Helicobacter Pathogenesis: An Evolving Paradigm

Zoobiquity Research Symposium
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James G. Fox
Division of Comparative Medicine
MIT, Cambridge MA, USA
Q: Why does an Elephant paint its toes red?
A: To hide in a cherry tree!

Put into context: How one who studies pathogenesis of infectious diseases collaborates with gastroenterologists!
1982 Warren & Marshall grew the bacterium that would change the face of gastroenterology.

Gastroenterologists were not impressed.
The believers and the non believers

“...the thought that antibiotics may be more effective than conventional antacid treatment for peptic ulcer disease is intuitively offensive”

Discussed by Bartlett, Gastroenterology 1988, 94: 229-238
Colonization of the stomachs of various animal species by *Helicobacter pylori*

<table>
<thead>
<tr>
<th>Animal</th>
<th>Establishment Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Would not establish</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Would not establish</td>
</tr>
<tr>
<td>Ferret</td>
<td>Would not establish</td>
</tr>
<tr>
<td>Cat</td>
<td>Would not establish</td>
</tr>
<tr>
<td>Pig</td>
<td>Would only establish in germfree piglets. Mononuclear infiltrate only</td>
</tr>
<tr>
<td>Monkey</td>
<td>Established with associated active gastritis</td>
</tr>
</tbody>
</table>
Histopathologic illustration of gastric ulcer and chronic inflammation, loss of fundic glands in a ferret with *H. mustelae*. H&E Stain.
Pure culture of *H. mustelae* showing rod-shaped morphology and long polar and lateral flagella. A) Transmission electron microscopy. B) Phase contrast microscopy. C) Bacteria penetrating into epithelial cells. Scale bars= 0.2 µm (A) 0.5 µm (C), 0.1.

(Modified from Fox JG, Lee A. The role of Helicobacter species in newly recognized gastrointestinal tract diseases of animals. Lab Anim Sci 1997; 47:222.)

*H. mustelae* in mucosal crypt of stomach.
Warthin-Starry stain
### Severity of gastritis related to ferret age

<table>
<thead>
<tr>
<th>Age group (months)</th>
<th>Total no. of ferrets</th>
<th>Minimal or no inflammation</th>
<th>Mild chronic gastritis</th>
<th>Moderate to severe chronic gastritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3</td>
<td>22</td>
<td>13 (59)</td>
<td>9 (41)</td>
<td>0 (-)</td>
</tr>
<tr>
<td>3-12</td>
<td>27</td>
<td>14 (52)</td>
<td>9 (33)</td>
<td>4 (15)</td>
</tr>
<tr>
<td>&gt;12</td>
<td>33</td>
<td>3 (9)</td>
<td>12 (36)</td>
<td>18 (55)</td>
</tr>
</tbody>
</table>

There was a statistically significant difference between age groups in the severity of gastritis ($P<0.001; \chi^2 = 28$).

Fox et al, 1993

Fox JG, 1994
From Wiley, 2014

- Biology and Diseases of the Ferret, 3rd Edition
- Thorough revision and update, provides current, comprehensive reference on the ferret
- Only book to focus on characteristics that make the ferret an important research animal
- Ten completely new chapters from previous edition

Eds: James G. Fox, Robert P. Marini
“Helicobacter felis”

Natural habitat is the cat or dog. Microaerophilic, urease positive, and induces gastritis in dog and mouse.
### Gastric Classification of GF Mice Colonized with *H. felis*

<table>
<thead>
<tr>
<th>Time</th>
<th>Gastritis classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preinfection</td>
<td>No inflammation</td>
</tr>
<tr>
<td>1 week</td>
<td>No inflammation</td>
</tr>
<tr>
<td>2-4 weeks</td>
<td>Acute</td>
</tr>
<tr>
<td>8-10 weeks</td>
<td>Active chronic</td>
</tr>
<tr>
<td>20-50 weeks</td>
<td>Chronic</td>
</tr>
</tbody>
</table>

Lee, Fox, Otto, Murphy; Gastroenterology 1990
Divergent Responses to *H. pylori* Infection

**Chronic *H. pylori* Infection**

- **Antral predominant Gastritis**
  - Acid, little or no atrophy
  - Low risk of gastric ca
- **Corpus predominant Gastritis**
  - Acid, gastric atrophy
  - High risk of gastric ca
- **Mild Mixed Gastritis**
  - Normal Acid
- **DU disease**
- **No significant disease**
- **Gastric Ca**

*? Bacterial ? Environment ? Host*
Gastric Cancer- Facts

- 4th leading cause of malignancy
- Variable global incidence
- High prevalence in Asia
- Decreased incidence in North America
- Males have a 2 fold higher risk than females
- 2ND leading cause of cancer related death in males and 4th in women

Source: American Cancer Society, Global Cancer Facts and Figures, 2007pub
Rudolph Ludwig Carl Virchow was a pathologist who developed the cell theory and applied it to explain the causes of diseases.

- First to note leukocytes in neoplastic tissues
- These infiltrates reflected the origin of cancer at sites of chronic inflammation
<table>
<thead>
<tr>
<th>Infectious agent</th>
<th>IARC classification</th>
<th>Cancer site/cancer</th>
<th>Number of cancer cases</th>
<th>% of cancer cases worldwide</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. Pylori</em></td>
<td>1</td>
<td>Stomach</td>
<td>490,000</td>
<td>5.4</td>
</tr>
<tr>
<td>HPV</td>
<td>1, 2A</td>
<td>Cervix and other sites</td>
<td>550,000</td>
<td>6.1</td>
</tr>
<tr>
<td>HBV, HCV</td>
<td>1</td>
<td>Liver</td>
<td>390,000</td>
<td>4.3</td>
</tr>
<tr>
<td>EBV</td>
<td>1</td>
<td>Lymphomas and nasopharyngeal carcinoma</td>
<td>99,000</td>
<td>1.1</td>
</tr>
<tr>
<td>HHV-8</td>
<td>2A</td>
<td>Kaposi sarcoma</td>
<td>54,000</td>
<td>0.6</td>
</tr>
<tr>
<td><em>Schistosoma haemotobium</em></td>
<td>1</td>
<td>Bladder</td>
<td>9,000</td>
<td>0.1</td>
</tr>
<tr>
<td>HTLV-1</td>
<td>1</td>
<td>Leukaemia</td>
<td>27,000</td>
<td>0.1</td>
</tr>
<tr>
<td>Liver Flukes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Opisthorchis viverrini</em></td>
<td>1</td>
<td>Cholangiocarcinoma (biliary system)</td>
<td>800</td>
<td></td>
</tr>
<tr>
<td><em>Clonorchis sinensis</em></td>
<td>2A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total infection-related cancers</td>
<td>1,600,000</td>
<td>17.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total cancers in 1995</td>
<td>9,000,000</td>
<td>100</td>
</tr>
</tbody>
</table>

The burden of cancer caused by infectious agents worldwide.
Group 1 = carcinogenic to humans
Group 2A = probably carcinogenic to humans
*Applies only to cervical cancer
Normal

Non-atrophic gastritis

Multifocal atrophic gastritis

Intestinal metaplasia, complete type

Intestinal metaplasia, incomplete type

Dysplasia

Correa’s Model of Gastric Carcinogenesis

Adenocarcinoma, intestinal type

Courtesy of P. Correa
Helicobacter infection in INS-GAS Mice

- ↑ H. felis/pylori colonization in fundus
- INS-GAS H. felis infected mice had ↑ gastrin than uninfected INS-GAS mice
- Severe foveolar hyperplasia, atrophy, metaplasia, dysplasia
- At < 8 mos
  - 85% intramucosal carcinoma, 54% submucosal invasion, 46% intravascular p = < 0.05

Gastric pathology in Helicobacter felis-infected INS-GAS tg mice at 7 months post infection

Wang et al., 2000; Fox et al 2003
Mouse Model of *Helicobacter* spp. Induced Gastric Adenocarcinoma

- **Mouse Stomach - Corpus**
- **Acute gastritis**
- **Atropic gastritis**

- **Pseudopyloric metaplasia**
- **Pseudopyloric metaplasia, oxyntic atrophy and glandular dysplasia**
- **Epithelial defects/ surface epithelial dysplasia**

- **High grade dysplasia and metaplasia**
- **Carcinoma**

Modified from Rogers & Fox
Mucus in different areas of the rodent gut is colonized by different spiral-shaped bacteria.
Helicobacter-Associated Hepatitis and Hepatocellular Neoplasms in Control A/JCr Male Mice

Number

Hepatitis

Tumors

May-July 1989 (n=47)  December 1992 (n=12)

0  1  12

1 (2%)  (100%)  11 (92%)

Fox et al 1994
Ward et al 1994
H. hepaticus in A/J mouse liver and colon
Examples of *H. hepaticus* in mutant mice with rectal prolapse

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Background</th>
<th>PCR</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>nu/nu</td>
<td>NCr (outbred)</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>nu/nu</td>
<td>Balb/C</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>nu/nu</td>
<td>C3H</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>SCID</td>
<td>C.B-17TM</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>P53&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>C57BL/6</td>
<td>+*</td>
<td>+</td>
</tr>
<tr>
<td>TCR αβ &lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>C57BL/6</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>U-PA &lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>C57BL/6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C3H/HeJBi&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Distinct Helicobacter sp. (by 16S nucleotide sequence)*  
**One of several mice tested, purchased directly from Jackson Labs

Mouse rectal prolapse
### H. hepaticus- and H. bilis-associated IBD in mice

<table>
<thead>
<tr>
<th>Genetic status of mice</th>
<th>Type of defect</th>
<th>Pathology</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45RB (high)-reconstituted ICR defined flora scids</td>
<td>Reconstitution with naïve CD4+ T cell</td>
<td>Typhlocolitis</td>
<td>10</td>
</tr>
<tr>
<td>TCRα, B mutants</td>
<td>Abnormal T cell receptors</td>
<td>Typhlocolitis</td>
<td>88</td>
</tr>
<tr>
<td>Scid ICR-defined flora</td>
<td>Lack T and B cell</td>
<td>Typhlocolitis</td>
<td>89, 90</td>
</tr>
<tr>
<td>C57BL/IL-10&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>Knockout</td>
<td>Typhlocolitis</td>
<td>14, 18, 19, 36</td>
</tr>
<tr>
<td>129SvEv/Rag2&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>Knockout</td>
<td>Typhlocolitis, colon cancer</td>
<td>28, 56</td>
</tr>
<tr>
<td>C57BLRag2&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>Knockout</td>
<td>Typhlocolitis</td>
<td>14</td>
</tr>
<tr>
<td>IL-7&lt;sup&gt;-/-&lt;/sup&gt;/Rag2&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>Double Knockout</td>
<td>None</td>
<td>27</td>
</tr>
<tr>
<td>A/JCr</td>
<td>Normal</td>
<td>Typhlitis</td>
<td>3</td>
</tr>
<tr>
<td>Swiss Webster gnotobiotic</td>
<td>Normal</td>
<td>Enterocolitis</td>
<td>91</td>
</tr>
<tr>
<td>129SvEv/NF-κβ (p50&lt;sup&gt;-/-&lt;/sup&gt;p65&lt;sup&gt;+/&lt;/sup&gt;-)</td>
<td>Double Knockout</td>
<td>Typhlocolitis</td>
<td>92</td>
</tr>
<tr>
<td>mdr1a&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>Lack P-glycoprotein</td>
<td>Typhlocolitis</td>
<td>93</td>
</tr>
<tr>
<td>SMAD3&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>Knockout</td>
<td>Typhlocolitis, colon cancer</td>
<td>94</td>
</tr>
</tbody>
</table>

*Fox et al, Mucosal Immunol, 2011*
Survey of academic and commercial mouse colonies

30 of 34 commercial and academic institutions in Canada, Europe, Asia, Australia, and the U.S. have mouse colonies infected with *Helicobacter* spp. This is a prevalence of 88%.

Taylor et. al., 2007
Mouse Helicobacter spp. dendrogram depicting phylogenetic location based on 16S rRNA sequence similarity values.

Formally Named:
- H. hepaticus
- H. bilis
- H. rodentium
- H. typhlonius
- H. muridarum
- H. mastomyrinus
- H. muricola
- H. pullorum
- H. trogontum

Nine additional clusters of novel Helicobacter species

- Helicobacter sp. MIT 01-6451 {EF373968}
- Helicobacter sp. MIT 01-3083
- Helicobacter sp. MIT 01-6452
Novel helicobacter sp. MIT 01-6541 in IL10-/- mice

Infected Cecum/ICC

A higher magnification image showing prominent mucosal and submucosal inflammation, abundance of hyperplastic and dysplastic glands in the mucosa and cluster of horizontally spreading high grade invasive glands. Bar: 160uM
An Animal Model of Helicobacter Induced Disease: Colon Cancer

Is there a human counterpart?
Taxonomy

- Formally named helicobacter species: 27
- An additional 35 or more awaiting formal naming
- Classification based on phenotypic, biochemical and molecular techniques
  - 16SrRNA sequencing
  - DNA: DNA hybridization
  - Protein analysis
Table. Non *H. pylori* helicobacters isolated from the GI tract of humans (as of 2014)

<table>
<thead>
<tr>
<th>Species</th>
<th>Other Hosts</th>
<th>Other sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>“<em>H. rappini</em>” <em>/H bilis</em></td>
<td>Sheep, rats, dog, mice</td>
<td>Blood (humans)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver (sheep and rats), stomach (dogs)</td>
</tr>
<tr>
<td><em>H. canis</em> *</td>
<td>Dog, cat</td>
<td>Blood (humans)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver (dog)</td>
</tr>
<tr>
<td><em>H. cinaedi</em> *</td>
<td>Hamster, rhesus monkey, dog</td>
<td>Blood, soft tissue, joints (humans) Liver (monkey)</td>
</tr>
<tr>
<td><em>H. fennelliae</em></td>
<td>Dog, macaque</td>
<td>Blood</td>
</tr>
<tr>
<td><em>H. pullorum</em> *</td>
<td>Chicken, mice</td>
<td>Liver (chicken)</td>
</tr>
<tr>
<td><em>H. canadensis</em></td>
<td>Geese</td>
<td>NR</td>
</tr>
<tr>
<td><em>H. winghamensis</em></td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

* Some data suggest zoonotic potential

**NR

Modified from Fox, Gut 2002
Helicobacter spp. and In Vivo Studies: the Saga Continues
One Health

Comparative medicine / Translational medicine

Zoonotic infections
- Bacterial infections
- Viral infections
- Antimicrobial resistance
- Vector-borne infections
- Parasite infections
- Food safety
- Bio threats
- Global health

Intervention
- Vaccines and therapeutics
- Vector control
- Surveillance
- Sanitation

Public health
- Veterinary medicine
- Human medicine
- Molecular and microbiology
- Health economics

Eco-system health
- Individual health
- Population health

Environmental health

In collaboration with One Health Initiative Autonomous pro bono team