokines with chemoattractive and pro-inflammatory functions. The proteolysis activity resulting from the host immune response causes tissue damage and is thus responsible for the painful urination exhibited by Naser.

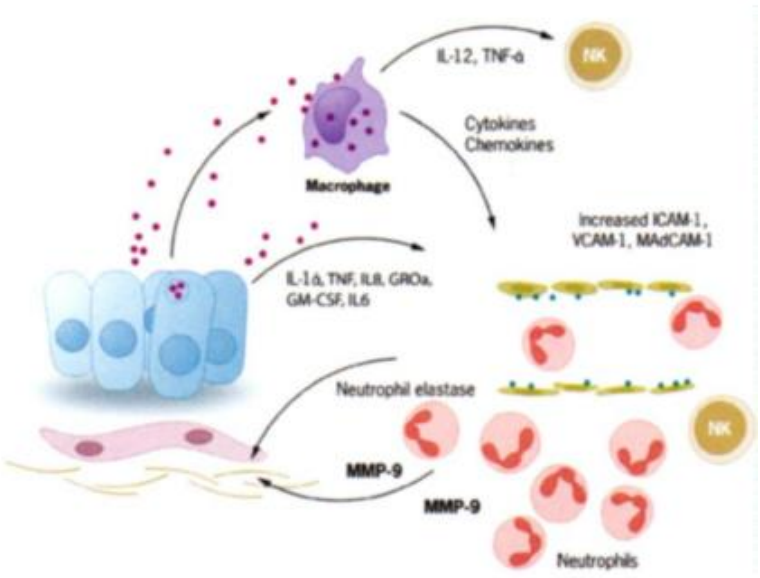

Figure 6. Host immune response to *C. trachomatis* infection. Image reproduced from Darville & Hiltke (2010).

B) *N. gonorrhoeae*

Lipooligosaccharide (LOS) of the *N. gonorrhoeae* outer membrane is responsible for the inflammation and tissue damage exhibited in gonorrhoea infections due to its ability to trigger inflammatory responses from the host. *N. gonorrhoeae* causes urethritis, specifically

gonorrhoea, by infecting the mucus membranes or the urethra. After entry into the host, LOS contributes to tissue damage by inducing the release of proteases and phospholipases, leading to proteolysis and cell membrane breakdown (Todar, 2008). Furthermore, the presence of LOS within host cells stimulates tumor-necrosis factor release which augments cell damage (Todar, 2008). The dysuria that Naser experiences is due to tissue damage and the associated inflammation of urethral tissues which causes swelling, heat, and pain in the region. In addition, Naser exhibits green discharge, characteristic of gonorrhoea infection. During infection, the host immune response causes local neutrophilic activity and lysis of phagocytes, thus resulting in purulent discharge (Morse, 1996).

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