PATH 417

Q4 THE IMMUNE RESPONSE

FRANK LAM CHECK LAST PAGE NEISSERIA

1. Host Response

WHAT ELEMENTS OF THE INNATE AND ADAPTIVE (HUMORAL AND CELLULAR) IMMUNE RESPONSE ARE INVOLVED IN THIS INFECTION?

Innate Response

Innate Response
Anatomical Barrier

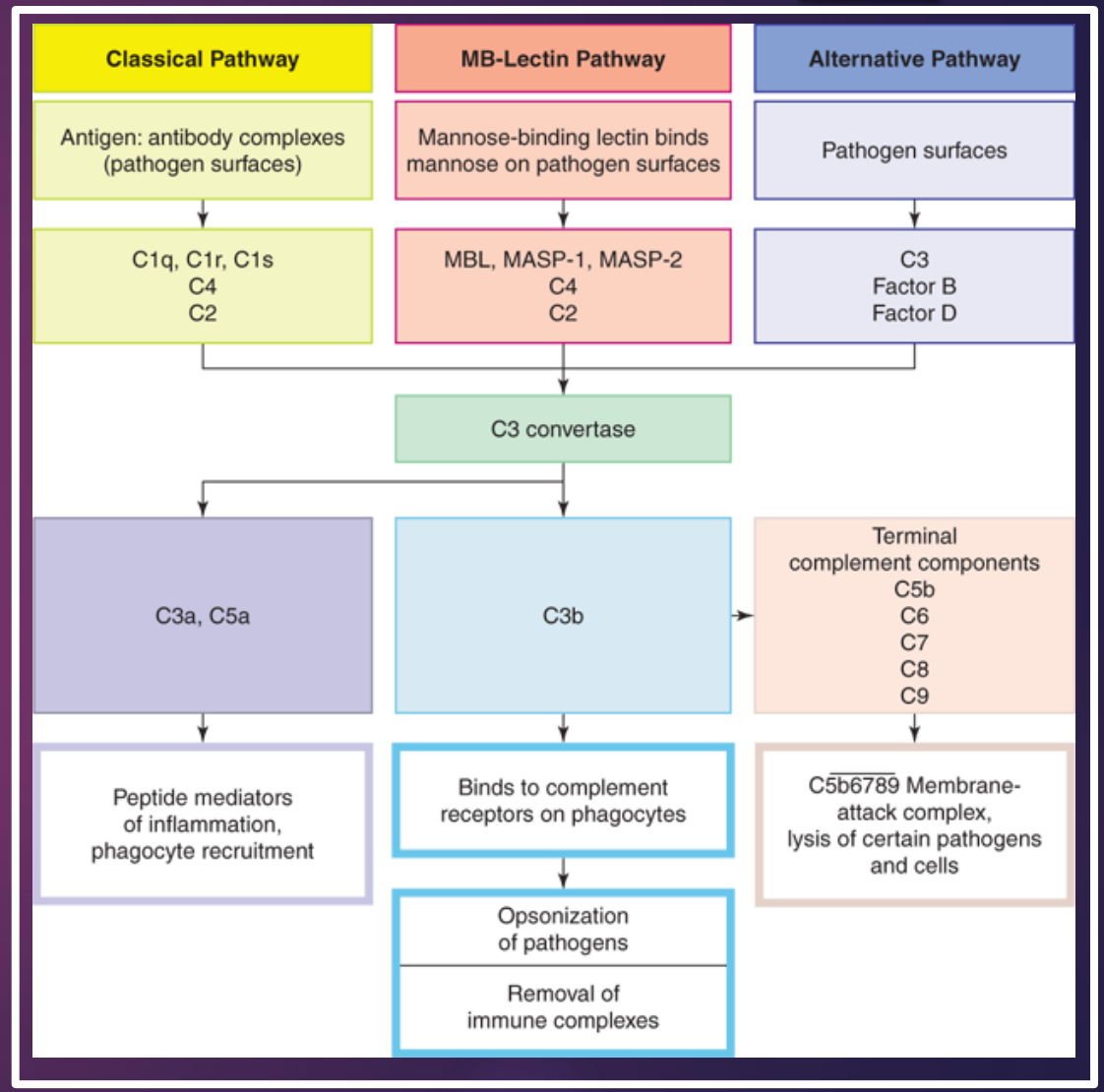
- Target of N. gonorrhoeae & C. trachomatis is the columnar epithelial cells in Nasar's urethra
  - The **mucus layer** is the first anatomical line of defense at the urethral epithelium[1]
  - Some of the defensive properties of the mucous layer include:
    - Mucins – Hydrophilic glycoprotein that is negatively charged, making it more difficult for bacteria to adhere[2]
    - Defensins – Secreted by epithelia and leukocytes, they are antimicrobial peptides that permeabilize the bacterial cell membrane which inhibits RNA, DNA and protein synthesis[1,3]
    - Lysozymes – Enzyme that can digest proteoglycan[1]

Innate Response
Complement System

- Complement is a set of ~20 proteins that act to[4]:
  1) Lyse bacterial cells
  2) Enhance phagocytosis (**opsonization**)
  3) Upregulates mediators that induce proinflammation and neutrophil attraction
- Complement is activated along 3 pathways that lead to these effects[4]:
  - Classical pathway - Activated through antibody-antigen complexes
  - MB-Lectin pathway – Mannose-binding lectin binds to mannose on bacterial surface
  - Alternative pathway – Activated by bacterial cell wall components such as lipopolyssacharide (LPS)
- N. gonorrhoeae and C. trachomatis is likely to trigger all 3 pathways

Innate Response
Complement System

- All pathways lead to the generation of **C3 convertase** which activates the effectors for the various biological effects of complement[4]
- C5b,6,7,8,9 – Cytolysis of cell via membrane attack complex[4]
- C3b – Opsonizes bacterium and required for some immune cells to phagocytose bacteria[1]
- C3a, C4a, C5a – degranulation of mast cells which increases vascular permeability



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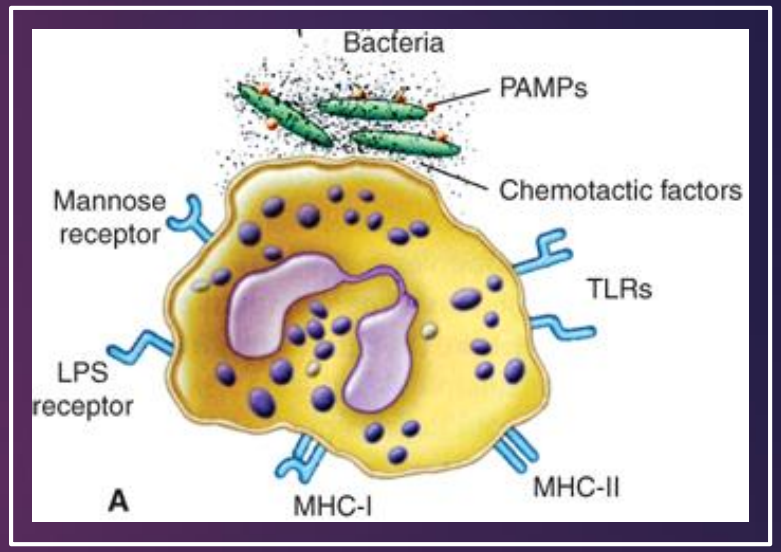
Innate Response
Cellular Mediated

- Invariant Pathogen-associated molecular patterns (PAMPs) on bacteria is recognized by host Pattern-recognition receptors (PPRs) which are present in the cell membrane, extracellularly and within cytoplasmic compartments[2]
- Prominent examples of PAMP and PPR complexes include peptidoglycan with Toll-Like receptor 2 (TLR-2) and LPS with TLR-4[1]
  - Binding to TLRs trigger pathways that lead to cytokine induction and recruitment of immune cells
    - A few important cytokines: TNF-a, GM-CSF, IL-1, IL-6, IL-8, and MCP-1



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Innate Response
Cellular Mediated

- Some of the functions of cellular mediated cytokines:
  - **IL-1** – Mediates polymorphonuclear leukocyte (PMN response) which includes recruitment of neutrophils[1,4]
  - **IL-8** – Attracts PMNs and T-cells (chemotaxis)[4]. Induces degranulation of PMNs[1]
  - **MCP-1** – Chemotaxis of macrophages[5]
  - **TNF-a** - Helps neutrophils kill more effectively by activating phagocytosis and respiratory burst[4]
  - **GM-CSF** – Granulocyte macrophage colony stimulating factor. Promotes macrophage proliferation[5]

Innate Response
Cellular Mediated

- Generally the cellular mediated response is about attracting immune cells such as macrophages and neutrophils to the site of injury so that they can kill the pathogen
- This is mediated through phagocytosis and **respiratory burst**
  - PMNs and macrophages can ingest the bacteria by invaginating their cell wall around the bacteria, forming a phagosome[4]
    - IgG and/or C3b that has bound to the bacteria (opsonization) can be recognized by Fc receptors on PMNs and macrophages, enhancing the effectiveness of phagocytosis[4]
  - Both kill the pathogen by fusing lysosomal granules containing degradative enzymes such as proteases and lipases with the phagosome[4]
  - They are also exposed to **hypochlorite**, **superoxide radicals** and **hydrogen peroxide** which damage cell walls and membranes, killing the bacteria[4]

Innate Response Cellular Mediated

C. Trachomatis | TLR-2

- Triggered primarily by C. trachomatis inclusion[6]
  - Leads to upregulation of mRNA expression for IL-1a, IL-6, IL-8, Growth regulated oncogene (GROa) and GM-CSF[6] in epithelial cells

N. Gonorrhoeae | TLR-4

- Triggered primarily by lipooligosaccharide (LOS) of N. gonnorhoeae[7]
  - Leads to upregulation of TNF-a, TGF-β, GM-CSF, IL-1a, IL-1β, IL-6, IL-8, IL-12, and MCP-1 in epithelial cells[5]

Although C. trachomatis can trigger TLR-4 pathways, it primarily acts via the TLR-2 pathway. Both TLR-2 and TLR-4 pathways lead to a similar response[8].

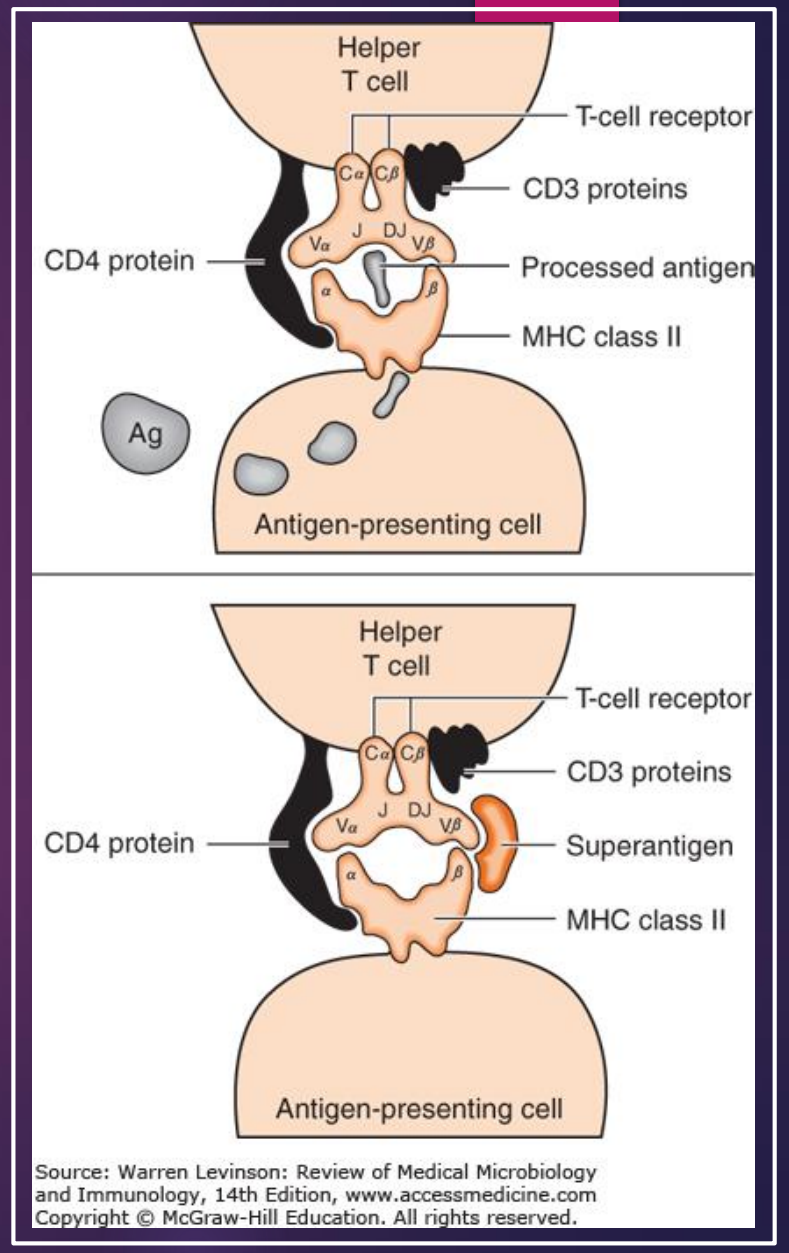
Adaptive Response

Adaptive Response
Cell-mediated Immunity

- Unlike innate immunity, an adaptive response possesses long-term memory and mounts a **specific response** to a pathogen[1]
- **Cell-mediated immunity** is one of two branches of the adaptive response[1]
- Helper T (CD4+) and Cytotoxic (CD8+) T-cells are the main players in this response[4]
  - Before they can be put into play, they must first be **ACTIVATED** by an antigen-presenting cell (APC) such as a macrophage, B-cell or dendritic cell[4]
  - Foreign proteins or bacteria are ingested by APCs, then its fragments are presented on the surface of the cell membrane by forming a complex with MHC. These are known as **antigens** as they can stimulate an immune response[1]

Adaptive Response
Cell-mediated Immunity

- Dendritic cells are the primary APC for presentation and travel to the lymph node, where it presents the antigen to a T-cell with a matching receptor to the antigen[4]
- Different T-cells respond to different MHC classes and also have different functions[4]
  - MHC I – Bind to peptides that are generated in the cytoplasm. Ubiquitous across nucleated cells[1]
  - MCH II – Bind to fragments from outside of the cell. Only on certain cells such a macrophages, B cells and dendritic cells[1]
- After presentation, the T- cells become activated[4]



Adaptive Response
Cell-mediated Immunity

Helper T (CD4+)[1]

- Recognize MHC II complexes
- Branch off into **T_H1** or **T_H2** pathways
  - **T_H1** - Cell-mediated immunity
  - **T_H2** – Humoral immunity. Produces antibodies
- **T_H1** cells enhance macrophages via gamma interferon, increasing production of oxidative radicals[4]
- N. gonorrhoea and C. trachomatis infections favor $T_H1$pathways

Cytoxic (CD8+)[1]

- Recognize MHC I complexes
- Kills specific cells expressing the epitope they are targeting [1]
- Release several cytotoxins:
  - Perforin – Open a channel in the membrane[4]
  - Granzymes – proteases that degrade cell membrane, causing contents to leak and killing cell[4]

Adaptive Response
Humoral Immunity

- Naiive B cells produce antibodies in the humoral immunity branch of the adaptive response[4]
- First they must be activated by T_H2 cells before they can begin producing antibodies[4]
- Antibodies can bind bacteria with the corresponding antigen to cause number of effects[4]:
  - Neutralization – Blocking cellular adhesion[7]
  - Opsonization[4]
  - Complement activation[4]
- IgG and IgA are the primary antibodies produced to combat a C. trachomatis infection and function to opsonize and lyse the cell

2. Host Damage

WHAT DAMAGE ENSUES TO THE HOST FROM THE IMMUNE RESPONSE?

Neisseria gonorrhoeae
Host damage

- N. gonorrhoeae infections results in damage to Nasar's epithelial cells lining his urethra[1]
- This is caused by the **inflammatory response** that N. gonorrhoeae triggers and is characterized by pain in the urethra, a burning sensation while urinating and purulent discharge[1]
- The LOS component of N. gonorrhoeae illicits a strong innate immune response, upregulating NF-κB transcription, and consequently IL-1, IL-6, IL-8, MCP-1, GM-CSF and TNF-a production[5].
  - IL-1, IL-8, MCP-1 recruit macrophages and neutrophils to the site and GM-CSF promotes marcrophage proliferation[4]
- IL-8 induces granulation of neutrophils while TNF-a enhances phagocytosis and respiratory burst in macrophages and neutrophils, enhancing their killing power[4]
- Peptidoglycan fragments released by N. gonorrhoeae also stimulate the immune response and contributes to the damage[9]

Neisseria gonorrhoeae
Host damage

- When macrophages and neutrophils engage in phagocytosis and respiratory burst, degradative enzymes such as elastase, and reactive oxygen species such as hydrogen and superoxides that are released also damage healthy tissue[1,4]
- The accumulation of neutrophils and macrophages at the site of infection combined with their killing action damages healthy tissues at the urethral epithelium, causing pain
- The waste products of the immune response – dead neutrophil, bacterial, host cells and fluids result in the exudate that comes out of Nasar's penis

Chlamydia trachomatis
Host damage

- The signs and symptoms of a C. trachomatis infection is very similar to N. gonorrhoeae because they both damage the host by triggering a strong innate immune response[1]
- Though they illicit the same immune response, the inclusion body of C. trachomatis when it takes its intracellular form is what is mainly responsible for the immune response[6]
- TLR-2 is predominantly expressed less than the TLR-4 in endothelial cells, which may cause a weaker response compared to N. gonorrhoeae[6]

	C. trachomatis	N. gonorrhoeae
LPS/LOS response	Weaker	Stronger
Primary immunogenic trigger	Inclusion body	LOS
Inflammatory path	TLR-2	TLR-4

- The TLR-2 pathway still leads to the same innate immune response, and so the mechanism of damage is the same as N. gonorrhoeae:
  - Inflammatory cytokines lead to the recruitment of macrophages and neutrophils to the site
  - Their destructive enzymes and reactive oxygen species cause collateral damage to healthy tissues

3. Bacterial Invasion

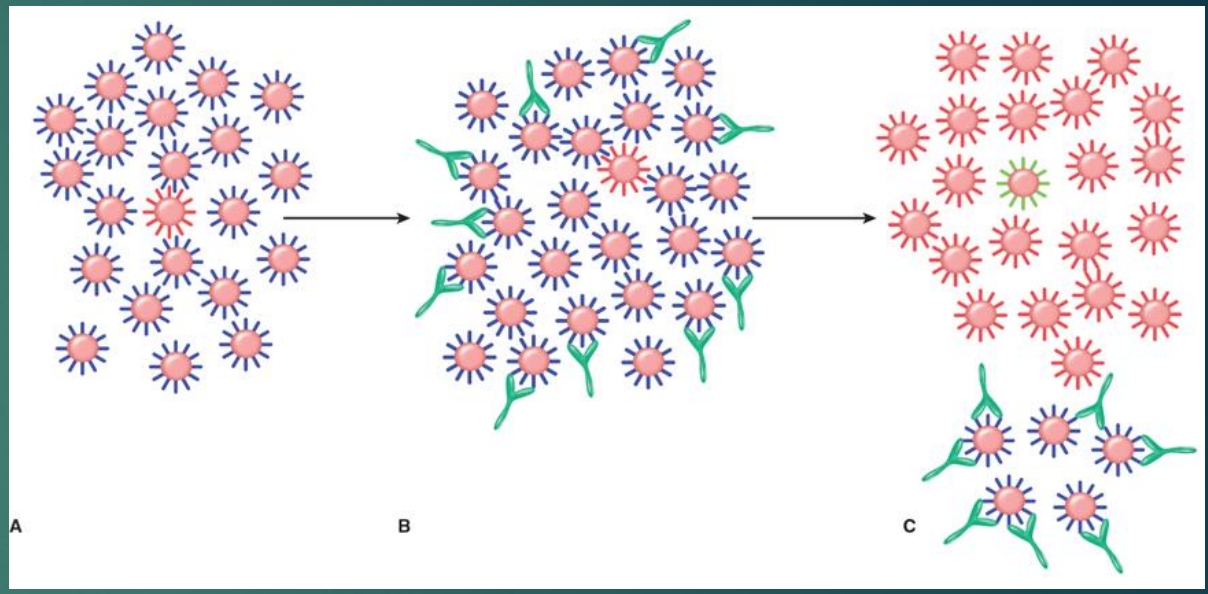
HOW DO THE BACTERIA ATTEMPT TO EVADE THESE HOST RESPONSE ELEMENTS?

Neisseria gonorrhoeae Methods of evasion

- LOS can bind to sialytated acid in the serum to form a sialylated capsule, which prevents anti-LOS antibodies from binding[11]
- Catalase production is upregulated when inside of a phagocyte. Catalase is an enzyme that can degrade hydrogen peroxide[1,4]
- Produces IgA1 protease that cleaves secretory IgA which functions to inhibit bacterial adhesion[4,7]
- RMP is an outer membrane protein of N. gonorrhoeae. When RMP is bound to an antibody it can *inhibit* antibodies against LOS and Por, enhancing virulence[9]
- Pili and porin can reduce fusion with phagosomes[13]
- Porins can bind to complement components such as C4b and factor H to downregulate complement response[14]
- Pili and Opa can disrupt phagocytosis[1]

Neisseria gonorrhoeae Methods of evasion

- Pili, LOS, porins, Opa proteins all express antigenic variation. This makes it harder for it to be targeted by the adaptive immune system[12]
  - The figure to the right shows that antibodies have recognized one variation of antigen (blue) but the remaining bacteria proliferated and now predominantly express a different antigen variation (red) allowing it to evade the immune system



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Chlamydia trachomatis Methods of evasion

- The obligate intracellular nature of C. trachomatis[6] means that it can avoid many host defenses such as complement, antibodies, defensins and phagocytes while it is inside the host cell
- Chlamydial protease-like activity factor (CPAF) can be injected into the host cell which helps to inhibit host cell apoptosis until it has completed its replication cycle[1]
- CPAF can degrade transcription factors for MHC I and II expression, reducing the chances of chlamydial proteins being presented as antigens[6]
- The extracellular elementary body (EB) form of C. trachomatis is not affected by antibodies that bind to it and doesn't hinder its ability to infect host cells[6]
- Tail-specific protease (Tsp) can cleave host NF-kB, which drastically cuts down on the proinflammatory cytokines that NF-kB regulates[16]
- Under duress – exposure to antibiotics or proinflammatory cytokines can put C. trachomatis into a persistent state, forming aberrant bodies (ABS). Staying in a dormant state allows it to evade the immune system until conditions become more favorable[17]

4. Outcome

IS THE BACTERIA COMPLETELY REMOVED, DOES THE PATIENT RECOVER FULLY AND IS THERE IMMUNITY TO FUTURE INFECTIONS WITH THESE CANDIDATE AGENTS?

Neisseria gonorrhoeae Outcome

- **Is the bacteria completely removed?**
  - Yes! But antibiotics are likely needed as N. gonorrhoeae is highly effective at evading the host's immune system
  - Dual treatment therapy is recommended by the CDC to combat mixed infections and antibiotic resistance[18]
    1. Ceftriaxone 250mg injected intramuscularly in a single dose
    2. Azithryomycin 1g ingested orally in a single dose
  - Ceftriaxone is a cephalosporin beta lactam antibiotic that works by binding to key enzymes needed for bacterial peptidoglycan synthesis[9,20]
  - Azithryomycin binds to the 50s ribosomal subunit of bacteria which disrupts protein synthesis[21]

Neisseria gonorrhoeae Outcome

- **Does Nasar recover fully?**
  - Given the early stages of his infection, and no evidence that the infection has spread to other locations, this would be considered an uncomplicated urogenital infection[1]. If left untreated this could spread to his epididymitis and possibly lead to infertility[18]. If Nasar completes his antibiotic regimen N. gonorrhoeae should be eliminated from his system. The inflammatory immune response will stop, allowing his epithelial cells to heal.
- **Is there immunity to future infections?**
  - No. Repeat infections do generate an antibody response, but it is weak[1]. Due to N. gonorrhoeae's immense antigenic variation in the pili, Opa proteins and LOS, forming a lasting immunity is very difficult[1].

Chlamydia trachomatis Outcome

- **Is the bacteria completely removed?**
  - Yes! Nasar will need to use antibiotics to help eliminate C. trachomatis from his body as the immune response may not be enough to overcome C. trachomatis' evasion strategies.
  - Antibiotics recommended by the CDC[19]:
    - Azithryomycin 1g ingested orally in a single dose is the recommended treatment
    - Doxycycline 100g taken orally 2x/day for 7 days is an alternative that is equally as effective
      - Doxycycline is a tetracycline antibiotic, and acts to inhibit protein synthesis in bacterial cells[22]

Chlamydia trachomatis Outcome

- **Does Nasar recover fully?**
  - Nasar detected the infection early on and received treatment promptly. As there are no indicators that the infection has spread to other areas, he will make a full recovery.
- **Is there immunity to future infections?**
  - Somewhat. Immunity has a chance to develop over a long period of time but it may not be full immunity[1]. Cell mediated T_H1 seems to be the most effective immunity. One reason why there is only partial immunity may be the ability of C. trachomatis to assume a persistent state[1].

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