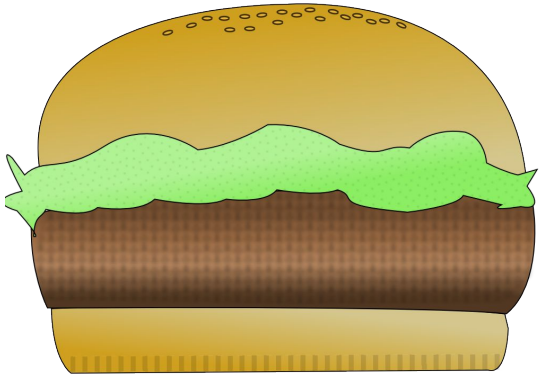
Bacterial Gastroenteritis

The Immune Response

The Patient's Situation

10-year-old Ronnie McDonald has developed abdominal cramps, bloody diarrhea 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 neighbor'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 bloodwork.

Periumbilical tenderness: tenderness or pain in the umbilical area of the abdomen (around the navel).



What's Wrong with Ronnie?

Taking in consideration Ronnie's signs and symptoms of bloody diarrhea, abdominal cramps, and a fever, it is likely that he is experiencing bacterial gastroenteritis, an inflammation of the stomach and intestines caused by bacterial pathogens.

This condition can be caused by a variety of bacteria such as:

- Campylobacter jejuni
- E. coli
- Salmonella
- Shigella
- Staphylococcus
- Yersinia

These bacteria are commonly found contaminating food or water products in cases where the meat or poultry came into contact with the bacteria when the animal was processed, when someone preparing the food did not wash their hands or utensils properly, or when meats or eggs are undercooked. Either of these cases could have occurred that led to the contamination of the hamburgers that Ronnie ingested, which further led to his condition.

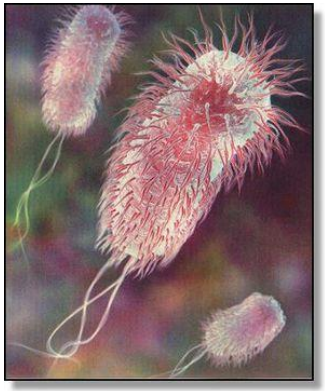
Due to the fact that Ronnie's condition seems to have arisen after eating the possibly undercooked hamburgers , the likely pathogen in this scenario is *E. coli* O157:H7 (an Enterohemorrhagic *E. coli* or EHEC serotype). This bacterium produces an 'invasive' toxin, Shiga-like toxin, that catalytically inactivates 60S ribosomal subunits of most eukaryotic cells, blocks mRNA translation and thus causes cell death. This process explains the presence of blood in the stool of the patient and the abdominal pain.

Escherichia coli 0157:H7

Escherichia coli 0157:H7 is a pathogenic serotype of Escherichia coli. It is classified as a Gram-negative, facultative anaerobe.

Pathogenic *E .coli* evolved from commensal *E. coli* through the acquisition of multiple virulence factors such as toxins, adhesins, and secreted effector proteins that modulate host responses. It is an example of an enterohemorrhagic pathogen as it produces an invasive toxin, Shiga-like toxin, which damages the gastrointestinal tissue and causes bloody diarrhea (condition known as hemorrhagic colitis). It is named Shiga-like toxin due to its similarity to the activity of the toxins produced by *Shigella dysenteriae.*

Although this serotype is infectious in humans, it is a commensal bacterium found in the intestinal tracts of animals such as cows, goats, sheep, and deer.
Therefore, although exposure to this bacterium is commonly associated with ingestion of undercooked meat, infections can also arise following the ingestion of animal feces-contaminated foodstuffs.



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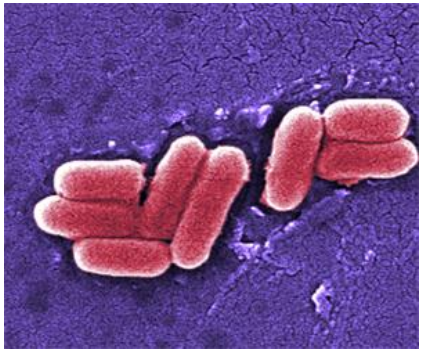

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Host Response: Innate Immune Response

One of the first lines of host defense against *E. coli* 0.157:H7 infection is through the activation of the innate immune system. The innate immune system refers to the nonspecific defense mechanisms that come into effect either immediately or within hours of the antigen's entry in the body. These mechanisms include physical barriers such as skin, chemicals in the blood, acidity of the stomach and immune system cells that attack foreign cells in the body[5]. The innate immune response uses pattern recognition receptors present on innate immune cells, such as natural killer cells, mast cells, eosinophils, basophils, macrophages, neutrophils, and dendritic cells to detect pathogen-associated molecular patterns expressed by microbes to elicit a protective antimicrobial immune response.

In the particular case of the human body attempting to combat the colonization and infection by *E. coli* 0157:H7, the following comprises the innate immune response:

- Gastric acidity is one the most vital first lines of defense since 99.9% of incoming bacteria will be killed within 30 min at the normal gastric pH of 1.5 to 3.5. Exposure to these acidic conditions will inhibit the activity and growth of this pathogenic organism.
- Intrinsic motility patterns of the gastrointestinal tract prevent adherence of the bacterium.
- The presence (and preservation) of the normal flora of the large intestine may also contribute to decreasing the risk of infection by creating competition for pathogens for attachment sites and nutrients, and also inhibit their activity or kill bacteria through the production of toxic metabolites.
- The mucous membrane of the gastrointestinal tract also serves as a innate defense system: goblet cells produce mucous, composed of polysaccharides and proteins, which prevents bacterial adherence, rapid turnover of mucosal cells also prevents bacterial attachment, tight junctions between mucosal cells prevent bacterial penetration, and paneth cells, located in the crypts of small and large intestines, produce peptides which are toxic to bacteria.

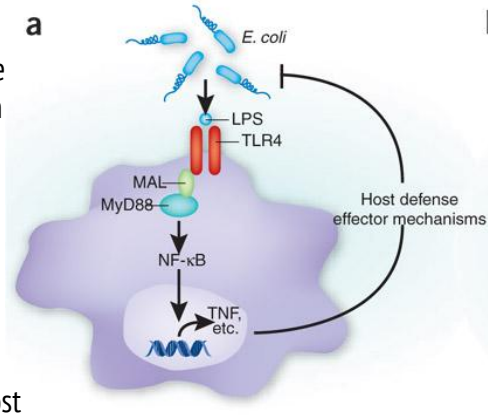
Host Response: Innate Immune Response

Besides the properties of the gastrointestinal tract which aim to defend the organ system from bacterial infection, there are also nonspecific immune cells that recognize antigenic characteristics of the pathogens and consequently mount an immune response. These immune cells are located immediately below the epithelial layer of the GI tract in the lamina propria.

- innate immune cells, such as natural killer cells, mast cells, eosinophils, basophils, macrophages, neutrophils, and dendritic cells possess antigen pattern recognition receptors, Toll-like receptors (TLRs) and Nod-like receptors (NLRs), which recognize pathogen-associated molecular patterns. TLRs are a family of membrane-bound receptors that recognize specific microbial components such as the lipopolysaccharide and flagellin of the *E. coli*. In contrast, NLRs detect other microbial products, such as peptidoglycan, in the cytoplasm of the host cell. The activation of leukocytes surrounding the intestine by *E. coli* leads to their general activation and secretion of interleukin-12, which activates nearby macrophages, T cells, and natural killer T cells to secrete IFNγ.

- An important signalling cascade that is activated to clear the microbial pathogen is the interferon-gamma (IFNγ), Jak 1, 2, Stat1 signal transduction pathway. IFNγ secreted by the immune cells binds to the IFNγ receptors ubiquitously expressed in all host cells and activates the Jak 1, 2, Stat1 signal transduction pathway, resulting in the activation of up to 2000 IFNγ-stimulated genes in host cells, to initiate an anti-microbial state in the body. Activated genes include, for example, inducible nitric oxide synthase (iNOS), monocyte chemoattractant protein-1 (MCP-1), lymphocyte adhesion protein ICAM-1, and Interferon regulatory factor (IRF) -1, as well as increased MHC II expression.

- Histamine release by mast cells and prostaglandins production due to release of interleukins and tumor necrosis factors (TNF-α) result in an inflammatory state in the GI tract, which causes swelling, fever, and pain.



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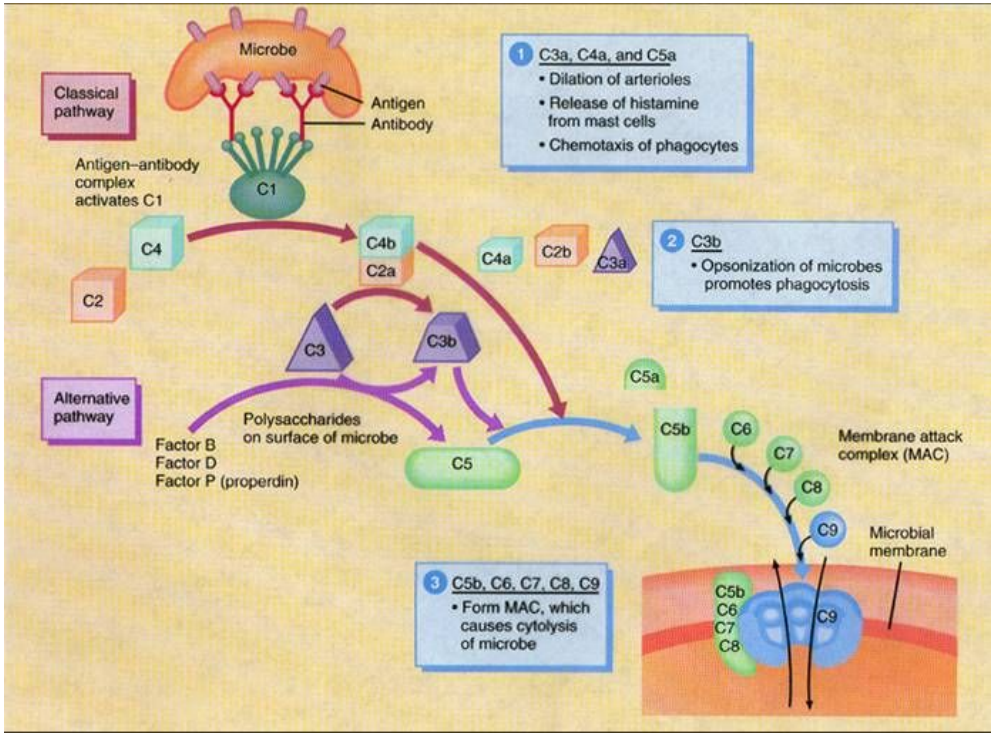
Host Response: Innate Immune Response

The complement system is also a part of the innate immune system and causes biochemical cascades which aim to eliminate the pathogen. There are three pathways of complement activation and the alternate pathway is the one most prevalent in innate immune responses. There are three ways in which the complement system protects against infection. First, it generates large numbers of activated complement proteins that bind covalently to pathogens, opsonizing them for engulfment by phagocytes bearing receptors for complement. Second, the small fragments of some complement proteins act as chemoattractants to recruit more phagocytes to the site of complement activation, and also to activate these phagocytes. Third, the complement components damage certain bacteria by creating pores in the bacterial membrane.

- tumor necrosis factor (TNF-α) released by the immune cells also enhances activation of a protein called p65 which is a key mediator of NF-κB inflammatory signaling, causing inflammation associated with the innate immune response.



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Host Response: Adaptive Immune Response

Some of the innate immune cells act as linkers of innate immunity to adaptive immunity. Cells such as dendritic cells and macrophages, once exposed to the pathogen, become activated and then go on to activate adaptive immune cells, the T lymphocytes and the B lymphocytes, initiating the adaptive immune response. Within the gastrointestinal tract, the innate and adaptive cells can be found clustered in compartments of patches of lymphoid tissue, which are distributed throughout the gastric and intestinal mucosa. These mucosal immune compartments make up the gut-associated lymphoid tissue (GALT) often referred to as the gastrointestinal tract's immune system.

- The GI tract also possesses M cells. These cells are found overlying organised lymphoid follicles of the small and large intestine and play a central role in the initiation of mucosal immune responses by transporting microorganisms to the underlying lymphoid tissue (GALT). The pathogens find their way inside host cells via processes such as phagocytosis but are also engulfed by antigen presenting cells, such as the dendritic cells and macrophages, which process and present the antigen on major histocompatibility complex (MHC) molecules on their cell surface.

- Since *E. coli* O157:H7 is an extracellular pathogen, cell mediated adaptive immunity doesn't play as great of a role in containing and eliminating the infection as does humoral immunity. Nevertheless, since the bacterium does release toxins which are taken up by the gut epithelial cells, these toxins can be degraded and presented on MHC molecules (class 1) on the cell's surface. CD8+ T cells or cytotoxic T cells recognize the antigenic peptides presented by the MHC molecules, becoming activated and releasing cytokines which leads to apoptosis of the infected cells.

- Humoral immunity also plays a great role in the adaptive immune response against *E. coli* O157:H7. After engulfment, a particular antigenic sequence in presented on the MHC molecules (class 2) of antigen presenting cells which then bind to and activate CD4+ T helper cells. These activated T cells then go on to activate naive B lymphocytes which undergo clonal expansion and differentiate into antibody producing plasma cells or memory B cells specific for the antigenic sequence. Upon secondary exposure to the bacterium, the memory B cells are capable of converting to plasma cells, secreting antibodies and mounting a humoral response quicker and greater than before. The most common type of antibodies secreted by the plasma cells are IgA antibodies, which exist in a dimeric form and are are found in abundance in the lumen of the gastrointestinal tract.

Host Response: Adaptive Immune Response

The IgA antibodies produced by the B lymphocytes can perform a variety of functions.

- They can recognize and attach to invading antigens through the recognition of a specific antigenic sequence thereby neutralizing the pathogen and preventing it from binding to receptors on host cells.
- They can bind to the antigen and increase its uptake by phagocytic cells (opsonization).
- The attachment of the antibodies to the antigens and therefore, the formation of an antibody-antigen complex can activate the classical pathway of the complement system. The activation of the complement system then allows for recruitment of inflammatory cells, opsonization of pathogens, and direct killing of pathogens by lysis.

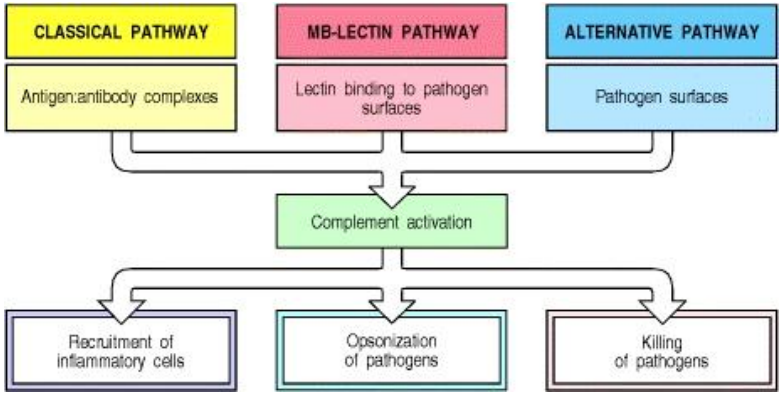

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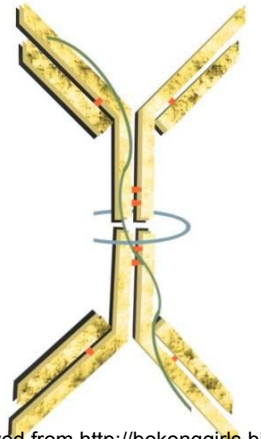
Figure 2: Antibody IgA



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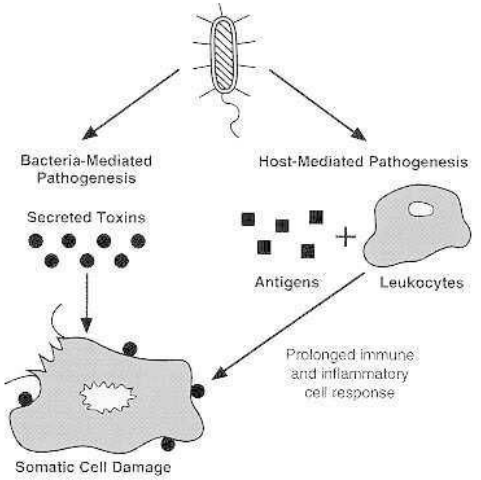
Immune Response: Damage to the Host

The damage to the host that results from *E. coli* O157:H7 infection is both pathogen-mediated and also host-mediated. The host-mediated damage results due to the immune response that the body mounts against the pathogen.

Since this strain of *E.coli* is classified as an extracellular pathogen, much of the tissue damage that ensues is a result of the innate immune responses rather than cell-mediated immunity. Humoral immunity does not cause damage to the host as the antibodies produced by the B lymphocytes are specific for the antigen and not for host cells. Additionally, as there are always antibodies present in the body system even when there are no bacterial infections, it shows that their presence does not have damaging effects on the body.

On the other hand, innate immune responses do not have the same targeted actions or specificity as the adaptive immunity branch aforementioned. Upon exposure to the antigen, a state of inflammation results from the innate immune system, in which there is activation of various immune cells such as macrophages, natural killer cells, and other leukocytes, followed by immense production of inflammatory mediators. Cytotoxic substances are produced within the immune cells when the pathogen is phagocytosed. These molecules however can leak out of the phagocytic cells and into the environment. The tissue damage in *E. coli* O157:H7 infections is therefore caused by toxic factors released from these immune cells, damaging the nearby host cells. Sometimes cases can result where the host response is so intense that host tissues are destroyed, allowing bacteria the capability to proliferate even further.



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Bacterial Evasion

As a successful human pathogen capable of causing infection and disease in the body, *E. coli* O157:H7 has evolved various mechanisms to subvert specific host innate immune responses.

- The first obstacle that the bacterium faces is getting past the acidic conditions of the stomach. The *E. coli* bacterium is protected from the stomach acid by a protein called HdeA. One way that acid kills bacteria is by causing the proteins in them to unfold and stick together (protein aggregation). It is very difficult for bacteria to dissolve these protein clumps leading to decreased activity of the pathogen and eventual death. Like other proteins, HdeA unfolds when exposed to acid, but the unfolding process that inactivates most other proteins activates HdeA. Once unfolded, this protein molds itself to fit other bacterial proteins that have been made sticky by acid-induced unfolding. This prevents the unfolded proteins from sticking together and forming clumps and thus, leads to the ability of the bacterium to survive the acidic conditions of the stomach.
- As observed during the body's innate immune response against *E.coli* O157:H7, IFNγ plays an important role in triggering an antibacterial response in the host. IFNγ production by macrophages, Natural Killer T cells and neutrophils triggers an antimicrobial state in host cells by tyrosine phosphorylation of the signal transducer and activator of transcription-1 (Stat-1) molecule, leading to dimerization, translocation to the nucleus, binding to the gamma-activating sequence (GAS), and downstream up-regulation of up to 2,000 pro-inflammatory genes. The two major groups of Shiga-like toxins, Stx1A and Stx2A, secreted by the *E. coli* O157:H7 cells suppress IFNγ-mediated Stat-1 tyrosine phosphorylation and therefore, inhibit IFNγ mediated cellular activation and the associated immune response.

Bacterial Evasion

- The bacterium secretes a protein called NleH1 that directs the host immune enzyme IKK-beta to alter specific immune responses. This process not only helps the bacterium evade elimination by the immune system, it also works to prolong the survival of the infected host, enabling the bacterium to persist and ultimately spread to unaffected individuals.
- *E. coli* O157:H7 also produces the enzyme catalase-peroxidase which aids in the bacterium's colonization of the host intestine by reducing oxidative stress brought upon by the innate immune cells.
- It also produces zinc metalloprotease, an endopeptidase (breaks down proteins) that inhibits the regulation of host inflammation pathways and the complement system.
- E. coli O157:H7 infection suppresses the inflammation pathway through NF-κB inflammatory signaling by inhibiting p65 activation. It does this through injecting effector molecules, NleB, NleC, NleE and NleH, into the host cells.

The Outcome after Infection

With treatment, such as consistent hydration and rest, most people show improvement within five to seven days after the onset of an *E. coli* O157:H7 infection and make a full recovery. The infectious bacteria previously colonizing the gastrointestinal tract are completely removed through the collective actions of the innate and adaptive immune responses of the body.

After infection with the bacterium, the body develops immunological memory towards the infectious agent. In the humoral adaptive response during primary infection, the B lymphocytes, after recognition of the antigen and activation by helper T cells, proliferated and differentiated into antibody-secreting plasma cells and memory cells. If this particular bacterium were to re-infect the host, these presence of differentiated antibodies and memory B lymphocyte will allow for the body to create an immune response more quicker and more robust than before.

- In secondary and subsequent immune responses, any persisting antibodies produced by the B cells that differentiated in the primary response are immediately available to bind the newly introduced antigen. If there is sufficient preexisting antibody to clear or inactivate the pathogen, it is possible that no immune response will ensue.
- However, if there is a trace excess of antigen, B cells whose receptors are specific for the antigen will clonally expand and differentiate into plasma cells to produce antibodies targeting the antigen. Thus, the higher-affinity memory B cells are efficiently stimulated in the secondary immune response as they recognize the specific antigenic sequence of the invading bacterium. In this way, there is a quicker and greater production of antibodies.

Additionally, if the particular antigenic sequence that is recognized by the memory B cells is shared among other serotypes of *E. coli*, there is a possibility that there may be protective immunity against other strains of the bacterium and prevention against their associated infections.

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