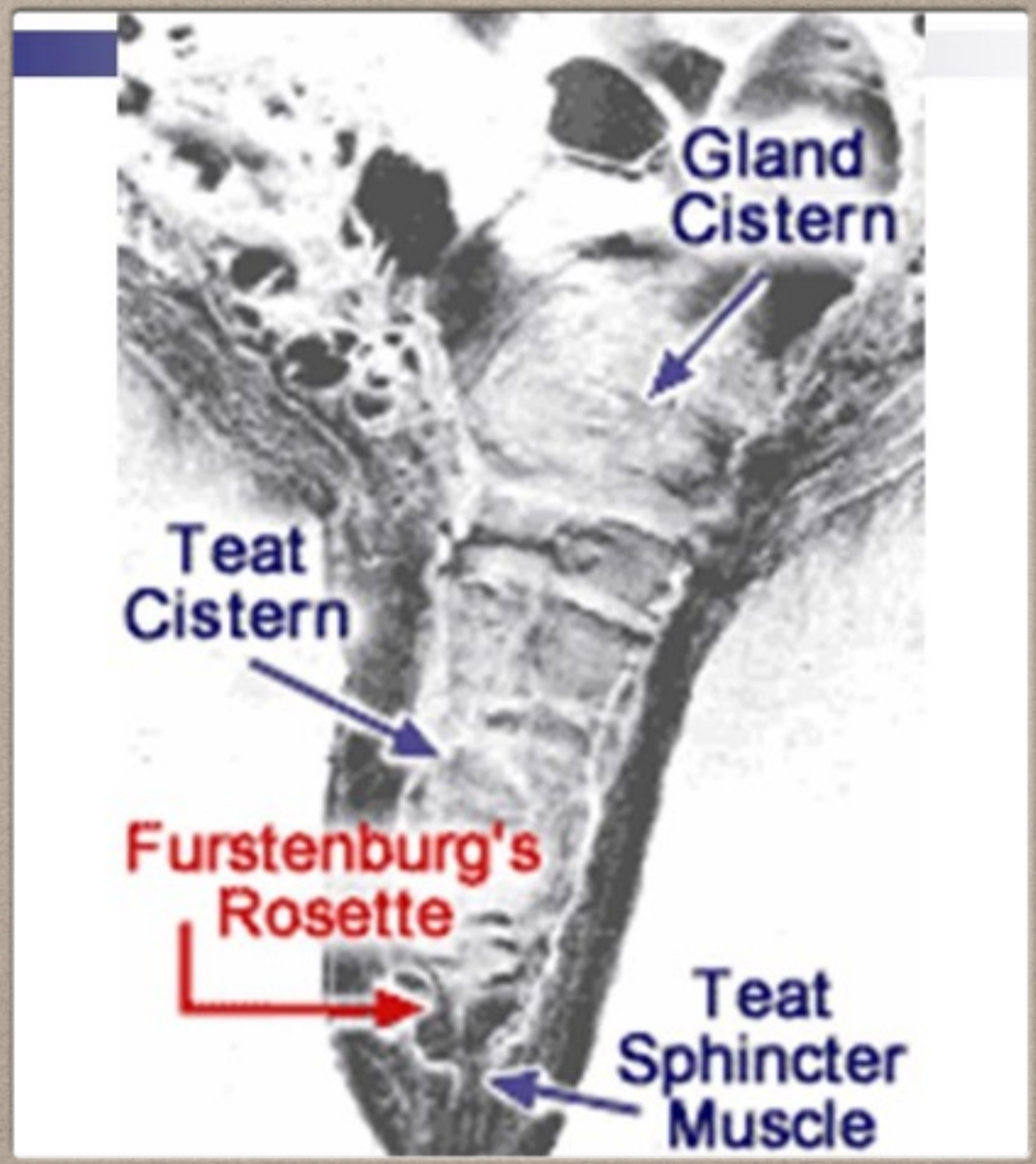
CASE 4 - IMMUNE RESPONSE

PATH 417A SUMMARY -
MICHELLE YANG (23634141)

#1 - HOST RESPONSE: WHAT ELEMENTS OF THE INNATE AND ADAPTIVE (HUMORAL AND CELLULAR) IMMUNE RESPONSE ARE INVOLVED IN THIS INFECTION?

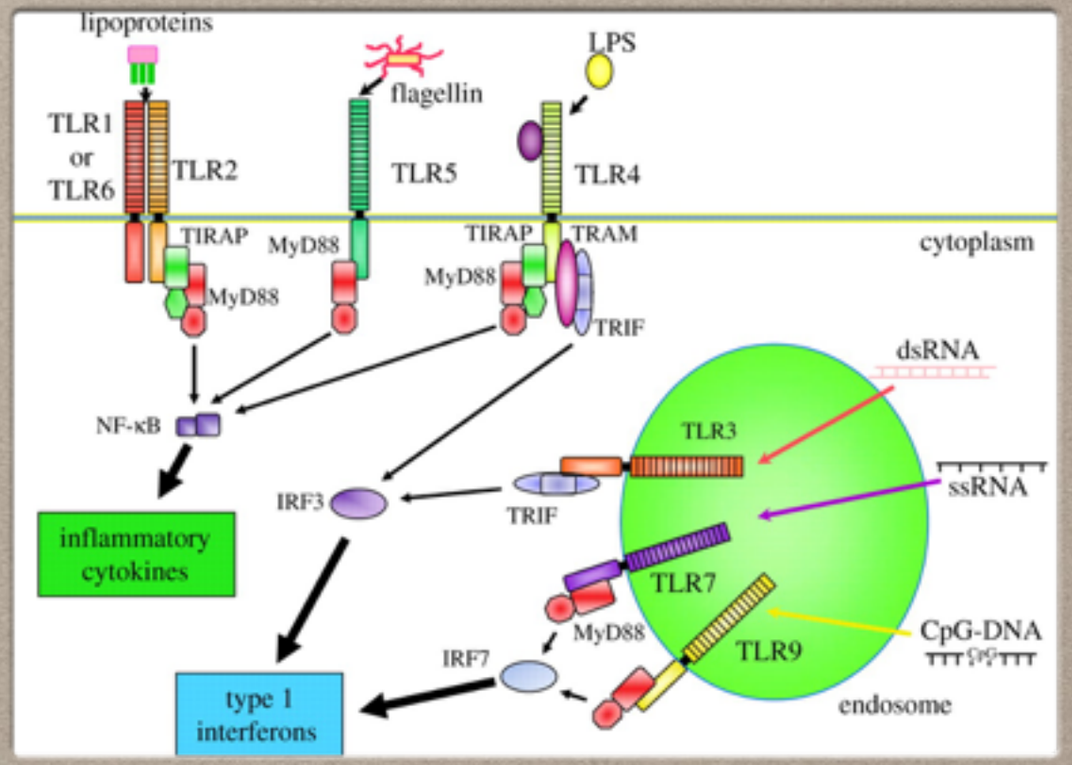
FIRST LINE OF DEFENCE - PHYSICAL BARRIER

- Teat sphincter muscle blocks the entrance of the duct by maintaining it tightly close
- Chemical component of the sqamous epithelium of the duct filled with keratin physically blocks bacteria from invading the host through a plug creation
    - also chemically attacks the pathogen by latering the cell wall with the help of fatty acids composing the keratin
- Protects host between milking periods and enables the bacteria to reach the cisterna gland
- Detectable to the immune system of the mammary glands once it gets through these barriers and into the periductal lymphatic system



BEGINNING OF INNATE IMMUNE RESPONSES - DETECTION BY IMMUNE SYSTEM: PAMPS AND PRRS

- Pattern recognition receptors (PRR) found on innate immune cells (ie. macrophages, neutrophils, dendritic cells)
- Types of PRR: Toll-like (TLR), NOD-like (NLR)
- Recognize pathogen associated molecular patterns (PAMP) to trigger immune response
  - TLR4 -> lipopolysaccharide (recognize gram-negative bacteria, like E.coli)
  - TLR2 -> peptidoglycan (PGN) and lipoteichoic acid (LTA) (recognize gram-positive bacteria, like Staphylococcus aureus)
  - Other recognition structures of Staphylococcus aureus: mannose-binding lectins (MBL), ficolins, and complement molecules

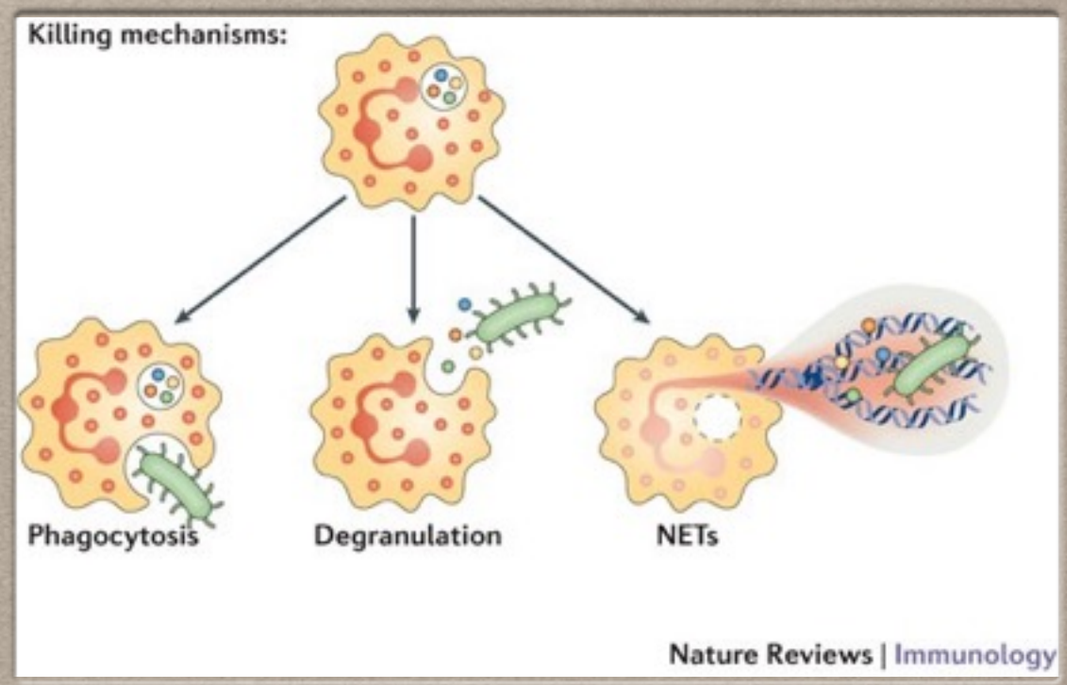


INFLAMMATORY RESPONSES THAT RESULT FROM MASTITIS

- Increase in concentration of eicosanoids
    - prostaglandin E2 (PGE2), prostaglandin F2 alpha (PGF2a) and thromboxane B2 (TXB2): generally increases permeability of vascular tissues and its blood flow enabling the infiltration of leukocytes in the site of infection, or induce fever
    - eicosanoids, prostaglandins D2 and 15-Deoxy-Delta-12,14-prostaglandin J2 (15 d-PGJ2): they block the activation of NFkB (a transcription factor enhancing the expression of pro-inflammatory factors) and therefore inhibit the leukocytes infiltration
- Uroplasminogen: conducts vascular alteration, facilitate the diapedeses of leukocytes and thus enhance the leukocytes' infiltration

INNATE IMMUNE RESPONSES: ROLE OF NEUTROPHILS AND MACROPHAGES

- Neutrophils recruited by cytokines and complement components (C5a and C3a) into the mammary gland tissue
    - 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
- Release chemokines, prostaglandins and leukotrienes - enhance inflammation through recruiting more cells and increasing vascular permeability
- Form a neutrophil extracellular trap (NET): physically blocks the pathogen through the release of nuclear and granular material from the neutrophil
- 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)

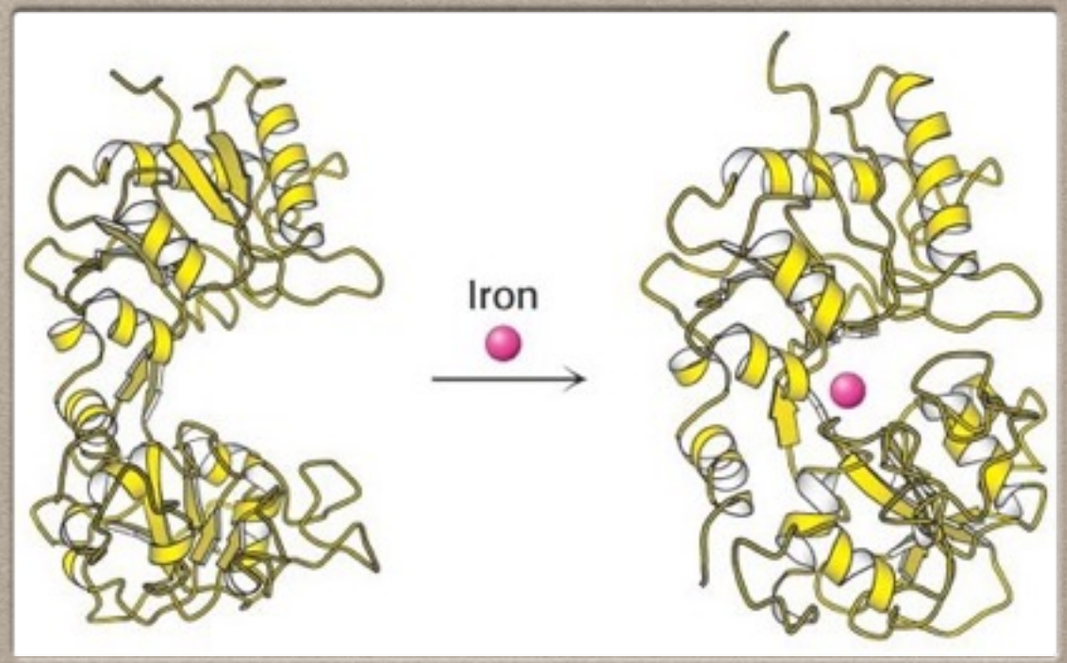


INNATE IMMUNE RESPONSES: ROLE OF EPITHELIAL CELLS AND OTHER HOST CELL RESPONSES SEEN IN INNATE RESPONSES

- Epithelial cells in mammary glands release TNF-α, IL-6, and chemokine IL-8 after bacterial adhesion
- Activate several PRR 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: produced by γδ T cells in early stages of infection; stimulates release of antibacterial proteins, cytokines, and chemokines, which target neutrophils

INNATE IMMUNE RESPONSES: THE ROLE OF LACTOFERRIN

- Antimicrobial factor found in the mammary glands produced by epithelial cells and leukocytes
- Alters the milk's composition to transform it to an unsuitable environment for the bacteria
- Deprives bacteria from iron by sequestering iron in the milk with the help of bicarbonate
- Low in healthy conditions, high during infection



COMPLEMENT SYSTEM - FACTORS C3B AND C3BI

MAJOR COMPONENTS OF THE INNATE IMMUNE SYSTEM THROUGH THEIR ROLE OF BACTERIA'S OPSONIZATION THAT WILL LATER BE PHAGOCYTOSED. IT WILL ALSO CREATE PORE ON THE BACTERIA'S SURFACE LEADING IT TO ITS DEATH.

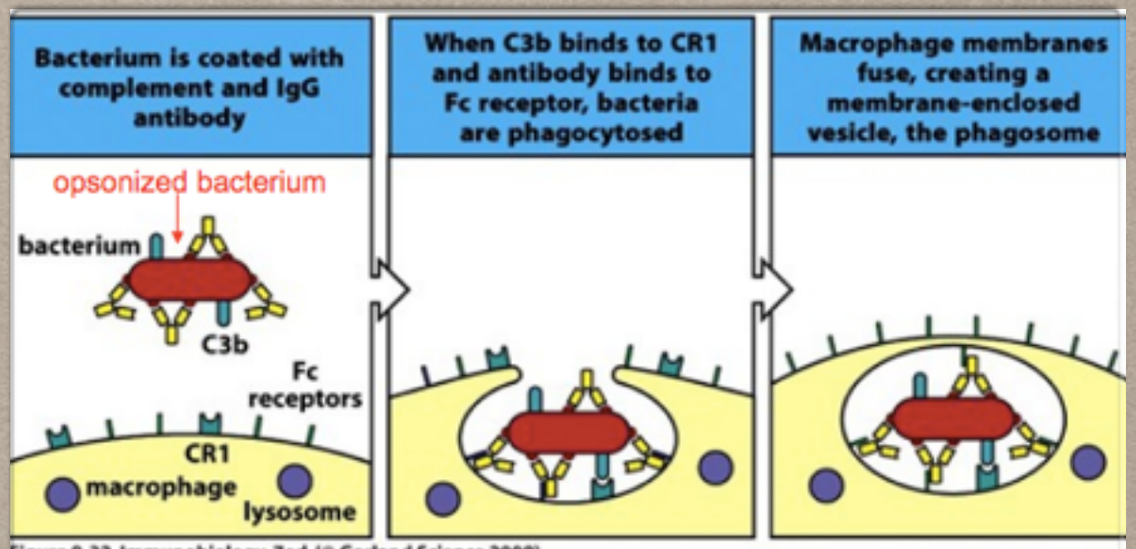
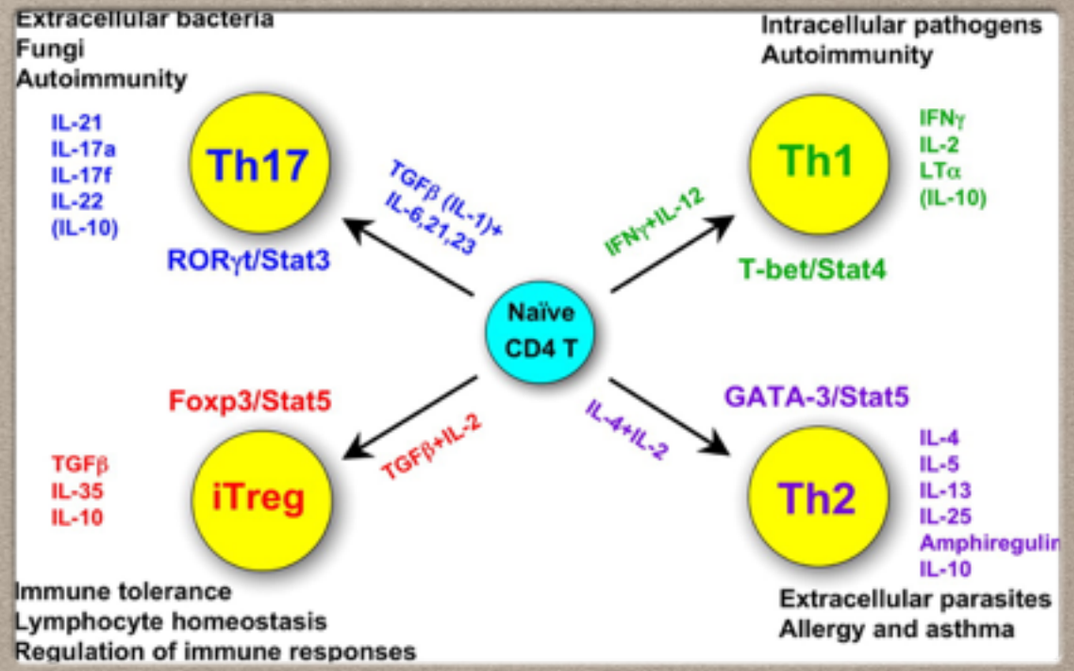


Figure 9-32 Immunobiology, 7ed. (© Garland Science 2008)

-both C3b and iC3b (fragment of C3b) are opsonins

ADAPTIVE IMMUNE RESPONSES

- Bacterial antigens are recognized by major histocompatibility complex class II (MHCII) molecules on professional antigen-presenting cells (ie. Dendritic cells, macrophages, Bcells)
- Both 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
- Bcells differentiate into cells that provide long term memory of the pathogen or cells that produce large amounts of antibody against S. aureus
- Opsonizing antibodies help neutrophils in phagocytosis of bacteria



MAMMOGENESIS AND OTHER ENVIRONMENTAL FACTORS THAT CAN AFFECT IMMUNE RESPONSE

- Female undergoes mammogenesis during pregnancy
- Involves cell modifications, like more proliferation and new organization in the tissue
- Includes higher concentration in IL-4, IL-10 and TNFalpha (pro-inflammatory cytokines)
- Presence of the cytokines allows maintenance of homeostasis and can upregulated pro-inflammatory factors in case of infection
- Cell composition of the milk: mostly composed of macrophages but lymphocytes, neutrophils and mammary epithelial cells are also found in healthy conditions. Leukocyte composition is changed with an increase in the neutrophil concentration when pathogen reaches the gland

#2 - HOST DAMAGE: WHAT DAMAGE ENSUES TO THE HOST FROM THE IMMUNE RESPONSE?

MOST DAMAGE IS CAUSED BY HOST IMMUNE RESPONSES

- Excess amounts of ROS and RNS secreted from macrophages: cellular components can be denatured causing necrosis and apoptosis
- M1 macrophages: generate proteases such as lysozyme and pro-inflammatory lipids such as cyclo-oxygenase and lipoxygenase
    - Result: red discoloration, swelling, and breast abscesses
- (Note: M2 downregulates inflammation with secretion of IL-4, IL-10, IL-13)
- Neutrophil damages:
    - Secrete proteases such as elastase which can both inactivate bacterial toxins and damage the host
    - Imperfect migration away from infection site after immune signals from chemokines dissipate, driving chronic inflammation and damage due to continued ROS and protease release

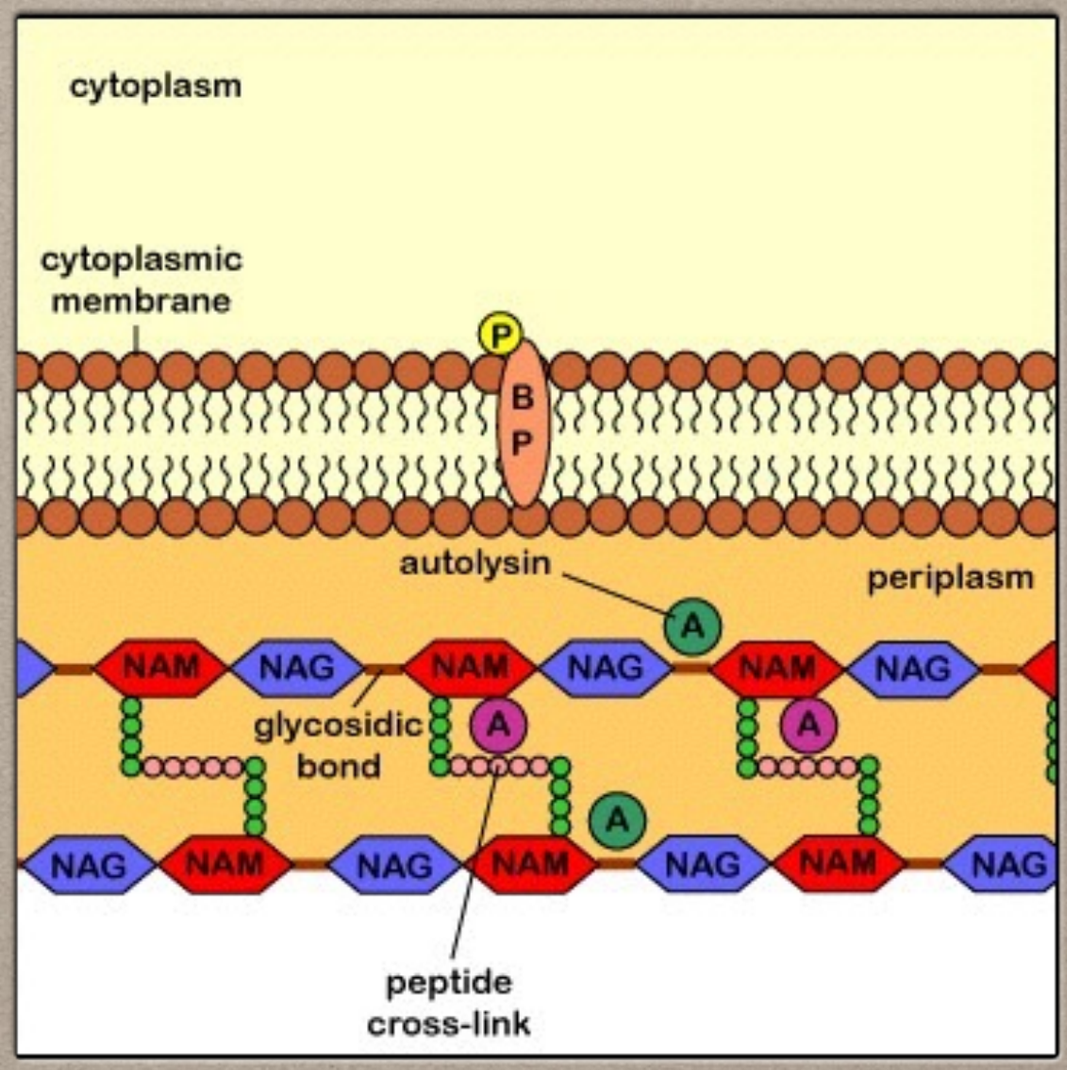
#3 - BACTERIAL EVASION: HOW DOES THE BACTERIA ATTEMPT TO EVADE THESE HOST RESPONSE ELEMENTS?

MANIPULATION OF INNATE IMMUNE RESPONSES - NEUTROPHIL EXTRAVASATION AND CHEMOTAXIS INTERFERENCE

- Staphylococcal superantigen-like proteins (SSLs) slow down the rate of bacterial clearance and phagocytosis by binding to components of the innate immune system
    - SSL7 binds complement factor C5 and IgA with high affinity, inhibiting the end stage of complement activation
- Production of extracellular adherence protein (Eap) can inhibit leukocyte migration
- ICAM-1 blocking prevents neutrophils from squeezing through endothelial cells of the blood vessel wall to enter the damaged tissue

MANIPULATION OF INNATE IMMUNE RESPONSES - COMPLEMENT SYSTEM INTERFERENCE

- Aureolysin (secreted Zn-dependent protease):
  - Cleaves C3 to generate C3a and C3b
  - Inhibits C5b from associating with other complement proteins to form the membrane attack complex (MAC)
- Thick peptidoglycan layer that protects it from killing via MAC
- Genes encoding for capsular polysaccharides (type 5 and 8 in particular) expressed in some strains that help protect the bacterium from digestion

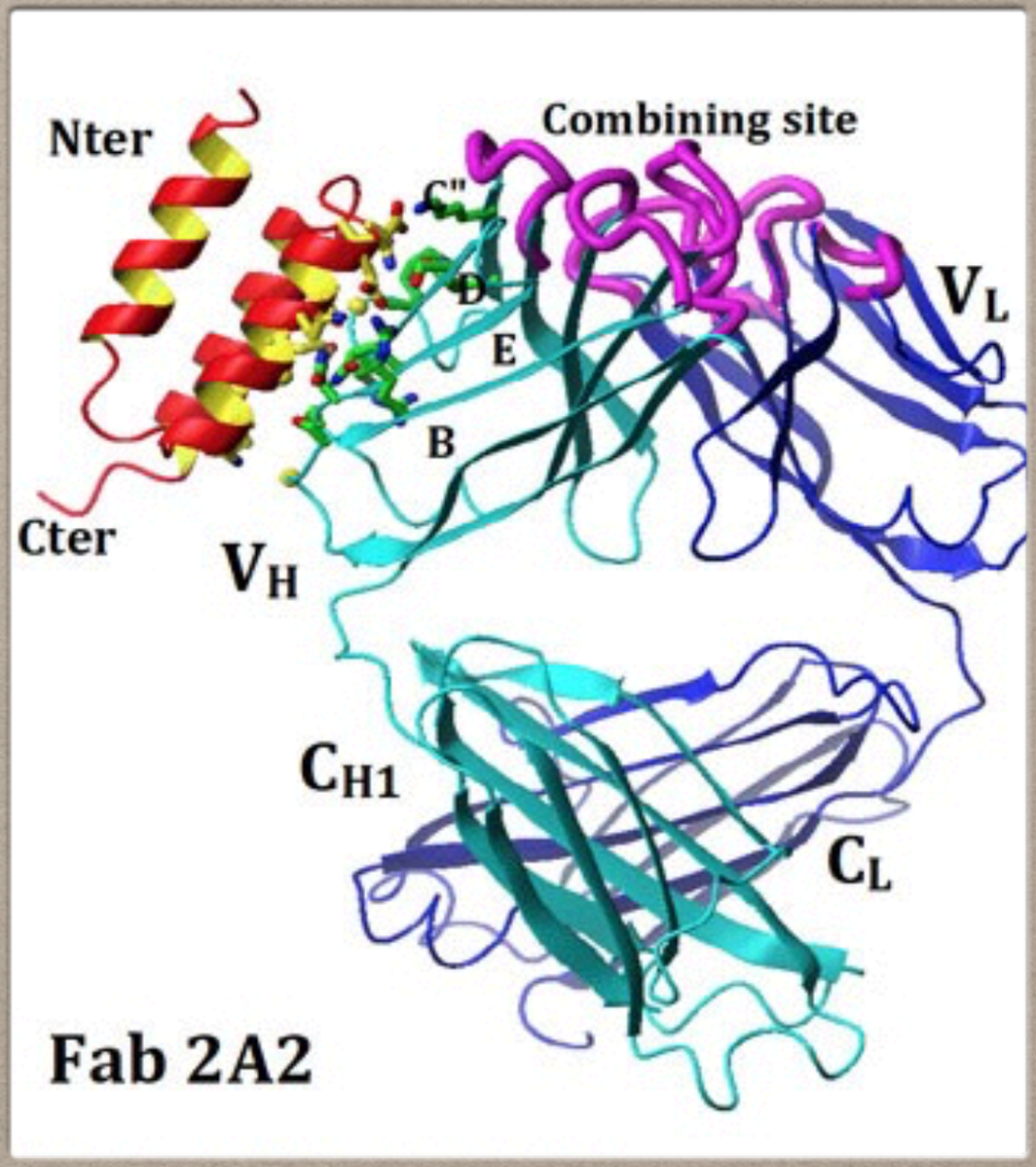


MANIPULATION OF INNATE IMMUNE RESPONSES - ANTIMICROBIAL PEPTIDES (AMP) AND REACTIVE OXYGEN SPECIES (ROS)

- Peptidoglycan acetylation (OatA) and D-alanylation of teichoic acids (DltABCD): provide for staphylococcal resistance against AMPs and lysozyme mediated killing
- Siderophores (bind and acquire iron from host): may facilitate establishment of infection
  - Significantly higher in all strains involved in mastitis
  - Helps evade siderocalin (Scn), a mammalia lipocalin-type protein that prevents iron uptake by pathogenic bacteria

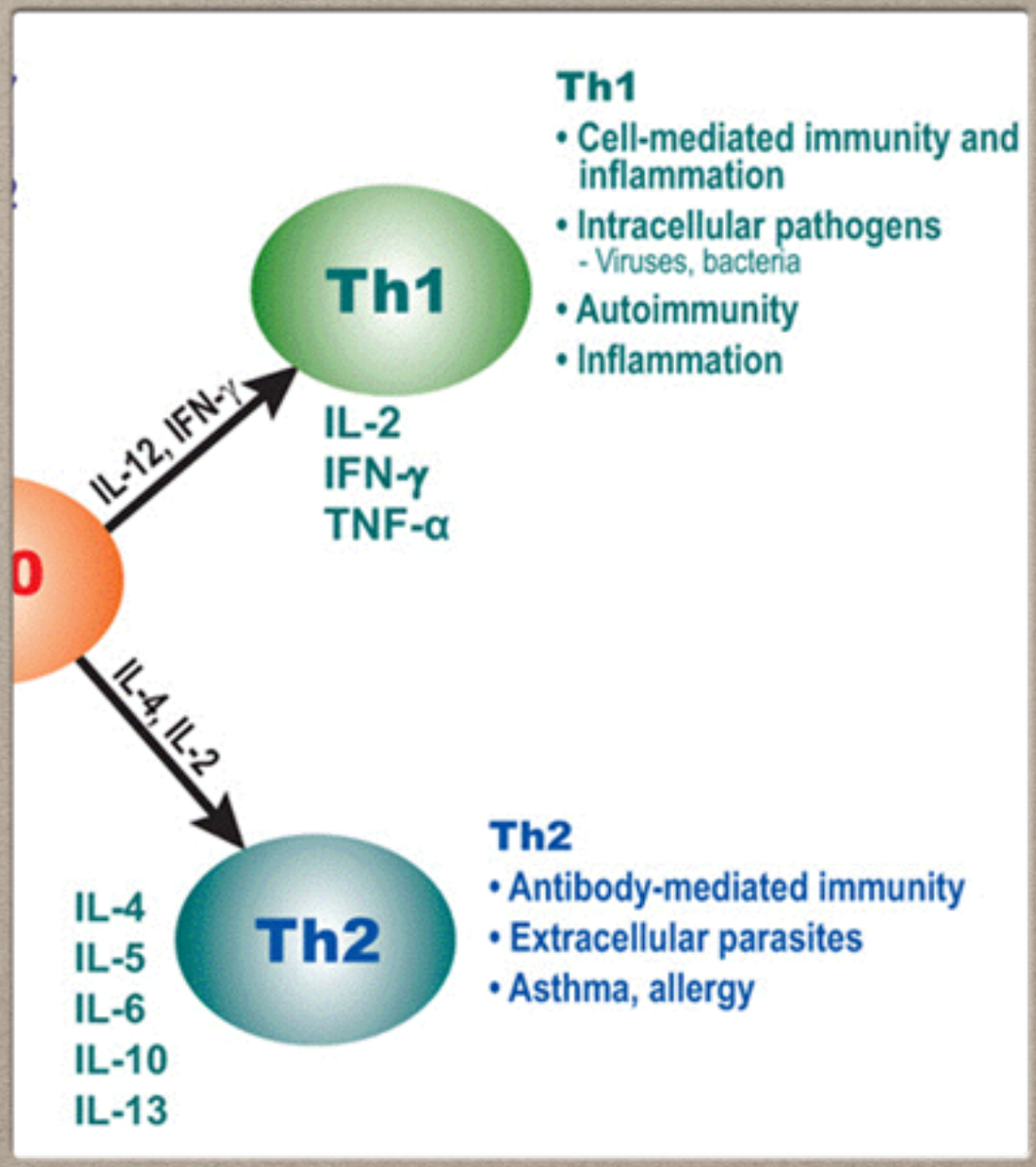
MANIPULATION OF ADAPTIVE IMMUNE RESPONSES

- S.aureus evade the humoral response through polyclonal activation of B cells by staphylococcal protein A (SpA)
- Binding to the Fcy domain of immunoglobulins (Igs) prevents opsonophagocytic killing of Staphylococcus bacteria while binding to the Fab domain of Igs leads to clonal activation of B cells
- SpA binding to Bcells also results in down regulation of certain Bcell receptors and co-receptors, limiting proliferation and inducing apoptotic cell death (decreases amount of memory cells)



MANIPULATION OF ADAPTIVE IMMUNE RESPONSES

- Staphylococcal superantigens bypass the conventional MHC-restricted antigen presentation and processing, significantly increasing Th1 cell activation
- Skews the immune response heavily towards a Th1 type of response, delaying development of antigen-specific antibodies.
- Deplete local concentrations of IL-2, limiting the progression of protective T cell responses



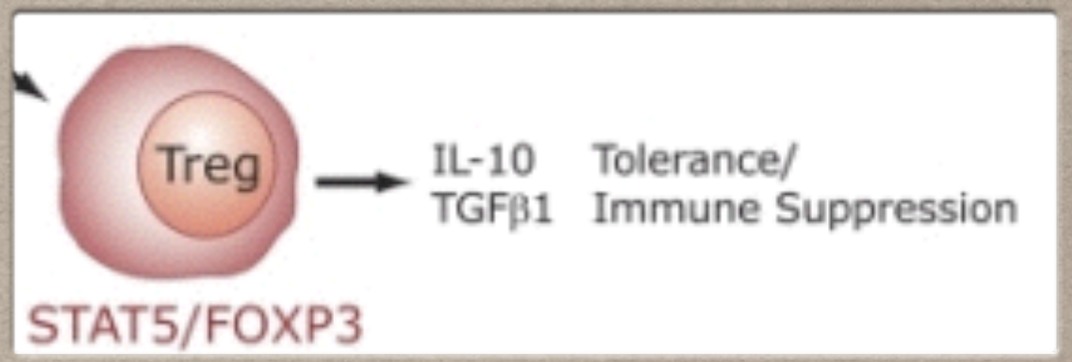
#4 - OUTCOME: IS THE BACTERIA COMPLETELY REMOVED, DOES THE PATIENT RECOVER FULLY AND IS THERE IMMUNITY TO FUTURE INFECTIONS FROM THIS PARTICULAR BACTERIA?

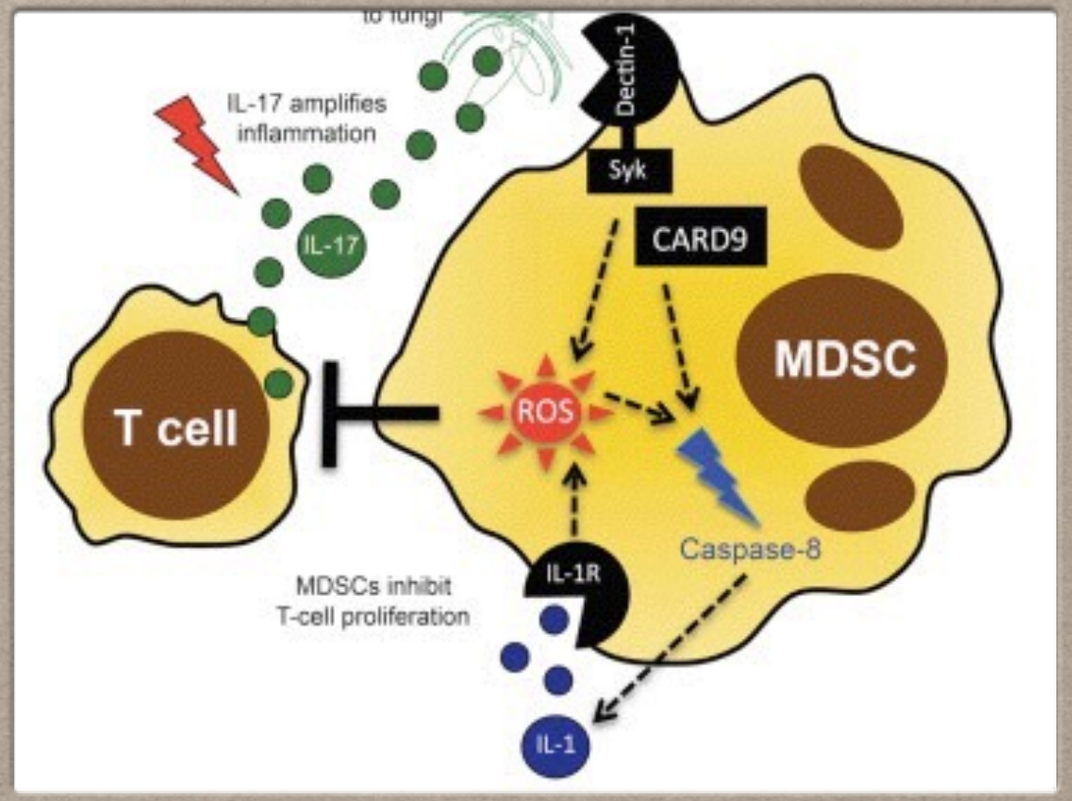
BACTERIAL CLEARANCE: PROBLEMS OF REMOVAL IN S.AUREUS INFECTIONS

- Bacteria may not be completely cleared
- Acute mastitis can evolve into chronic and subclinical mastitis with persistence of the bacteria in the mammary gland
- In the bovine model, S. aureus predominantly results in subclinical mastitis and chronic infection
- The ability of S. aureus to persist in the mammary gland may be explained by a number of host and bacterial properties:
  - type of immune response by the host
  - expression and modification of virulence factors
  - presence of biofilms
  - existence of small colony variants (SCV)
  - intracellular existence of the bacteria in epithelial cells and macrophages

BACTERIAL CLEARANCE: PROBLEMS OF REMOVAL IN S.AUREUS INFECTIONS - TYPE OF IMMUNE RESPONSE BY THE HOST

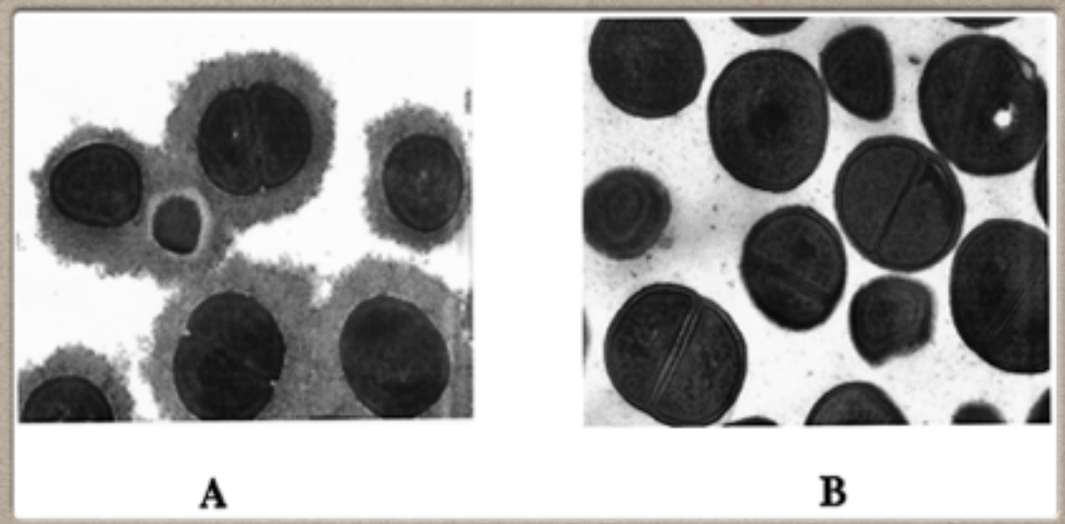
- Induce a much weaker immune response in the breast compared to Gram-negative pathogens like E. coli
- Less NF-kB signaling and a delayed secretion of inflammatory cytokines
- No activation of the NF-kB factor complex, although the infection will trigger this cascade in professional immune cells
- Greater likelihood that S. aureus infections can establish colonies and biofilms and resist a host immune response
- May also trigger immune dampening soon after infection.
    - Increase TGFB1 (inflammatory antagonist (anti-inflammatory)) and IL-10
- Chronic infection may blunt Tcell reactivity generally as a result of myeloid-derived suppressor cells (MDSCs) with some contribution from Treg cells





BACTERIAL CLEARANCE: PROBLEMS OF REMOVAL IN S.AUREUS INFECTIONS - EXPRESSION AND MODIFICATION OF VIRULENCE FACTORS

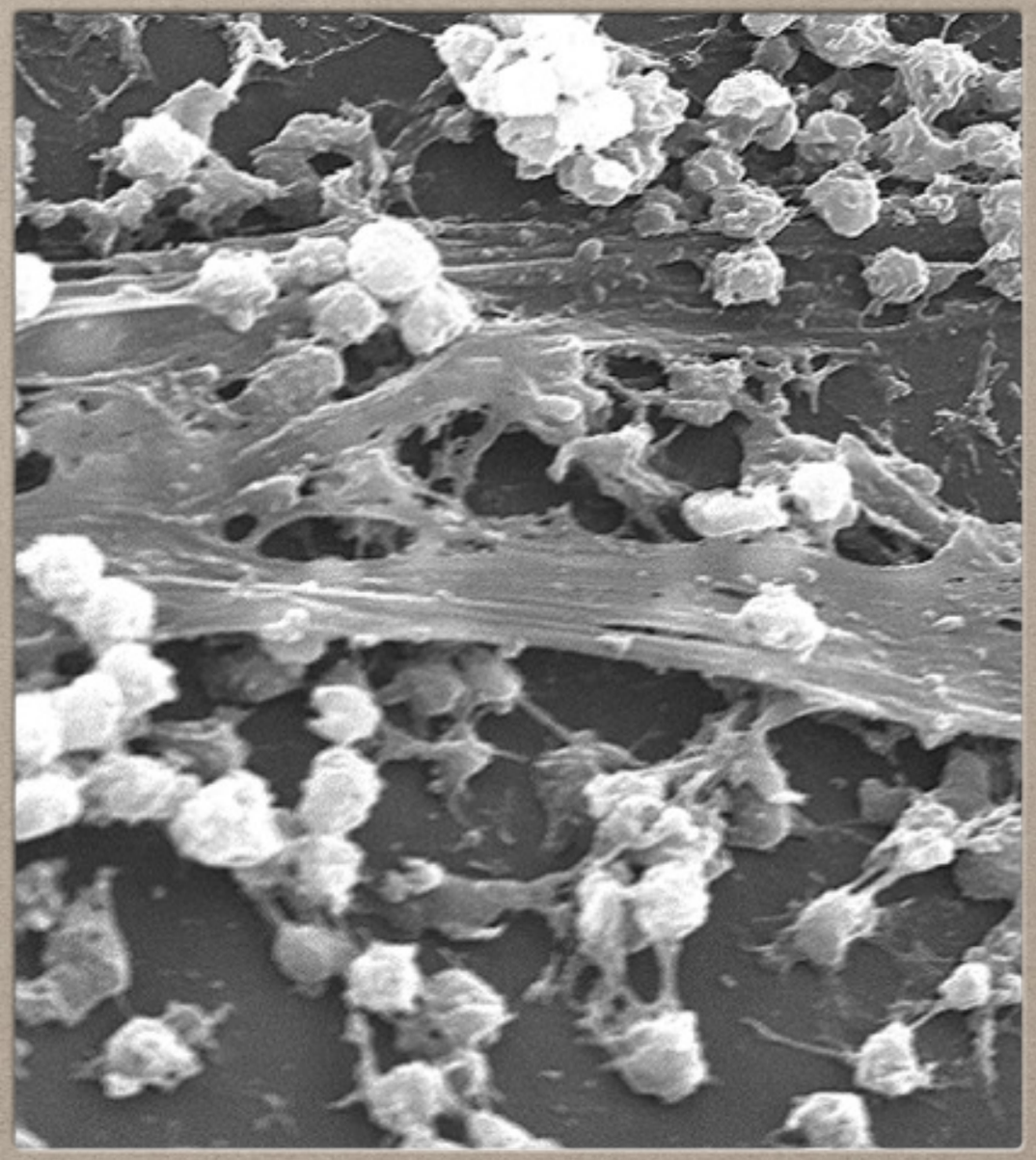
- Capsular polysaccharides (CP): may enhance virulence and extracellular survival by inhibiting phagocytosis
    - Absence of CP expression results in greater persistence of the bacteria in the mammary gland (specific to the mammary gland)
    - Presence of CP results in greater neutrophil and monocyte leukocyte infiltration in the mammary gland of infected mice compared to mice infected with acapsular strains (Result in more inflammation but also greater clearance)
- Acapsular S. aureus may have enhanced adhesive abilities and become internalized into epithelial cells more often than capsular strains (may be related to greater access to adhesion receptors in the absence of a capsule)
    - May avoid immune clearance by internalization within mammary epithelial cells
    - Antibodies to capsular proteins may enhance clearance, but will create selective pressures for an acapsular subpopulation



A: capsular, B: acapsular

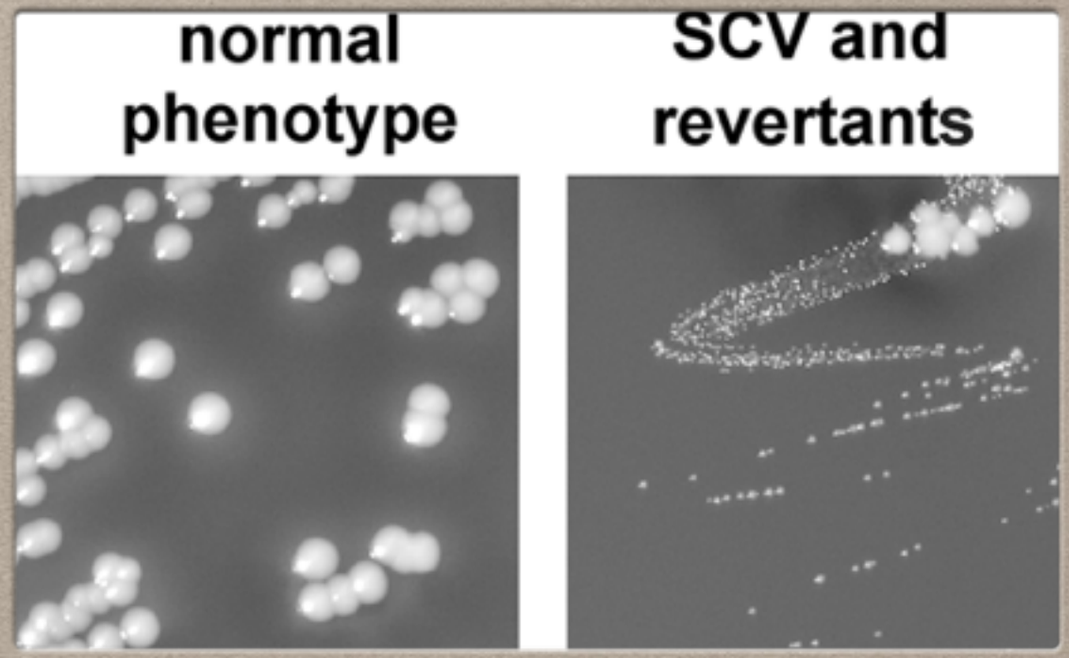
BACTERIAL CLEARANCE: PROBLEMS OF REMOVAL IN S.AUREUS INFECTIONS - PRESENCE OF BIOFILMS

- The ability to produce biofilm in the mammary gland has been shown for S. aureus, although not all strains are capable
- Genetic and environmental factors influence S. aureus biofilm formation
- Two toxins involved in establishing biofilms: alpha and beta toxins (can also contribute to mammary epithelial cell damage)
- Biofilms can be established in deep-seated pockets of infection in the alveoli of the mammary gland
- Enable bacterial cells to evade the antibiotic and host defense mechanisms (dense extracellular matrix and exterior layer of cells will shield the interior layer)



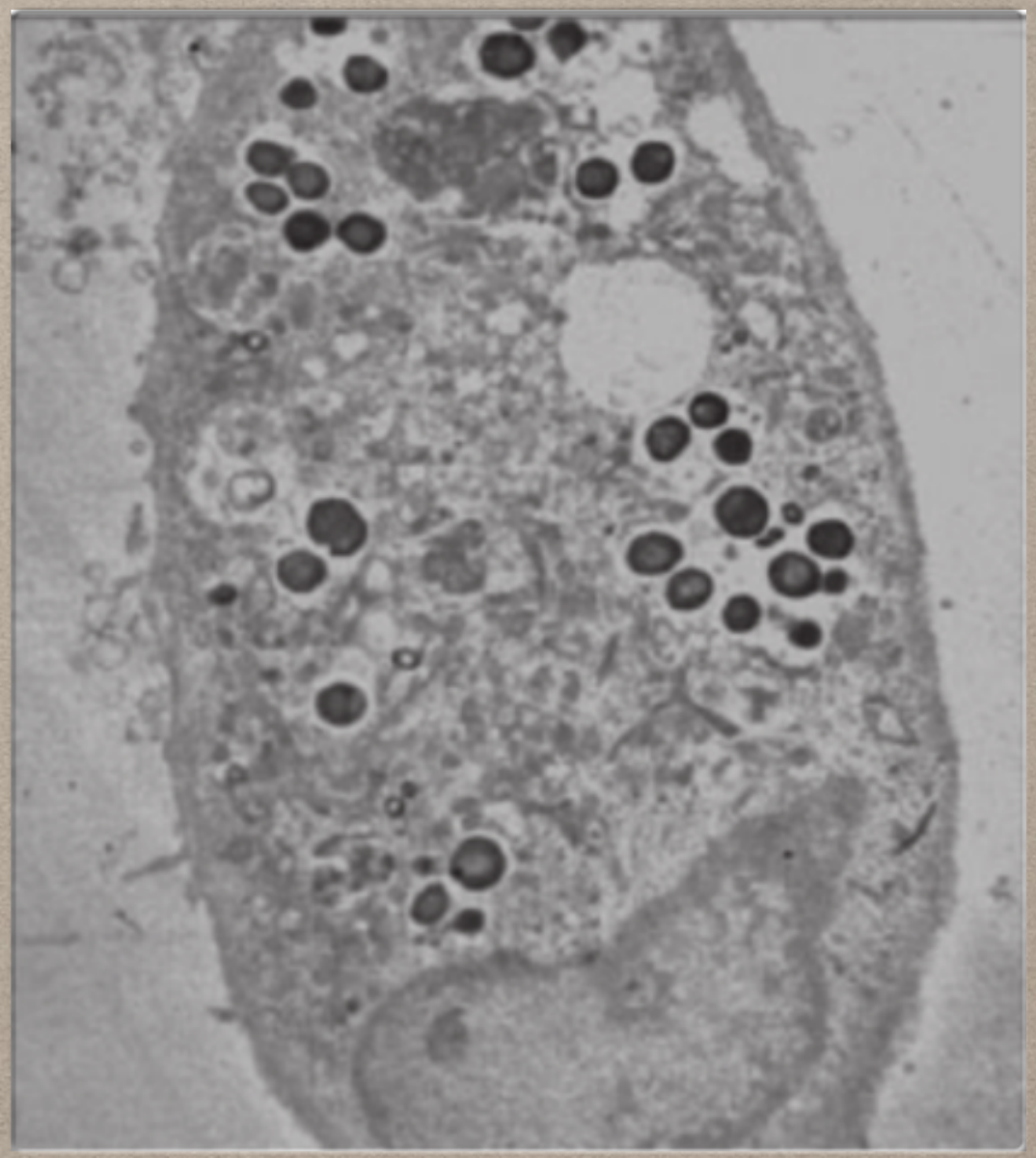
BACTERIAL CLEARANCE: PROBLEMS OF REMOVAL IN S.AUREUS INFECTIONS - EXISTENCE OF SMALL COLONY VARIANTS (SCV)

- SCV: slow-growing subpopulations of bacteria with contribute to bacterial persistence, mutants with deficient metabolic pathways (often related to the electron transport chain)
- May be more persistent and avoid detection by immune cells compared to wild type bacteria
- May confer antibiotic resistance, especially to antibiotics that are only effective when bacteria are dividing
- Typically display decreased respiration, decreased haemolytic activity, decreased coagulase activity and increased resistance to aminoglycosides (all linked to electron transport)
- Seem to have an increased ability to persist intracellularly (protect them from antibiotics)
    - Produce less alpha toxin, able to live intracellularly without lysing the host cell
    - Enables persistence in the host without inducing apparent inflammation



BACTERIAL CLEARANCE: PROBLEMS OF REMOVAL IN S.AUREUS INFECTIONS - INTRACELLULAR EXISTENCE OF BACTERIA IN EPITHELIAL CELLS AND MACROPHAGES

- S. aureus is a facultative intracellular pathogen
- Ability to survive intracellularly may contribute to relapse and prolonged course of some infections
- Can invade mammary epithelial cells, as well as endothelial cells, and fibroblasts
- Found enclosed in membrane bound vacuoles in the cytosol
- Antibody-mediated immune response alone is not protective in this case. Cell-mediated immune response is also necessary.



RECOVERY AND IMMUNITY

- Antibiotic treatment and even surgical drainage are often necessary to cure infections
- No vaccine available
- Combination of antibiotic treatment and host immune responses should clear bacterial infection from host
- BUT: Bacteria can return if not completely cleared

RECOVER AND IMMUNITY - EFFECTS OF BREASTFEEDING TECHNIQUE

- 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

- If a mother completely stops breastfeeding because of the infection and pain that occurs, further milk stasis may be promoted, worsening the situation

- If the S. aureus infection causes abscesses or severe tissue damage, this may compromise milk production permanently which may be due to the fact that damaged alveoli epithelial cells are replaced with non-milk secreting epithelial cells

RECOVERY AND IMMUNITY - RE-INFECTIONS OF THE DISEASE AND PROTEIN A

- Initial infection can lead to memory cells, but re-infection leads to a decrease in long lived plasma cells (LLPCS) by Protein A
- Protein A binds Bcells with Ig, leading to an inability of cells to survive in the bone marrow and differentiate into LLPCs
- Binding of Protein A to B cells also results in downregulation of certain Bcell receptors and co-receptors, limiting proliferation and inducing apoptotic cell death (Depletion of Bcells, creating a weak immune response against re-infection)