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

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

Bacterial activation of the host immune response

Subclinical

Innate/Acquired
Clearance mechanisms

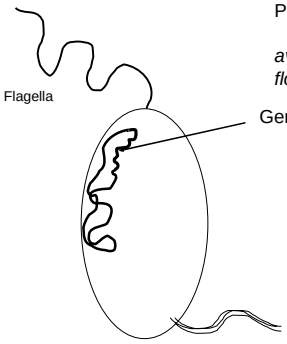
Symptomatic

"Appropriate"

Resolution

Excessive

Septic Shock
Death

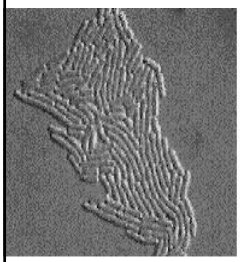


Prototypic *E.coli*

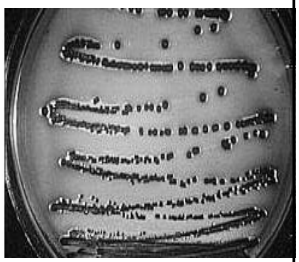
avirulent in gut - commensal flora

Genomic DNA

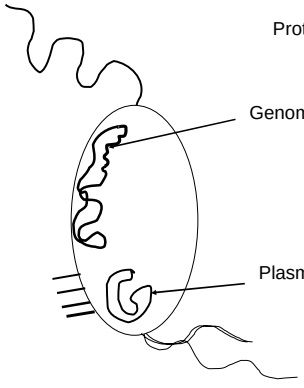
Sex pilus



E.coli - lining up for lunch….



E. coli - Eosin-methylene blue plate



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

Pathogenesis of infection

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

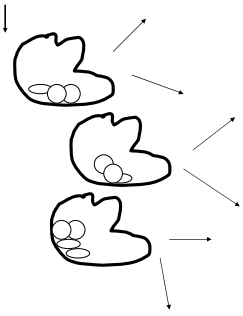
Immune response – Overactive –lots of PMN's
Excessive inflammation

Related to polymorphisms in specific Toll like receptors
Genetic predisposition

Microbial Factors

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

IL-8 - PMN chemokine



PMN mediated damage

Reactive oxygen intermediates
Superoxide
Neutrophil elastase

Toxic products

PMN's

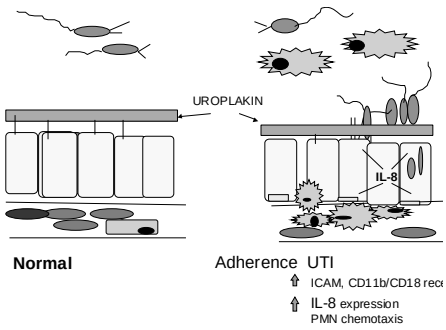
How do *E.coli* become pathogenic in the gut ?

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 !



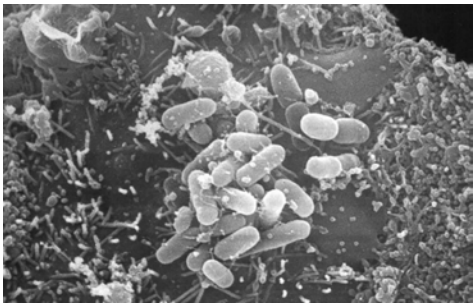
E.coli and diarrheal disease

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

E. coli - bundle forming pilus !

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

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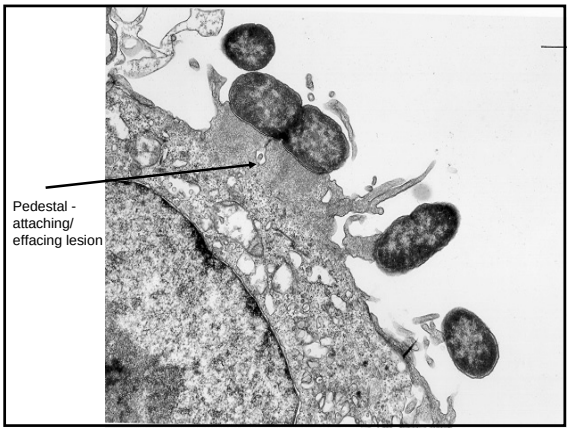


Enteropathogenic *E. coli*

Activate rearrangements of the actin cytoskeleton

Stimulate phosphorylation of specific substrates

Interferes with the normal barrier function of the gut mucosal cell

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

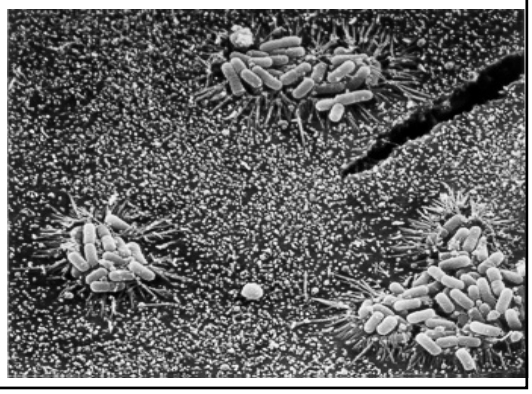
light blue - sep genes type III secretion system components

red encode the outer membrane adhesin intimin and its receptor Tir

teal blue - encode chaperones of secreted proteins

green encode -esp secreted proteins

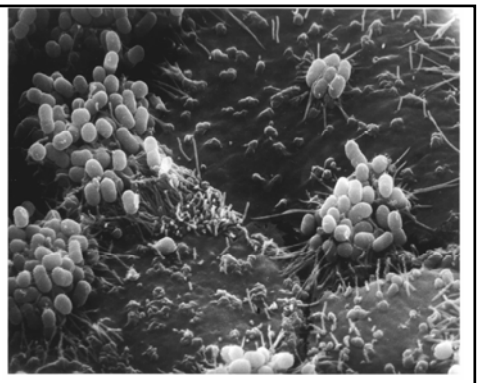
Enteropathogenic *E.coli*



Enterohaemorrhagic *E.coli*

Pilin mediated attachment
Pedestal formation – type III secretion
+ cytoxin

HUS - small blood vessel damage
affects glomeruli
platelets
renal failure



Scanning EM - EPEC

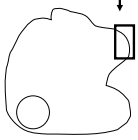
SEPSIS

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

↓

LPS - endotoxin - actively shed

↓



Toll like receptors and adaptor proteins

TLR4

CD14 + LBP

↓

NF-kB activation

Macrophage or T cell (epithelial cells also)

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

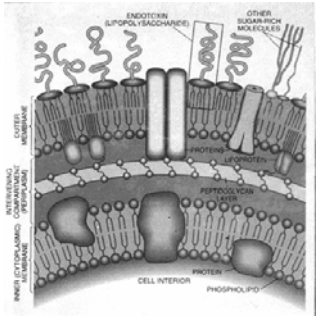
Secretory diarrhea

Lipopolysaccharide = Endotoxin

Gram negative bacteria – activate immune cells

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

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



LPS

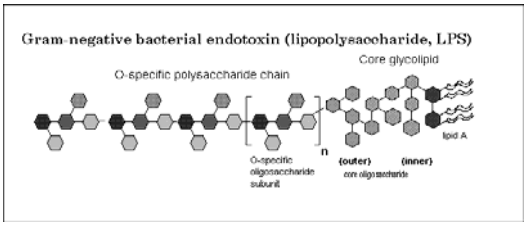
Peptidoglycan

Cytoplasmic membrane

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

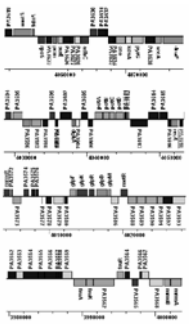
E.coli sepsis - organism in a normally sterile

- Rapidly cleared - no immune response
- Large inoculum - Activation of host cytokine expression

How are gram negative bacteria cleared from the blood ?

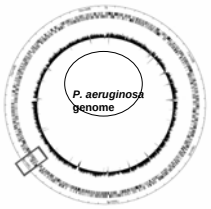
↓

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



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

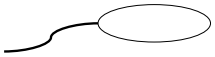
Flagella -motility
immune activation

Hemolysins - Phospholipases - cleave host components

Proteases -

Siderophores - pigments - scavenge iron

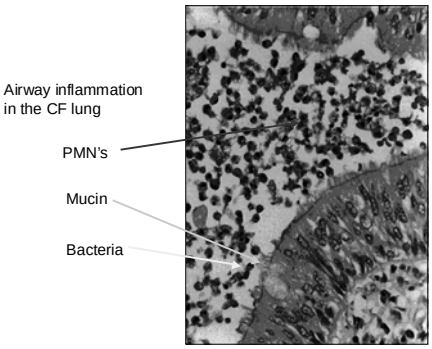
Flagella -



Motility - swim toward a desired carbohydrate
Ligands - for mucins

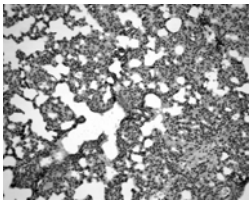
→ clearance

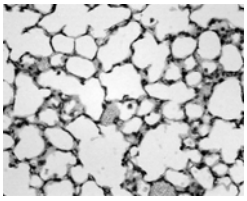
Ligand for macrophages -



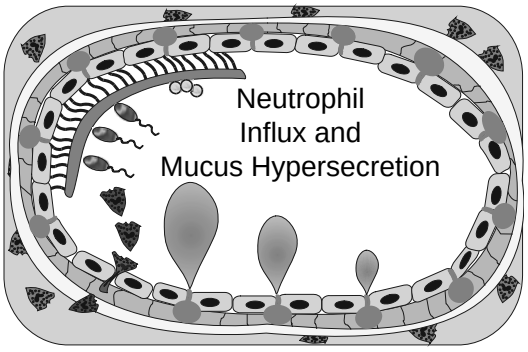
PA1244 - wild type

DB103 – mutant – lacks flagella





Analysis of these pathways – Identify mutants



Flagella

Multiple interactions with the host

Highly immunostimulatory → MUCIN

NF-kB → IL-8

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)

Two-component signaling cascades

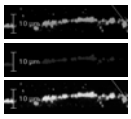
Insures the expression of the multiple virulence genes appropriate for the environment

- Motility
- Pili
- Metabolic activation for growth

Pathogenicity island - often geographically linked
facilitates transmission

Human airway cells

Flagella
TLR5
Merge



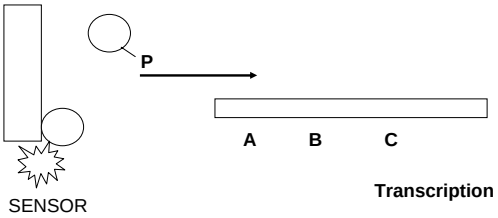
Airway – superficial stimulus is sufficient to activate inflammation
Apical display of the toll like receptors

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

Two component signaling -

Coordinate regulation of virulence genes

In response to a given environmental signal:



Intracellular pathogen
Salmonella enteriditis

Gut pathogen – Need INVASION to activate inflammation

Flagella → Ipaf → IL-1
→ IL-18

Monomeric flagellin – interacts with intracellular receptors

Mutations in these receptors – Inflammatory bowel disease

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

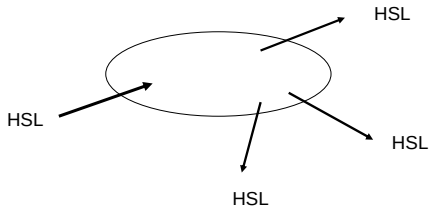
Actively express groups of genes to facilitate persistence within the host:
(iron scavenging, immune evasion)

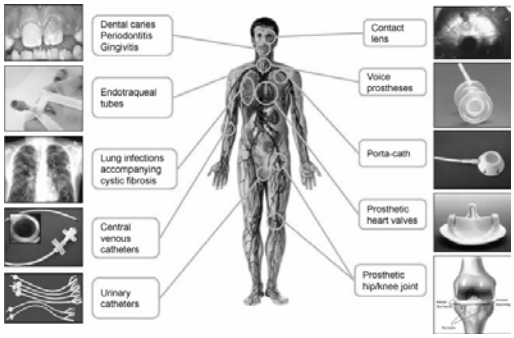
Communities - **Biofilms**

Quorum sensing systems

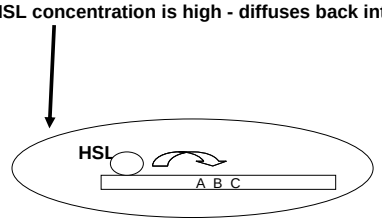
- Coordinate expression of virulence factors -
- Regulation via transcriptional activation
- Secretion of small diffusable molecules - organize the bacterial population to respond
- Biofilm production - *SLIME*

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

Quorum sensing -

How do communities of bacteria coordinate their activities?

Examples - Biofilms - dental plaque
Airway colonization
Infected intravenous catheters

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 -