The use of specific IgY to control *Helicobacter pylori* infection in human: some study results in Japan

Sa V. Nguyen, Ph. D

Immunology Research Institute in Gifu
Agenda

- *H. pylori* urease and IgY (Ovalgen-HP)
- Laboratory experiments
- Volunteer trials
H. pylori urease and IgY
Urease - an important pathogenic factor of *H. pylori*

As an enzyme: degrades urea to form NH$_3$ and this reaction changes local environment from acid (pH 2-3) to neutral side (pH 7)

\[
\text{Urea} \xrightarrow{\text{Urease}} \text{NH}_3 + \text{CO}_2
\]

As an adhesin: pH-dependent adherence to gastric mucin with optimal binding at pH 2.5-3.5
H. pylori urease: other information

- The most abundant protein of *H. pylori* (up to 15% of the bacterial cell).
- Essential for colonization (urease-negative strains are unable to colonize the stomach of nude mice).
- Induces proinflammatory cytokines.
Production of Urease-Specific IgY (Ovalgen – HP)

1. Cultivation of *H. pylori* NSP335 in BHI broth for 72 hr at 37°C
2. Collection of cell biomass
3. 2-step affinity purification
4. Collection of purified urease
5. Collection of egg yolk for IgY (Ovalgen-HP) production
6. Accumulation of urease-reactive IgY in eggs of immunized chicken
7. Mixing of urease with CFA to immunize poultry
IgY modes of action

1. Urease inactivation: IgY inhibits urease activity resulting in growth inhibition.
2. Adherence inhibition: IgY prevents *H. pylori* from binding to gastric mucin.
3. Causes damages to cell surface structure making Hp more vulnerable to medicines (having synergetic effect when combined with drugs).
Changes to *H. pylori* by IgY and probiotic

![Images of H. pylori with and without IgY and L. gasseri](image-url)

- **H. pylori**
- **Hp + L. gasseri**
- **Hp + IgY**
- **Hp + IgY + L. gasseri**
Laboratory experiments
Animals: NS:Hr/ICR hairless mice, 8 weeks old
Challenge: H. pylori strain NSP335, $10^9$ CFU/mouse
Groups: 0.25, 2.5, 25% egg powder with IgY (Ovalgen HP) in feed and controls
Period: 10 weeks
Assessment: Bacterial count in gastric tissue

**Exp 1: Effect of anti-urease IgY in a mouse challenge model**

- **F**: 2-day fasting
- **IgY feeding**
- **Autopsy**

**Timeline:**
- **Challenge**
- **1 week**
- **10 week**
### Results

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$\log_{10}$ CFU / 0.1g gastric tissue / mouse</th>
<th>Negative mouse / Total mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected control</td>
<td>$0.00\pm0.00$</td>
<td>$6 / 6$</td>
</tr>
<tr>
<td>Infected control</td>
<td>$3.64\pm0.39$</td>
<td>$0 / 10$</td>
</tr>
<tr>
<td>0.25% Ovalgen HP</td>
<td>$3.08\pm0.79$</td>
<td>$0 / 10$</td>
</tr>
<tr>
<td>2.5% Ovalgen HP</td>
<td>$1.26\pm1.47^{**}$</td>
<td>$5 / 10^#$</td>
</tr>
<tr>
<td>25% Ovalgen HP</td>
<td>$0.94\pm1.53^{**}$</td>
<td>$7 / 10^{##}$</td>
</tr>
</tbody>
</table>

**; p<0.01 compared to positive control group value, one-way Anova test

#, ##; p<0.05 and 0.01 compared to positive control group, chi-square test
Exp 2: Effect of anti-urease IgY in a Mongolian gerbil challenge model

Control group

-  

F + IgY  F + IgY

H. pylori group

-  

H. pylori inoculation  

F + IgY  F + IgY

IgY: 25 mg/g  
F: Famotidine 0.16 mg/g

(Helicobacter 10:43-52, 2005)
Results

Specific anti-\textit{H. pylori} IgG in serum

\begin{itemize}
\item Control
\item \textit{H. pylori}
\end{itemize}

Cut off value

\begin{itemize}
\item Control
\item \textit{H. pylori}
\end{itemize}

\begin{itemize}
\item n.d.
\item n.d.
\item n.d.
\end{itemize}

\begin{itemize}
\item \textit{H. pylori} colonization in stomach
\end{itemize}

(Helicobacter 10:43-52, 2005)
Inflammation of gastric mucosa

Uninfected control

Infected untreated

Infected & F+IgY treated group

(Helicobacter 10:43-52, 2005)
Anti-urease IgY (Ovalgen-HP) reduced *H. pylori* colonization in stomach of infected mice and Mongolian gerbils.

IgY in combination with famotidine facilitated the excretion of *H. pylori* and reduced gastric inflammation in Mongolian gerbils.
Volunteer clinical trials in Japan
Volunteer trial No.1
(J Dairy Sci. 2004 Dec;87(12): 4073–9)

Subjects: 22 H. pylori - positive volunteers

Test material: Ovalgen HP (1.5 g in capsule; 3 times/day for 4 weeks)

Assessment: UBT (0, 1 month)
Results
(J Dairy Sci. 2004 Dec;87(12):4073-9)
Volunteer trial No.2
(Food and Development vol 38 No 11, 2004)

Volunteers: 16 *H. pylori* - positive volunteers

Test material: Yogurt containing 2.0g of Ovalgen HP (Yolk liquid)

Dosage: 2 cups of yogurt / day

Period: 2 months

Assessment: UBT & HpSA (0, 1, 2, 3M)
Results

(Food and Development vol 38 No 11, 2004)
Volunteer trial No. 3


Protocol

Volunteer: 17 Hp-positive volunteers

Treatment: Ovalgen HP 0.9 g/day for 4 weeks.

Evaluation: UBT test
## Results

<table>
<thead>
<tr>
<th>Volunteer No</th>
<th>Before</th>
<th>After</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>66.4</td>
<td>49.9</td>
</tr>
<tr>
<td>2</td>
<td>62.9</td>
<td>46.3</td>
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<tr>
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<td>5.3</td>
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<td>4</td>
<td>57.7</td>
<td>19.9</td>
</tr>
<tr>
<td>5</td>
<td>46.6</td>
<td>29.9</td>
</tr>
<tr>
<td>6</td>
<td>44.2</td>
<td>32.4</td>
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<tr>
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<td>4.4</td>
</tr>
<tr>
<td>17</td>
<td>6.9</td>
<td>9.1</td>
</tr>
</tbody>
</table>

** ** *p* < 0.01
Ovalgen-HP was effective in reducing *H. pylori* colonization in infected animals and *H. pylori* - positive human volunteers.

Ovalgen-HP could be useful in treatment of antibiotic resistant *H. pylori* infection.
Acknowledgement

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