HISTORY OF THE DISEASE

Cholera Pandemics

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<tbody>
<tr>
<td>I</td>
<td>1817-1823</td>
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<tr>
<td>II</td>
<td>1829-1850.....reached England in 1832</td>
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<tr>
<td>III</td>
<td>1852-1859.....John Snow/epidemiology USA</td>
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<td>IV</td>
<td>1863-1875</td>
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<tr>
<td>V</td>
<td>1881-1883......isolation by Koch</td>
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<td>VI</td>
<td>1889-1923</td>
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<tr>
<td>VII</td>
<td>1961..............01 El Tor Biotype, initiated in Indonesia</td>
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<tr>
<td>VIII (?</td>
<td>1992..............0139 Bengal Strain</td>
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Cholera (the disease, not necessarily the organism) was confined to an endemic focus in the floodplain of the Ganges, in Bengal, during the periods between pandemics.

In 1991, for the first time in 100 years, cholera arrived in the Western Hemisphere from its focal center in Asia. Cases were first reported in Peru, and epidemics throughout South and Central America rapidly followed.

THE ORGANISM

*Vibrio cholera* is a well defined species by both classic biochemical, and DNA homolgy. It is a Gram-negative bacterium that belongs to the gamma subdivision of the family Proteobacteriaceae, making it a distant cousin of E. coli. Whereas most organisms in the genus *Vibrio* are marine bacteria, *V. cholerae* appears adapted to brackish water where they may represent a ubiquitous member of the bacterial flora. In aquatic environments *V. cholerae* may persist by attachment to algae or other eukaryotic microorganisms. *V. cholerae* strains vary widely in pathogenicity.

The *V. cholerae* genome has been completely sequenced. It consists of 2 "megareplicons:

- 2.9 Mb (2770 ORF)
- 1.1 Mb (1115 ORF)
<table>
<thead>
<tr>
<th>Barriers to Infection</th>
<th>Virulence Factors</th>
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<tbody>
<tr>
<td>Sanitary Infrastructure</td>
<td>Chemotaxis</td>
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<tr>
<td>Aesthetics, religion and morals</td>
<td>Protease (mucinase)</td>
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<tr>
<td>Gastric acidity</td>
<td>Pilus mediated adhesion</td>
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<tr>
<td>Intestinal motility</td>
<td>Exotoxin</td>
</tr>
<tr>
<td>Mucus</td>
<td>Porin switching ?</td>
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<tr>
<td>Epithelial cell replacement</td>
<td></td>
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<tr>
<td>Indigenous microflora</td>
<td></td>
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<tr>
<td>Immune response (secretory IgA)</td>
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<tr>
<td>Lysozyme, lactoferrin, peroxidase</td>
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**Sensory Transduction and Coordinate Regulation of Virulence Functions**

Host-specific gene expression is regulated by a transmembrane signalling protein, ToxR.

*toxR* is a gene, constitutively expressed, encoding a 294 residue (32.5 kD) transmembrane protein (ToxR) whose amino terminus is exposed to the periplasm and whose carboxy terminus is exposed on the cytoplasmic side of the pm. The carboxy terminus is a DNA binding domain which selectively regulates the transcription of at least 15 genes. The products of at least 13 of these genes are virulence factors (ie. required for disease).

The conformation of the DNA binding domain is modified according to conditions of pH, temperature, osmolarity and amino acid concentrations existing on the exterior of the pm. Thus, ToxR is a transmembrane, sensory transduction signaling protein that probably serves to detect when the bacterium has arrived in the small intestine.

*tcpA-G* ("toxin co-regulated pilus") these genes code for the production of pili mediating specific attachment of *V. cholera* cells to intestinal epithelium.
**acFA-D** code for “accessory colonization factors” which allow *V. cholera* cells, by a mechanism yet to be elucidated, to persist and multiply on the epithelial surfaces. (exopolysaccharide synthesis?)

**OmpT** and **OmpU** are outer membrane proteins whose role in virulence has not been defined. My guess is that they are analogous to OmpC and OmpF in *E. coli*.

**ctxA,B** code for the subunits of cholera exotoxin. The *ctxAB* operon is encoded on the *V. cholera* chromosome within an element that resembles a complex transposon, within the genome of a lysogenic bacteriophage. This element is subject to gene amplification during residence of the cells in the intestinal tract. Control of toxin gene amplification has not been studied. The promoter region has 3-8 (depending on the strain) direct repeats of the sequence TTTTGAT which are required for ToxR mediated activation of transcription.

Holotoxin (87 kD): 5 B subunits + 1 A Subunit  
B Subunit (11 kD): product of *ctxB*; mediate specific binding to Gm1 ganglioside receptors on epithelial cell surface; form transmembrane channel which allows A Subunit to enter target cell; B Subunits do not enter target cell. Strongly immunogenic.  
(Suggested as basis for drug delivery system.)

A Subunit (29 kD): product of *ctxA*; enters target cell; enzymatically catalyzes ADP ribosylation of the GTP-binding regulatory subunit of Adenyl Cyclase; weakly immunogenic.

Both the A and the B polypeptides are synthesized with short amino-terminal leader sequences to drive export from bacterial cell.

ORF for A subunit is upstream from B. Relative amounts of A and B polypeptides is regulated at translational level by ribosome binding efficiency.

**TOXIN INTERNALIZATION**

- receptor mediated endocytosis (RME) – **NO**  
- fluid-phase macropinocytosis – **NO**  
- fluid-phase micropinocytosis – **NO**  
- hydrophobic diffusion – **NO**  
- receptor-mediated direct membrane translocation – **YES**

• Initially, a single B subunit of the holotoxin binds to a single Gm1 ganglioside receptor molecule. Lateral diffusion proceeds until all five B subunits have bound a corresponding Gm1 receptor.  
• Lateral redistribution of toxin-receptor complexes lead to aggregation of complexes (“patching and capping”). Antitoxin antibodies (secretory IgA) inhibit prior to this phase .  
• Internal hydrophobic associations of B subunits with each other are replaced by hydrophobic interactions of B subunits with integral membrane components.  
• The B subunits become embedded in the membrane, forming a hydrophilic channel for the entry of the A subunit.  
• The A subunit is proteolytically cleaved as it traverses the membrane to form two fragments, A₁ (amino terminal) and A₂. Cleavage is required for activation of toxin activity.  
• Enzymatic activity directed against the host cell resides in the A₁ subunit.
Presumably A₁ crosses the membrane channel in an elongated, unfolded conformation and refolds in the cytoplasm.
ENZYMATIC MECHANISM

- CtxA is an NAD N-glycosyltransferase
- It ADP-Ribosylates the Gα protein of a G-Protein-mediated signalling pathway
- The ADP-Ribosylated Gα protein cannot hydrolyze bound GTP, so AC is "locked" in its active state.
- Intracellular cAMP levels are abnormally elevated, causing overstimulation of a second-messenger pathway leading to ion imbalance and water loss.
Cholera toxin exhibits the following features which are typical of bacterial AB$_5$-type protein exotoxins in general:

1. A + B subunit structure with one subunit responsible for binding/internalization and the other subunit enzymatic,

2. entry into target cells by receptor mediated translocation,

3. ADP-ribosylation of sensitive cell component.

The binding, entry and action of bacterial exotoxins resembles in many respects the processes by which protein hormones interact with cells.

Cholera toxin 80% similar to E. coli enterotoxin.

**PHYSIOLOGICAL EFFECTS AND CLINICAL SYMPTOMS**

- Elevated cAMP levels in epithelial cells alters ion transport.
- Na$^+$ uptake decreases and Cl$^-$ efflux increases.
- Increasing salt concentration in intestinal lumen causes osmotic water loss.
- Clinical manifestation is profuse watery diarrhea ("rice water stool") leading to electrolyte imbalance and dehydration.
- Patients may lose up to 20 liters of fluid per day; excreted fluids contain high concentrations of ions and V. cholera.
- Death by dehydration can occur within 4 hr of onset of symptoms; mortality averages 50% without treatment.

**Treatment**

<table>
<thead>
<tr>
<th>ORH for Moderate Cases (WHO formulation)</th>
<th>NaCl</th>
<th>3.5 g/L</th>
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<tbody>
<tr>
<td></td>
<td>KCl</td>
<td>1.5 g/L</td>
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<tr>
<td></td>
<td>NaHCO$_3$</td>
<td>2.5 g/L</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>20.0 g/L</td>
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Glucose stimulates absorption of Na$^+$ by intestinal epithelium. Sucrose is an acceptable substitute for glucose and is more readily available. Crude cane sugar and many fruits contain significant amounts of K$^+$.

Patients severely debilitated by dehydration require i.v. fluid replacement.

With timely fluid and electrolyte replacement patients generally recover in 5-7 days. Antibiotic therapy is generally not indicated.

Tetracycline has been recommended to limit excretion of V. cholera by infected individuals and as a prophylactic measure for their close associates. However, after a 5-month mass prophylaxis trial in Tanzania the incidence of Tet$^R$ isolates increased from 0% to 75% due to the rapid spread of a broad host range R plasmid.
Vaccines based on killed cells or toxoids (artificially inactivated toxin) have been of limited use due to logistical problems, transient immunity (<6 months) and instability of A-subunit mutants induced by classic methods. Immunity acquired by contracting the disease itself is superior to that afforded by any vaccine currently available. Research is now directed towards developing a live cell vaccine based on genetically engineered, stable A-subunit deletion strains.

Discussion Questions

1. Describe the nature of the molecular system that regulates host-specific gene expression in *Vibrio cholera*.

2. Give a detailed description of the structure and function of colera toxin, or of any other HMW protein exotoxin (botulism, diptheria, tetanus).

3. What is the evidence that gastric acidity is a barrier to colonization by *V. cholera* ingested in contaminated water?

   See p. 1035.
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Cholera 1832

Epidemic Cholera in the Americas 
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A fine web site on 19th Century London physician John Snow: 
www.ph.ucla.edu/epi/snow.html

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Alternative mechanism of cholera toxin acquisition by Vibrio cholerae: 
generalized transduction of CTX Phi by bacteriophage CP-T1 
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