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
  - Metacysts - 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 abscesses
- 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 passer
- 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, vomica

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>Entamoeba coli</th>
<th>Endolimax nana</th>
<th>Iodamoeba bütschlii</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>trophozoite</strong></td>
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Details of these commensals are covered in the text.
Entamoeba histolytica Diagnosis

- Microscopic
  - Detection in stool samples
    - Classic stains
    - Multiple samples tested
    - Blood and mucus present
  - Culturing of samples - time
    - histolytica vs. dispar
    - impracticle

- Molecular
  - ELISA - immunological based via specific lectins
    - histolytica vs. dispar
  - PCR-based methods
    - 100x more sensitive
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>
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<tbody>
<tr>
<td>GalNAc lectin</td>
<td>Adherence to mucin/cells, serum resistance</td>
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<tr>
<td>Fibronectin/collagen</td>
<td>Adherence to extracellular matrix</td>
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<tr>
<td>Receptors</td>
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<tr>
<td>Cysteine proteinases</td>
<td>Invasion through the extracellular matrix</td>
</tr>
<tr>
<td>Amoebapore</td>
<td>Lysis of target cells</td>
</tr>
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<td>Phospholipases</td>
<td>Lysis of target cells</td>
</tr>
<tr>
<td>Cytoskeleton</td>
<td>Adhesion plates, endocytosis, motility</td>
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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.
**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.

**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
Amoebapores - pore-forming proteins (~5 kDa)
Surface cysteine proteases? Still unclear
Gal/GalnAc Lectin Signaling

Determinants of invasion: correlation with variation in disease

Colonial Epithelium

MUC2 - predominant secreted mucin
Allelic variation!

Bacterial flora - influence balance
between trophozoite vs. cysts formation.
Some bacterial combinations promote better.

Free-living opportunistic amoeba

Naegleria fowleri

Enter through the olfactory
nerve or leptomeningeal arteries
in healthy individuals.

Cysts and trophozoites in CSF

Aspergillus and
Balamuthia mandrillaris

Enter through lower respiratory tract
or through leptomeningeal arteries
causing granulomatous encephalitis
in individuals with compromised immune system.

Cysts and trophozoites in tissue

\( MUC2 \) - predominant secreted mucin
Allelic variation!

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

**Geographic Range:** Cosmopolitan
- Found throughout the world in freshwater.
  - Three life forms: amoeba, flagellate, cyst
- Infections generally occur around thermal pools where the 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 the 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.

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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