CASE 4: IMMUNE RESPONSE

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

INNATE IMMUNITY – PHYSICAL BARRIERS

Teat Sphincter

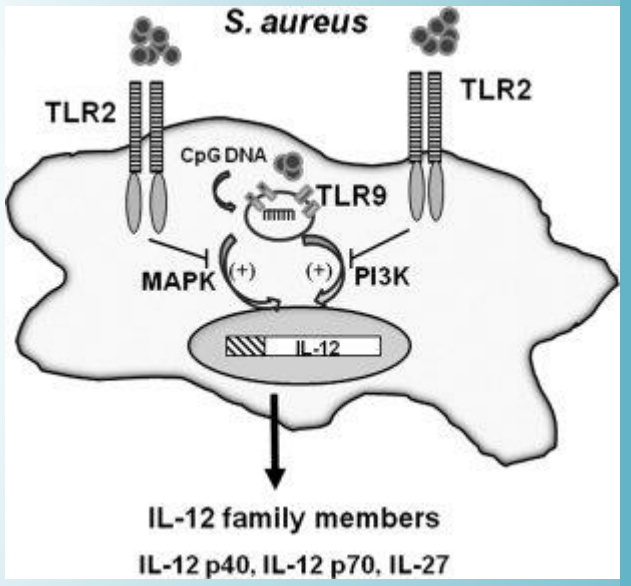
- A which muscle blocks the entrance of the duct by maintaining it tightly close

Keratinized Epithelium of Duct Canal

- Keratin creates a plug to physically block bacteria from entering
- Fatty acids in the keratin chemically attack the cell wall of bacteria

INNATE IMMUNITY – PRR AND PAMP

- Once the bacterium enters the periductal lymphatic system, it is detected by the mammary glands
- Innate immune cells such as macrophages, neutrophils or dendritic cells display receptors which recognize foreign bodies
- Pathogen recognition receptors (PRR) are either toll-like or nod-like and
- PRRs are responsible for recognizing PAMP (pathogen associated molecular pattern) or DAMP (damage associated molecular pathogen)
- Once PAMPs or DAMPs have been recognized by immune cells, there are activated and initiate other immune responses such as inflammation
- Different pathogens are recognized by different PRR's – *S. aureus* features such as peptidoglycan (PGN) and lipoteichoic acid (LTA) are recognized by TLR2

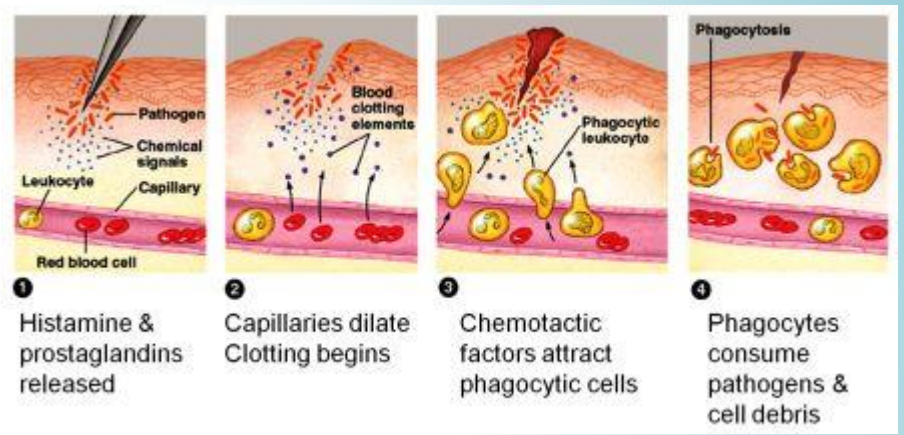


Toll-like receptor 2 (TLR2)-TLR9 crosstalk dictates IL-12 family cytokine production in microglia, by Monica Mariani, 2012 , retrieved April 5th

INNATE IMMUNITY – PRO INFLAMMATORY CYTOKINES

- Pregnant women, such as Elizabeth, undergo mammogenesis – in which cell modifications lead to a higher concentration of pro-inflammatory cytokines such as IL-4, IL-10 and TNFalpha
- In the case of an infection, these cytokines are able to upregulate components of the inflammatory response

INNATE IMMUNITY – INFLAMMATORY RESPONSE



Biology Exams 4 U, Retrieved April 5th

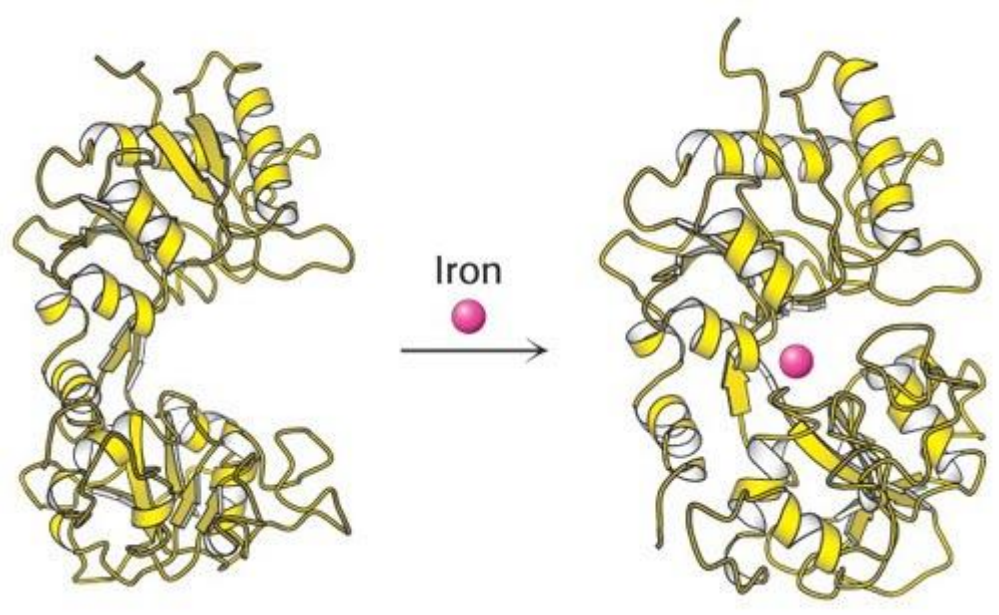
- Increase in concentration of Eicosanoids
  - Some which are pro-inflammatory
    - E2 (PGE2), prostaglandin F2 alpha (PGF2a) and thromboxane B2 (TXB2) increase the permeability of vascular tissues and its blood flow, recruiting leukocytes to the site of infection, or they induce fever. The vascular alteration is also conducted by uroplasminogen, a molecule that facilitate the diapedeses of leukocytes and thus enhances leukocyte recruitment
  - Others which resolve inflammation
    - prostaglandins D2 and 15-Deoxy-Delta-12,14-prostaglandin J2 (15 d-PGJ2) have the opposite effect: they inhibit leukocyte infiltration by blocking the activation of NFkB (a transcription factor which upregulates the expression of pro-inflammatory factors)

INNATE IMMUNITY - NEUTROPHILS

- In milk samples from individuals with mastitis infections, there is a higher concentration of neutrophils than in health milk samples
- cytokines and complement components (C5a and C3a) recruit neutrophils from the circulatory system into the mammary gland
- Inflammatory components induce the neutrophils to perform bactericidal functions such as release of defensins, reactive oxygen species and antibacterial peptides such as cathelicidins, hydrolases, proteases, and lysozymes
- neutrophils also release chemokines prostaglandins and leukotrienes which increase vascular permeability and recruit cells, leading to an increase in the inflammatory response
- A neutrophil extracellular trap (NET) is a physical barrier for pathogens and is formed by nuclear and granule material from neutrophils

INNATE IMMUNITY – LACTOFERRIN PROTEIN

- Antimicrobial factor secreted by epithelial cells and leukocytes
- Creates an undesirable environment for bacterial growth by depriving them of iron
- Lactoferrin sequesters iron in the milk, with the help of bicarbonate
- The concentration of lactoferrin remains low in healthy conditions and only increases under infection



Lactoferrin is able to sequester iron

Lecture 3: Introduction to Proteins; Amino Acids, the Building Blocks of Proteins, retrieved from http://cbc.chem.arizona.edu/classes/bioc460/spring/460web/lectures/LEC3_AAs/LEC3_AAs.html

INNATE IMMUNITY – COMPLEMENT FACTORS

- Complement factors C3b and C3bi
- C3b and C3bi opsonize bacteria for phagocytosis
- Damage bacterial surface by making pores

Opsonization / Phagocytosis

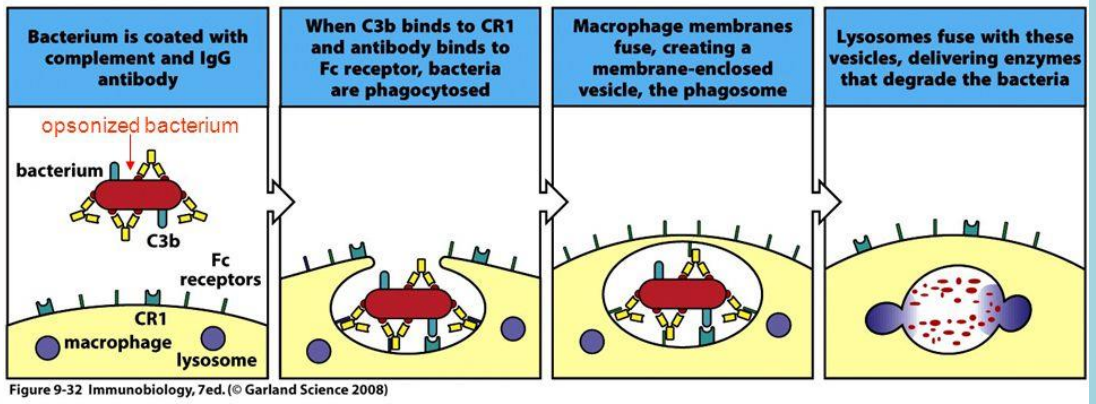


Figure 9-32 Immunobiology, 7ed. (© Garland Science 2008)

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

-CR3 and CR4 also mediate phagocytosis

Complement, by S. Barbour, Retrieved from http://slideplayer.com/slide/3375469/

ADAPTIVE IMMUNITY

- activated after the innate immune system
- major histocompatibility complex class II (MHCII) molecules on professional antigen-presenting cells recognize
  - B lymphocytes, and macrophages
- Lymphocytes such as CD4+ T regulate the immune response
  - activate CD8+ cytotoxic T cells and NK cells
  - facilitate B cell differentiation by releasing the cytokine IL-2
- CD8+ cytotoxic T cells eliminate pathogens

ADAPTIVE IMMUNITY

- Th1 cells secrete IFN-γ which enhances phagocytosis ability by switching neutrophils to IgG2 isotope
- The2 cells provide antibody-mediated immunity
- Th17 cells produce IL-17, IL-21, IL-22, and IL-26, which recruit neutrophils and form abscesses
- B cells can act as antigen presenting cells, they can also differentiate into cells that provide long term memory of the pathogen or produce large amounts of antibody against S. aureus
- Opsonization of bacteria by antibodies makes phagocytosis by neutrophils more effective

HOST DAMAGE: WHAT DAMAGE ENSUES TO THE HOST FROM THE IMMUNE RESPONSE?

DAMAGE FROM IMMUNE RESPONSE

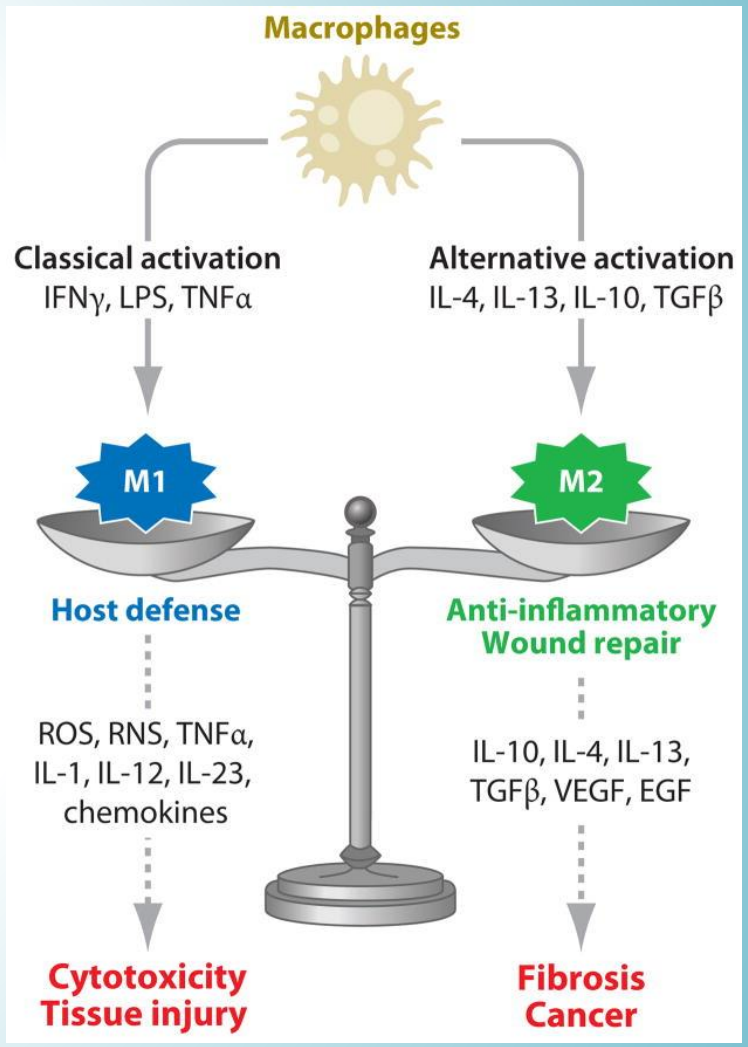
- Most research concludes that the damage which occurs during a mastitis infection is a result of the immune response and not bacterial action
  - There was no correlation between the number of bacteria and the level of damage to the host
  - Not one species of bacteria was responsible for causing damage in particular

DAMAGE TO HOST FROM INNATE IMMUNITY

- When neutrophils are activated, they secrete proteases which mainly inactivate bacterial toxins but can also damage the host
- Chemokine signals recruit neutrophils to the site of infection and neutrophils leave when the signals are no longer present; however, this movement of neutrophils can cause chronic inflammation
- Continual protease and ROS release by neutrophils can damage the host

DAMAGE TO HOST FROM ADAPTIVE IMMUNITY

- Activation of macrophages can lead to damaging the host
  - M1 macrophages are activated by PAMPS, IFN-y and TNF-a and they secrete IL-12 and IL-23 to increase the inflammatory Th-1 response;
  - M1 macrophages produce lysozymes and pro-inflammatory lipids such as lipoxygenase
  - Also increase inflammation by secreting ROS and RNS species which in excess amounts denature cellular lipids, proteins and DNA – leading to necrosis and apoptosis
  - M2 macrophages downregulate inflammation by secreting anti-inflammatory cytokines such as IL-4, IL-10 and IL-13



Macrophages and tissue injury: agents of defense or destruction?, by Laskin DL, 2011, retrieved April 5th

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

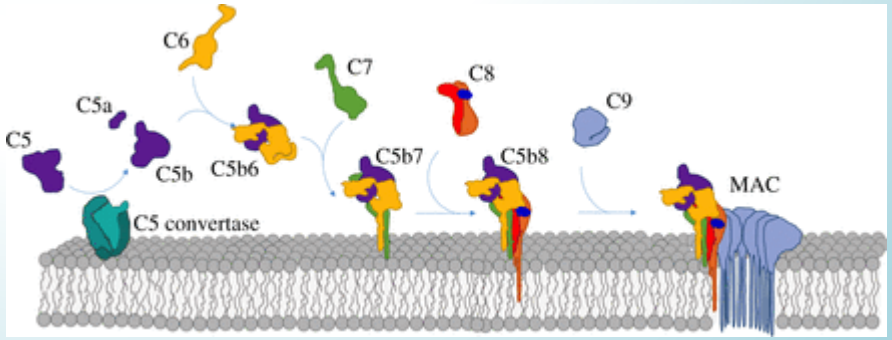
MANIPULATION OF INNATE IMMUNE RESPONSE

- S. aureus secretes specialized proteins called staphylococcal superantigen-like proteins (SSLs) which interfere with neutrophil functions such as extravasation and chemotaxis
- SSLs slow down the rate of bacterial clearance and phagocytosis by binding to components of the innate immune system
  - SSL7 binds to complement factor C5 and IgA with high affinity, and inhibits the last stage of complement activation
- Extracellular adherence protein (Eap) is made up of four β-grasp-like domains and associates with ICAM-1, inhibiting the migration of leukocytes
- ICAM-1 prevents neutrophils from squeezing through endothelial cells of the blood vessel wall to enter the damaged tissue.

MANIPULATION OF INNATE IMMUNE RESPONSE

- *S. aureus* secretes many proteins that interfere with complement directed opsonization
- a Zn-dependent protease, aureolysin, cleaves C3 to generate C3a and C3b;
  - C3b is prevented by from accumulating on the S. aureus surface by complement factors I and H which either degrade C3b or bind to it
  - The membrane attack complex (MAC) is not able to form because C5b is inhibited from interacting with other complement proteins
- The thick peptidoglycan layer of a gram positive bacteria such as S. aureus helps it evade attack from the MAC
- Certain strains of S. aureus encode for capsular polysaccharides which help protect the bacterium from digestion

The Membrane Attack Complex (MAC)



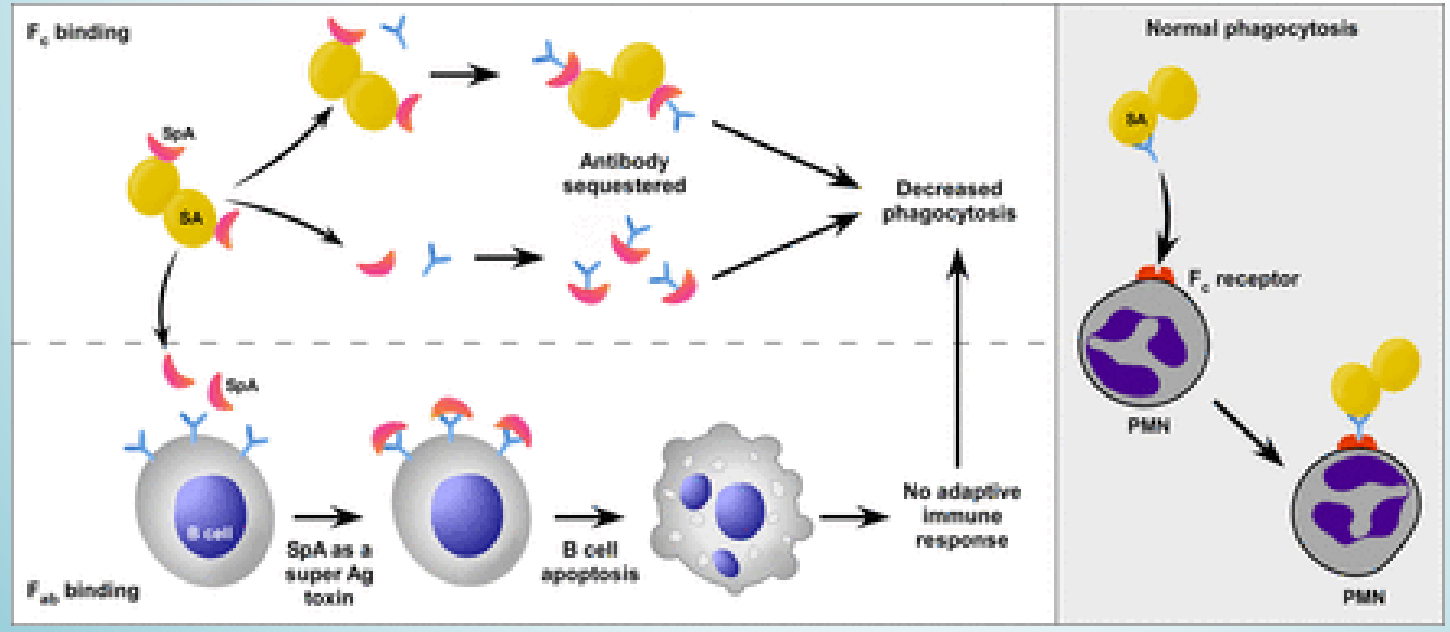
The mystery behind membrane insertion: a review of the complement membrane attack complex, by Charles Bayly-Jones, 2017, retrieved on April 5th

MANIPULATION OF INNATE IMMUNE RESPONSE

- An increase in the prevalence of antimicrobial peptides and reactive oxygen species creates an unfavourable environment for S. aureus, however S. aureus has a few mechanisms to evade this
- Peptidoglycan acetylation (OatA) and D-alanylation of teichoic acids (DltABCD) protect S. aureus from antimicrobial-peptide and lysozyme mediated killing
- Bacteria can overcome the lack of iron by producing siderophores which bind and acquire iron from the host
  - Siderophores help facilitate the infection and are produced at high levels in mastitis causing strains of bacteria

MANIPULATION OF ADAPTIVE IMMUNE RESPONSE

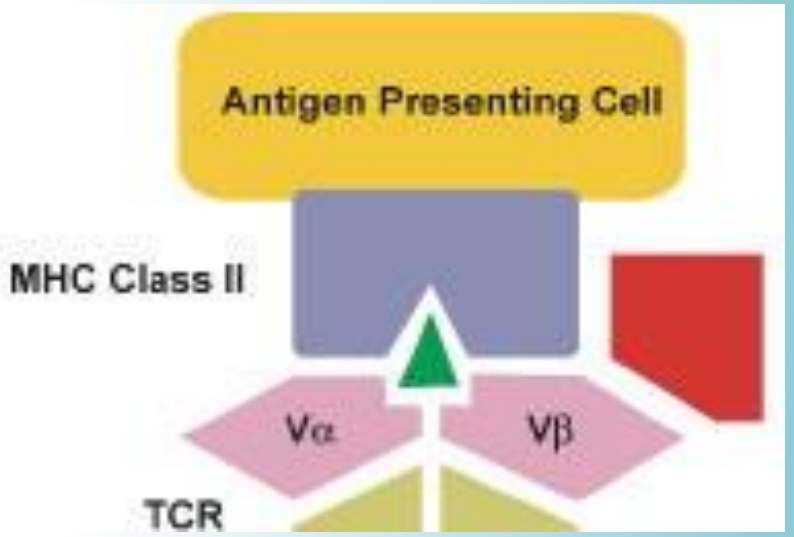
- S. aureus can manipulate the humoral immune response through SpA
  - SpA is a secreted protein by S. aureus with five immunoglobulin-binding domains,
  - SpA binds to the Fcy domain of immunoglobulins (Igs) and prevents opsonophagocytic killing of Staphylococcus bacteria
  - when SpA binds to the Fab domain of Igs it leads to clonal activation of B cells.
  - SpA can also bind to B cells and downregulate the expression of certain B-cell receptors and co-receptors; this induces apoptosis in B cells and leads to a decrease in their abundance and host memory of infection



Staphylococcus aureus Protein A Promotes Immune Suppression, retrieved from http://mbio.asm.org/content/4/5/e00764-13/F1.expansion.html

MANIPULATION OF ADAPTIVE IMMUNE RESPONSE

- S. aureus can manipulate T-cell mediated immune responses
  - S. aureus secretes superantigens - toxic shock syndrome toxin and staphylococcal enterotoxins, which are able to escape MHC presentation
  - Superantigens induce the production of Th1 cells which depletes local IL-2 concentrations and manipulates the immune response to delay the development of antigen-specific antibodies and limit protective T cell responses



Superantigens: a brief review with special emphasis on dermatologic diseases, By Lakhan Singh Solanki, 2008, Retrieved on April 5th

S. AUREUS – ADDITIONAL VIRULENCE FACTORS

- fibronectin-binding proteins and collagen-binding proteins promote adherence to host cells
- evasion factors inhibit neutrophil migration to the site of infection, induce neutrophil lysis, and inactivate the complement system
- Certain virulence factors are able to render leukocyte migration and phagocytosis ineffective

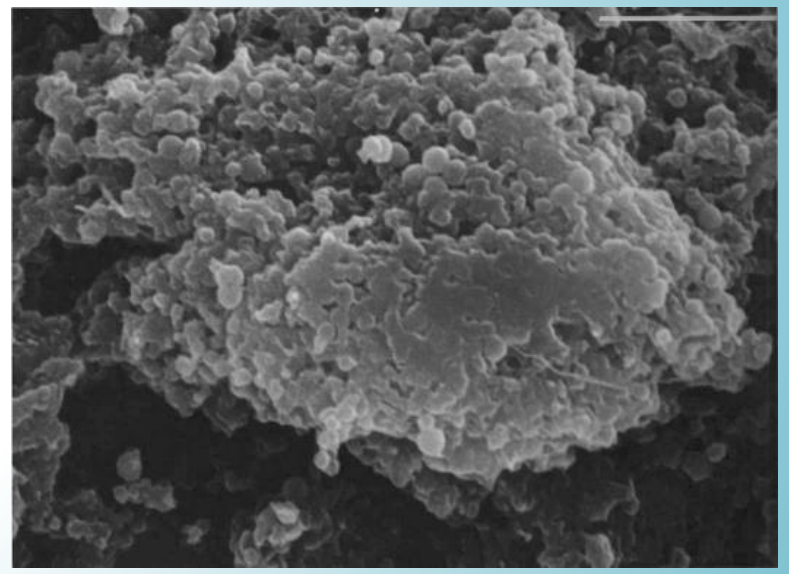
OUTCOME: IS THE BACTERIA COMPLETELY REMOVED, DOES THE PATIENT RECOVER FULLY AND IS THERE IMMUNITY TO FUTURE INFECTIONS FROM THIS PARTICULAR BACTERIA?

BACTERIAL CLEARANCE

- Certain mastitis infections are resolved without the need of antibiotic treatment
- Adequate drainage from the breast can flush out the pathogen
- immune response and the antibacterial properties of human milk can clear the infection

BACTERIAL PERSISTENCE

- S. aureus is often able to persist in the mammary gland
  - Acute infections can develop into chronic and subclinical mastitis
- S. aureus induces a much weaker immune response in the breast compared to Gram-negative pathogens
  - less NF-kB signaling and a delayed secretion of inflammatory cytokines
  - Results in S. aureus being able to establish colonies and biofilms
- Biofilms establish extracellular survival of S. aureus by providing protection from antibiotics and other host factors
  - Biofilms are also composed of two toxins which attack host cells



Staphylococcus aureus biofilms
Properties, regulation and roles in human disease, by Nathan Archer, 2011, Retrieved on April 5th

BACTERIAL PERSISTENCE

- Small colony variants (SCVs) contribute to persistence since these bacteria are under slow growth conditions and can survive unfavorable environment created by the host
  - Also gain antibiotic resistance against antibiotics which only target dividing bacteria
- Dampen the immune response by increasing TGFB1 production in cells
- Absence of capsular polysaccharide (CP) enhances persistence of S. aureus in mammary glands
  - CP results in greater neutrophil and monocyte leukocyte infiltration to the mammary gland

RECOVERY AND IMMUNITY

- Appropriate breast feeding techniques must be continued for full recovery
  - Improper breast feeding or stopping breast feeding can lead to chronic inflammation and persistence of milk stasis
  - Extreme damage to breast tissue could result in no more milk production since milk producing epithelial cells become replaced by non milk producing epithelial cells
- Finishing a course of antibiotic treatment along with adequate immune responses from Elizabeth's body should be adequate for recovery (given that the patient is not immunocompromised)
- Despite activating an adaptive immune response, there usually isn't immunity generated against future S. aureus infections
  - Due to S. aureus causing a depletion of B cells and therefore more difficult to create a strong humoral defense