Pathogens: Nature and Transmission

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Module 2: pathogens and host immunity
- Existing and emerging infectious diseases
- Principles of microbial transmission and host responses
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Section A

Introduction
Infectious Disease

- Disease caused by replicating agents transmissible to humans from another person, an animal, or the environment
## Infectious Disease Health Burden: All Nations

The following table presents the leading causes of mortality from infectious diseases in 2001, measured in millions:

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mortality (in Millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory infections</td>
<td>3.9</td>
</tr>
<tr>
<td>AIDS</td>
<td>2.9</td>
</tr>
<tr>
<td>Diarrhoeal diseases</td>
<td>1.9</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>1.6</td>
</tr>
<tr>
<td>Malaria</td>
<td>1.1</td>
</tr>
<tr>
<td>All infectious diseases</td>
<td>~16.4 (32%)</td>
</tr>
</tbody>
</table>

Source: Communicable Diseases 2002, WHO 2003
## Infectious Disease Health Burden: All Nations

### Leading Infectious Causes of Cancer, 2000

<table>
<thead>
<tr>
<th>Infectious agent</th>
<th>Cancer site</th>
<th>Cases (number)</th>
<th>% Due to Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human papilloma virus</td>
<td>cervix</td>
<td>471,000</td>
<td>100%</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>liver</td>
<td>306,800</td>
<td>55%</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>liver</td>
<td>175,600</td>
<td>31%</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>stomach</td>
<td>442,000</td>
<td>50%</td>
</tr>
</tbody>
</table>

Source: *Communicable Diseases* 2002, WHO 2003
Encouraging Trends

- Crude death rate (infectious disease) USA 1900–1996

We have not yet applied existing tools to many infectious diseases with effective interventions (polio, measles)

Many of the remaining infectious disease problems are refractory to existing tools (HIV, malaria)

Emerging diseases (HIV, SARS, new influenza strains, drug resistant pathogens) illustrate that, as a public health issue, infectious diseases will always be important
Application of **existing** tools to remaining “controllable” infectious disease

Development of **new** tools applicable controlling refractory and emerging infectious disease

- The development of new tools will be critically dependent upon knowledge of the biology of disease and pathogen
A Generalized Infectious Cycle

Environment

In or on human host

Continued
A Generalized Infectious Cycle

- Invasion
- Proliferation
- Evasion of defenses
- Exit
- Pathogenesis

In or on human host

Environment

Continued
A Generalized Infectious Cycle

- Survival
- Transit (via water, food, air, contact, vectors)
- Multiplication or development in intermediate hosts, reservoirs or environmental niches

\[ \text{Environment} \]

\[ \text{In or on human host} \]

- Invasion
- Proliferation
- Evasion of defenses
- Exit
- Pathogenesis
Infectious Agents

- Prions
- Viruses
- Bacteria (prokaryotes)
- Eukaryotes
  - Fungi
  - Protozoan parasites (single cells)
  - Metazoan parasites (multicellular)
Prions

- Creutzfeldt-Jacob disease (CJD), vCJD, Mad Cow (and early-onset Alzheimer’s disease?)
- Single protein molecules (or small aggregates) (PrP)
- No nucleic acid
  - Prion protein encoded by cellular DNA
- Aberrant folding propagates
Fungi

- Eukarotes (contain defined nuclei)
- Fairly closely related (biochemically) to humans
- Fungal disease comparatively rare
  - Seen mostly in the immunocompromised
- Intractable: drugs poor
- No vaccines

Candida albicans
Metazoan Parasites

- Multicellular eukaryotes
- Agents of many important diseases (mostly tropical)
  - Schistosomiasis
  - Filariasis
- Drug treatment available for a several diseases
- No vaccines
- Transmission control central
Section B

Viral Pathogens
Viruses

- Obligate intracellular parasites
  - Perform replicative functions only in living cells
    - Dependent upon host cells for:
      - Energy
      - Biochemical precursors (amino acids, nucleosides)
      - Protein synthesis
      - Nucleic acid synthesis (to varying extents)
    - Transmitted as metabolically inert particles (virions)
Viruses

- Virions all have:
  - Nucleic acid (DNA or RNA)
    - 5,000 to 250,000 bases (human: $3 \times 10^9$)
    - 3 to 100+/- genes (human: 50,000)
  - Protective protein coat
    - 1 to 50 different types, a few to 1000s of copies of each
  - Mechanism for specific attachment to host cells
    - (Commonly targets for immunity)
- And may also have:
  - Membranes derived from the host cell
  - Enzymes (HIV reverse transcriptase, for example)
  - Specialized attachment structures
Virion Morphology

- Human adenovirus (colds, DNA)
- Ebola virus Zaire (hemorrhagic fever, RNA)

Continued
Virion Morphology

T4 virus of *E. coli*,
( DNA)
Poliovirus

- Etiologic agent of paralytic poliomyelitis
- Wild polio eradicated in the West
  - 1,919 cases worldwide in 2002 (down from ~350,000 in 1988)
- Decrease due to effective vaccines and successful immunization campaigns
Poliovirus: Infectious Cycle

- Transmission by fecal-oral route via contaminated water
- Primary replication/multiplication is in lymphoid cells (specialized cells of the immune system) especially in the gut
- Virus shed primarily into the gut, excreted in the feces
- Some virus also enters the blood and reaches other susceptible cells
- These include anterior horn cells (motor neurons), which innervate muscle
- Destruction of these cells can result in paralysis
  - Disease is not a consequence of an essential step in the virus’s life cycle
Poliovirus Virion

- 30 nM diameter virion contains 60 copies each of four proteins (encoded in the viral RNA)
- Viral RNA is a single strand with mRNA (+) polarity, is about 8000 bases long, and encodes 11 proteins
- Virion is non-enveloped and contains no enzymes
1. Attachment to cell via specific receptor (Vpr) on cell membrane

2. Virus entry (endocytosis); extrusion of RNA into cytoplasm

3–5: Translation of viral RNA; processing of polyprotein; formation of RNA replicase protein

Source: Adapted from Flint et al., *Principles of Virology*, ASM Press, 2000
8-10. Replication of viral RNA
11. Continued translation and processing; formation of virion proteins
12. Assembly of (+) RNA and virion proteins into new virions
13. Virion release into the gut

Source: Adapted from Flint et al., *Principles of Virology*, ASM Press, 2000
Potential Targets: Viruses

- Entry
  - Immunization
- Uncoating
  - Drugs (amantadine)
- Nucleic acid synthesis
  - Drugs (nucleoside analogs—e.g., AZT)
- Translation
  - Interferons
- Protein processing
  - Drugs (protease inhibitors)
Section C

Bacterial Pathogens
Bacteria Are Living Cells

- Living cells
  - Membrane bound; with or without cell walls
  - Genetic material is DNA
  - Produce energy
  - Produce biochemical precursors
  - Produce and translate RNA to form proteins
  - Replicate DNA
  - Reproduce by cell division
  - Respond to environmental signals
Bacteria

- Bacteria are prokaryotes ("before nuclei")
  - Biochemically, they are distantly related to their eukaryotic hosts (such as humans)
    - This underlies successful antibacterial drug treatments
- Bacteria are transmitted either as metabolically active cells or dormant forms called spores
- Bacteria can be professional pathogens (e.g., TB), but many are opportunistic or facultative pathogens
Etiologic agent of cholera, a severe epidemic diarrheal disease

There are both pathogenic and non-pathogenic V. Cholerae strains
- Close relatives are normal estuarine organisms

Transmission to humans happens occasionally from this source
- During epidemics, transmission is fecal-oral via water

Infection control is by cleanliness of water and food
- A good vaccine is not available
V. Cholerae: Infectious Cycle

- Bacteria are ingested in contaminated water or food
- Bacteria pass through the stomach to the gut, where they colonize the surface of the small intestine
  - Ability to colonize the gut is the key determinant of ability to cause disease
  - Colonization is dependent on a number of identified and unidentified genetic “virulence factors”
  - *V. cholerae* is non-invasive and does not cause much tissue damage (differing from some other bacterial and viral pathogens)
V. Cholerae: Infectious Cycle

- Multiplication occurs on the surface of the gut.
- Cholera toxin induces diarrhea, washing organisms out into the environment
  - Pathogenesis greatly increases efficiency of transmission
- Death is from hypotensive shock and circulatory collapse
Cholera toxin (CT) induces diarrhea by stimulating secretion in the intestine

- CT secreted by *V. cholerae* in response to colonization
- CT binds to intestinal cells (R subunit)
- CT A subunit enters cell
- A1 indirectly activates a cellular pump that causes Cl⁻ and HCO₃⁻ secretions across epithelium; water, K⁺, and Na⁺ are secreted passively
Effects of the toxin are independent of the continued presence of the bacteria (for a few days)
- Antibiotics are not immediately effective in treating disease
Cholera Treatment

- Treatment is by fluid/salt replacement
- Salt solutions are not effective due to poor uptake
- IV infusions of salt solutions are effective
  - Expensive
  - Require trained personnel
  - Involve risk of other infection
- Discovery in the 1960s of glucose-dependent co-transport of Na\(^+\) and water in the intestinal epithelium
- Oral rehydration solution now standard (glucose or starch plus appropriate salts)
  - Inexpensive
  - Skilled personnel not required
  - Immediately available (no hospitalization required)
Virulence is the ability of an organism to cause disease.
Most pathogens exist in forms with varying virulence:
- Not all *V. cholerae* are virulent, nor are the large number of closely related Vibrios.
A variety of genetically-determined properties of *V. cholerae* underlie virulence:
- Ability to move through mucus
- Ability to attach to intestinal epithelium
- Ability to produce CT
- Ability to evade pre-existing immunity
  - For example, when El Tor *V. cholerae* was replaced with O139
Virulence Determinants

- Mechanisms/proteins/genes are of interest
  - Identification of virulent strains in the environment
  - Targets for intervention (chemotherapeutic or immune)
  - Targets for attenuation (vaccine design)
    ▶ CVD 111 *cholerae* O1 El Tor Ogawa Δ (ctx, zot, cep, ace), ins (ctxB, mer')
    - Prediction of emergence of virulent strains

- Identification of virulence genes
  - Genomics: comparison of DNA sequence of known virulent and non-virulent strains
  - Construction and characterization of mutant strains in model systems
## Relevant Genes Associated with Virulence and Environmental Properties in *V. Cholerae*

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Function</th>
<th>Location</th>
<th>Virulence (v)/(e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>aphAB</td>
<td>Regulatory proteins</td>
<td>chromosome</td>
<td>v</td>
</tr>
<tr>
<td>chi genes</td>
<td>Chitinase homologues</td>
<td>chromosome</td>
<td>e</td>
</tr>
<tr>
<td>£rC</td>
<td>Flagellar transcriptional regulator</td>
<td>chromosome</td>
<td>v/e</td>
</tr>
<tr>
<td>irgA</td>
<td>Iron-regulated outer membrane protein</td>
<td>chromosome</td>
<td>v</td>
</tr>
<tr>
<td>msh genes</td>
<td>Type IV pili (mannose-sensitive hemagglutinin)</td>
<td>chromosome</td>
<td>e</td>
</tr>
<tr>
<td>ompU, T</td>
<td>Outer membrane porins</td>
<td>chromosome</td>
<td>v/e</td>
</tr>
<tr>
<td>rfb genes</td>
<td>O-antigen biosynthesis</td>
<td>chromosome</td>
<td>v/e</td>
</tr>
<tr>
<td>rtxA</td>
<td>&quot;Repeats in toxin&quot; toxin, cross-links cellular actin</td>
<td>chromosome</td>
<td>?</td>
</tr>
<tr>
<td>toxRS</td>
<td>Transmembrane regulatory proteins</td>
<td>chromosome</td>
<td>v/e</td>
</tr>
<tr>
<td>wav genes</td>
<td>LPS core oligosaccharide synthesis</td>
<td>chromosome</td>
<td>v/e</td>
</tr>
<tr>
<td>vps genes</td>
<td>Exopolysaccharide synthesis</td>
<td>chromosome</td>
<td>e</td>
</tr>
<tr>
<td>ace</td>
<td>M13 gene VI homologue formerly ‘accessory enterotoxin’</td>
<td>CTXP phage</td>
<td>v</td>
</tr>
<tr>
<td>cep</td>
<td>M13 gene VIII homologue formerly ‘core-encoded pili’</td>
<td>CTXP phage</td>
<td>v</td>
</tr>
<tr>
<td>ctxAB</td>
<td>CT subunits A, B</td>
<td>CTXP phage</td>
<td>v</td>
</tr>
<tr>
<td>orfU</td>
<td>M13 gene III homologue</td>
<td>CTXP phage</td>
<td>v</td>
</tr>
<tr>
<td>rst genes</td>
<td>regulation, integration, replication</td>
<td>CTXP phage</td>
<td>v</td>
</tr>
<tr>
<td>zot</td>
<td>M13 gene I homologue formerly ‘zonula occludens toxin’</td>
<td>CTXP phage</td>
<td>v</td>
</tr>
<tr>
<td>acfABC</td>
<td>Accessory colonization factors, function unknown</td>
<td>VPI</td>
<td>v</td>
</tr>
<tr>
<td>aldA</td>
<td>Aldehyde dehydrogenase ToxT-activated</td>
<td>VPI</td>
<td>?</td>
</tr>
<tr>
<td>tagA</td>
<td>ToxT-activated gene</td>
<td>VPI</td>
<td>?</td>
</tr>
<tr>
<td>tcpA</td>
<td>Toxin co-regulated pili major subunit (type IV pili)</td>
<td>VPI</td>
<td>v</td>
</tr>
<tr>
<td>tcpPH</td>
<td>Transmembrane regulatory proteins</td>
<td>VPI</td>
<td>v</td>
</tr>
<tr>
<td>toxT</td>
<td>Virulence transcriptional activator</td>
<td>VPI</td>
<td>v</td>
</tr>
</tbody>
</table>
**Virulence and Mobile Genetic Elements**

- Half of *V. cholerae* virulence-associated genes lie on mobile genetic elements
  - DNA sequences that can be transmitted from one strain to another (or across species barriers)
    - Vibrio Pathogenicity Island (Toxin co-regulated Pili)
    - CTX phage (CT)
- Populations that interact to produce cholera epidemics include humans, Vibrio, viruses, and other mobile genetic elements
  - Transfer of mobile virulence determinants may underlie emergence of virulent strains
Section D

Eukaryotic Pathogens/Malaria
Eukaryotic Pathogens

- Living cells
  - Membrane bound; with or without cell walls
  - Genetic material is DNA
  - Produce energy
  - Produce biochemical precursors
  - Transcribe and translate RNA
  - Replicate DNA
  - Reproduce by cell division
  - Respond to environmental signals

- Unlike bacteria, eukaryotic pathogens are (biochemically) rather closely related to humans
  - Complicates chemotherapy
Malaria: The Disease

- Responsible for about 300 million acute cases/year and about 1 million deaths (mostly children)
- Acute malaria includes periodic fever, diarrhea, aches
- Most deaths result from severe malaria (severe anemia, hypoglycemia, circulatory collapse) or cerebral malaria
  - The unifying pathogenic mechanism is reduced tissue oxygenation (anemia, sequestration)
Malaria: Pathology

- Etiologic agent is a single-cell eukaryotic parasite of the genus *Plasmodium*
  - *Falciparum* (severe malaria)
  - *Vivax*
  - *Ovale*
  - *Malariae*
- Vector-borne
  - Transmitted by female mosquitoes of the genus *Anopheles*
- Complex life cycle

Photo source: (www.dpd.cdc.gov/dpdx/images/ParasiteImages/M-R/Malaria/malaria_LifeCycle.gif)
Malaria Life Cycle

Continued
Malaria Life Cycle

1. Mosquito takes a blood meal (injects sporozoites)
2. Human Liver Stages: Liver cell
3. Ruptured schizont
4. Schizont
5. Human Blood Stages: Immature trophozoite (ring stage)
6. Ruptured schizont
7. Mature trophozoite
8. Mature gametocyte
9. Immature gametocyte
10. Ookinete
11. Oocyst
12. Ruptured oocyst

\[ \text{Liver cell} \rightarrow \text{Infected liver cell} \rightarrow \text{Exo-erythrocytic Cycle} \rightarrow \text{Human Blood Stages} \rightarrow \text{Erythrocytic Cycle} \rightarrow \text{Human Liver Stages} \]

\[ \text{A} = \text{Infective Stage} \]
\[ \text{B} = \text{Diagnostic Stage} \]
Malaria Control: The Vector

- Vector essential for transmission (Ronald Ross)
  - Control of breeding sites (water)
  - Insecticide use (residual sprays in/around houses)
  - Reduction in exposure (bed nets, screens, repellants)

An. gambiae

Continued
Malaria Control: The Vector

- Difficulties of target control
  - Target species must be determined and programs must be designed
    - Not all mosquitoes (nor all anopheles) transmit malaria
    - Species differ in breeding, feeding, resting behaviors
  - Costs of vector control
    - Programs are hard to sustain when the problem appears to be solved
  - Vectors develop insecticide resistance (with inappropriate use)
  - Political opposition to insecticide use/environmental modification
Complications of vector control: in some areas, huge reductions in vector populations would be needed to affect transmission (1000x)

- Prevalence of Parasitemia*
- Vectorial Capacity†

*Proportion of human population infected
†Number of potentially infectious contacts per person per day
Malaria Control: Drugs

- Chloroquine
  - Discovered prior to WW II
  - Cheap and effective
  - Non-toxic to humans
  - Acts against the blood stage (merozoites) by interfering with heme detoxification
  - Resistance to chloroquine developed in the 1960s, and some degree of resistance is now seen in all endemic regions in Africa
- Mefloquine
- Primaquine
- Pyrimethamine
- Artemesinin (Quinhaosu)
Malaria Control: Vaccination

- No effective malaria vaccines exist
- Major efforts to produce vaccines against multiple life stages are underway
  - Sporozoites (infection-blocking)
  - Hepatic stages (early post-infection)
  - Blood stages (reduce symptoms)
  - Gametes/gametocytes (transmission-blocking)
- Technologies include vaccination with recombinant protein, DNA, and vectored vaccines
Using these three strategies in combination, it should be possible to substantially reduce malaria:
- Vaccination
- Development of new drugs
- Mosquito control

The biology of infectious organisms underlies prevention and treatment strategies.

Knowledge of the ecology of infectious organisms provides insight into population-based control strategies.