Pray for Michael Sullivan
The Road to Perdition
Of Aggregatibacter actinomycetemcomitans
TOM HANKS
PAUL NEWMAN
JUDE LAW
Localized Aggressive (Juvenile) Periodontitis

Edward T. Lally, DMD, PhD
► Thomas Evans Building (1915).
 ► Robert Schattner Center (2002).
Localized Aggressive (Juvenile) Periodontitis

- Primary dentition is not involved
- Peripubertal onset
- Minimal clinical evidence of inflammation or plaque formation
- Molar/incisor involvement
- Familial and racial predilections
- Vigorous humoral immune response to this organism
- Disease “burns out” in late teens
- Simple flora--*A. actinomycetemcomitans* is the primary cultivatable organism
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GREAT SALE of SLAVES

JANUARY 10, 1855

THERE Will be Offered For Sale at Public Auction, at the SLAVE MARKET, CHEAPSIDE, LEXINGTON, All The SLAVES of JOHN CARTER, Esquire, of LEWIS COUNTY, KY., on Account of His Removal to Indiana, a Free State. The Slaves Listed Below Were All Raised on the CARTER PLANTATION at QUICK'S RUN, LEWIS COUNTY, KENTUCKY.

3 Bucks Aged from 20 to 26, Strong, Able-bodied
1 Wench, Sallie, Aged 42, Excellent Cook
1 Wench, Lize, Aged 23 with 6 mo. old Picinniny
17 Bucks Aged from twelve to twenty, Excellent

TERMS: Strictly CASH at Sale, as owner must realize cash, owing to his removal to West. Offers for the entire lot will be entertained previous to sale by addressing the undersigned.

JOHN CARTER, Esq.
Po. Clarksville, Lewis County, Kentucky

AUCTION!

Will be sold at Public Auction, July 27th, 1855, the following articles of property:

THE GIRL BETSEY!

Betsey is a very likely girl; large, intelligent, handy and healthy. She is acquainted with all kinds of house work, and is a good breeder. Second, SARAH;

Said girl is very neat, handsome and intelligent; a good cook and seamstress, and would make a good housekeeper for a single man. Third, THE BOY FRANK.

Said boy is 22 years old, healthy, well made, and able to endure much labor, drinks but little, and is as bright as "niggers" generally are. There would be no trouble with him on a cotton plantation. All the above are warranted sound in mind and limb.

ALSO, AT THE SAME TIME AND PLACE,
4 Plantation Whips, 2 Branding Irons, 4 Pistols, 3 rifles, 7 Hand-cuffs, 1 Gag, 2 Bull Dogs, and 6 Blood Hounds.

Also, at the same time and place,
300 copies of "Letter of Inquiry by a Northern Presbyter"
All which will be sold without reserve to the highest bidder.

NERO LEGREE.
Hanover, N. H. edf. 17.
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A. actinomycetemcomitans

A pioneer colonizer
Aggregatibacter actinomycetemcomitans

- Member of the Family *Pasteurellae*, a HAP(M) organism
- Non-enteric, fermenting, Gram negative coccobacillus
- Only *Actinobacillus* species routinely cultured from man.
- A pioneer colonizer (4 months) of the oral cavity
- Comprised of five serotypes (a-e)-- Serotype *b* is associated w/ LJP.
- Catalase-positive
- Serum-resistant
- Produces leukotoxin (LtxA) and cytolethal distending toxin (Cdt)
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- Only Actinobacillus species routinely cultured from man
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Virulence factors of *A. actinomycetemcomitans*

Factors that promote colonization and persistence in the oral cavity
- Adhesins
- Invasins
- Bacteriocins Antibiotic resistance

Factors that interfere with the host's defenses
- Leukotoxin
- Cytolethal distending toxin
- Chemotactic inhibitors
- Fc-binding proteins

Factors that destroy host tissues
- Cytotoxins
- Collagenase
- Bone resorption agents
- Stimulators of inflammatory mediators

Factors that inhibit host repair of tissues
- Inhibitors of fibroblast proliferation
- Inhibitors of bone formation
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- Only *Actinobacillus* species routinely cultured from man
- A pioneer colonizer (4 months) of the oral cavity
- Produces leukotoxin (LtxA) and cytolethal distending toxin (Cdt)
1. 116 kDa member of repeats-in toxin (RTX) family of bacterial toxins.

2. Specifically kills human leukocytes including neutrophils, NK cells, B cells and T cells.

3. Toxin receptor is LFA-1, a β2 integrin
Leukotoxin operon

Promoter → LtxC → LtxA → LtxB → LtxD

Acylase

Structural Toxin Gene

Secretion
LtxA Production and Aa strains

“Minimally” Leukotoxin Operon

Leukotoxin Operon

Δ 530 bp
Highly leukotoxoxic strains of Aa are cultured from patients with active disease

- Haraszthly *et al.* found this strain in 55% of LJP patients (N=71).
- Haubek *et al.* found that highly leukotoxotoxic Aa strains were exclusively associated with LJP.
- Bueno *et al.* found that individuals infected with highly leukotoxotoxic Aa were 22.5 times more likely to develop LPJ than subjects with the minimally leukotoxotic strain.
Relative sensitivity of *Actinobacillus actinomycetemcomitans* LTX to primate PMNs*

<table>
<thead>
<tr>
<th>Species</th>
<th>LTX Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>100</td>
</tr>
<tr>
<td>Chimpanzee</td>
<td>27</td>
</tr>
<tr>
<td>Baboon</td>
<td>19</td>
</tr>
<tr>
<td>Gorilla</td>
<td>19</td>
</tr>
<tr>
<td>Mangabe</td>
<td>16</td>
</tr>
<tr>
<td>Spot-nose guenon</td>
<td>16</td>
</tr>
<tr>
<td>Pig-tailed Macaque</td>
<td>16</td>
</tr>
<tr>
<td>Liontailed Macaque</td>
<td>14</td>
</tr>
<tr>
<td>Orang-utan</td>
<td>10</td>
</tr>
<tr>
<td>Debranza’s guenon</td>
<td>4</td>
</tr>
<tr>
<td>Diana</td>
<td>2</td>
</tr>
<tr>
<td>Gibbon</td>
<td>&lt; 0.1</td>
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<tr>
<td>Grivet</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Vervet</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Marmoset</td>
<td>&lt; 0.1</td>
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<tr>
<td>Cebus</td>
<td>&lt; 0.1</td>
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<tr>
<td>Spider</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Squirrel</td>
<td>&lt; 0.1</td>
</tr>
</tbody>
</table>

*(% control)*
Effect of LtxA on Target Cells

Normal

RTX treated
Hoechst 33342 Fluorescence

A

D

B

C

7-AAD Fluorescence

10

1

10

1

10

2

10

2

10

3

10

3

10

4

10

4

D

C

B

A

Graph showing fluorescence levels with areas A, B, C, and D labeled.

Scale bars indicated in images.
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A. actinomycetemcomitans infections are clonal infections which show vertical transmission through families.
Differences in the Ltx promoter structure are associated with clinical disease

PCR analysis of dental plaque samples indicating the presence of both highly leukotoxic and minimally leukotoxic *A. actinomycetemcomitans* in the same individual. PCR using the leukotoxin primers was performed on a pooled dental plaque sample taken from each of 7 subjects. Lane 1, molecular weight markers; lanes 2, 3, 4, and 8, the 1022 base pair product indicates the presence of minimally leukotoxic *A. actinomycetemcomitans*; lanes 6 and 7, the 492 base pair product indicates the presence of highly leukotoxic *A. actinomycetemcomitans*; lane 5, the 1022 and 492 base pair products indicate the presence of both highly leukotoxic and minimally leukotoxic *A. actinomycetemcomitans*. 
Differences in the Ltx promoter structure is linked to the production of toxin.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Promoter classa</th>
<th>$10^6 \text{LD}_{50} \text{b}$</th>
<th>Sourcec</th>
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<tbody>
<tr>
<td>JP2</td>
<td>JP2</td>
<td>2.5</td>
<td>N. Taichman</td>
</tr>
<tr>
<td>652</td>
<td>652</td>
<td>50</td>
<td>B. Shenker</td>
</tr>
<tr>
<td>Y4</td>
<td>652</td>
<td>50</td>
<td>N. Taichman</td>
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<tr>
<td>NCTC 9710</td>
<td>652</td>
<td>25</td>
<td>N. Taichman</td>
</tr>
<tr>
<td>ATCC 29523</td>
<td>652</td>
<td>$&gt;$50</td>
<td>N. Taichman</td>
</tr>
<tr>
<td>ATCC 29524</td>
<td>652</td>
<td>25</td>
<td>N. Taichman</td>
</tr>
<tr>
<td>060192</td>
<td>652</td>
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<td>B. Shenker</td>
</tr>
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<td>HK890</td>
<td>652</td>
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<td>M. Kilian</td>
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<td>25</td>
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<td>HK910</td>
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<td>HK968</td>
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<td>M. Kilian</td>
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<tr>
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<td>$&gt;$50</td>
<td>M. Kilian</td>
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</tr>
<tr>
<td>HK909</td>
<td>JP2</td>
<td>3</td>
<td>M. Kilian</td>
</tr>
<tr>
<td>ER</td>
<td>JP2</td>
<td>3</td>
<td>M. Kilian</td>
</tr>
</tbody>
</table>

a Promoters were classified as 652-like or JP2-like on the basis of results of PCR amplification of the promoter region as described in Materials and Methods. Strains which amplify a 470-bp fragment were classified as JP2-like; those which amplified a 1,000-bp fragment were classified as 652-like.

b $\text{LD}_{50}$ is defined as the number of bacterial cells required to lyse 50% of $2 \times 10^5$ HL60 cells in 1 h at 37°C.
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Cellular defects attributed to patients with LAP

- Decreased chemotaxis
- Altered superoxide production
- Decreased $\text{Ca}^{2+}$ mobilization
- Decreased phagocytosis
- Defective fMLP-mediated chemotaxis
- Decreased diacylglycerol kinase activity
Reconciling the *A. actinomycetemcomitans* mutant and genetic defect lines of experimentation: Unifying hypothesis or shotgun wedding?

- a virulent strain of *A. actinomycetemcomitans* has found and selectively colonized individuals with the LAP immune cell deficiency
- the JP2 mutant is ubiquitous but colonization proceeds in individuals with a selective immune defect
The alternative hypothesis

Vertical transmission of an organism
Actinobacillus pleuropneumoniae (APP)

**Signs:** Depend on stage of disease

**Peracute:**
- Sudden onset of severe illness
- Pigs found dead with no clinical signs of disease
- Fever, off feed, difficulty breathing, open mouth breathing
- Bloody foam coming from nose and/or mouth

**Acute:**
- Fever, depression, off feed
- Coughing
- Difficulty breathing, open mouth breathing

**Chronic:**
- Off feed
- Decreased average daily weight gain
Necropsy findings

- Blood, necrotic debris, and fibrin around lungs
- Blood tinged fluid in the thorax
- Bloody foam in trachea and lung airways
- Abscesses
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