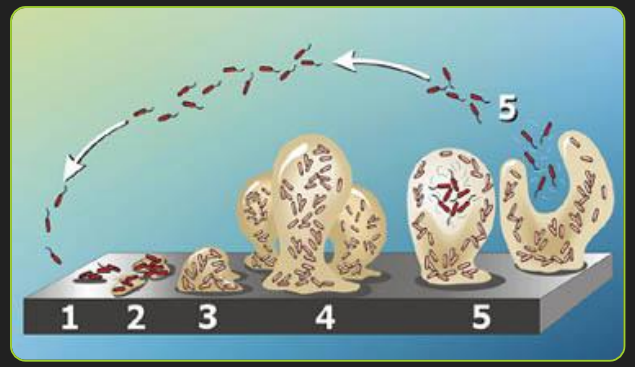
Bacterial Pathogenesis

Pseudomonas aeruginosa by Laura Ramirez

Environmental Conditions

- Environment: soil, water or any surface that is in contact with these, common in hot tubs
  - Can survive high concentrations of salt, dye and weak antiseptics, hence wide range of habitats
  - Resistant to weak-antiseptics, therefore present in many creams, disinfectants and sinks
- Residence: can colonize skin surfaces, stool, auditory and eye canal and throat, pulmonary, cardiac and brain tissue
- Temperature: 25° C to 37° C for optimal growth
- Opportunistic bacteria
  - Burn wounds are ideal due to loss of skin barrier, poor circulation to the wound and under performing immune response and healing

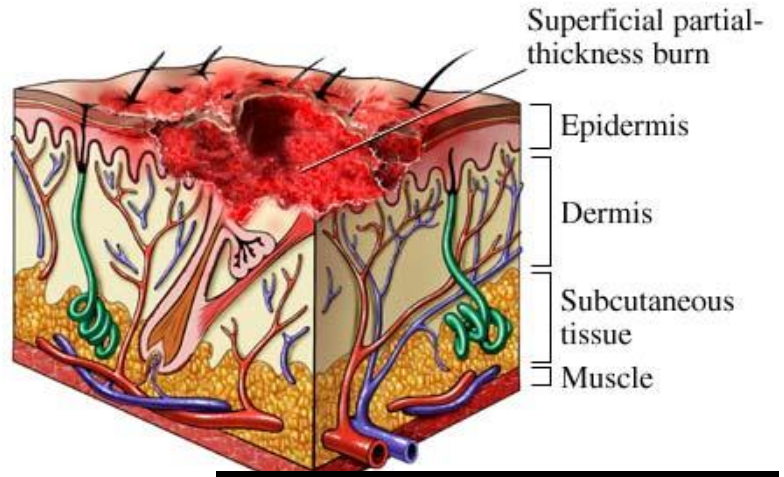
Bacterial Characteristics



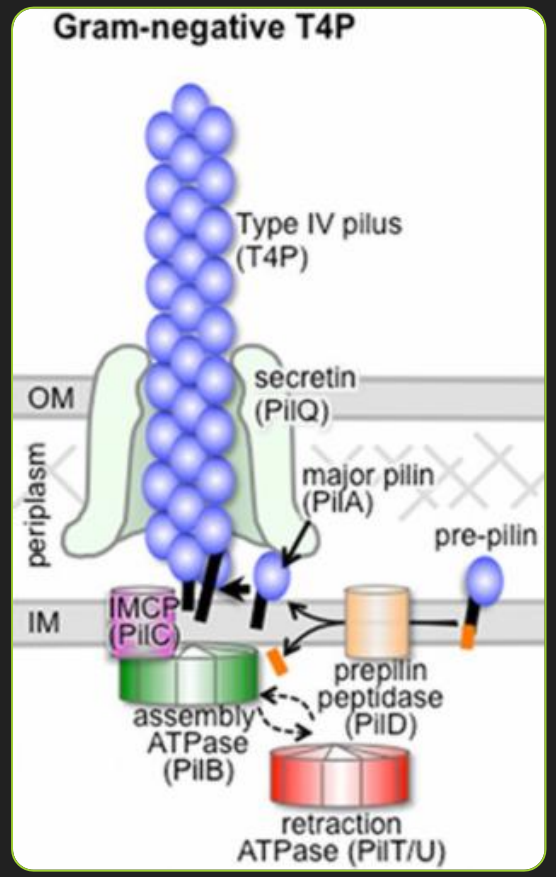
- Gram negative aerobic bacteria with single polar flagellum
- Has mucoid capsule that consists of alginate, repeating polymers of polysaccharide
  - Allows biofilm formation to assist with adherence to cell surface, as well as protection from host defenses
- Extracellular polymeric substances (EPS):
  - Biofilm: closely-structured organized elements which increase *P. aeruginosa* adherence to surfaces, organic or inorganic. Makes up 50-90% of EPS
  - Contributes to resistance to chemical and mechanical forces, like toxic substances and antibiotics or flowing water, respectively.

P. aeruginosa Entry

- Common Entry Pathways: Skin, gastrointestinal, respiratory and genitourinary tracts
- Mostly adheres and infects immunocompromised cells
  - Burn wounds have increased probability of bacterial infection due to damaged epidermal tissue
  - Fc receptor is reduced at burn sites, which reduces polymorphonuclear leukocyte mobility and therefore, infection prevention effectiveness
- Virulence factors:
  - Adhesins
  - Biofilm
  - Protease enzymes
  - Pyocyanin
  - Toxins



P. aeruginosa Virulence Factors



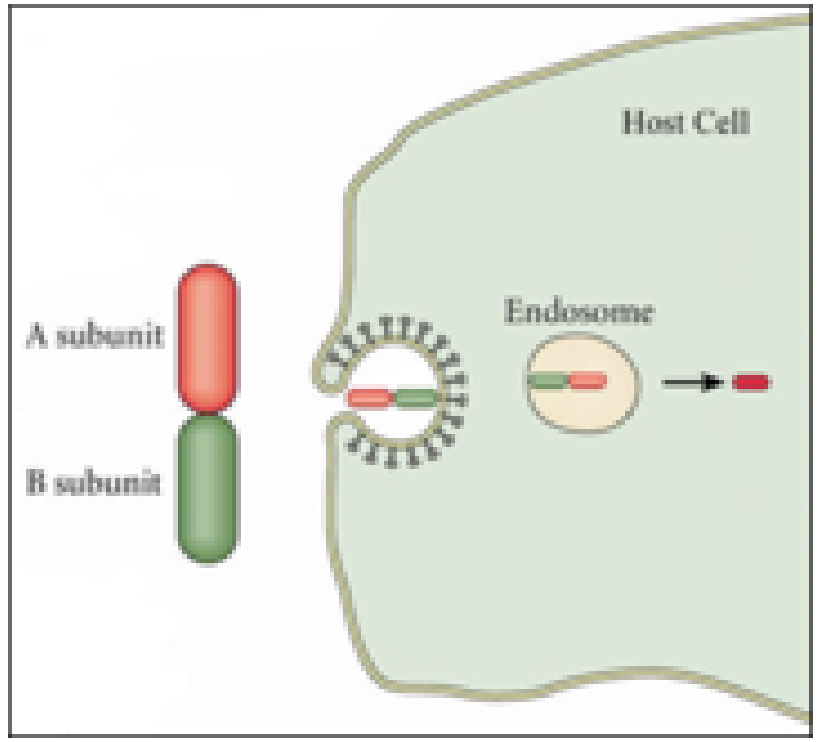
- **Type IV Pili (adhesin):** the principle adherence factor responsible for making contact with host epithelial cells
  - Can reversibly assemble or disassemble to direct bacteria
  - The pili interacts with carbohydrate and sialic acid receptors on human epithelial cells
  - Causes cascade of events to increase intracellular cAMP concentration
    - Leads to downregulation of directional movement machinery and upregulation of adhesin factors that facilitate attachment
  - Mediates biofilm formation
- **Biofilm**: Progressed from alginate which is secreted mucoid exopolysaccharide
  - Can attach tightly onto surfaces and becomes resistant to forces experienced by epithelial cells due to alginate's negatively charged functional groups which influence electrical charge, steric hinderence, hydrophobicity and electrostatic interactions
  - Bars host immune cells from interacting with or destroying bacterial colonies

P. aeruginosa Virulence Factors

- **Elastase and Alkaline Protease:** help with adherence, colonization and evasion of host immunity
  - Degrade fibronectin, a glycoprotein found on host epithelial surfaces that acts to prevent bacteria from colonizing the epithelial layer. This leads to host epithelia vulnerability
  - Exposes pili receptors and facilitates adhesion onto membrane
  - Destroy host proteins such as structural proteins which disrupt physical barriers.
  - Inhibit monocyte chemotaxis and phagocytosis which allow the pathogen to stabilize and adhere
- **Pyocyanin:** zwitterionic compound
  - Due to its positive and negative charge at physiological pH it can penetrate host plasma membrane
  - Host immune cells and tissue are destroyed or suppressed when oxygen free radicals are produced during redox reactions
  - Harbors antimicrobial activity against other bacterial species, which dampen the microflora within the host

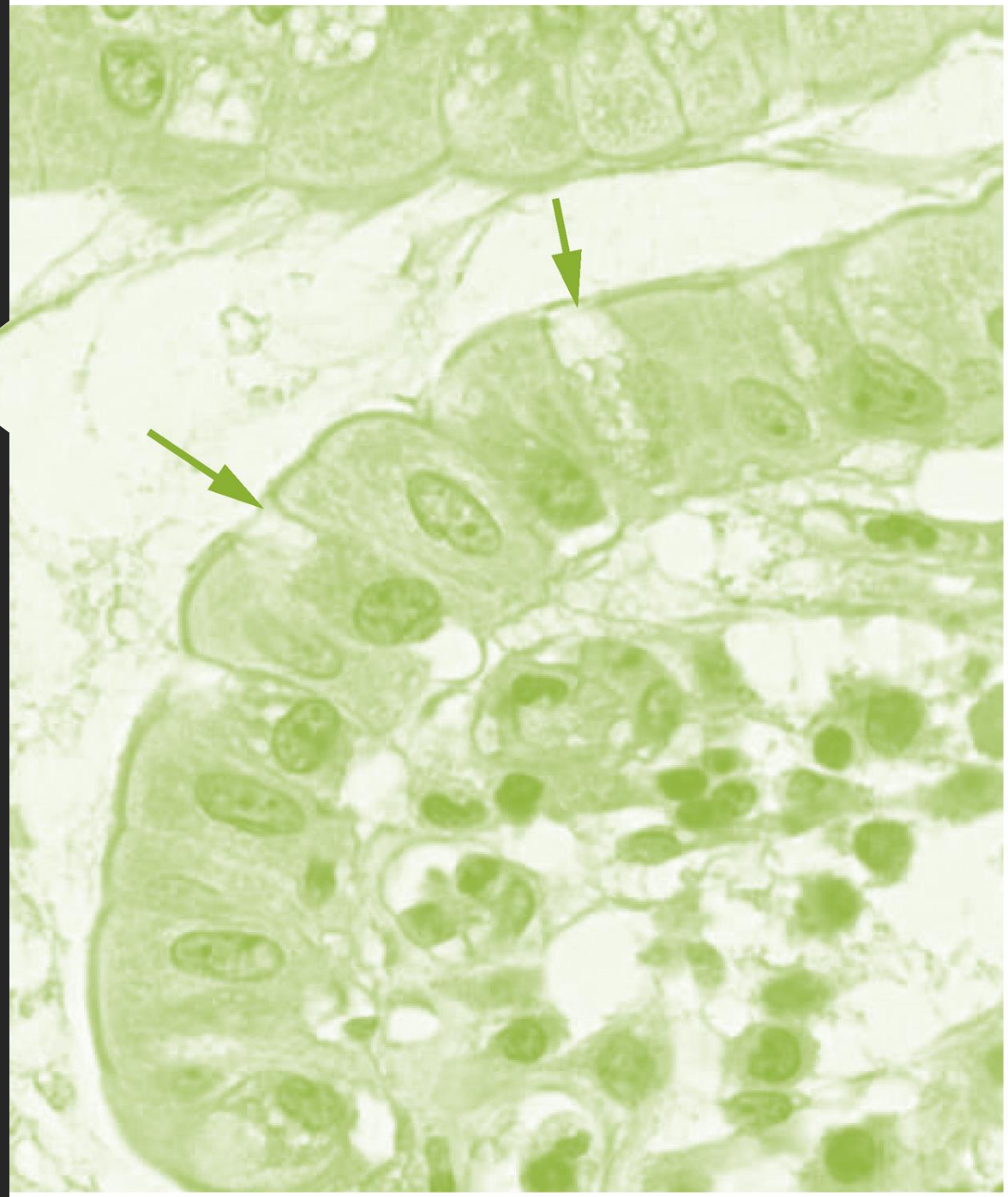
P. aeruginosa Virulence Factors

- **Toxins:** exoenzyme A and exoenzyme S
  - Exoenzyme A: has two subunits, A and B; potent cytotoxin that ribosylates host EF-2 inhibiting protein translation after entry into host.
    - Subunit B binds to the host cell membrane, the AB subunit complex internalizes into the cell via receptor-mediated endocytosis
    - Subunit A separates from subunit B, then proceeds into cytoplasm of the cell and prevents translation of proteins by inactivating EF-2 which prevents elongation process of protein translation.
    - Results in decreased immune response and tissue necrosis
  - Exoenzyme S: holds intrinsic ADP-ribosyltransferase activity that can inhibit phagocytic engulfment and destroys normal function of host organs via apoptosis



P. aeruginosa and Host Factors

- **Host interactions:** the innate system is activated and fights *P. aeruginosa* with neutrophils, which can cause the pus, we see in Marian.
  - Type IV pili allows N-glycan binding to the apical surface which activates phosphatidylinositol 3-kinase/ Akt pathways and entry of the bacteria at the apical surface
  - The flagella was required for basolateral surface binding by activating epidermal growth factor receptor, adaptor protein Shc and PI3K/Akt
  - Fibronectin on mucosal epithelial cells act as defense barrier to prevent adhesion and colonization but these are degraded by elastase and alkaline protease

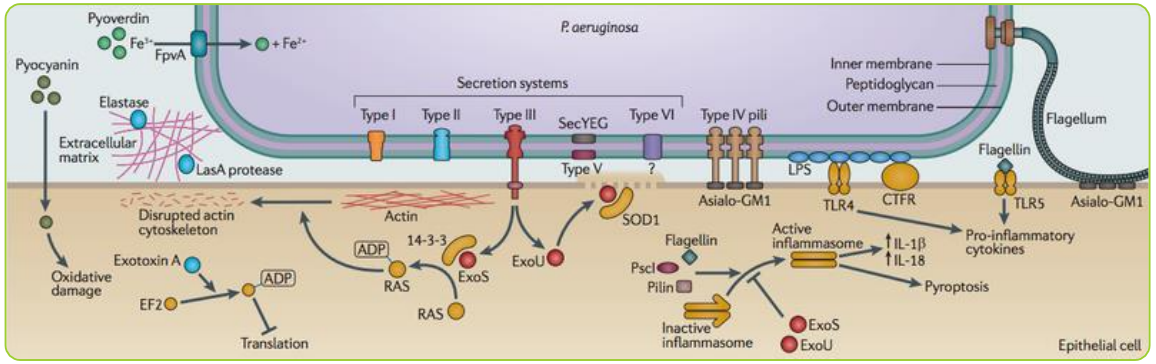


P. aeruginosa Invasion

- To invade tissue, various extracellular enzymes and toxins damage host epithelial cells and allow phagocytic invasion
  - Exotoxin A catalyzes ADP-ribosylation of elongation factor 2, which inhibits protein synthesis, causes local tissue damage and promotes invasion
  - Type 3 secretion system: injects toxins directly into host cells and translocates effector proteins from the bacteria into the host via membrane pores. Allows bacteria to break epithelial later and antagonizing wound healing and contributing to cell injury
    - Exoenzyme S is produced in burnt tissue and inhibits function of phagocytic cells
    - Exoenzyme U causes rapid death of host eukaryotic cells through loss of plasma membrane integrity
- Hemolysins break down lipids and lecithin and cytotoxin that is toxic to neutrophils and host cells
- Proteases and elastase also contribute to tissue damage and invasion
  - Elastase cleaves collagen, IgG, IgA and complement and lysis fibronectin and exposes receptors for further bacterial attachment. Disrupts epithelial cell tight junctions and interferes with mucociliary clearance
  - Protease interfere with flagellin signaling through host TLR5 by degrading free flagellin and assist in pathogen avoidance

P. aeruginosa Internalization, Multiplication and Dissemination

- **Internalization:** after adhering to epithelial cells, *P. aeruginosa* may be internalized with the help of Src-like tyrosine kinases
- **Multiplication:** aided by the biofilm and occurs within corneal cells after becoming internalized even though it is considered an extracellular pathogen
- **Dissemination:**
  - May occur via penetration through the epithelium, followed by invasion of the bloodstream. In the blood, *P. aeruginosa* is resistant to phagocytosis due to its mucoid capsule and LPS which are associated with increased immunogenicity.
  - Endocarditis ensues by direct invasion from the blood
  - Following endothelium invasion, the bacteria can survive and multiply and can travel to any site through the bloodstream.



P. aeruginosa Damage

- Majority of damage attributed by host responses against *P. aeruginosa* is mediated by neutrophils of the innate immune system
- Serine proteases cathepsin G and proteinase 3, which kill bacterial cells indiscriminately, also affect host tissue proteins which damage and contribute to inflammation
- Exotoxin A causes direct damage by deactivating elongation factor 2 which leads to necrosis
- Host signal transduction is interrupted when Type 3 Secretion System is activated which can also lead to necrosis
  - ExoU, one of the effector proteins, disturbs the host cell membrane and causes irreversible damage to the host cells, this leads to irreversible damage to the host cell, resulting in necrosis and lysis. ExoS triggers apoptosis
- Pyocyanin disrupts cellular antioxidant defenses through catalase inhibition which causes oxidative damage