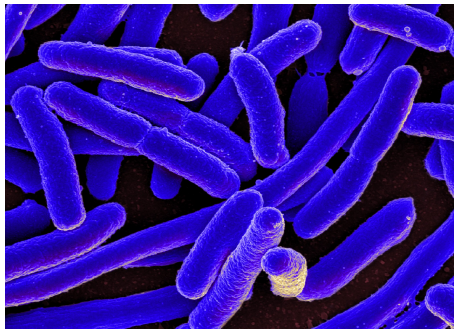
Ronnie’s Case



Img. DiseaseOutbreakControl

The Immune Response

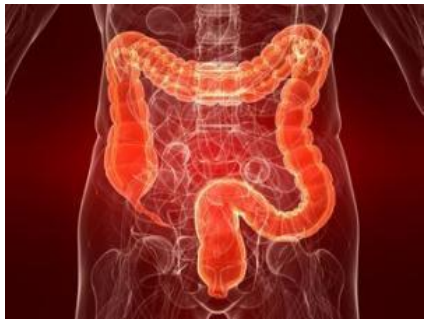
Isabella Aversa

The Case

Img. Pediatric Association

10-year-old Ronnie McDonald has developed abdominal cramps, bloody diarrhoea and a low grade fever. His parents take him to see the family doctor. The doctor asks about what Ronnie may have eaten in the past week and his parents recall that last weekend at a neighbour's barbecue they were concerned that the hamburgers may not have been cooked thoroughly and Ronnie had eaten two burgers. The doctor performs a physical examination noting no rebound tenderness just some mild periumbilical tenderness. He asks the parents to collect a stool sample for the Microbiology Laboratory and to take Ronnie to the local lab for some routine blood-work.

Case Possibilities



Img MayoClinic

Symptoms:

Bloody diarrhoea, abdominal cramps, low-grade fever, mild periumbilical tenderness.

Given his symptoms it is likely that Ronnie has a gastrointestinal (GI) infection that may have been caused by pathogens present in the undercooked hamburgers that he consumed.

However, further tests must be done in order to confirm the cause of his malaise.

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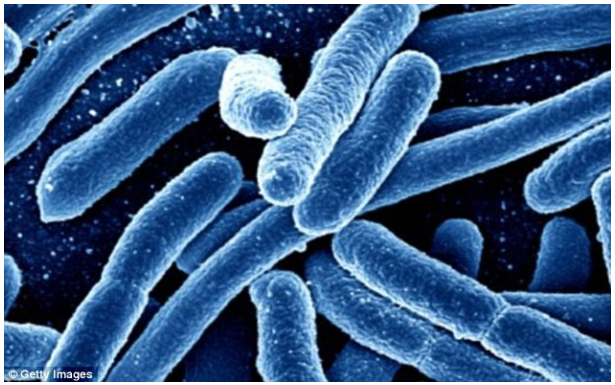
The Pathogen

The most likely causative pathogen:



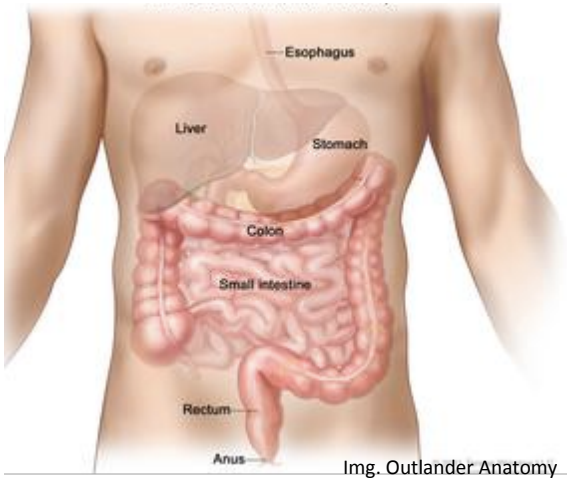
Img. E.coli DailyMail

Escherichia Coli (E.coli)

- Gram-negative, rod-shaped bacteria
- Normally inhabits the lower intestine
- E.coli O157:H7 strain is pathogenic and can cause food poisoning leading to abdominal cramps, bloody diarrhea, fever and vomiting.
- This strain is prevalent worldwide and can be passed on though contaminated food, water and via improper sanitary practices.

GI tract

- Gastrointestinal (GI) tract is an organ system which digests food to extract the nutrients needed by the body and expels the remains.
- The GI tract is primarily a tube extending from mouth to anus.
  - Includes: esophagus, stomach, intestines.
- GI tract also includes associated organs:
  - Liver, gallbladder, pancreas.



Img. Outlander Anatomy

Ronnie's symptoms of diarrhea, and periumbilical tenderness point to the possibility of the him having an infection in the GI tract.

Question 1

Host response: what elements of the innate and adaptive (humoural and cellular) immune response are involved in this infection?

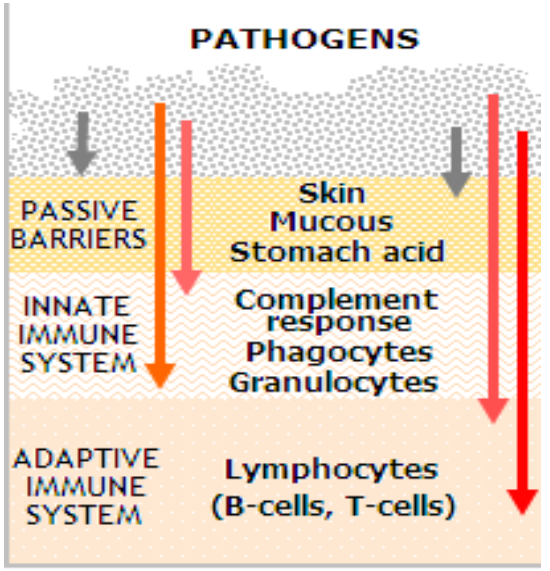
3 Main Mechanisms for Defense

There are 3 main mechanisms that are present in the intestines to defend against these pathogens:

i. Mucosal Barrier

ii. Innate Immune Response

iii. Adaptive Immune Response



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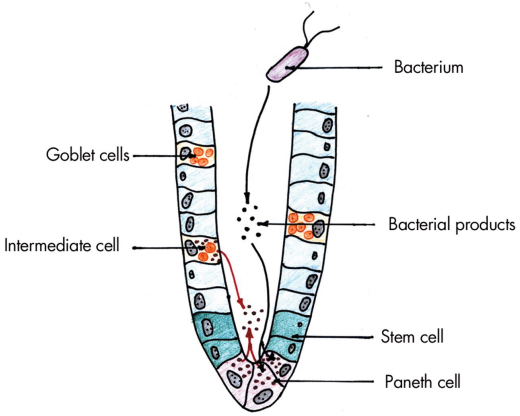
1. Mucosal Barrier



Img. Mucosal Crypt
BMJ

Parietal cells in gastric mucosa secrete HCl

- Acidity can kill most bacteria

<u>Mucosal lining consists of:</u>

Intestinal epithelial cells: Line the mucosal epithelium

- Tight junctions preventing pathogen entry into lamina propria (LP)

Paneth cells are specialized epithelial cells that produce:

- Antimicrobial peptides (RegIII), defensins, lysozomes that disrupt bacterial membranes
- Cathelicidins which bind to LPS and result in pore-formation
- Lactoferrin: takes up free iron preventing bacterial growth

Goblet cells: Produce mucus (glycoproteins) which contains:

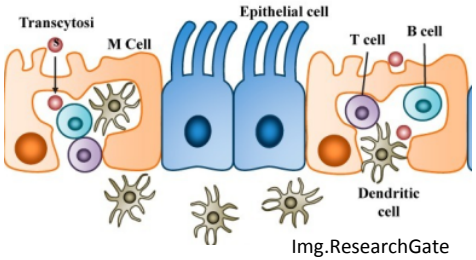
- Mucin protects mucosal epithelial surface and prevents bacterial attachment
- Antibacterial substances such as lysozyme and lactoferrin

1. Mucosal Barrier Cont.

Mucosal lining consists also of:

M cells: Epithelial cells that bring sample antigens from gut lumen into LP where they are sampled by the innate immune system.

- Act as a bridge between the first and second line of defense



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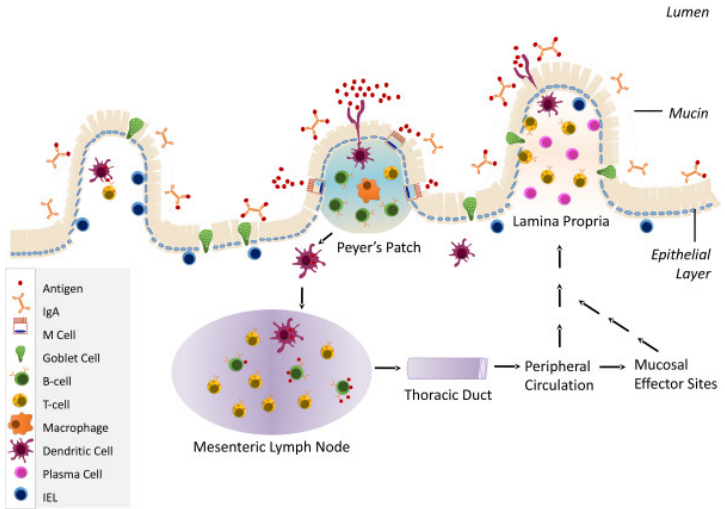
Other intestinal factors:

- Bile products can also act as barriers for pathogen entry into LP.
- Commensals (non-pathogenic bacteria) out-compete some pathogens trying to enter.

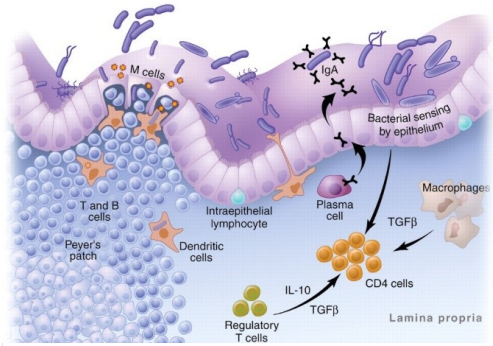


Img.GALT- ResearchGate

Gut-Associated Lymphoid Tissue

Once inside Lamina Propria (LP):

- LP is thin, loose connective tissue layer underneath the mucosal epithelium.
- **Different cell types can enter LP**:
  - T and B cells
  - Dendritic cells (DC), macrophages (MQ)
  - Neutrophils, eosinophils, mast cells



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GALT (Gut-associated lymphoid tissue):

Component of the mucosal-associated lymphoid tissue to protect the body from invasion.

- **Peyers Patches (PP)** consisting of leukocytes that regulate the inflammatory response if pathogen invades or is transported by an M cell.
- Antigen presenting cells (APC) in GALT phagocytose pathogen, migrate to mesenteric lymph nodes and present it to naïve CD4+ T , which are helper cells for B cells and CD8+ T cells maturing into effector cells such as:
- **CD8+ cytotoxic T** cells that kill the pathogen.
- **T regulatory cells (CD4+)** that keep in check the immune response.
- **Plasma B cells** that secrete antibodies against the pathogen.

2. Innate Response

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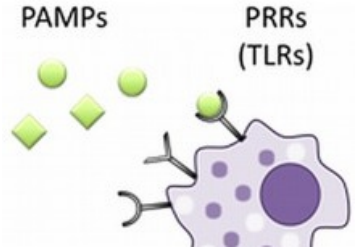


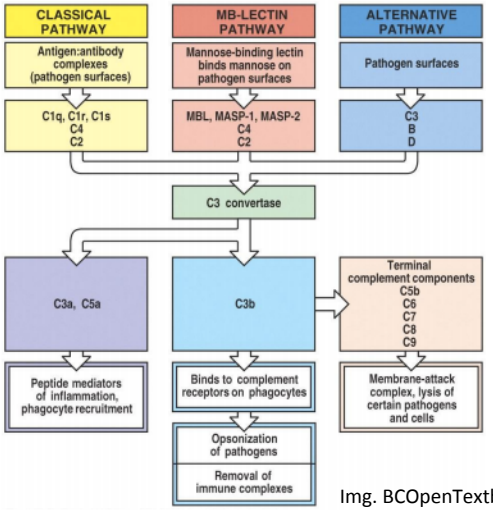
<u>Second line of defense</u>

- Activated by **PAMPs** (pathogen-associated molecular patterns) like LPS and flagellin
- Recognized by **PRRs** (pattern recognition receptors) expressed on immune cells:
  - Toll-Like Receptors (TLRs): expressed on many immune cells (MQ) and detect endosomal and extracellular PAMPS
    - Ie. TLR4 recognizes LPS.
  - NOD-like Receptors: present of a variety of immune cells and detect cytosolic PAMPs which leads to the recognition of intracellular bacteria.
    - Ie. NOD1 recognizes intracellular gram-negative peptidoglycan
- The stimulation of PRRs causes a signaling cascade that results in activation activation of transcription factors (NFkB) in MQ
- In turn, this results in production of pro-inflammatory cytokines and chemokines: IL-6, IL-1beta, but also regulatory cytokines (IL-10)
  - This stimulates the innate response, recruiting more immune cells like neutrophils and innate components such as complement.

2. Innate Response Cont.

Complement Cascade:

- Activated during an immune response
- 3 pathways: Classical, Lectin, Alternative.
- Formation of C5 convertase same for all
- C3 convertase causes inflammatory responses, opsonization, and bacterial lysis through the formation of Membrane Attack Complex (MAC)

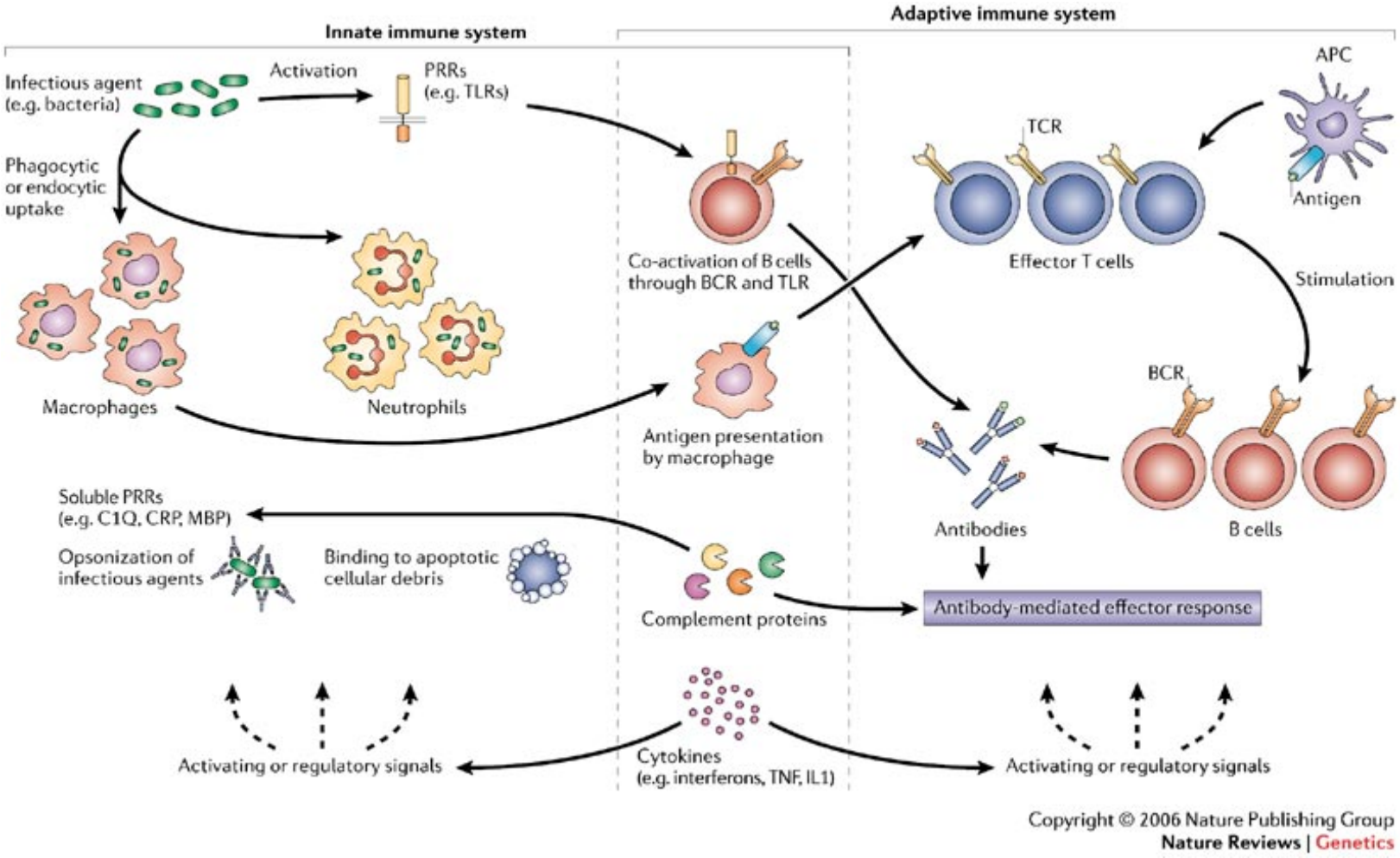


Img. BCOpenTextbooks

E. coli:

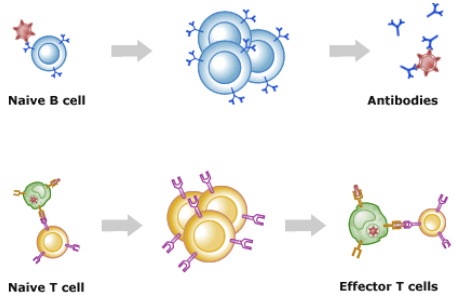
- Binds to endothelial cells facilitated by its pili, and secretes proteins through its T3SS such as EspA that allow for adhesion.
- E.coli's intimin surface protein then binds to Tir protein expressed on host cells.
- The bacteria's PAMPs bind to TLRs on MQ after being transcytosed by M cells resulting in MQ activation and pro-inflammatory cytokine production.
- Recruitment of immune cells such as neutrophils, DC and the production of complement helps to eliminate some of the bacteria via phagocytosis and bacterial lysis.

Innate and Adaptive Immune Response



Img. Nature.com

3. Adaptive Response



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Third line of defense and is more <u>specific</u>!

- Activated upon breach of both mucosal barrier and innate system.
- Initiated when APC, like DC capture bacteria (E.coli or Salmonella), migrate and present it to naïve T cells causing them to mature.

MHC –Bacterial peptide complexes on APC presented to the specific T cells receptors (TCR) together with costimulatory signals provided by APCs (CD80/86) binding CD28 on T cell

→ Cause T cell activation and proliferation and cytokines (IL-2, IL-4 etc..) release

→ In turn, T cells help naïve B cells get activated (via CD40L-CD40 interaction and IL-4) to produce antigen-specific antibodies towards E.coli.

<u>Activated Cells in Adaptive Response:</u>

T cells: CD4+ T helper cells, CD8+ cytotoxic T cells and T regulatory cells

B cells: that produce antibodies such as IgG, IgA.

Produce memory cells leading to long-lasting immunity.

Adaptive Response Cont.

E.Coli:

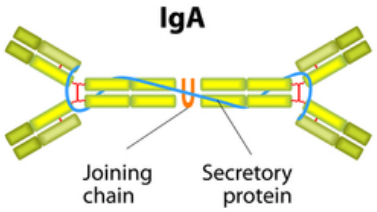
- CD4+ T cells help prime other naïve T cells (CD8+) to be E.coli-specific.
- CD8+ E.coli-specific T cells start killing off E.coli.
- Plasma B cells start producing E.coli-specific antibodies (ie. IgA) which bind to E.coli antigens on its surface.

- This causes opsonization allowing for E.coli to be phagoyctosed.

- Cytokines are also produced as a response (IL-1, IL-6)
- Memory B and T cells are produced protecting against future re-infection.

<u>Cell-mediated:</u> T cells killing

<u>Humoral:</u> Antibody production (plasma B cells)



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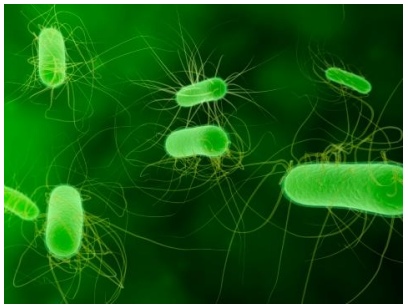


Img. Patel

Question 2

Host damage: What damage ensues to the host from the immune response?

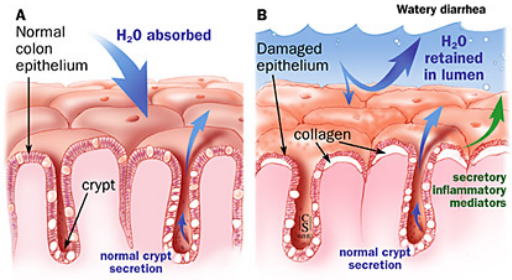
Host Damage

The damage by the immune system from the host is as a result of the bacterial invasion that triggered it.

1. Diarrhea

The invasion of the intestinal mucosa by E.coli bacteria results in the disruption of the flora"

- Can secrete toxins that cause epithelial cells damage
- Resulting in the release of large amounts of water due to less water absorption causing diarrhea.
- Ex: E.coli secretes Lipid A, an endotoxin that causes diarrhea.



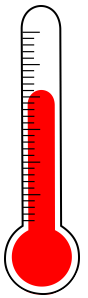
Img. JHMICAL

2. Blood in diarrhea

- The inflammatory response results in intestinal epithelial cell death.
- Due to:
  - Pro-inflammatory cytokines released such as IL-1, IL-6
  - The recruitment of immune cells such as macrophages and neutrophils
- Results in killing of bacteria and bacteria-infected cells that can be killed off or die as a result of the pathogen invading them.
- Leads to bleeding: Mucosal epithelium breaks down = vasculature around ruptures and releases blood.
- Shiga toxin cleaving of subunit A causing cessation of protein synthesis by epithelial cells, leading to host cell death and breakdown the mucosal lining leading to bleeding

Img. PedAssociation

Host Damage (cont.)



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3. Abdominal Cramps + Periumbilical Tenderness

- Macrophages, DC and neutrophils arrive at infection site
- They contribute to inflammation and loss of barrier function through the release of inflammatory cytokines and other chemicals.

The symptoms that Ronnie is experiencing can be due to the cell death in GI tract due to the cytokines released by the host cells and tissue damage.

- E.coli causes inhibition of protein synthesis due to the shiga toxin.
- E.coli's alpha-hemolysin toxin results in host cell membrane disruption which contributes to pain.
- Ruptured cells can release their contents into the environment, further damaging surrounding tissue causing pain.

4. Fever

The fever that Ronnie experiences is as a result of the pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α).

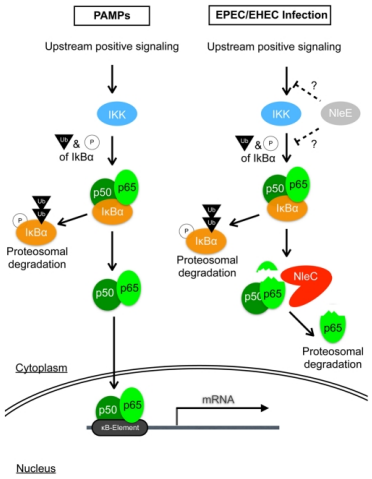
- These cytokines are released in response to the bacterial infection act on the CNS to increase body temperature.
- Endotoxins (Lipid A) secreted by E.coli also can induce fever.

Question 3

Bacterial Evasion: How do the bacteria attempt to evade the host response elements?

E.coli Bacterial Evasion

E. Coli is able to evade the immune system via a number of virulence factors.

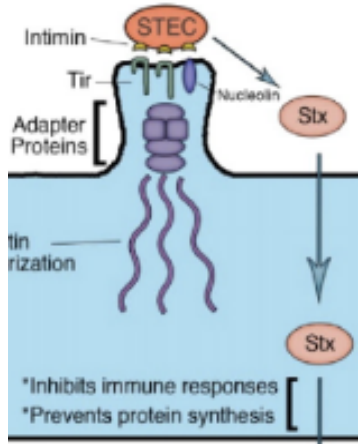


Img. Meyaard

E.coli secretes **NLeC** and **NLeE proteases**

- NLeC-induces degradation of p65 subunit of NFkB in cytoplasm
- Less p65 enter the nucleus →less cytokine gene transcription
- Results in impaired cytokine (e.g. IL-8) production
- Benefitting E.coli

This hijacking results in more bacterial replication within the host before immune cells are able to recognize the bacteria.



Img. AS Microbiology

E.coli also has **Shiga toxins (Stx)**:

- Suppresses IFNgY Stat-1 tyrosine phosphorylation = inhibiting MQ activation
- Prevents host cell protein synthesis via the cleaving of subunit A = cell death

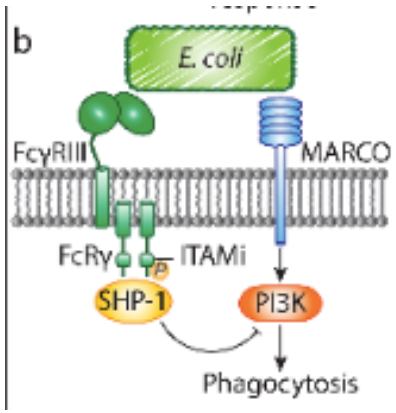
Bacterial Evasion: E. coli

E.Coli exploits **ITAM signalling**:

- allowing it to escape phagocytosis through engagement of FcyRs and altering ITAMi signaling.
- Serves to resist clearing of bacterial pathogen by the host.

E.Coli dampens **TLR signalling**:

- inserts Tir (virulence factor) into epithelial cell membrane to attenuate TLR responses and pro-inflammatory cytokine release.



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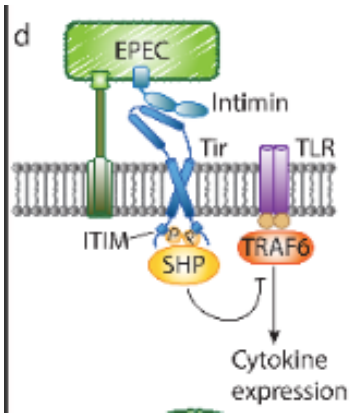

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Question 4

Outcome: Is the bacteria completely removed, does the patient recover fully and is there immunity to future infections with this candidate agent?

Outcome: E.Coli

E.coli is an inhabitant of human and mammals intestines,

- The majority of the strains are non-pathogenic.
- Some are non-pathogenic in one species (cattle), but pathogenic in others (humans).
- Harmful bacteria can be completely removed from the host, resulting production of memory cells protecting host from re-infection.
- T cells and antibody-producing B cells against E.coli can help control and eliminate the infection.

Infection is often easily treatable with E.Coli O157:H7 with individuals often only needing adequate amounts of rest and hydration (orally or intravenously).

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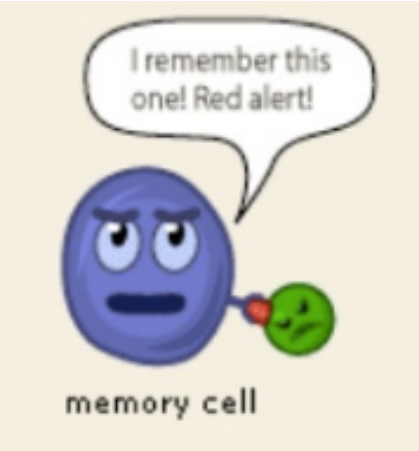
Img. OpenClipArt

Outcome: E.Coli

The memory cells can be either **T or B memory cells**.

- In case of re-infection the response against this bacterial strain will be a lot quicker.
- If it is a different strain of E.coli these memory cells may not be able to elicit such a fast response due to different surface antigens present that may not be recognized by host



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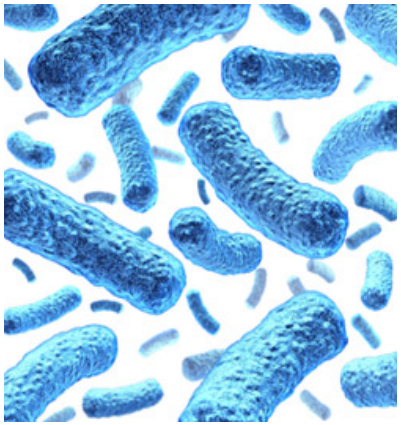

Img.DiseaseControl

Future Prevention

In the future it very important for Ronnie to learn how to prevent pathogenic E.coli infections:

- Washing his hands properly before eating
- Making sure his food is not contaminated or old
- His food is cooked with clean water and materials.
- Avoiding eating raw foods

All the above can help prevent Ronnie getting re-infected by E.coli.

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Summary

In Ronnie's case he had symptoms that pointed to the infection being possibly due to the bacteria E.coli. However, it is important that Ronnie get further tests done in order to confirm the cause of his illness and be able to take the appropriate measures (e.g. appropriate antibiotics to which bacteria are sensitive) to get better quickly.

Img. OpenClipArt

Additional References

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