INTRODUCTION

Brucella spp are intracellular parasites and, as discussed in the previous article, they have evolved methods by which they evade detection by immune systems and establish chronic infections. Development of an adequate immune response is critical to the control of Brucella spp infections and in order to understand the host response to Brucella spp infection this article will discuss immunity to Brucella spp infections. Classically, the host immune response is functionally divided into innate or non-specific and adaptive or specific immunity. This article will deal with the innate immune system and the adaptations that Brucella spp have evolved to evade this.

INNATE IMMUNITY

The innate immune response is activated within minutes to hours after tissue damage or infection and targets pathogens non-specifically. However, it does not improve or get faster with future exposure to the pathogen but certain aspects of the response improve with age. For example, the ability of neutrophils to phagocytize bacteria is better in adult cattle compared to calves in the first week of life and certain complement proteins are present in lower concentrations in young calves than in adults. The main function of the innate response would be to limit bacterial spread through bactericidal mechanisms and secondly induce the dynamic adaptive immunity. The latter functions would be to clear the infection by antigen mediated lymphocytic clones.

The major components of the innate immune system are:

Physical Barriers - These include the skin, mucosa, secretory molecules (various chemokines and cytokines, complement system and opsonins) and fluids such as saliva and tears. The main function of physical barriers is to prevent pathogens such as viruses, bacteria, and parasites from entering the body and causing infection. Many cells that are not considered to be ‘immune cells’, such as the epithelial cells that line the mammary gland, urogenital tract, and respiratory tract, have been shown to participate in innate immunity by expressing certain cell surface markers or secreting molecules that can protect an animal against infection directly, or indirectly by activating immune cells.

Complement is part of the innate immune system that does not change over an individual’s lifetime but it can be brought into action by the adaptive immune system.
The complement system consists of a number of small proteins found in the blood which are synthesized in the liver, and in addition are produced by tissue macrophages, blood monocytes, and epithelial cells of the urogenital and gastrointestinal tract. Complement enhances phagocytosis of antigens by opsonisation, attracts macrophages and neutrophils by chemotaxis, contributes to lysis of foreign cells and causes clumping of antigenic agents.

Opsonins effectively mark antigens and promote phagocytosis by masking the negative charge on cell walls of pathogens thus enhancing their destruction by natural killer cells amongst others. Opsonin molecules include non-specific antibodies such as IgG and IgA. IgM is may also be considered to be an opsonin and part of the complement system.

Cytokines are immunomodulating agents such as interleukins and interferons and regulate host responses to infection, inflammation and trauma. Some are proinflammatory while others are anti-inflammatory in order to control excessive inflammatory response which in itself may be a virility factor of certain pathogens. Virtually all nucleated cells, but especially endo/epithelial cells and resident macrophages particularly those near the interface with the external environment, are potent producers of IL-1, IL-6 (chronic inflammation and other immunoresponses, such as fever and acute phase proteins) and TNF-α (cellular immune responses).

Cytokines can be classified structurally or functionally. Functional classification is more practical and divides immunological cytokines into those that enhance cellular immune responses, type 1 (INF-γ, TNFα, etc.), and type 2 (TGFβ, IL-4, IL-10, IL-13, etc.), which favor antibody responses.

Cytokines serve important functions in regulating neutrophil activity. Two important cytokines are (interleukin 1) IL-1 and (tumor necrosis factor α) TNF-α. TNF-α is involved with neutrophil apoptosis, chemotaxis, and respiratory burst. IL-1 is important primarily in chemotaxis but also functions in phagocytosis and respiratory burst. Cytokines must bind to their respective receptors on the surface of neutrophils to be effective.

Cellular components – All cells of the immune system have their origin in the bone marrow. The myeloid progenitor (stem) cell in the bone marrow gives rise to erythrocytes, platelets, neutrophils, monocytes/macrophages and dendritic cells whereas the lymphoid progenitor (stem) cell gives rise to the natural killer cells and T and B lymphocytes. Important cells of the innate immune response include the phagocytic cells (macrophages, dendritic cells, neutrophils) and the innate lymphocyte subsets (natural killer cells and T cells).

Selectins are a family of cell surface adhesion molecules that are produced as a part of the inflammatory response of innate immunity. E and P-selectin are produced by the endothelial cells that line the vessel walls of the circulatory system. They attract lymphocytes to sites of inflammation, which are indicative of the presence of pathogens, and are utilized by both the innate and adaptive immune systems.

P-selectin is expressed on the surface of capillary endothelial cells when these cells are stimulated by bacterial components, such as lipopolysaccharide, or molecules released by damaged tissues. This glycoprotein then binds to L-selectin, which is expressed on the surface of passing neutrophils binding these cells to the endothelium from where they move through the basement membrane by a process known as diapedesis making them the first cells to arrive at damaged tissues and move from the blood towards the site of infection via a process known as chemotaxis with the objective being phagocytosis and neutralisation of the pathogen.

Phagocytosis of microbes is facilitated by various host cell phagocytosis promoting pattern recognition receptors which recognise certain pathogen associated molecular patterns (PAMPs), such as lipopolysaccharide [LPS], peptidoglycan [PG], lipoproteins and DNA that are termed pattern recognition receptors (PRRs) (e.g. toll-like receptors TLRs).
Phagocytosis leads to the activation of innate immune cells, and the expression of pro-inflammatory mediators and co-stimulatory molecules that initiate adaptive immunity. Pattern recognition receptors include the membrane bound toll like receptors that sense nucleic acid molecules in the extracellular compartment or endosomal lumen. Toll-like receptor 4 (TLR-4) helps neutrophils recognize gram-negative bacteria while toll-like receptor 2 (TLR-2) binds primarily to gram-positive bacteria.

Gomes et al (2012) reported that B. abortus contains an unusual lipid A composing the LPS molecule, which is important for evading the host immune system during the early stages of infection. In addition, when entering the host intracellular space, B. abortus displays several strategies to avoid the cellular killing mechanism. One of the virulent factors involved in such action is the Type IV secretion system (T4SS). After B. abortus is phagocytosed, T4SS is induced during phagosomal acidification, leading to the translocation of effector proteins into host cytosol. This process is essential for bacteria to subvert lysosome fusion and to create Brucella-containing vacuoles (BCV), which are permissive for replication and interaction with the endoplasmic reticulum (ER).

Barquero-Calvo et al (2007) proposed that Brucella sp has developed a strategy through PAMPs reduction to reach a replication niche before activation of adaptive immunity systems. They found that Brucella sp did not induce pro-inflammatory responses in mice and only slightly induced proinflammatory cytokines, consumed complement, and were strongly resistant to bactericidal peptides. They further stated that the lack of proinflammatory responses was not due to conspicuous inhibitory mechanisms mediated by the invading Brucella sp or its products. Activated 24 h post-infection macrophages did not kill Brucella sp and intracellular replication did not interrupt the cell cycle or cause cytotoxicity.

Conde-Alvarez et al (2012) reported that the lipopolysaccharide (LPS) of the gram-negative bacterium Brucella sp lacks a marked pathogen-associated molecular pattern, and it has been postulated that this delays the development of immunity, creating a gap that is critical for the bacterium to reach the intracellular replicative niche. They showed that Brucella sp escapes recognition in early stages of infection by expressing a shield against recognition by innate immunity in its LPS core and they identified this as a novel virulence mechanism in intracellular pathogenic gram-negative bacteria.

Pollak et al (2012) reported that outer membrane vesicles (OMVs) released by some gram-negative bacteria have been shown to exert immunomodulatory effects that favour the establishment of the infection. OMVs were shown to inhibit the cytokine response of THP-1 cells to B. abortus infection. Their results suggests that OMVs from B. abortus exert cellular effects that promote the internalization of these bacteria by human monocytes, but also down regulate the innate immune response of these cells to Brucella sp infection. These effects may favour the persistence of Brucella sp within host cells.

Kuehn and Kesty (2005) described OMVs of naturally produced bacterial vesicles as discrete, closed OMV blebs produced by growing cells, not products of cell lysis or cell death. They described different types of secretion systems but in nearly every case these structures are secreted products that enhance the survival of bacteria or damage the host primarily through interaction with prokaryotic and eukaryotic cells.

Intravenously injected recombinant human interleukin-1 alpha (IL-1 alpha) given to mice 4 h before infection with Brucella abortus 19 depressed the growth of bacteria in the spleen and liver. However, the same dose (10(5) U) or a 10-fold higher dose was not able to decrease numbers of bacteria when given to chronically infected mice.

In work published by Oliveira et al (2011) the importance of IL-1 and interleukin-1 receptor-associated kinase 4 (IRAK-4) in host innate immune response against B. abortus was shown to be critical to trigger the initial immune response but not in later phases of infection.
Once neutrophils have phagocytosed the pathogen they then proceed to destroy it with respiratory (oxidative) burst and antimicrobial peptides. The respiratory burst generates oxidants to destroy pathogens. When neutrophils bind to bacteria, they consume more oxygen, and the NADPH oxidase enzyme complex is activated. The NADPH oxidase enzyme complex is comprised of 5 subunits with one of them called the neutrophil cytosolic factor 1 (NCF1). In the process of respiratory burst, the NADPH oxidase enzyme complex produces a superoxide anion. This superoxide anion is then converted into hydrogen peroxide via the enzyme superoxide dismutase (SOD1). Myeloperoxidase (MPO) then converts the hydrogen peroxide to bactericidal compounds, such as hypohalides, using intracellular halide ions. In addition to the respiratory burst process, neutrophils also produce antimicrobial peptides to destroy pathogens. One example is bactericidal/permeability-increasing protein (BPI), which destroys gram-negative bacteria by damaging the inner membrane after binding to their lipopolysaccharide.

*Brucella sp* regulate gene expression in response to low oxygen tension and two high-oxygen-affinity terminal oxidases, namely cytochrome C and bd ubiquinol, are induced and are required for intracellular survival of *B. abortus* and *B. suis*. Another strategy for anaerobic respiration in this pathogen is the use of nitrate as an electron acceptor rather than oxygen. Recently a FixL-like sensor FlbS of Brucella abortus which binds haem and which is necessary for survival within eukaryotic cells has also been described.

Gomes *et al* 2012 suggest that virulent *Brucella sp* inhibit macrophage death thus avoiding exposure to the more hostile extracellular environment. In an ideal situation, the neutrophils use all of these tools to destroy invading pathogens quickly, and the affected animal shows no sign of illness.

Carvalho Neta *et al* (2008) indicated that *B. abortus* modulates the innate immune response by trophoblastic cells, suppressing the expression of pro-inflammatory mediators during the early stages of infection that is followed by a delayed and mild expression of pro-inflammatory chemokines, which is similar to the profile of chemokine expression in the placentomes of experimentally infected cows. They suggested that this trophoblastic response is likely to contribute to the pathogenesis of *B. abortus*-induced placentitis.

Macrophages and dendritic cells act as antigen presenting cells (APC) and link the innate immune system with the adaptive immune system. Activated macrophages and dendritic cells perform pathogen uptake and process the antigenic material into peptides, presenting them in association with major histocompatibility (MHC) class II and I molecules to CD4+ T helper (Th) lymphocytes and CD8+ cytotoxic T lymphocytes (CTLs), respectively. T cells recognise peptide–MHC complexes via their specific receptors, T-cell receptors (TCRs).

**Inflammatory response**

The main innate immune response is inflammation, which increases the blood flow to the site of infection or trauma thus bringing cells to attack and destroy pathogens. If the pathogen is not eliminated quickly, white blood cells start producing several substances that trigger an inflammatory response that is needed to recruit additional immune cells to fight the infection. If the invading pathogen is not rapidly eliminated and the inflammation process persists, it leads to fever. Increased body temperature helps with infection control and elimination because some viruses and bacteria do not survive higher temperatures, whereas many immunological processes become further stimulated. However, prolonged inflammation and fever are harmful to the animal’s overall health; therefore the infection needs to be eliminated quickly to prevent further immune complications.

If the health challenge is not eliminated by the innate immune system, the adaptive system takes over. This transition is mediated by some of the white blood cells from the innate system, which “introduces” the foreign agent to specific cells from the adaptive system for activation of this immune response. Collectively, innate immune responses aim
initially to limit microbial spread using intracellular bactericidal mechanisms and, in parallel, to induce a dynamic adaptive immunity, which is mediated by antigen specific lymphocyte clones responsible for the clearance of the infection.


**CONCLUSION**

Brucella has developed sophisticated evolutionary mechanisms in order to ‘hide’ from, or manipulate, cellular immunity, and to achieve intracellular persistence. It has been shown that the innate immune system’s components interact with the adaptive immune system to improve adaptive responses. It has been suggested that if we can find ways to manipulate the innate immune system, we may not only improve the animals’ baseline resistance to infection, but we might also be able to improve the cow’s future resistance to infections by improving immune memory, which is the result of the adaptive immune response.

The following schematic from P. Skendros & P. Boura illustrates the immune response to Brucella infections

- **Ag**: antigen
- **APC**: antigen-presenting cell
- **B7**: CD80/CD86 co-stimulatory molecules
- **BCV**: Brucella-containing vacuole
- **CTL**: cytotoxic lymphocyte
- **IFNγ**: interferon gamma
- **IL**: interleukin
- **MHC II**: major histocompatibility complex type II
- **TCR**: T-cell receptor
- **TLR**: Toll-like receptor
- **TNFa**: tumour necrosis factor alpha
Phagocytosis and/or pattern recognition receptor (e.g. TLR) signalling lead to the activation of APC and the priming of naïve CD4+ T lymphocytes towards a Th1 phenotype (innate immunity).

The Th1 cytokines (TNFα, IFNγ) enhance the anti-Brucella mechanisms of macrophages (Mφ) and induce the CD8+ CTL-mediated cytotoxicity against Brucella-infected Mφ (specific cellular immunity). Innate lymphocytes are early producers of IFNγ, linking innate to specific immunity.

The Th2 response activates B lymphocytes (B) for antibody production, facilitating the phagocytosis of Brucella through opsonisation (specific humoral immunity). The Th2 cytokines (e.g. IL-10) inhibit the action of Th1 cytokines (e.g. IFNγ) and vice versa.

Which one of the following statements are correct?

1. The main function of innate immunity to bacteria is:
   a. To phagocytose bacteria
   b. To neutralise bacteria through antibodies
   c. To present antigens to the adaptive immune system for antibody production
   d. To limit spread through bactericidal means and induce adaptive immunity
   e. None of the above

2. Epithelial cells of the urogenital tract
   a. Are not involved in innate immune response
   b. Do participate in innate immune response
   c. Are phagocytic in nature
   d. Produce stem cells for lymphocyte production
   e. Are resistant to Brucella sp infections

3. Immunoglobulin A is
   a. An antibody found in the blood stream
   b. Included in the opsonin system
   c. A chemotactic agent
   d. An immunomodulating agent
   e. None of the above

4. Cytokines are
   a. Pro-inflammatory agents
   b. Anti-inflammatory agents
   c. Phagocytic agents
   d. None of the above
   e. Both anti-inflammatory and pro-inflammatory

5. Important cellular components of the innate immune system include
   a. Red blood cells
   b. Hepatocytes
   c. Phagocytic cells
   d. Epithelial cells
   e. All of the above

6. Interleukine 1 (IL-1)
a. Is important in chemotaxis
b. Is important for presenting Brucella antigen to the adaptive immune system
c. Is not part of the innate immune system
d. Is found on the surface of only endothelial cells
e. Is an antibody

7. Brucella spp are

a. Intracellular parasites
b. Extracellular parasites
c. Survive only in trophoblasts
d. Survive in epithelial cells
e. None of the above

8. The respiratory oxidative burst

a. Inactivates the NADPH oxidase enzyme complex
b. Increases chemotaxis
c. Generates oxidants to destroy pathogens
d. Enhances opsonisation
e. Enhances phagocytosis by macrophages

9. The function of complement is to

a. Enhance phagocytosis of antigens by opsonisation
b. Supress chemotaxis of macrophages
c. Suppress chemotaxis of neutrophils
d. Prevent lysis of foreign cells
e. Prevent clumping of antigenic agents

10. Brucella sp respond to loss oxygen lesions by

a. Regulating gene expression
b. Inducing cytochrome C and bd ubiquinol
c. Using nitrate as electron acceptor
d. Binding haem by Fix L-like reception
e. All of the above

REFERENCES


Gene Mayer, PhD Microbiology and Immunology, University of South Carolina School of medicine.


