CASE 4: HUMAN MASTITIS

Immune Response Questions

A Summary

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INNATE AND ADAPTIVE IMMUNE RESPONSES

INNATE IMMUNE RESPONSES

- Provides a physical barrier
  - Teat sphincter contributes to this protection
  - Muscle blocks entrance of the duct by remaining tightly closed
  - Chemical components of stratified squamous epithelium of the duct
    - Fills in the duct with keratin
    - Keratin physically blocks bacteria from invading, acts like a plug
    - Also chemically attacks the pathogen - alter their cell wall by using fatty acids composing the keratin
    - Physical and chemical blocks protect the host between milking periods
- After bacteria gets through the sphincter and keratinized epithelium barriers, they move through the duct canal and into the periductal lymphatic system
  - Detected by the immune system of the mammary glands

INNATE AND ADAPTIVE IMMUNE RESPONSES

INNATE IMMUNE RESPONSES: RECOGNITION OF PATHOGENS

- Pathogens are recognized by receptors
  - Pathogen Recognition Receptors (PRRs) are found on macrophages, neutrophils, or dendritic cells
- PRRs can be sub-categorized into
  - Toll-like Receptors (TLR)
  - NOD-like Receptors (NLR)
- PRRs recognize pathogen associated molecular patterns (PAMPs) or damage associated molecular pattern (DAMPs)
  - Activation of receptors lead to an immune response through intracellular signaling cascades
    - This would lead to the increased expression of pro-inflammatory molecules }(ie. cytokines)

INNATE AND ADAPTIVE IMMUNE RESPONSES

INNATE IMMUNE RESPONSESI HUMAN MASTITIS

MASTITIS

- Can be caused by a variety of bacteria
  - Gram-negative (like Escherichia coli)
  - Gram-positive (Staphylococcus aureus)
- Different pathogens will activate different receptors
  - TLR-4: Binds to lipopolysaccharide (gram-negative bacteria)
  - TLR-2: Bindns to peptidoglycan (PGN) and lipoteichoic acid (LTA) (gram-positive bacteria, S. aureus)
    - S. aureus can also be recognized through mannose-binding lectins (MBL), ficolins, and complement molecules

INNATE AND ADAPTIVE IMMUNE RESPONSES

INNATE IMMUNE RESPONSES

MAMMOGENESIS DURING PREGNANCY

- Proliferation and new organization in the tissue
  - Result in higher concentrations of IL-4, IL-10, and TNFalpha (pro-inflammatory cytokines)
  - Cytokines would allow the maintenance of homeostasis and can also be upregulated to pro-inflammatory factors in case infection occurs

INFLAMMATION

- A response to the pathogen’s presence
- During mastitis, eicosanoids concentration increases (some being pro-inflammatory, while others being inflammation-resolving

INNATE AND ADAPTIVE IMMUNE RESPONSES

INNATE IMMUNE RESPONSES

PRO-INFLAMMATORY EICOSANOIDS

- Will increase permeability of vascular tissues and blood flow
  - Leads to infiltration of leukocytes in the site of infection, or induced fever
    - Prostaglandins, PG → PGE2, PGF2a
    - Thromboxane, TX → TXB2

INFLAMMATION-RESOLVING EICOSANOIDS

- Block the activation of NFkB (a transcription factor that enhances the expression of pro-inflammatory factors)
  - Leads to inhibited leukocyte infiltration
    - Prostaglandins, PG → PGD2, PGJ2

INNATE AND ADAPTIVE IMMUNE RESPONSES

INNATE IMMUNE RESPONSES

MILK CELL COMPOSITION

- Also plays a role in pathogen-eradication
- Mostly consisted of macrophages (lymphocytes, neutrophils, and mammary epithelial cells)
    - BUT when a pathogen reaches the gland, the leukocyte composition is altered with a consequential increase in neutrophil concentration

NEUTROPHILS

- Are recruited by cytokines and complement components (C5a and C3a)
- Migrate from the circulatory system into the mammary gland tissue
- Inflammatory cytokines cause neutrophils to become bactericidal through the release of defensins, reactive oxygen species, and antibacterial peptides
    - Antibacterial peptides: cathelicidins, hydrolases, proteases, and lysozymes

INNATE AND ADAPTIVE IMMUNE RESPONSES

INNATE IMMUNE RESPONSES

MORE ON NEUTROPHILS

- Release chemokines: prostaglandins and leukotrienes
  - Which enhance inflammation (recruit more cells increase vascular permeability further)
- Have the ability to form neutrophil extracellular trap (NET)
  - NETs physically block the pathogen through the release of nuclear and granular material from the neutrophil

MACROPHAGES

- Phagocytose bacteria in the tissue environment
- Release pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-a) and interleukin 1 beta (IL-1B)

INNATE AND ADAPTIVE IMMUNE RESPONSES

INNATE IMMUNE RESPONSES

EPITHELIAL CELLS IN THE MAMMARY GLANDS

- Release TNF-a, IL-6, and chemokine IL-8 after exposure to bacteria
- After, endothelial cells of mammary gland vasculature upregulate cellular adhesion molecules (E-selectin), intercellular adhesion molecule 1 (ICAM-1), and vascular cellular adhesion molecule 1
    - These facilitate the entry of immune cells of the infected site
- Produce Lactoferrin protein, an antimicrobial factor that alters the milk's composition to make it more challenging for the bacteria to invade
    - Lactoferrin deprives bacteria of iron, with the use of bicarbonate
    - Lactoferrin concentration increases when infection occurs

INNATE AND ADAPTIVE IMMUNE RESPONSES

INNATE IMMUNE RESPONSES

COMPLEMENT FACTORS: C3b and C3bi

- Major components of the innate immune system
- Opsonize bacteria that will later be phagocytosed
- Create a pore on bacteria's surface leading to its death

ADAPTIVE IMMUNE RESPONSES

MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II (MHCII)

- Recognizes antigens on professional antigen-presenting cells like B lymphocytes and macrophages
- Lymphocytes (like CD4+ T helper cells and CD8+ cytotoxic T cells regulate the immune response and eliminate damaged cells respectively
    - CD4+ Th cells activate CD8+ Th cells and NK Cells
    - And they also facilitate B cell differentiation by releasing cytokines (IL-2)
- Th17 cells produce: IL-17, IL-21, IL-22, and IL-26(recruit neutrophils)

DAMAGE TO HOST

BACTERIAL DAMAGE TO HOST

- Mostly a result of the host immune response to bacterial infection, not the pathogens causing direct damage to the host
  - This fact is based on two pieces of evidence
    - 1) They did not find a direct correlation between bacterial counts and levels of host damage
    - 2) They did not find any one specific species of bacteria to responsible for causing severe symptoms (bacteria can invade through cracks on the skin, *Pseudoomonas* spp. And *S. aureus*)
- Damage can be caused by Inflammation response
  - Macrophages can be activated by PAMPs, IFN-y, and TNF-a → They then secrete IL-12 and IL-23 to promote an inflammatory Th-1 response
  - Inflammatory responses can also result from macrophage production of reactive oxygen and nitrogen species (ROS and RNS)

DAMAGE TO HOST

BACTERIAL DAMAGE TO HOST

ROS and RNS Species

- ROS are generated by NADPH oxidase
- RNS are generated by NOS-2
- ROS and RNS secretion in excess amounts leads to the denaturation of cellular components such as lipids, proteins, and DNA
    - Happens through the process of necrosis and apoptosis

MASTITIS: Red Discolouration, Swelling, and Breast Abscesses

- Result from M1 macrophages generating proteases such as lysozyme and pro-inflammatory lipids
- M2 macrophages function to downregulate inflammation
    - Anti-inflammatory cytokines include IL-4, IL-10, and IL-13

DAMAGE TO HOST

BACTERIAL DAMAGE TO HOST

ACTIVATION OF NEUTROPHILS

- Neutrophils would secrete proteases such as elastase
  - Would inactivate bacterial toxins
  - Would damage the host
- Neutrophils are drawn to infection sites through chemokine gradients, and they will migrate away when these signals dissipate
  - Mechanism is imperfect → can drive chronic inflammation and damage due to continued ROS and protease release

BACTERIAL EVASION: INNATE IMMUNE RESPONSE

BACTERIAL ATTEMPT TO EVADE HOST RESPONSE ELEMENTS

MANIPULATION OF INNATE IMMUNE RESPONSE

- Staphylococcal Superantigen-Like proteins (SSLs)
  - *S. aureus* interferes with neutrophil extravasation and through the secretion of specialized proteins, SSLs
  - SSLs slow down the rate of bacterial clearance and phagocytosis
    - Mechanism: They bind to the components of the innate immune system
    - For example: SSL7 binds complement factor C5 and IgA with high affinity, and hinders the latter part of complement activation
- Extracellular Adherence Protein (Eap)
  - Production of Eap inhibits leukocyte migration
  - Eap: made up of four B-grasp-like domains and associates with ICAM-1
    - Blocking ICAM-1 prevents neutrophils from squeezing through endothelial cells of the blood vessel wall (can’t get to damaged tissue)

BACTERIAL EVASION: INNATE IMMUNE RESPONSE

BACTERIAL ATTEMPT TO EVADE HOST RESPONSE ELEMENTS

S. AUREUS RELEASE PROTEINS THAT INTERFERE WITH COMPLEMENT PROTEINS DIRECTING OPSONIZATION

- AUREOLYSIN (a secreted Zn-dependent protease)
  - Cleaves C3 to generate C3a and C3b
  - Complement factors I and H bind or degrade C3b to prevents its accumulation on staphylococcal surface
    - It also inhibits C5b from associating with other complement proteins to form the membrane attack complex (MAC) → In this case, MAC might not be that effective because *S. aureus* is a gram-positive bacteria with a thick peptidoglycan layer

BACTERIAL EVASION: INNATE IMMUNE RESPONSE

BACTERIAL ATTEMPT TO EVADE HOST RESPONSE ELEMENTS

PEPTIDOGLYCAN ACETYLATION (OatA) and D-ALANYLATION OF TEICHOIC ACIDS (DltABCD)

- Provide staphylococcal resistance against antimicrobial-peptide and lysozyme mediated killing

SIDEROPHORES (potential pathogenic trait)

- Molecules that bind and acquire iron from the host
- Presence of siderophores was significantly higher in strains involving mastitis
- Mechanism enables bacteria to evade siderocalin (Scn), a mammalia lipocalin-type protein (prevents iron uptake by pathogenic bacteria)

BACTERIAL EVASION: ADAPTIVE IMMUNE RESPONSE

BACTERIAL ATTEMPT TO EVADE HOST RESPONSE ELEMENTS

S. AUREUS EVASION OF HUMORAL RESPONSE

- Polyclonal activation of B cells by Staphylococcal protein A (SpA)
  - SpA is a secreted protein with five immunoglobulin-binding domains
    - Fcy domain: Binding to this domain prevents opsonophagocytic killing of Staphylococcus bacteria
    - Fab domain: Leads to clonal activation of B cells
      - SpA binding to B cells results in downregulation of certain B-cell receptors and co-receptors
      - This limits proliferation and induce apoptotic cell death
      - This can lead to decreased production of sufficient memory cells needed to prevent future infections

BACTERIAL EVASION: ADAPTIVE IMMUNE RESPONSE

BACTERIAL ATTEMPT TO EVADE HOST RESPONSE ELEMENTS

- Manipulation of T-cell mediated immune responses
  - *S. aureus* strains secrete various superantigens including toxic shock syndrome toxin and staphylococcal enterotoxins
  - Staphylococcal Superantigens: ability to bypass the MHC-restricted antigen presentation and processing
    - This significantly increases Th1 cell activation
    - Production of cytokine producing Th1 cells → superantigens can skew immune responses (heavily Th1 type)
      - DELAYS development of antigen-specific antibodies

- Virulent factors: promote adherence to host cells
  - Fibronectin-binding proteins, collagen-binding proteins, iron-regulated surface determinants, ECM-binding proteins, and surface proteins

OUTCOME: BACTERIAL CLEARANCE

CLEARANCE OF BACTERIAL PATHOGEN

- Properties of human milk as described earlier can clear the infection alone in some cases of mastitis
- *S. Aureus* cases are complicated may not be completely cleared
    - Acute mastitis can evolves into chronic and subclinical mastitis, and the bacteria may persist in the mammary gland
        - Persistence may be the result of bacteria capability to
            - Modify virulence factors
            - Create biofilms
            - Small colony variants (SCV)
            - Exist in intracellular spaces like epithelial cells and macrophages

OUTCOME: BACTERIAL CLEARANCE

CHALLENGE OF CLEARING S. AUREUS

- *S. aureus* is a Gram-positive bacteria
  - That induces a weaker immune response compared to those induced by gram-negative pathogens
- Bacteria can trigger an immune dampening right after infection
    - This would result in an increase in TGFB1 (an inflammatory antagonist, would prevent inflammation) and IL-10
- Chronic infection may blunt T-cell reactivity
  - This is a result of myeloid-derived suppressor cells (MDSCs) and some influence of T-regulatory cells
- Capsular Polysaccharides (CP)
  - Can enhance virulence and extracellular survival by inhibiting phagocytosis
- Acapsular *S. aureus* may develop enhances adhesive abilities, and become more prone to being internalized into epithelial cells
  - May avoid immune clearance by internalization within mammary epithelial cells

OUTCOME: BACTERIAL CLEARANCE

CHALLENGE OF CLEARING S. AUREUS

- Ability to form biofilms and resist a host immune response
  - Genetic and environmental factors influence biofilm formation
  - Two toxins are involved in establishing biofilms
    - Alpha and Beta toxins
  - Biofilms can be established in deep-seated pockets of infection → In the alveoli of the mammary gland
  - Biofilms can allow bacterial cells to evade the antibiotic and host defense mechanisms
    - The dense extracellular matrix and exterior layer of cells will shield the interior layer

OUTCOME: BACTERIAL CLEARANCE

CHALLENGE OF CLEARING S. AUREUS

- Formation of Small Colony Variants (SCVs)
  - Slow-growing subpopulations of bacteria will enhance their ability to survive and persist in the environment
    - Increased ability to persist intracellularly
    - Can introduce the possible ability to avoid detection by immune cells
    - May enhance antibiotic resistance
  - SCVs display: decreased respiration, decreased hemolytic activity, decreased coagulase activity, and increased resistance to aminoglycosides (which are all linked to electron transport)

- *S. aureus* as Facultative Intracellular Pathogens
  - Surviving intracellularly: may contribute to relapse and prolonged infections
  - Can invade mammary epithelial cells, endothelial cells, and fibroblasts
  - Antibody-mediated immune response not effective in this case
    - Cell-mediated immune response is also necessary

OUTCOME: PATIENT RECOVERY AND IMMUNITY

ANTIBIOTICS

- Paired with surgical drainage are often necessary to cure infection
- Combination of effective antibiotic treatment PLUS intact host immune response is required for bacterial clearance
- I not cleared completely or effectively, the bacteria may come back

VACCINE

- No vaccine developed and available

BREASTFEEDING TECHNIQUE

- Improper breastfeeding technique plays a big role in effective recovery
  - If not corrected, it may continue to cause milk stasis and pressure on breast
- Mother stopping breastfeeding because of the infection and pain, may lead to further milk stasis getting promoted = worse situation
- Abscesses and tissue damage: may lead to non-milk secreting epithelial cells because of damaged alveoli epithelial cells (compromised milk production)

OUTCOME: PATIENT RECOVERY AND IMMUNITY

RE-INFECTIONS AND PROTEIN A

- Initial exposure and developed memory cells
  - Re-infection may lead to a decrease in long-lived plasma cells (LLPCS) by Protein A
    - PROTEIN A: Binds with B-cells with immunoglobulins, leading to an inability of cells to survive in the bone marrow and differentiate into LLPCs
    - Binding of Protein A to B cells:
      - Results in downregulation of som B-cell receptors and co-receptors
        - This would limit proliferation → which would induce apoptotic cell death (Depletion of B-cells)
        - Next re-infection would induce a weaker immune response