Mastitis- S.Aureus

The Immune Response

Innate Immunity

Physical barrier

- first line of defense (sphincter and keratinized epithelium of the duct canal). Those two first encounters protect the host between milking periods and enables the bacteria to reach the cistern gland.
- The bacterium then passes into the periductal lymphatic system, it is detected by the immune system of the mammary glands. The pathogen recognition receptors (PRR) are found on cells from the innate immune system such as macrophages, neutrophils or dendritic cells.

PRR

Toll-like receptors (TLR) or NOD-like receptors (NLR)- recognize pathogen associated molecular patterns (PAMPs) or damage associated molecular pattern (DAMPs)

Innate Immunity

Type of pathogen causing the Mastitis will activate different receptors.

- TLR-4 will bind to lipopolysaccharides, thus recognize gram-negative bacteria, whereas TLR-2 will identify gram-positive bacteria.
- S. aureus is detected by TLR-2 that binds peptidoglycan (PGN) and lipoteichoic acid (LTA).
- Other recognition structures for S. aureus include mannose-binding lectins (MBL), ficolins, and complement molecules.

Inflammatory response during mastitis

- The concentration of Eicosanoids increases during Mastitis.
- Some eicosanoids are pro-inflammatory (prostaglandin E2 (PGE2), prostaglandin F2 alpha (PGF2a) and thromboxane B2 (TXB2) that will generally increase the permeability of vascular tissues and its blood flow enabling the infiltration of leukocytes in the site of infection, or induce fever) .
- Others help resolve the inflammation (prostaglandins D2 and 15-Deoxy-Delta-12,14-prostaglandin J2 (15 d-PGJ2) have the opposite effect: they block the activation of NFkB (a transcription factor enhancing the expression of pro-inflammatory factors) and therefore inhibit the leukocytes infiltration).

The cell composition of the milk

- plays a role in the pathogen eradication
- composed of macrophages but lymphocytes, neutrophils and mammary epithelial cells are also found.
- When the pathogen reaches the gland, the leukocyte composition is changed with an increase in the neutrophil concentration.

Neutrophils and Macrophages

- **Neutrophils** migrate from the circulatory system into the mammary gland tissue, recruited by cytokines and complement components (C5a and C3a). further induced by the inflammatory cytokines to be bactericidal through the release of defensins, reactive oxygen species and antibacterial peptides such as cathelicidins, hydrolases, proteases, and lysozymes. They also release chemokines prostaglandins and leukotrienes that enhance inflammation.
- **Macrophages** phagocytose bacteria in the tissue environment, and release pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β).

Epithelial cells

- release TNF-α, IL-6, and chemokine IL-8 after bacterial adhesion
- activate several PRRs to induce inflammation
- endothelial cells of mammary gland vasculature upregulate cellular adhesion molecules (E-selectin), intercellular adhesion molecule 1 (ICAM-1) and vascular cellular adhesion molecule 1 to facilitate entry of immune cells of the infected site. IL-17 is produced by γδ T cells in early stages of infection; IL-17 stimulates release of antibacterial proteins, cytokines, and chemokines, which target neutrophils.

Lactoferrin Protein and Complement Factors

- Alters the milk's composition to transform it to an unsuitable environment for the bacteria.
- Its role in the infection is put forward by the change in its concentration, from low when healthy conditions are met, to high when infection occurs.
- The **complement factors C3b and C3bi** are major components of the innate immune system through their role of bacteria's opsonization that will later be phagocytosed.

Adaptive Immune Response

- Bacterial antigens are recognized by major histocompatibility complex class II (MHCII) molecules on professional antigen-presenting cells, such as B lymphocytes, and macrophages.
- Lymphocytes such as CD4+ T helper cells and CD8+ cytotoxic T cells regulate the immune response and eliminate damaged cells
- CD4+ T helper cells activate CD8+ T helper cells and NK cells and facilitate B cell differentiation by releasing the cytokine IL-2.
- IFN-γ secreted by Th1 cells switches neutrophils to the IgG2 isotope with enhanced phagocytosis, while Th2 cells drive antibody-mediated immunity. Th17 cells produce IL-17, IL-21, IL-22, and IL-26, which recruit neutrophils and form abscesses.
- Opsonizing antibodies help neutrophils in phagocytosis of bacteria.

Damage from Host Immune Response

- All damage caused by host response because no direct correlation between the bacterial counts and levels of damage. No one species of bacteria was responsible for causing severe symptoms
- Bacterial invasion will trigger the adaptive immune system causing **activation of macrophages**. Macrophages can be phenotypically divided into two subpopulations, M1 and M2. In response, they secrete IL-12 and IL-23 to promote an inflammatory Th-1 response.

Damage Caused by Host Response

- When excess amounts of ROS and RNS are secreted, cellular components such as lipids, proteins and DNA can be denatured causing necrosis and apoptosis. M1 macrophages also generate proteases such as lysozyme and pro-inflammatory lipids such as cyclooxygenase and lipoxygenase. The result of these molecules can be seen in mastitis as red discoloration, swelling, and breast abscesses. M2 macrophages function to downregulate inflammation. IL-4, IL-10 and IL-13 are secreted which are anti-inflammatory cytokines.
- The innate immune system also causes the activation of neutrophils. These cells secrete proteases such as elastase which can both inactivate bacterial toxins and damage the host.

Bacterial Evasion

S. aureus interferes with neutrophil extravasation and chemotaxis

- secretion of specialized proteins called staphylococcal superantigen-like proteins (SSLs). These proteins slow down the rate of bacterial clearance and phagocytosis by binding to components of the innate immune system.

S. aureus secretes many proteins that interfere with complement directed opsonization.

- Aureolysin, a secreted Zn-dependent protease, cleaves C3 to generate C3a and C3b. Complement factors I and H bind or degrade C3b, preventing its accumulation on the staphylococcal surface. It also inhibits the protein C5b from associating with other complement proteins to form the membrane attack complex (MAC).

Bacterial Evasion – S.Aureus

- Peptidoglycan acetylation (OatA) and D-alanylation of teichoic acids (DltABCD) help provide for staphylococcal resistance against antimicrobial-peptide and lysozyme mediated killing . The production of siderophores is also considered to be another potential pathogenic trait. Siderophores are molecules that bind and acquire iron from the host.

Manipulation of adaptive immune response

Polyclonal activation of B cells by staphylococcal protein A

- It has also been found that SpA binding to B cells results in the downregulation of certain B-cell receptors and co-receptors, thereby limiting proliferation and inducing apoptotic cell death. This can lead to decreased production of sufficient memory cells needed to prevent future infections.

S.aureus strains secrete various superantigens including toxic shock syndrome toxin and staphylococcal enterotoxins. Staphylococcal superantigens have the ability to bypass the conventional MHC-restricted antigen presentation and processing, significantly increasing Th1 cell activation. By inducing the production of cytokine producing Th1 cells, superantigens can skew the immune response towards a heavily Th1 type, delaying the development of antigen-specific antibodie

Virulent Factors – S.aureus

- promote the adherence to host cells to initiate infection, including fibronectin-binding proteins, collagen-binding proteins, iron-regulated surface determinants, ECM-binding proteins, and surface proteins.
- Possess factors that enable them to evade the innate immune responses related to leukocyte migration and phagocytic activity.
- Secrete evasion factors that impede neutrophil migration to the site of infection, induce neutrophil lysis, and inactivate the complement system.

Bacterial Clearance

- Infectious mastitis can resolve without antibiotics.
- Adequate breast drainage may flush out the pathogen or the mother's immune response and the antibacterial properties of human milk can clear the infection
- In the case of S. aureus infection, the bacteria may not be completely cleared. Acute mastitis can evolve into chronic and subclinical mastitis with persistence of the bacteria in the mammary gland
- Research suggests that in S. aureus related mastitis, there is less NF-kB signaling and a delayed secretion of inflammatory cytokines like TNF-alpha. One study suggested that S. aureus infection of mammary epithelial cells does not stimulate activation of the NF-kB factor complex, although the infection will trigger this cascade in professional immune cells . As a result, there is a greater likelihood that S. aureus infections can establish colonies and biofilms and resist a host immune response

Bacterial Clearance

- S. aureus can increase expression of TGFB1, which is an inflammatory antagonist (anti-inflammatory). Another immune dampener, IL-10 has been shown to be increased after challenge with S. aureus
- Biofilm formation by S. aureus are also significant in determining clearance of the bacteria. Biofilms enable bacterial cells to evade the antibiotic and host defense mechanisms because the dense extracellular matrix and exterior layer of cells will shield the interior layer.

Recovery and Immunity

- No vaccines available against S. aureus so antibiotic treatment and even surgical drainage are often necessary to cure infections .
- If the infected patients take the prescribed antibiotics timely and correctly, and is relatively immunocompetent, then the patient should be able to fully recover from the S. aureus infection, causing mastitis.
- Along with the help of antibiotics, the host innate and adaptive immune response against the bacteria should be enough to clear it from the host
- Breastfeeding technique also plays a big role in recovery from infection in Elizabeth's case. Improper breastfeeding practices may continue to cause milk stasis as well as pressure on the breast tissue, and in addition, chronic bacterial infections are likely and can lead to chronic inflammation

Immunity

- S. aureus infections generally fail to produce protective immunity although there may be detectable T and B cell responses.
- Patients tend to have weak protective immunity due to the Protein A, however this is via secondary infection .
- It has been found that initial infection with S. aureus can result in memory B cells, however, when re-infection occurs, Protein A decreases long-lived plasma cells (LLPCs) residing in the bone marrow