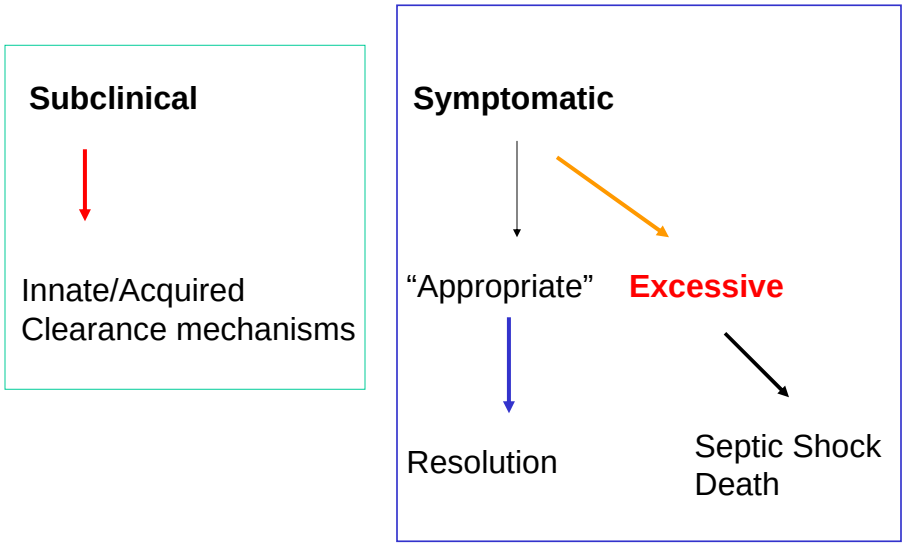
Microbial Pathogenesis

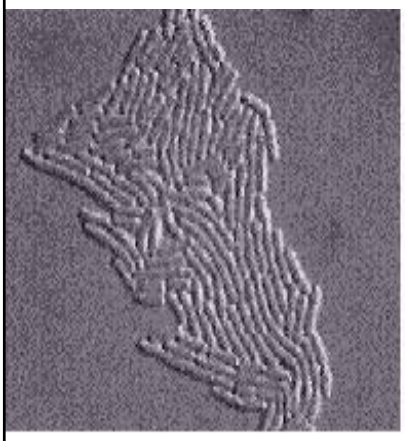
How do bacteria cause disease ?

1- Direct toxic effects – proteases – flesh eating bacteria

2- Activation of the host immune system –
Local – inflammation – PUS
Systemic – cardiovascular effects - SEPSIS

Bacterial activation of the host immune response





E.coli - lining up for lunch….

E. coli - Eosin-methylene blue plate

How do *E.coli* become pathogens ?

Commensal flora –

Acquire genes that cause disease –

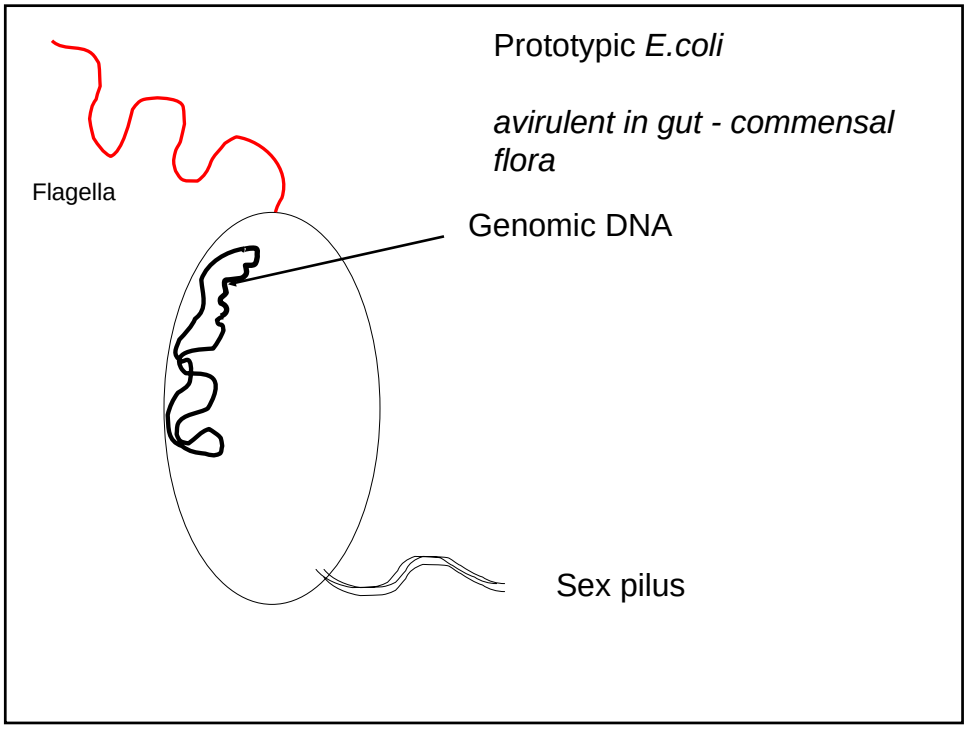
Colonization – attachment

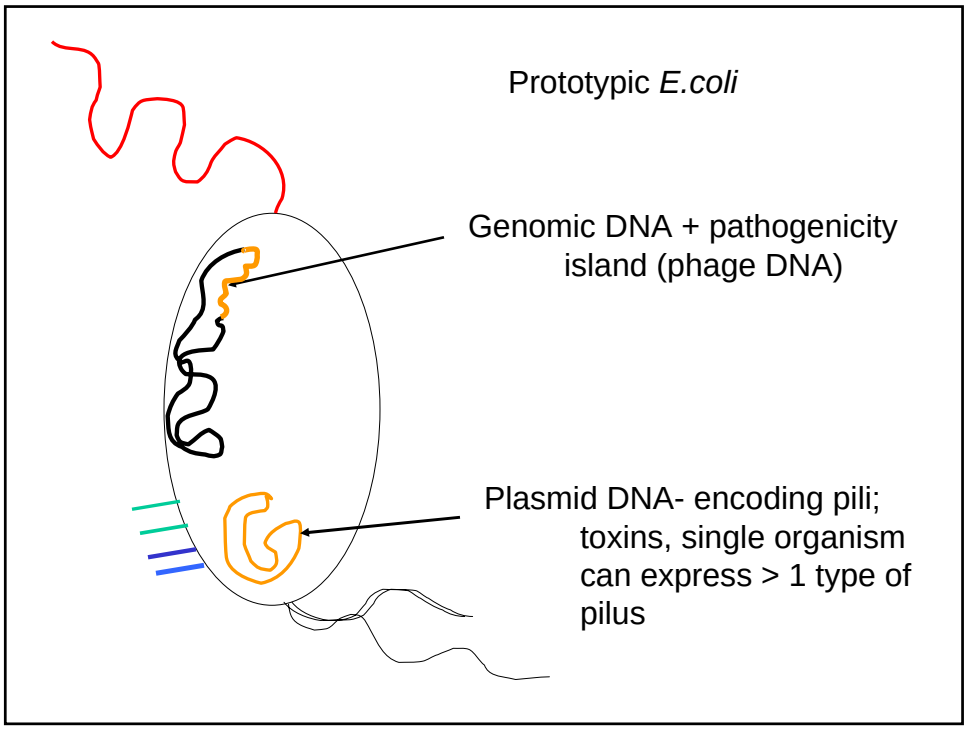
Toxin of some sort –

E. Coli – UTI's - urinary tract infection – activate a PMN response

Diarrheal disease – interact with gut epithelial cells – cause fluid secretion

Sepsis – get into the blood – activate immune cells


Prototypic *E.coli*
avirulent in gut - commensal flora
Flagella
Genomic DNA
Sex pilus


Prototypic *E.coli*
Genomic DNA + pathogenicity island (phage DNA)
Plasmid DNA- encoding pili; toxins, single organism can express > 1 type of pilus

Urinary tract infection : Adherence

Fecal contamination of the bladder, urethra (primarily in females)
Acquire a new organism from a partner

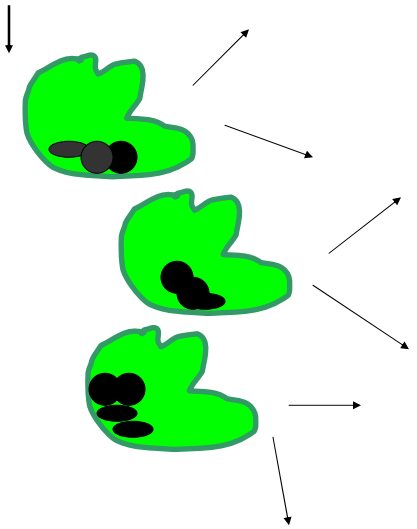
Organisms which express pili which bind to CHO

receptors on the uroepithelial cell - Gal-Gal - dissacharide ligand - Genetic susceptibility
- display the receptor - acquire the organism which expresses the type1 pilus

Ligand - Receptor interaction

→ Activates epithelial pro-inflammatory response

IL-8 - PMN chemokine

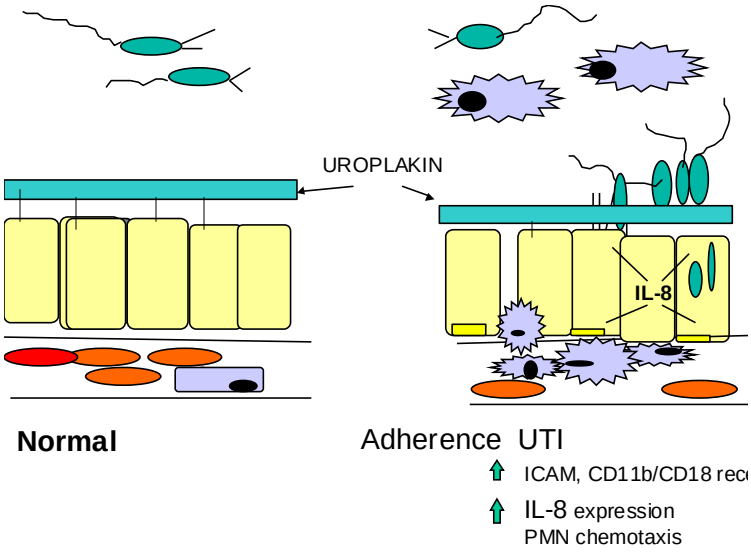


PMN mediated damage

Reactive oxygen intermediates
Superoxide
Neutrophil elastase

Toxic products

PMN's



Pathogenesis of infection

Host factors – Distribution of receptors – carbohydrates – related to blood group carbohydrates

Immune response – Overactive –lots of PMN's
Excessive inflammation

Related to polymorphisms in specific Toll like receptors
Genetic predisposition

Microbial Factors

Presence of appropriate ligands- flagella
Pathogenicity islands – groups of virulence-associated genes

How do *E.coli* become pathogenic in the gut ?

Acquisition of virulence genes:

From other *E.coli/Shigella* -

pili - Required to attach
(different pili for each type of disease)
- bundle forming pili
-*cfa* - colonization factor antigen

FECAL – ORAL contamination !

E.coli and diarrheal disease

Acquisition of virulence genes: **TOXINS**

ETEC - Toxigenic - secretory diarrhea – Traveler's diarrhea

EHEC - enterohemorrhagic - 0H157:H7 - toxigenic –

(Shiga toxin) – Hemolytic uremic syndrome
Interacts with platelets
Renal endothelial cells
(Jack in the Box hamburgers)

Enteropathogenic *E.coli* “pink” = gram negative rod
M. Donnenberg, Univ. Maryland

Enteropathogenic *E.coli*

Acquire the genes for virulence

Pathogenicity island
Ligand to attach + toxin

Bundle forming pilus

Pedestal formation - attaching/effacing lesion
LEE Locus -
Type III secretion genes SYRINGE GENES

Intimin expression - adhesin
Tir - bacterially encoded receptor

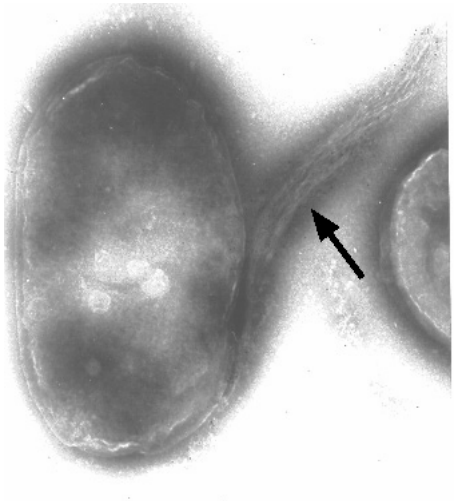
.

Enteropathogenic *E. coli*

Activate rearrangements of the actin cytoskeleton

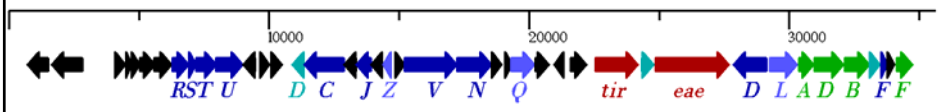
Stimulate phosphorylation of specific substrates

Interferes with the normal barrier function of the gut mucosal cell



Enteropathogenic *E.coli*

E. coli - bundle forming pilus !



Locus of Enterocyte Effacement (LEE) pathogenicity island
41 open reading frames
necessary and sufficient for attaching and effacing lesions

Genes - dark blue - esc genes- Yersinia type III secretion homologues

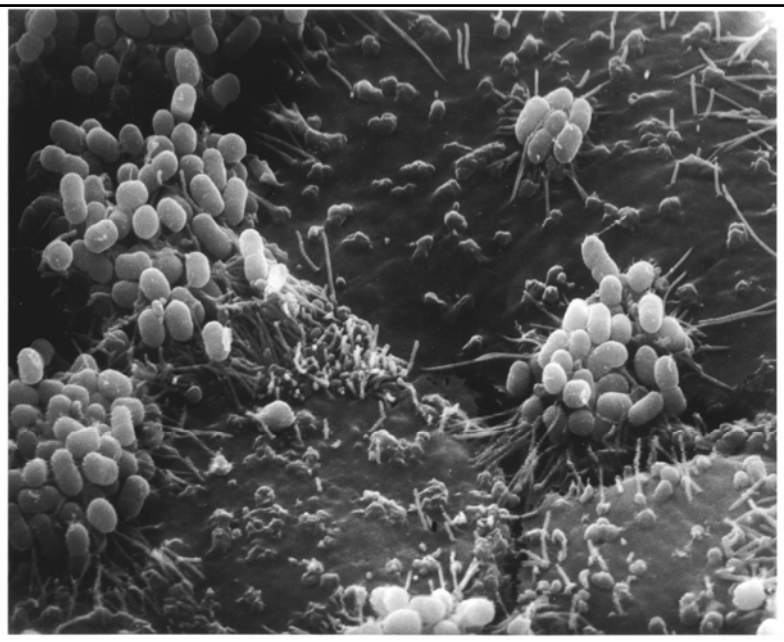
light blue - sep genes type III secretion system components

red encode the outer membrane adhesin intimin and its receptor Tir

teal blue - encode chaperones of secreted proteins

green encode -esp secreted proteins

Enteropathogenic *E.coli*



Scanning EM - EPEC

ETEC

Toxigenic *E.coli* - like cholera -

Attachment - *cfa* pilus

Toxin - ADP ribosylating enzyme

ADP-ribose transferred to GTP binding protein of adenyl cyclase

↑ Cl^- secretion
↓ Na^+ absorption

Secretory diarrhea

Enterohaemorrhagic *E.coli*

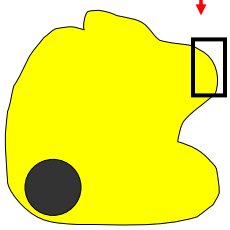
Pilin mediated attachment
Pedestal formation – type III secretion
+ cytoxin

HUS - small blood vessel damage
affects glomeruli
platelets
renal failure

SEPSIS

E.coli in the bloodstream - (perforated viscus etc)

LPS - endotoxin - actively shed



Toll like receptors and adaptor proteins

NF-kB activation

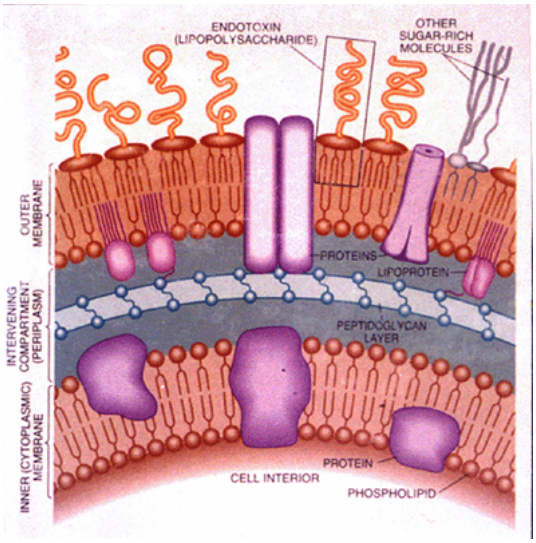
Macrophage or T cell (epithelial cells also)

Lipopolysaccharide = Endotoxin

Gram negative bacteria – activate immune cells

"toxicity" varies with species – **MICROBIAL FACTORS**

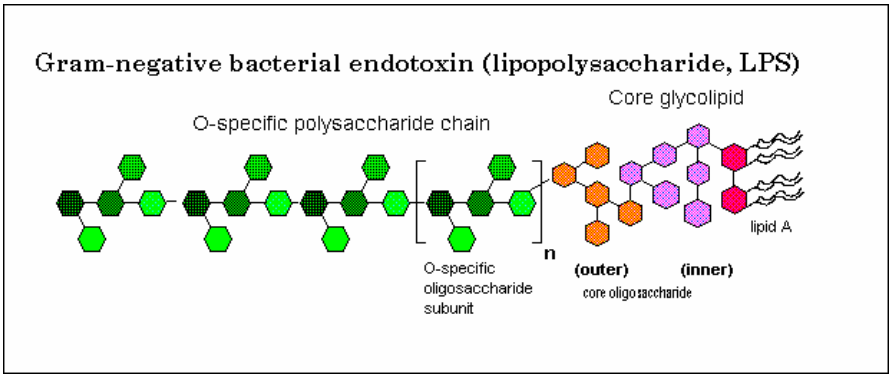
Clinical severity – dependent upon the **HOST response**



LPS

Peptidoglycan

Cytoplasmic membrane



E.coli sepsis - organism in a normally sterile

Rapidly cleared - no immune response

Large inoculum - Activation of host cytokine expression

How are gram negative bacteria cleared from the blood ?

Innate immune defenses -
"serum sensitive" - lysed by complement
opsonized - phagocytosed
reticuloendothelial clearance mechanisms

P.aeruginosa

Opportunistic pathogen –

Causes disease in impaired hosts

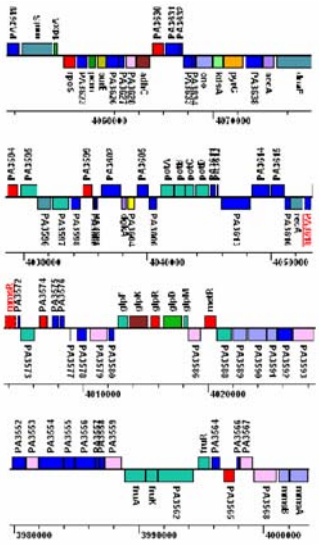
Opportunistic pathogens

Pseudomonas aeruginosa

- Genetically versatile bacteria
- Few growth requirements
- Rarely pathogenic in the normal host

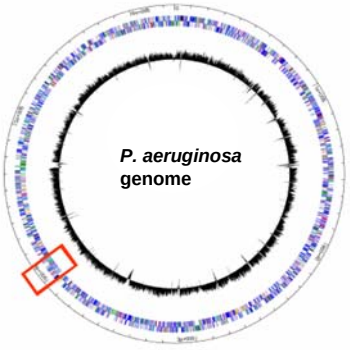
- Major pathogens in immunocompromised patients
- Special settings - cystic fibrosis

Genomic sequencing – compare genetic organization of pathogens and non-pathogens



Complete genome sequence of *Pseudomonas aeruginosa* PA01, an opportunistic pathogen

Nature (2000) 406:959-964



http://www.pseudomonas.com

Virulence factors:

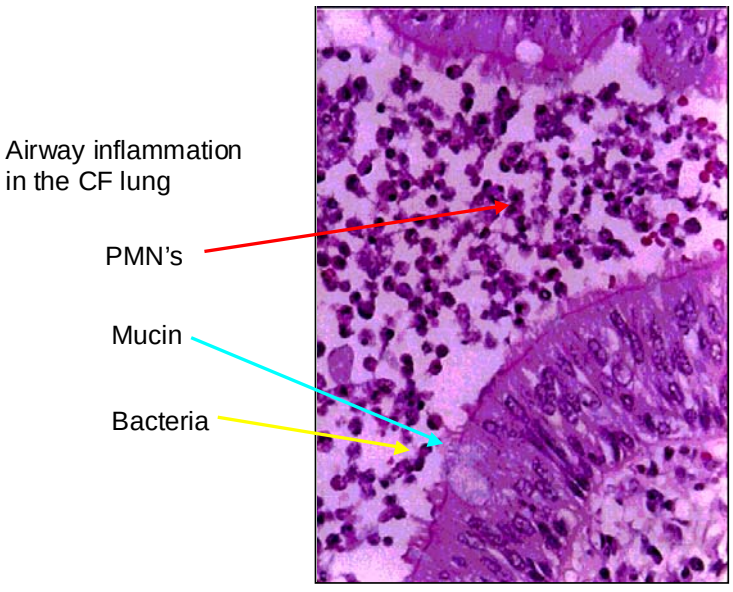
1- Turn on one group of genes in response to the environment to **ESTABLISH** an infection

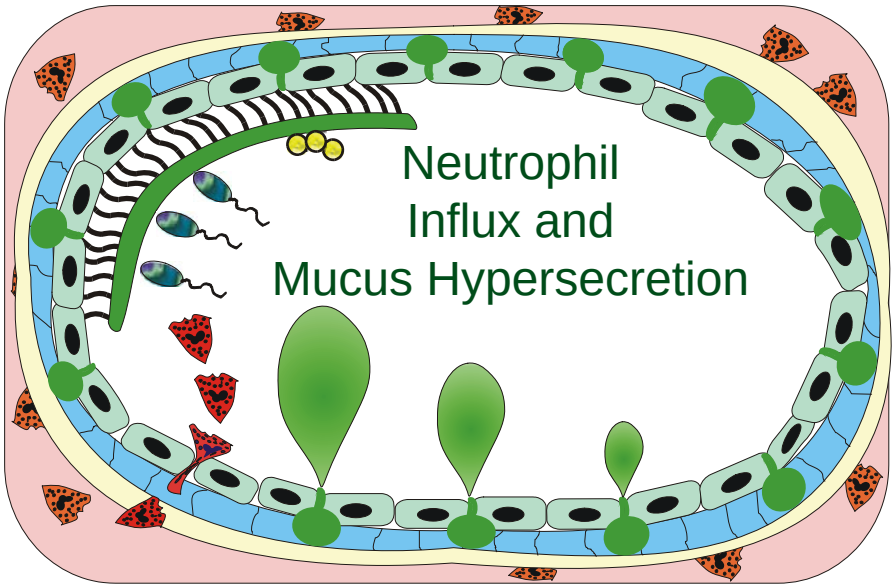
Flagella -motility
immune activation

Hemolysins - Phospholipases - cleave host components

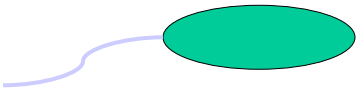
Proteases -

Siderophores - pigments - scavenge iron





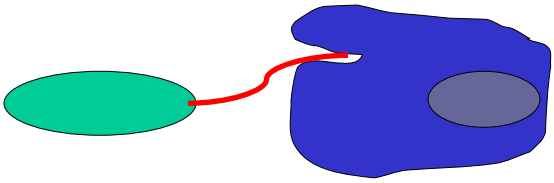
Flagella -



Motility - swim toward a desired carbohydrate
Ligands - for mucins

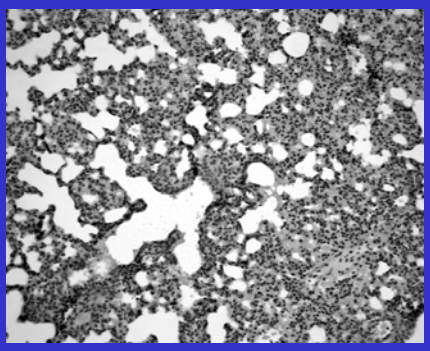
→ clearance

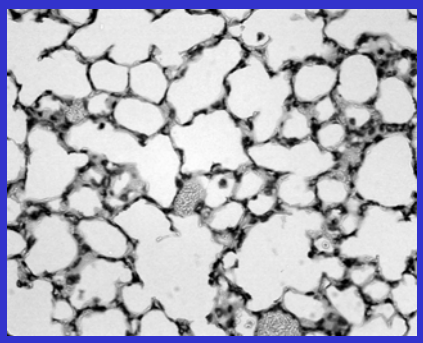
Ligand for macrophages -



PA1244 - wild type

DB103 – mutant – lacks flagella



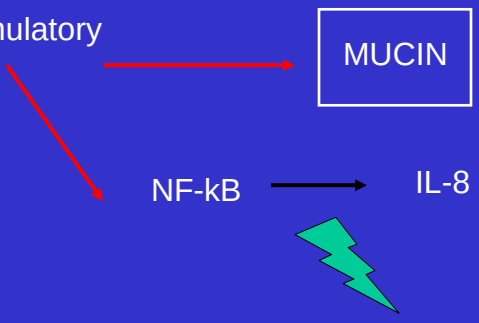


Analysis of these pathways – Identify mutants

Flagella

Multiple interactions with the host

Highly immunostimulatory



Recruits and activates PMN's

How are flagella signaled ??

Tissue dependent – Organism dependent

Extracellular pathogen – ***Pseudomonas aeruginosa***
Inhaled organism
airway epithelial cells

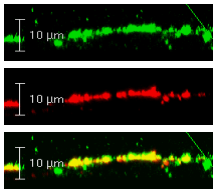
TLR -5 (toll like receptors)

Human airway cells

Flagella

TLR5

Merge



Airway – superficial stimulus is sufficient to activate inflammation
Apical display of the toll like receptors

Mutations in TLR's – associated with increased susceptibility
to specific bacterial infections

Intracellular pathogen
Salmonella enteriditis

Gut pathogen – Need INVASION to activate inflammation

Monomeric flagellin – interacts with intracellular receptors

Mutations in these receptors – Inflammatory bowel disease

Two-component signaling cascades

Insures the expression of the multiple virulence genes appropriate for the environment

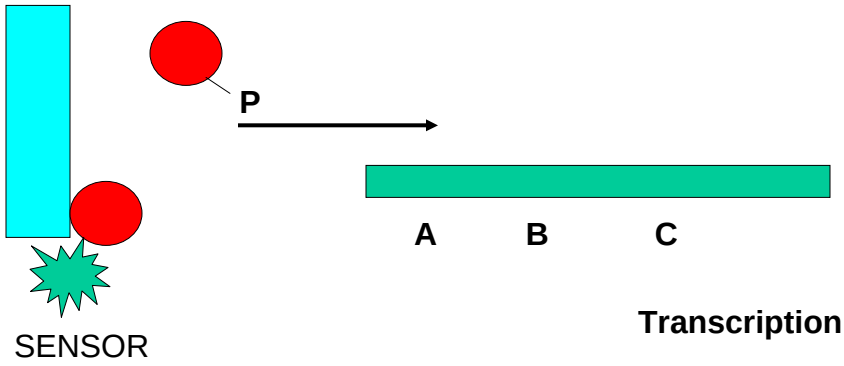
- Motility
- Pili
- Metabolic activation for growth

Pathogenicity island - often geographically linked
facilitates transmission

Two component signaling -

Coordinate regulation of virulence genes

In response to a given environmental signal:



Bacterial Adaptation –

Selection in vivo for “mutants” able to persist in the lung –

Less virulent – less “immunogenic”

don’t make flagella (ligand for macrophages)
flagella stimulate PMN’s

Actively express groups of genes to facilitate persistence within the host:
(iron scavenging, immune evasion)

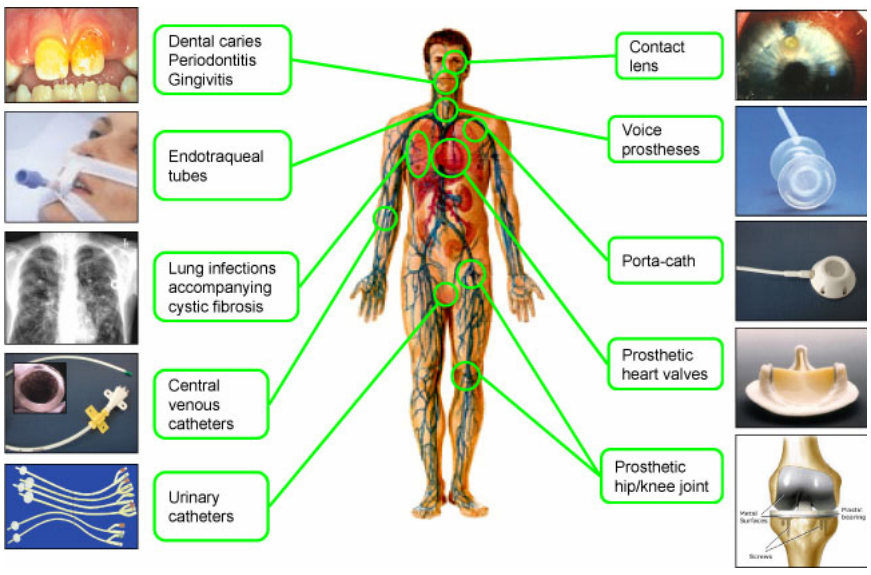
Communities - **Biofilms**

Quorum sensing systems

- Coordinate expression of virulence factors -
- Regulation via transcriptional activation

- Secretion of small diffusable molecules - organize the bacterial population to respond

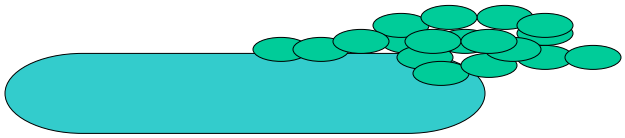
- Biofilm production - *SLIME*



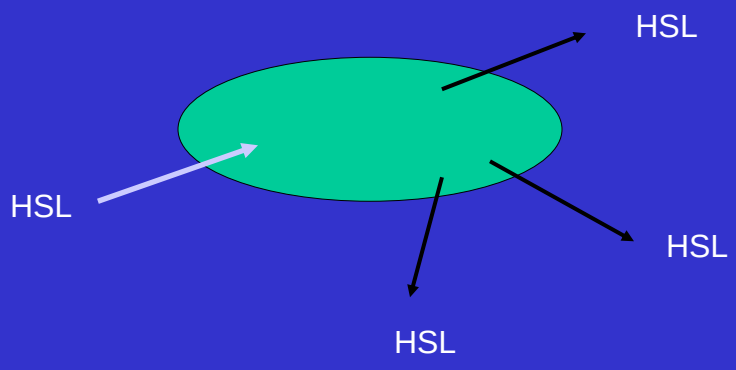
Quorum sensing -

How do communities of bacteria coordinate their activities?

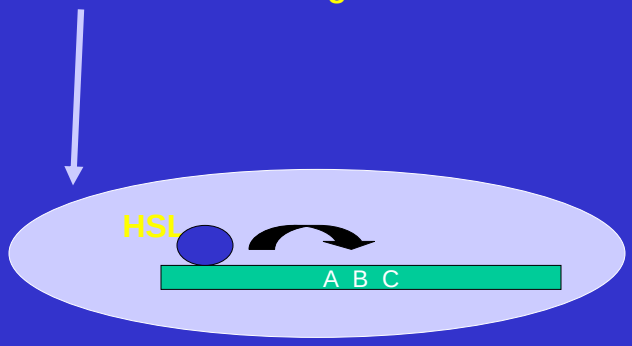
Examples - Biofilms - dental plaque
Airway colonization
Infected intravenous catheters



Acyl homoserine lactones - small highly diffusable molecules (gram negatives), others in gram positive organisms



HSL concentration is high - diffuses back into the bacteria



Transcriptional activation

Regulates the transcription of the same group of genes in many organisms at the same time – slime genes for example

Summary – Bacterial pathogenesis

Commensal flora –

Pathogens – acquire blocks of DNA – contribute to virulence

Microbial factors – adhesins
toxins

Host factors – Receptors – Tissue specific
Immune responses

Opportunists –
Adapt to the environment
Genetic flexibility – selection of mutants in vivo
Persist -