

## Bacterial Pathogenesis

How do bacteria cause disease?

### Which bacteria cause disease?

Commensal flora - oral streptococci

Pathogens - Group A Streptococcus

Pharyngitis

Impetigo

Rheumatic disease - secondary to immune response to the organism - cross reactive Abs

Commensal flora - *E. coli* - acquire virulence genes

*E. coli* -0157:H7 - toxigenic - hemolytic uremic syndrome

## Or is it the host response to the bacteria ?

Depends upon the host:

Coagulase negative Staphylococci - skin flora

Colonize catheters, prosthetic devices, neonates

Location of the organism:

Invasion into a normally sterile site:

*S. aureus* on the skin - colonization

bloodstream - bacteremia/sepsis

## *S. aureus* - Epidemiology

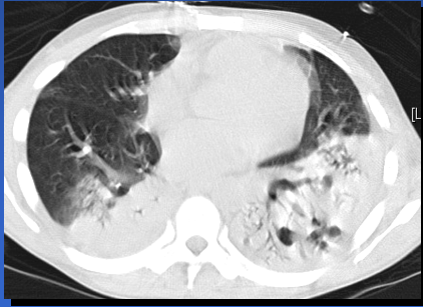


- The anterior nares is the primary site of colonization
- Colonization occurs in 20-40% of "normals"
- Infections are increased in colonized subjects usually with the colonizing strain
  - Colonization increased in IVDU, diabetics, HIV-infected
  - Elimination of carriage in high risk patients has been shown to reduce infection rates
- Most infections result from autoinoculation
  - 82-86% of cases *S. aureus* bacteremias (Von Eiff *et al.*, 2001)

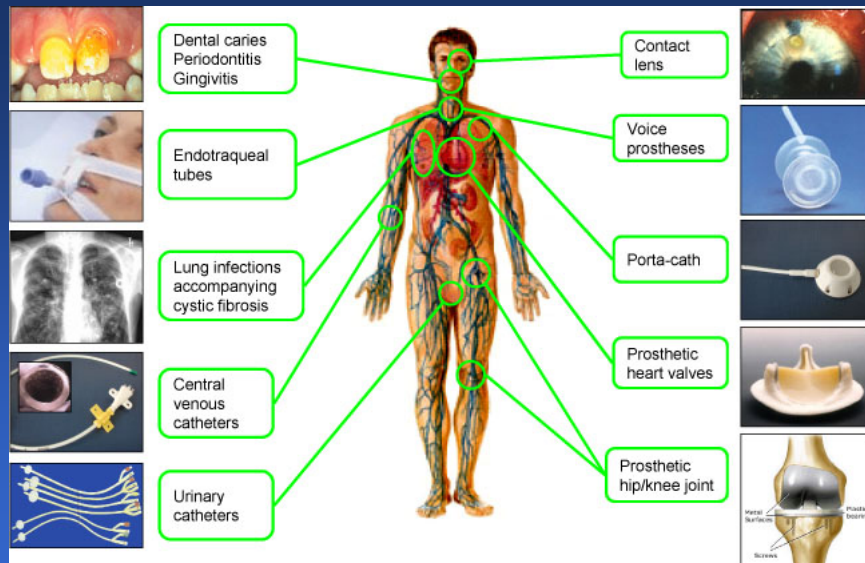
This is an official  
**CDC HEALTH ADVISORY**

Distributed via Health Alert Network  
Wednesday, January 30, 2008, 19:17 EST (07:17 PM EST)  
CDCHAN-00268-2008-01-30-ADV-N

**Influenza-Associated Pediatric Mortality and  
*Staphylococcus aureus* co-infection**

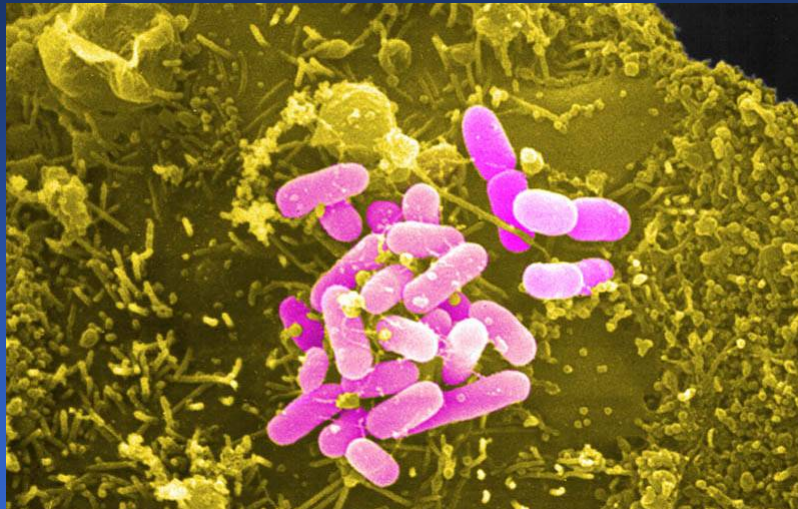


- 5 fold increase in pediatric influenza associated deaths 2006-07 due *S. aureus* bacteremia/pneumonia
- Of the 22 deaths associated with *S. aureus* 15 were caused by MRSA
- Similar data reported by *Hageman et al.* 2003-2004 influenza season



## How do bacteria cause disease ?

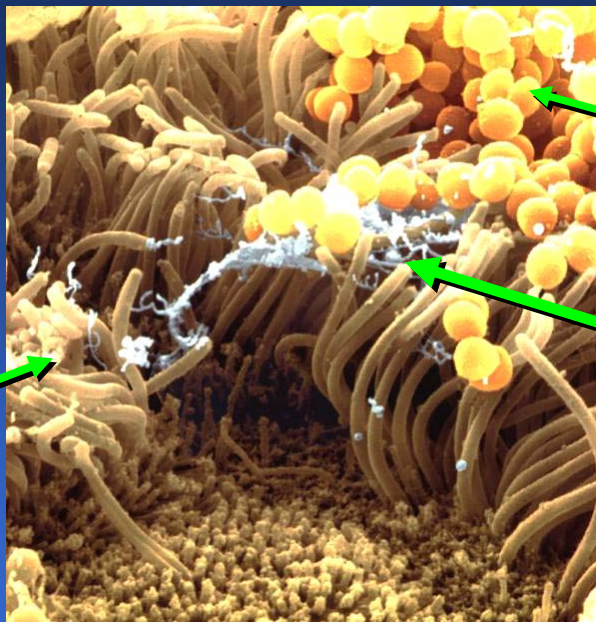
- 1 - Attachment
- 2 - Toxin expression
- 3 - Direct damage
- 4 - Activate host genes to cause damage
  - avoid immune recognition
  - look like the host





***E.coli***

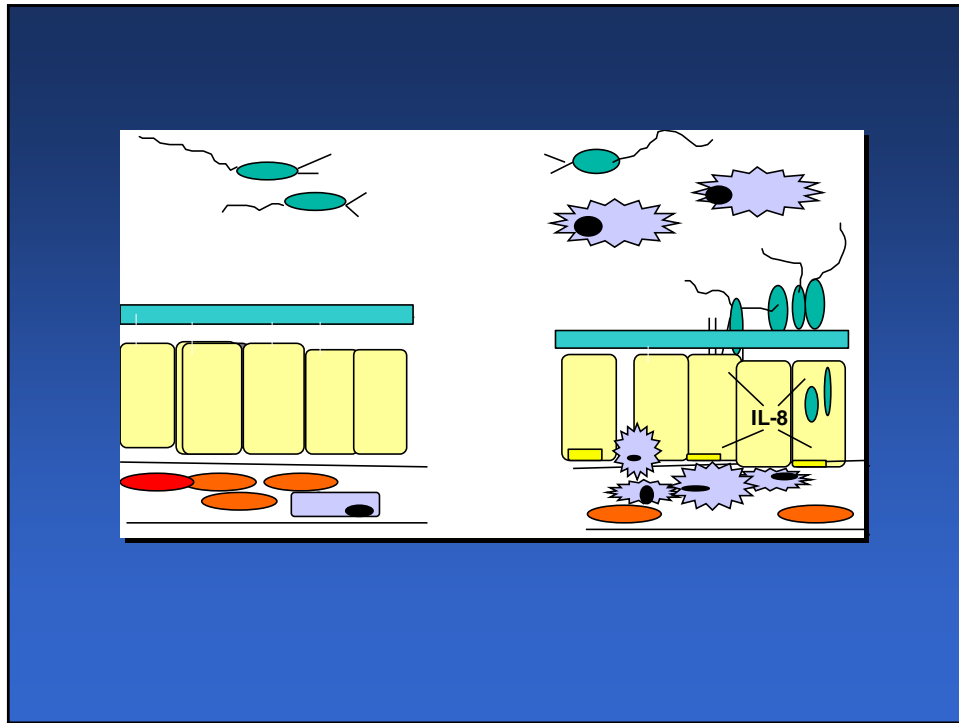
- tightly adherent
- type III toxin secretion



microvilli

bacteria

mucin



## Bacterial life styles

Extracellular - Environment  $\longrightarrow$  Host

Relatively hardy - resistant to extremes in temperature, can deal with various growth conditions - not fussy  
 some extracellular also are well adapted to live intracellularly- *Shigella*

Intracellular organisms (obligate) - *Chlamydia*

Predominantly intracellular - highly adapted to live within a macrophage - *Salmonella*, *Mycobacteria tuberculosis*

## How do bacteria “sense” the environment?

? - extracellular versus intracellular  
temperature, phosphate, glucose, magnesium etc.

Two component signal transduction

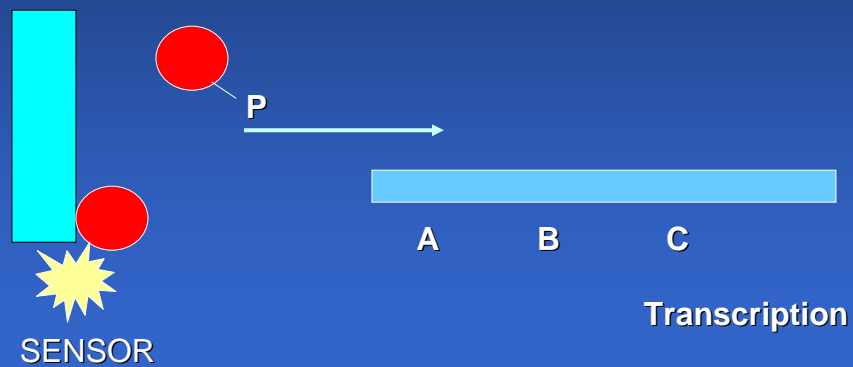
SENSOR COMPONENT - phosphorylation



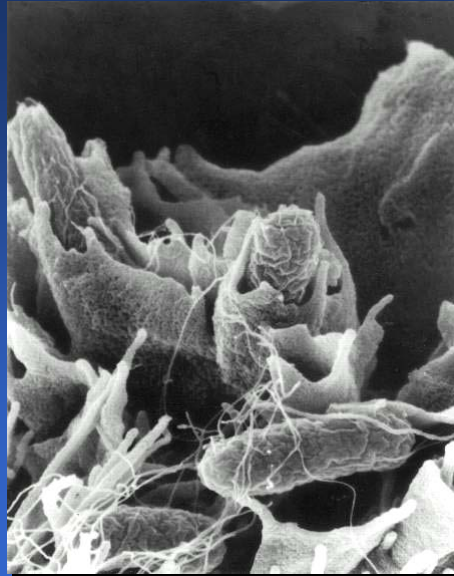
## Two component signaling

Coordinate regulation of virulence genes

In response to a given environmental signal



## Salmonella invading a gut epithelial cell



## Salmonella adaptation

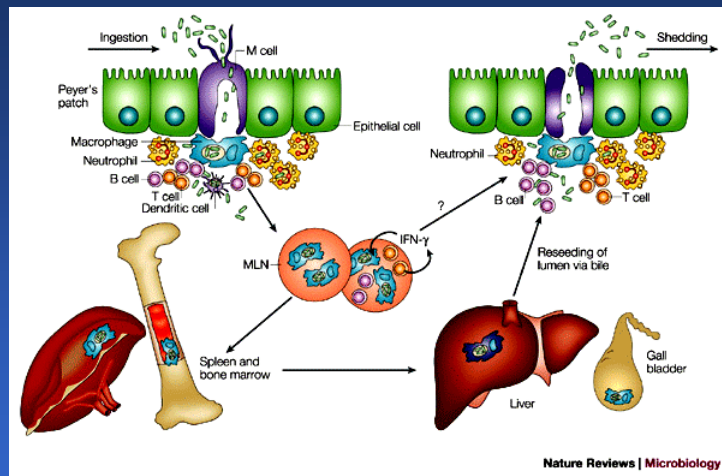
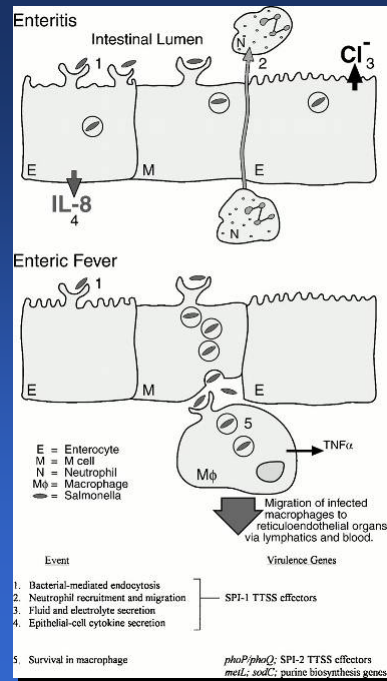
phoP/phoQ - 2 component signaling  
divalent cations

Changes in LPS

Affects susceptibility to antimicrobial peptides  
And antigenicity

Intracellular - T cell recognition for clearance

# Pathogenesis of Salmonella infection

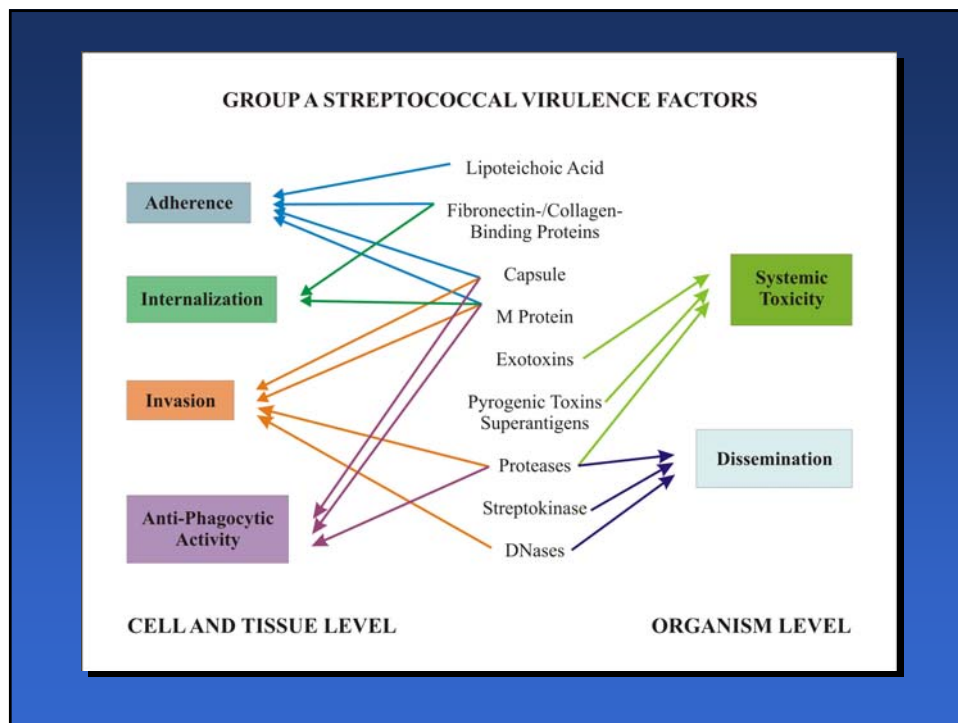


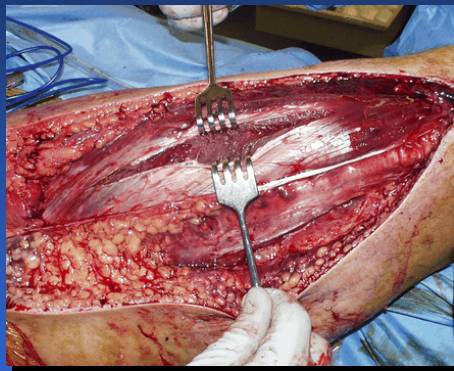
## Bacterial virulence factors

### What is a virulence factor ?

- 1 - Facilitate colonization – fimbriae, pili
- 2 - Thwart immune response – capsule, IgG binding
- 3 - Directly damage host tissues

*Streptococcus pyogenes* – Group A Strep







## TOXINS

Modify host components - ADP ribosylating enzymes

Activate the cells - cytokine expression

Alter tight junctions - allows invasion

Stop protein synthesis

Activate secretory systems - adenylyl cyclase  
*Vibrio cholera*

Induce apoptosis - airway epithelium – *Pertussis*

*Stop protein synthesis*

Toxins - Recognize eukaryotic receptors

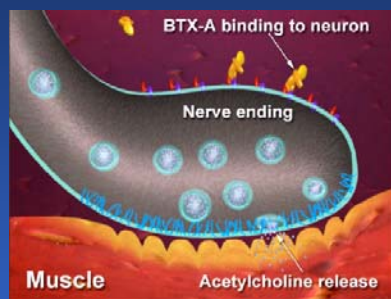
Cholera toxin

Activates Chloride (and H<sub>2</sub>O) secretion  
Via cAMP activation

*Clostridium botulinum* toxin - motor end plates

Spores - resistant to sterilization  
soil contaminants

Organic honey - infants ingest organisms -  
grows - produces toxin -  
Become floppy, lethargic -  
Infant botulism

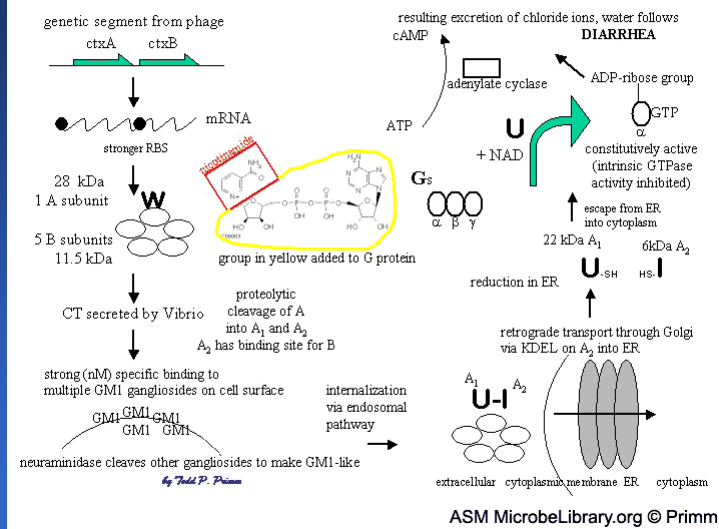




Infant botulism

Toxin prevents acetylcholine release

### Mechanism of action for cholera toxin



## Activation of host immune signaling

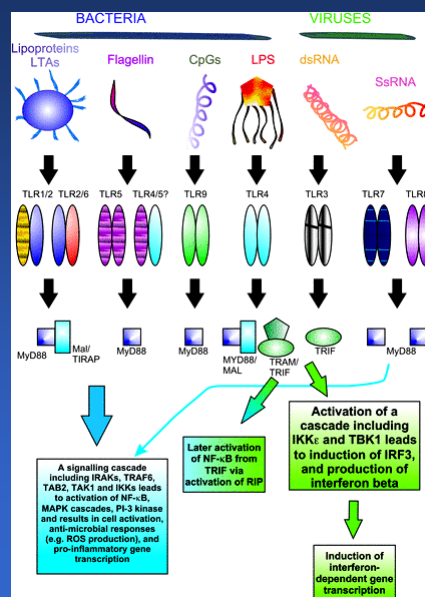
Innate immune responses

Toll like receptors and many others

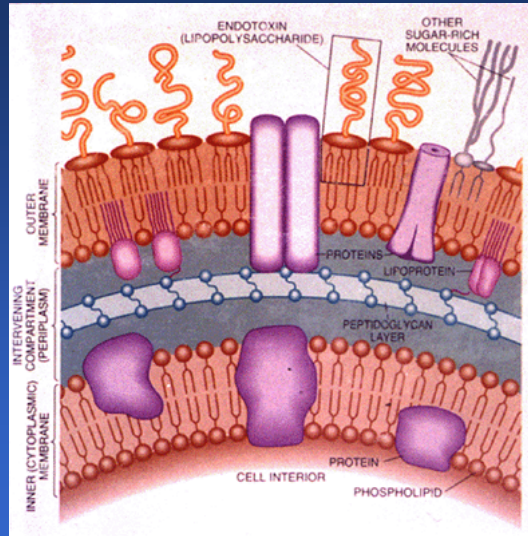
Activation of TLRs -  
Conserved signaling cascades  
Initiate inflammation

TLR polymorphisms -  
Genetic effects on disease susceptibility

## Toll like receptors - pattern recognition receptors



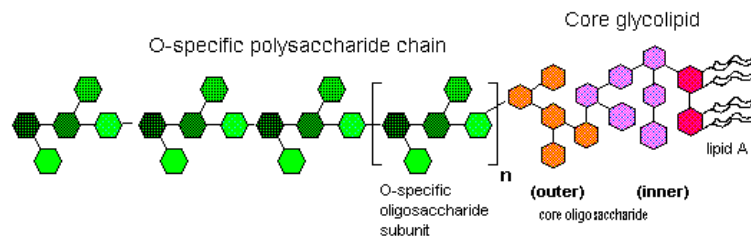
# LPS



Peptidoglycan

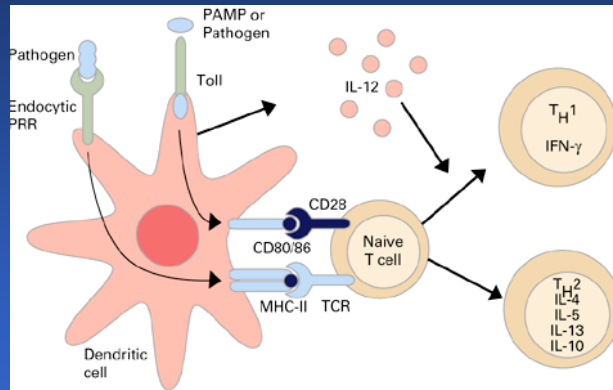
Cytoplasmic membrane

## Gram-negative bacterial endotoxin (lipopolysaccharide, LPS)



## Toll like receptors

- activating an innate immune response



## How do commensal flora cause disease?

Pattern recognition receptors -

Immuno-reactivity of shared bacterial components

Innate immune response -

If disordered - ? Role in autoimmune diseases  
inflammatory bowel disease

## TLR4 polymorphisms

**Proc Natl Acad Sci U S A.** 2007 Oct 16;104(42):16645-50.

TLR4 polymorphisms, infectious diseases, and evolutionary pressure during migration of modern humans.

Ferwerda B, McCall MB, Alonso S, Giamarellos-Bourboulis EJ, Mouktaroudi M, Izagirre N, Syafruddin D, Kibiki G, Cristea T, Hijmans A, Hamann L, Israel S, ElGhazali G, Troye-Blomberg M, Kumpf O, Maiga B, Dolo A, Doumbo O, Hermesen CC, Stalenhoef AF, van Crevel R, Brunner HG, Oh DY, Schumann RR, de la Rúa C, Sauerwein R, Kullberg BJ, van der Ven AJ, van der Meer JW, Netea MG.  
Department of Internal Medicine, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands.

Polymorphisms in Toll-like receptor 4 (TLR4) have been related to susceptibility to Gram-negative infections and septic shock. Here we show that two polymorphisms of TLR4, Asp299Gly and Thr399Ile, have unique distributions in populations from Africa, Asia, and Europe. Asp299Gly has evolved as a protective allele against malaria, explaining its high prevalence in sub-Saharan Africa. However, the same allele could have been disadvantageous after migration of modern humans into Eurasia, putatively because of increased susceptibility to severe bacterial infections. In contrast, the Asp299Gly allele, when present in co-segregation with Thr399Ile to form the Asp299Gly/Thr399Ile haplotype, shows selective neutrality.

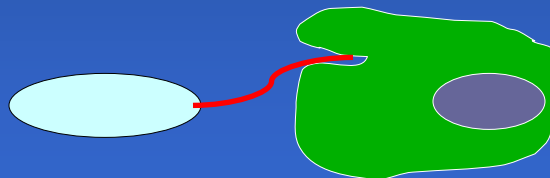
## Flagella



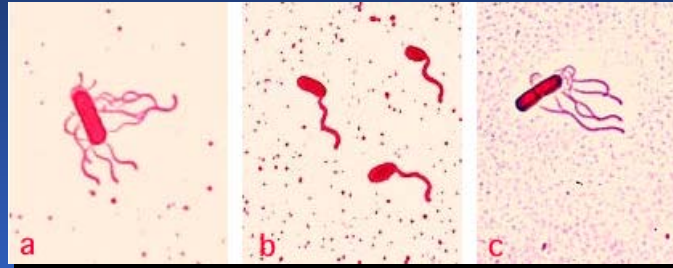
Motility - swim toward a desired carbohydrate  
Ligands - for mucins

→ clearance

Ligand for macrophages -

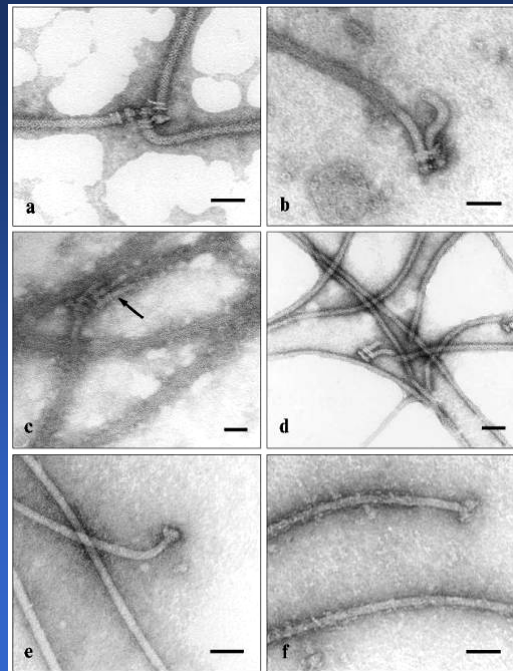


## Flagella

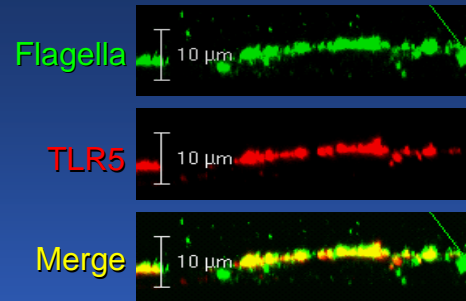


## Flagella

Electron micrograph



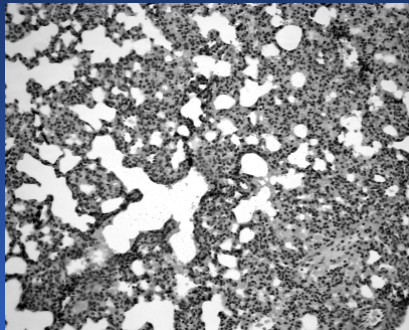
## Human airway cells



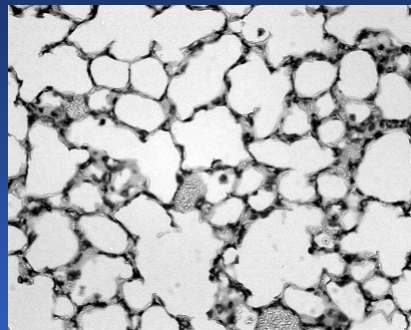
Airway - superficial stimulus is sufficient to activate inflammation  
Apical display of the toll-like receptors

Mutations in TLRs - associated with increased susceptibility  
to specific bacterial infections

PA1244 - wild type



DB103 - mutant (lacks flagella)



Analysis of these pathways – Identify mutants

## TLR5 Polymorphisms

**J Exp Med.** 2003 Nov 17;198(10):1563-72.

A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to legionnaires' disease.

Hawn TR, Verbon A, Lettinga KD, Zhao LP, Li SS, Laws RJ, Skerrett SJ, Beutler B, Schroeder L, Nachman A, Ozinsky A, Smith KD, Aderem A.  
Institute for Systems Biology, 1441 N. 34th St., Seattle, WA 98103, USA.

We show that a common stop codon polymorphism in the ligand-binding domain of TLR5 (TLR5392STOP) is unable to mediate flagellin signaling, acts in a dominant fashion, and is associated with susceptibility to pneumonia caused by *Legionella pneumophila*, a flagellated bacterium.

## 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

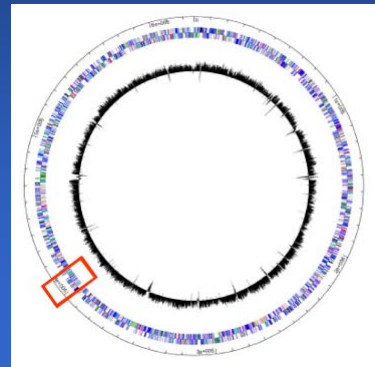
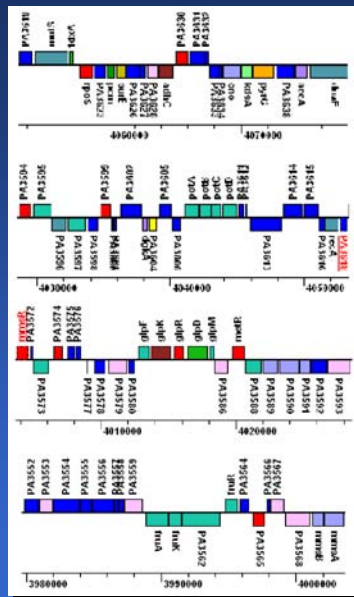
Genomic sequencing - compare genetic organization of pathogens and non-pathogens

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

G. K. Stover, X. O. Pham, A. L. Erwin, S. O. Mizoguchi, P. Warrener, M. J. Hickey, F. S. L. Brinkman, W. O. Hutnagle, D. J. Kowalik, M. Lagrou, R. L. Garber, L. Goltry, E. Tolentino, S. Westbrock-Wadman, Y. Yuan, L. L. Brody, S. R. Coulter, K. R. Folger, A. Kas, K. Lorch, B. Lim, K. Smith, D. Spencer, G. K.-S. Wong, Z. Wu, A. T. Paulsen\*, J. Reiser†, M. R. Saleri, R. E. W. Hancock‡, S. Lory§ & M. V. Olson¶

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 § Klinische Forschergruppe, Medizinische Hochschule Hannover, D-30623 Hannover, Germany  
 ¶ The Institute for Genome Research, 9712 Medical Center, Rockville, Maryland 20850, USA  
 ¶ Department of Biology, University of California at San Diego, 9500 Gilman Drive, La Jolla, California 92093-0116, USA  
 ¶ Department of Microbiology, University of Washington School of Medicine, Seattle, Washington 98195, USA

Nature (2000) 406:959-964



<http://www.pseudomonas.com>

## Virulence factors - *Pseudomonas aeruginosa*

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

2 - Persistence - turn ON genes to adapt to host immune response

