Bacterial Pathogenicity in a Historical and Experimental Perspective

Microbial Evolution and co-Adaptation

* A Workshop in Honor of Joshua Lederberg *
“The importance of bacteria as agents of infectious disease was clearly established by 1876, but this did not lead to much interest in their fundamental biology until sixty-five years later.”
DNA as the "Stuff of Genes": The Discovery of the Transforming Principle 1940-1944

Oswald Avery, Colin MacLeod, Maclyn McCarty

Lederberg noted that upon reading this paper he felt that this research was “unlimited in its implications.”

Beyond its details, the revolutionary contribution of Avery, MacLeod and McCarty was the refocusing on DNA by a generation of scientists that followed.
“The principal encouragement to think about genes in bacteria had come from Luria & Delbrück’s experiments on the statistics of mutation in \textit{E. coli}”
But bacteria were thought and taught to be “Schizomycetes,” that is, asexual, primitive plants.” and Microbiology was at the time the “last stronghold of Lamarckism”
The Discovery of Bacterial Conjugation

1946

Joshua Lederberg

“If bacteria could be crossed, a new repertoire of biological materials for experimental analysis would be available to physiological genetics and biochemistry. This work might also have important practical applications for vaccine improvement and the understanding of virulence—a latterday extension of Pasteur’s primitive techniques.”
Any piece of bacterial DNA can be incorporated into the phage. This type of transduction is termed generalized transduction.

“the possibility of gene recombination in the natural history of bacteria was presented by taxonomic tables of the species or serotypes of Salmonella”

The recombinant bacterium has a genotype \((\text{his}^+\text{lys}^-)\) that is different from recipient bacterial cell \((\text{his}^-\text{lys}^+)\).
The Watson – Crick Model of DNA
1957
Elie Wolman  F. Jacob
‘Rough Sex’
1961

Sydney Brenner
Discovery of mRNA
With Meselson and Jacob

2002
1961-1967

Cracking the Genetic Code

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In 1958 I Wondered What Made Organisms Pathogenic
Baron had worked with Lederberg and told me that Josh felt that if an experiment had more than 6 plates and 4 pipettes, it was over-designed.
Medical Microbiologists taught
A Pathogen is any organism that causes disease

A very famous scientist in the audience said
“Falkow, no one give a @%&$ about Typhoid, Or pathogens. Why Don’t You Work on Something Important. “

I said at a seminar at Cold Spring Harbor In 1964 that pathogens had evolved unique genetic traits that made them that way
The Episome Concept
(later to be called Plasmid, a term first coined by Joshua Lederberg)
Infectious Multiple Drug Resistance
‘Life is too short
To drink bad wine’

Julian Davies
1970
Not only could multiple resistance be transmitted by plasmids but also Toxins, Adhesins and, to some extent, Host Specificity
Construction of Biologically Functional Bacterial Plasmids In Vitro

(R factor/restriction enzyme/transformation/endonuclease/antibiotic resistance)

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Communicated by Norman Davidson, July 18, 1973
1975

DNA Sequencing

Fred Sanger

Wally Gilbert
1995

Hemophilus Influenzae Chromosome Sequence

Hamilton Smith, Claire Fraser, Craig Venter
Redefining Bacterial Pathogenicity Using the Tools of Molecular Genetics
All in All Pathogens are Impressive Cell Biologists

Pathogenic bacteria Interfere or Manipulate for Their Own Benefit Normal Function(s) of the Host Cell

- Adhesion to cell surface receptors (e.g., integrins) *(Bord. pertussis)*
- Manipulation of cytoskeleton by invasive organisms *(Salmonella spp.)*
- Manipulation of cytoskeleton by non-invasive organisms *(E. coli)*
- Inhibition of phagocytosis *(Yersinia spp.)*
- Membrane disruption *(Cl. perfringens)*
- Induction of cytokine release *(Hel. pylori)*
- Manipulation of actin skeleton to enable movement of organisms through cytoplasm *(M. tuberculosis)*
Horizontal Gene Transfer and Bacterial Pathogenicity

**Transposons:**
ST enterotoxin genes in *E. coli*

**Prophages:**
Shiga-like toxins in EHEC
Diptheria toxin gene, Cholera toxin
Botulinum toxins

**Plasmids:**
*Shigella, Salmonella, Yersinia*

**Pathogenicity Islands:**
Uro/Entero-pathogenic *E. coli*
*Salmonella typhimurium*
*Yersinia spp.*
*Helicobacter pylori*
*Vibrio cholerae*

Genomics has re-emphasized the importance of Mobile Genetic Elements in Bacterial Evolution
How Do We Define Virulence Genes and Their Function?

What is different about pathogens?
Salmonella infection

Monack et al. 2004 Nature Reviews Microbiology
Microarray-based negative selection strategy

1. **Mutant library**
   - Infect 50 129Sv mice IP 8x10^4

2. **Regrow on plates**
   - Mix probes hyb to array

3. **in vitro pool**
   - Label green
   - Label red

4. **in vivo pool**
   - Plate spleen & liver 1, 2, 3, 4, & 7 wks

5. **Pool Plate spleen & liver 1, 2, 3, 4, & 7 wks**
Time-dependent. Selection of Persistence genes

Yellow = absent
Blue/Blk = present

Pathogenicity Islands and LPS

Virulence Plasmid

Fimbral genes
SPI-1 of *Salmonella enterica*

Salmonella Pathogenicity Island 1

**Bacterial effector molecules**

host cell

PM

OM

IM
Microarray-based negative selection screen

Pooled Mutant library

Infect mice IP

Regrow on plates

in vitro pool

Mix probes hyb to array

in vivo pool, from spleens taken each week after infection

The Salmonella Pathogenicity Island 2 Type III secretion apparatus in the Salmonella cell envelope

Similar to: yscU T S R Q O N lcrD yscL J yscF espB espD espA yscE D C B barA glnG

26 kb

SPI 2 (30.5 centisomes)
sseI mutant is attenuated for establishing infection at systemic sites, liver and spleen

Bacterial Burden after Oral infection (34 days)

CFU/organ

WT SseI

sseI mutant

G. Govoni
Ssel

- Position specific iterative BLAST found homology to acetyltransferases including murine NAT2, human NAT1, and Salmonella NhoA.

- Contains catalytic triad: cysteine-histidine-aspartate

- No major homology to any known Protein

1  51% GC  142  33% GC  322
N  C
Identification of SseI function: Protein binding partners in the macrophage

Coomassie stain

10% mini-gel

4-20% gradient-gel

G Govoni, LM McLaughlin
**IQGAP1**

- **Cytoskeleton and cell motility regulator:**
  - binds actin and induces actin cross-linking through interaction with Arp2/3 complex and N-WASP and Cdc42.
  - binds CLIP-170, microtubule binding protein
  - localizes to the lamellipodia and filopodia and required for protrusion
  - Activated Cdc42 and Rac1 binds IQGAP1, and IQGAP1-inturn prolongs their activation by inhibiting slowing GTPase activity
    - Cdc42 and Rac1 are required for IQGAP1-mediated cell migration
SseI inhibits migration toward heat-killed S. typhimurium (ST) in infected BMDM

LM McLaughlin
The Host as a Reporter of Response to Infection
Isolate RNA
amplification
label
hybridize to mouse cDNA array

Gene expression in the peripheral blood from infected mice during acute and persistent infection

Lucy Thompson
Response to Salmonella Infection

SAM: naïve animals pre vs. post-challenge with WT
FDR: 0.93%, 2-fold change

334 induced
105 repressed
Network 1

Infiltration of neutrophils

Leukocyte extravasation signaling

JAK/STAT signaling

Interferon signaling
Early infection in 129sv mice,

- Primary and secondary responses are distinct
- Response to attenuated strain is less dramatic than WT
- Similar genes categories induced in this early infection as was seen in susceptible mice
- Immunity ‘Signature’
Induced in persistently infected mice (129sv): 336/3190 genes
Ten Months Postinfection

Major GO categories induced (DAVID):
- Antigen Presentation: MHCI plus Fc R IgG and IgE
- Defense response: beclin 1, IFN induced genes, G-CSFR, lysozyme, Phospholipase A2, MCP-1 receptor etc.
- Regulation of Cellular Processes: caspase 4, annexin A1, nudix, SOCS3, Bcl-2 like 1, fibrinogen-like protein 2 etc.
Most 6M samples moving towards "normal"

4 have a strong signature remaining

Typhoid T28
Typhoid T6M
Healthy C.
One of the least studied aspects of pathogenicity

Exit the Host
Exit the Host

Because transmission is the ultimate key to success
Mouse Models of Salmonella Infection

- **“Susceptible” Acute Model**
  - Resembles Enteric Fever
  - Higher proliferation of bacteria in RES organs
  - Death 5-7 days post infection
  - Limited to Innate Immune Response

- **“Resistant” Model**
  - Bacteria can persist for up to a year
  - MLN act as a reservoir
  - Immune Response involves:
    - Innate Adaptive
    - Ag specific

Denise Monack

![Graph showing CFU per spleen over time](image)
Experimental design to study horizontal transmission of serovar Typhimurium among mice

Orogastric infection of donor mice with $10^8$ cfus (wait 2-8 days for non-colonizing bacteria to pass through GI tract)

Naïve age matched mice

Co-mingle (5 mice/cage) for 1-60 days

Monitor fecal shedding of Typhimurium

Trevor Lawley

Infection and Immunity, 76 . 403-416 2008
Wild type S. typhimurium -5 0 5 10 15 20 25 30 35 40 45 50 55 60

1.0×10^0 1.0×10^1 1.0×10^2 1.0×10^3 1.0×10^4 1.0×10^5 1.0×10^6 1.0×10^7 1.0×10^8 1.0×10^9 1.0×10^10

days post-mingling

Shedding

naïve mouse

1.0×10^0 1.0×10^1 1.0×10^2 1.0×10^3 1.0×10^4 1.0×10^5 1.0×10^6 1.0×10^7 1.0×10^8 1.0×10^9 1.0×10^10

days post-mingling

IgA

OD405

1.0×10^0 1.0×10^1 1.0×10^2 1.0×10^3 1.0×10^4 1.0×10^5 1.0×10^6 1.0×10^7 1.0×10^8 1.0×10^9 1.0×10^10

days post-mingling

Colonization, 58 days

Cecum PP MLN Spleen Liver

1.0×10^0 1.0×10^1 1.0×10^2 1.0×10^3 1.0×10^4 1.0×10^5 1.0×10^6 1.0×10^7 1.0×10^8 1.0×10^9 1.0×10^10

cfu/g tissue

anti-Stm antibody titer

1.0×10^0 1.0×10^1 1.0×10^2 1.0×10^3 1.0×10^4 1.0×10^5 1.0×10^6 1.0×10^7 1.0×10^8 1.0×10^9 1.0×10^10

Cecum PP MLN Spleen Liver

séroconversion

naïve uninf.
Fecal Shedding of Salmonella typhimurium from Experimentally Infected Laboratory Mice

“Supershedders”

Remember that these mice are inbred
Supershedders are responsible for most transmission from animal to animal…

But…Bacteria from Supershedders Are No More Virulent or Transmissible Than Are Wild-Type Bacteria

Non-Genetic Factors at Play?
Perturbation of the Commensal Flora Increases the Incidence of ‘Super Shedders’
What is a Pathogen?**
What is the Difference Between a Pathogen and A Commensal?

Is Disease a Distraction?

##
The term pathogen is derived from the Greek παθογένεια, "birth of pain."
Steps in successful infection or How to Become a Successful Pathogen

- **Sex** comes before disease
  - acquire virulence genes

- **Sense** environment
  - and **Switch** virulence genes

- **Stick** to site of infection

- **Scavenge** nutrients
  - especially iron

- **Survive** stress

- **Stealth**
  - avoid immune system

- **Strike-back**
  - Neutralize innate immune

- **Set up**
  - In specific host niche

- **Scatter**
  - If necessary

OR, is it a Successful Commensal?
OR BOTH?

After Mark Pallen
What is the Difference Between a Pathogen and a Commensal?

- Pathogens possess the *inherent* ability to cross anatomic barriers or breach other host defenses that limit the survival or replication of other microbes and commensals.

- Therefore, most pathogens often establish themselves in a niche usually devoid of other stable microbial populations.

- These invasive properties are *essential* for their survival in Nature. And, are often host specific.
The Commensal Pathogens

- Some microorganisms are commonly found in cultures of the normal flora but also regularly cause human disease.
- These microbes have virulence determinants that suggest they regularly come in intimate contact with elements of the innate and adaptive immune system.
- Immunization against such microbes not only prevents human disease but also eliminates their ability to colonize the human host efficiently.

For example, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* b, *Streptococcus pyogenes*.
Prevalence of Endemic Carriage and Clinical Disease Caused by ‘Commensal’ Pathogens

Group A Streptococcus -- 3.2 cases per 100,000
(0-10% Asymptomatic Carriage Rate)

Group B Streptococcus -- 6.9 cases per 100,000
(4-18% Asymptomatic Genital Carriage Rate)

*Haemophilus influenzae* -- 1.3 cases per 100,000
(1.3-3.0% Asymptomatic Carriage Rate)

*Neisseria meningitidis* -- 0.8 cases per 100,000
(10-15% Asymptomatic Carriage Rate)

*Streptococcus pneumoniae* -- 21.9 cases per 100,000
(10-55% Asymptomatic Carriage Rate)
Are “Virulence Factors,” or “Pathogenic Determinants” really a subset of “Adaptive Factors”, the claws, fangs, fur, and scales that allow microbes to adapt to a particular niche. (But not necessarily designed to cause disease)?
Is Disease a Distraction?

- Human Medicine (and granting agencies) demand that we focus on disease and its cure or amelioration.

- But does this focus sometimes distract us from understanding the biology of the pathogen and the evolution of the host parasite relationship?
Many Human-Adapted Pathogens Cause Persistent Infection

- *Mycobacterium tuberculosis* and other *Mycobacterium* species
- *Treponema pallidum*
- *Chlamydia trachomatis*
- *Salmonella typhi*
- *Helicobacter pylori*
Which are Asymptomatic

- *Mycobacterium tuberculosis* - 90%
- *Salmonella typhi* – 80%
- *Bartonella henselae* (in preferred Cat host) -> 90%
- *Helicobacter pylori* - 80%
Infectious diseases
USA 1950-2000

We have met the enemy and he is us.

- Measles
- Mumps
- Crohn's disease
- Multiple sclerosis
- Type 1 diabetes
- Asthma

schizophrenia ˌskɪtsəˈfrɛnɪə; -ˈfrɛnɪə
noun
a long-term mental disorder of a type involving a breakdown in the relation between thought, emotion, and behavior, leading to faulty perception, inappropriate actions and feelings, withdrawal from reality and personal relationships into fantasy and delusion, and a sense of mental fragmentation.

• (in general use) a mentality or approach characterized by inconsistent or contradictory elements

.DERIVATIVES
schizophrenic ˌskɪtsəˈfrɛnɪk | ˌskɪtsəˈfrɛnɪk | ˌskɪtsəˈfrɛnɪk | -ˈfrɛnɪk

 adjective & noun
ORIGIN early 20th cent.: modern Latin, from Greek skhizein ‘to split’ + phrēn ‘mind.’
schizophrenia  

a long-term mental disorder of a type involving a breakdown in the relation between thought, emotion, and behavior, leading to faulty perception, inappropriate actions and feelings, withdrawal from reality and personal relationships into fantasy and delusion, and a sense of mental fragmentation.

• *(in general use) a mentality or approach characterized by inconsistent or contradictory elements*

*This is especially applicable to discussions of Microbial Pathogenicity*

.DERIVATIVES

schizophrenic |ˈfrenik| |ˈskɪtsəˈfrɛnək| |ˈskɪtsəfrinək| |ˈfrɛnək|

_adjective & noun_

ORIGIN early 20th cent.: modern Latin, from Greek *skhizein* ‘to split’ + *phrēn* ‘mind.’
Sit down before fact as a little child, be prepared to give up every preconceived notion, follow humbly wherever and to whatever abysses nature leads,...

Thomas Huxley