Amebas
Friend and foe

Facultative Pathogenicity of *Entamoeba histolytica*?

Confusing History
1875 Lösch correlated dysentery with amebic trophozoites
1925 Brumpt proposed two species: *E. dysenteriae* and *E. dispar*
1970's biochemical differences noted between invasive and non-invasive isolates
80's/90's several antigenic and DNA differences demonstrated
    • rRNA 2.2% sequence difference
1993 Diamond and Clark proposed a new species *(E. dispar)* to describe non-invasive strains
1997 WHO accepted two species

Family Entamoebidae
- Family includes parasites and commensals
- Species are differentiated based on size, nuclear substructures
  - *Entamoeba histolytica*
  - *Entamoeba dispar*
  - *Entamoeba coli*
  - *Entamoeba hartmanni*
  - *Endolimax nana*
  - *Iodamoeba bütschlii*

*Entamoeba histolytica*
one of the most potent killers in nature
Entamoeba histolytica

- worldwide distribution (cosmopolitan)
  - higher prevalence in tropical or developing countries (20%)
  - 1-6% in temperate countries
- Possible animal reservoirs
- Amebiasis - Amebic dysentery
  - aka: Montezuma's revenge

Taxonomy
- One parasitic species?
  - E. histolytica
  - E. dispar
  - E. hartmanni

Entamoeba Life Cycle - Direct

- Fecal/Oral transmission
- Cyst - Infective stage
  - Resistant form
- Trophozoite - feeding, binary fission

- Different stages of cyst development
  - Precysts - rich in glycogen
  - Young cyst - 2, then 4 nuclei with chromotoid bodies
  - Metacyst - infective stage
  - Metacystic trophozoite - 8

Excystation

- Cyst wall disruption
- Ameba emerges
  - Nuclear division 4 → 8
  - Cytokinesis
- Trophozoites go on to inhabit large intestine
  - Replicate via binary fission
Key Features of Trophozoites

- Shape - more ovoid
- 20-30 µm
- Psuedopods rapidly extend and withdraw
- 1 nucleus
  - Central endosome

Show movies here!
Will be posted on website

Keys Features of Cysts

- oval or spherical shape
- 10-20 µm
- distinct cell wall set apart from cytoplasm
- Young cysts - still contain chromatoid bodies
- Mature - quadrinucleated
  - Concentric endosome
  - Peripheral chromatin

Disease Manifestations

- Ulcer formation
- Ulcer enlargement
- Perforation of intestinal wall
- Local abcesses
- Secondary bacterial infections
- Occasional ameboma

ameboma = inflammatory thickening of intestinal wall around the abscess (can be confused with tumor)
Clinical Features and Symptoms

Range of Outcomes
- Asymptomatic/cyst
- Symptomatic nondysenteric
- Amebic dysentery
- Extraintestinal disease

Intestinal Symptoms
- Range
  - mild to intense, transient to chronic
- Nondysenteric
  - diarrhea, cramps, flatulence, nausea
- Dysenteric
  - blood/mucus in stools
  - cramps/pain
- Ameboma
  - palpable mass
  - obstruction

E. Histolytica Pathology

Healthy Intestine

E. Histolytica infected Intestine

Flask-shaped ulcer
Trophozoites at the boundary

Extraintestinal Amebiasis

Amebic Liver Abscess
- chocolate-colored ‘pus’
- necrotic material
- usually bacteria free
- lesions expand and coalesce
- further metastasis, direct extension or fistula
Pulmonary Amebiasis

- Rarely primary
- Rupture of liver abscess through diaphragm
- 2^ bacterial infections common
- Fever, cough, dyspnea, pain, vomiting

Cutaneous Amebiasis

- Intestinal or hepatic fistula
- Mucosa bathed in fluids containing trophozoites
- Perianal ulcers
- Urogenital (e.g., labia, vagina, penis)

Epidemiologic Risk Factors

- Prevalence
  - Lower socioeconomics
  - Crowding
  - Human fecal waste management
  - Endemic area
  - Communal living
  - Institutionalization

- Severity
  - Children, neonates
  - Malnutrition
  - Corticosteroid use
Intestinal Amoebae

Humans harbor 9 species of intestinal amoebae.

<table>
<thead>
<tr>
<th>Entamoeba histolytica</th>
<th>Entamoeba hartmanni</th>
<th>Endolimax nana</th>
<th>Iodamoeba bütschlii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyst</td>
<td>trophozoite</td>
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</table>

Details of these commensals are covered in the text.

Entamoeba histolytica Diagnosis

- **Microscopic**
  - Detection in stool samples
  - Class A stains
  - Multiple samples tested
  - Blood and mucus present
  - Culturing of samples - time
  - histolytica vs. dispar
  - impradil

- **Molecular**
  - ELISA - immunological based via specific lectins
  - histolytica vs. dispar
  - PCR-based methods
  - 100x more sensitive

Diagnostics

Comparison of Two Instruments for Detection of "Entamoeba Histolytica" in stool by Immunologic Methods. 1, 2, and 3.

[Image of diagnostic kit]

Compositions of each stage of treatment by PCR for diagnosis of intestinal amoebae.
Recognition of Host Cells

How is this process mediated?

Virulence factors

- Molecules that help:
  - Establish infection in host
  - Cause pathogenesis
  - Allow transmission from host to host
  - Evade host immune defenses

- General types of virulence factors
  - Adherence factors
  - Invasion factors
  - Endotoxins
  - Exotoxins
  - Siderophores

Amoebic Factors Implicated in Pathogenesis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Suggested role in pathogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>GalNAc lectin</td>
<td>Adherence to mucin/cells, serum resistance</td>
</tr>
<tr>
<td>Fibronectin/collagen Receptors</td>
<td>Adherence to extracellular matrix</td>
</tr>
<tr>
<td>Cysteine proteinases</td>
<td>Invasion through the extracellular matrix</td>
</tr>
<tr>
<td>Amoebapore</td>
<td>Lysis of target cells</td>
</tr>
<tr>
<td>Phospholipases</td>
<td>Lysis of target cells</td>
</tr>
<tr>
<td>Cytoskeleton</td>
<td>Adhesion plates, endocytosis, motility</td>
</tr>
</tbody>
</table>
**Amoebapores - virulence factor**

- Family of small (77 AA) proteins contained in secretory granules
- Similar in structure and function to NK lysins
- Used to kill bacteria and host cells
- Amoebapores insert into target membranes and form ion channels
- Amoeba mutants which make less amoebapores cause less disease in animal model studies

Originally 3 isoforms identified: A, B, and C.

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**Entamoeba virulence factor**

- Gal/GalNAc lectin is a multifunctional virulence factor
  - Lectin - proteins which specifically bind carbohydrates
  - Classification is based on carbohydrate specificity
  - Plays roles in adherence, cytolysis, invasion, resistance to lysis by complement, and encystation.

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**Gal/GalNAc Adherence Lectin**

- Heterotrimer
  - Heavy subunit (hgl)
    - 170kDa
    - Integral membrane protein
    - CRD: carbohydrate recognition domain
    - Cytoplasmic tail - signaling
  - Lipid-anchored light subunit (lgl)
    - 31-35kDa form - glycosylation
    - Covalent S-S bond to hgl
  - Intermediate subunit (igl)
    - 150 kDa lectin
    - Non-covalent association

30 igl homologues identified
GPI anchor

- Glycosylphosphatidylinositol
  - Glycolipid that anchors a protein to the cell surface
  - Roles in cell surface localization, signaling, surface molecule turnover

Man - mannose
GlcN - glucosamine

Fatty acid linkages
Variable chain length
C14–C22
(C18 is most common)

Possible pathogenic mechanism

Contact dependent killing of epithelial cell - lectin mediated
Breakdown of tissue (extracellular matrix) - cytolysis
Amoebopores - pore-forming proteins (~5 kDa)
Surface cysteine proteases? Still unclear

Gal/GalnAc Lectin Signaling

Determinants of invasion: correlation with variation in disease

MUC2 - predominant secreted mucus
Allelic variation

Bacterial flora - influence balance between trophozoite vs. cyst formation
Some bacterial combinations promote better...
**Naegleria fowleri**

- **Geographic Range:** Cosmopolitan
  - Found throughout world in freshwater.
  - Three life forms: amoeba, flagellate, cyst
  - Infections generally occur around thermal pools where population of amoeba is high.
  - Also very common in water above 80°F
  - Most cases of human infections are from the United States
    - Particularly from Florida, Texas, Colorado
  - Other countries reporting cases include Czech Republic, Mexico, Africa, New Zealand, and Australia.

**Pathology**

- Causes Primary Amebic Meningoencephalitis (PAM)
- Very rapidly causes the death of host
  - Rapid destruction of brain tissue
- Symptoms very similar to other types of meningitis and encephalitis.
  - Headaches, fever, stiff neck, etc. progressing to dementia and death.
  - But much less common and usually mistaken for more common bacterial and viral forms
**Acanthamoeba sp.**

- **Geographic Distribution:**
  - Cosmopolitan
  - Found in freshwater almost everywhere
  - Amoeba and cyst forms
  - Also found in soil, dust, sewage
  - Cannot survive in thermal pools

- **Location in Host:**
  - Most common in eye and skin. Rarely invades brain.

- **Pathology:**
  - Rarely causes damage in people with intact immune systems except contact lens wearers.

**Acanthamoeba**

- Most common cause of corneal ulcers and keratitis in contact lens wearers
  - Keratitis is an inflammation of the cornea
  - Can lead to blindness.
  - Most common in people who make their own saline solution.
  - May require abrasion by the contact lens