Association between *Helicobacter felis*–Induced Gastritis and Elevated Glycated Hemoglobin Levels in a Mouse Model of Type 1 Diabetes

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*Helicobacter pylori* infection has been described in association with increases in glycated hemoglobin (HbA1c) levels in patients with type 1 diabetes. The purpose of the present study was to use an animal model of *Helicobacter* infection to test, under controlled conditions, the hypothesis that infection is associated with high HbA1c levels. Diabetes was induced in C57BL/6 mice by administration of streptozotocin, and the mice were orally inoculated with *H. felis*. Six weeks after inoculation, infected mice (*n* = 10) showed gastritis scores significantly greater (*P* = .01) than those of uninfected mice (*n* = 10). HbA1c levels were significantly higher in infected mice with gastritis (11.6%; *n* = 6) than in infected mice without gastritis (8.4%; *n* = 4) or uninfected mice (7.6%; *n* = 10). It was concluded that gastritis induced by *H. felis* is associated with increased HbA1c levels in the mouse model of streptozotocin-induced diabetes.

Infections have been shown to destabilize blood glucose homeostasis in patients with diabetes mellitus (diabetes), sometimes resulting in severe metabolic disturbances such as ketoacidosis [1, 2]. Increased secretion of glucose counterregulatory hormones (epinephrine, glucagon, growth hormone, and cortisol) and production of proinflammatory cytokines result from infection and readily account for the hyperglycemia, lipolysis, and ketosis that occur during infection. Cytokines alone can stimulate the secretion of counterregulatory hormones and also may directly affect carbohydrate metabolism [3]. For example, tumor necrosis factor–α in vitro induces insulin resistance by antagonizing tyrosine phosphorylation of the insulin receptor [4].

*Helicobacter pylori* is one of the most common causes of chronic bacterial infection worldwide. Infection by this bacterium induces gastric inflammation and increased local and systemic levels of proinflammatory cytokines, such as tumor necrosis factor–α, interferon-γ, interleukin (IL)–1, IL-6, IL-8, and IL-18 [5, 6]. We previously reported [7] that 15.5% of our pediatric patients with type 1 diabetes were infected by *H. pylori* and that, even though infected individuals received higher daily doses of insulin than did uninfected patients (1.2 vs. 0.9 IU/kg/day), they still had higher levels of glycated hemoglobin (HbA1c) than their uninfected counterparts (14.9% vs. 11.8%). Additionally, in a follow-up study of the same cohort, we found that eradication of *H. pylori* infection was associated with a gradual decrease in HbA1c (from 13.6% to 11.7%) to a level comparable to that seen in uninfected control subjects (11.4%) [8].

To further explore the association between *Helicobacter* infection and severity of hyperglycemia, under controlled conditions, we used a mouse model of streptozotocin (STZ)–induced diabetes and *H. felis* infection. *H. felis* is the *Helicobacter* species that causes natural infections in mice and is analogous to *H. pylori* in humans.

**Methods**

**Study animals.** Pathogen-free 4-week-old, inbred, female C57BL/6 mice (Harlan Sprague Dawley) were housed in static microisolators. Mice were fed regular chow (Autoclavable Laboratory Chow 5010; Purina Mills) and tap water ad libitum.

**Induction of diabetes.** After 1 week of acclimatization, a solution of STZ (ICN Biomedicals) containing 15 mg/mL STZ in 0.005 M citrate buffer (pH 4.0) was freshly prepared under sterile conditions. A single dose of 0.2 mL of STZ per 20 g of body weight (equivalent to 150 mg of STZ per 1 kg of body weight) was administered intraperitoneally to each mouse [9].

**Diabetes management.** Once mice had developed diabetes (3–5 days after STZ injection), as demonstrated by findings of blood glucose levels persistently higher than 150 mg/dL, a fixed dose (0.5 U; U-100 insulin) of insulin NPH (HumulinN; Eli Lilly) was administered subcutaneously once daily. Random nonfasting glycemia was monitored weekly by measurement with a glucometer (One Touch II; LifeScan), using capillary blood obtained by clipping the end of each mouse’s tail. The purpose was to verify that the animals still had diabetes and still required insulin.
H. felis inoculation. Two weeks after insulin injections were started, cages of mice (3–4 mice per cage) were randomly assigned to receive either an inoculation of H. felis or a sham inoculation. H. felis 49179 (American Type Culture Collection) was grown at 37°C under microaerobic conditions (Bio-Chamber Type C1; Becton Dickinson) on 5% lysed sheep blood agar without antibiotics. A suspension was made in brain-heart infusion broth and was adjusted to a concentration of $10^8$--$10^{10}$ organisms/mL. An aliquot (0.2 mL) of the bacterial suspension was orally administered to each animal on 3 successive days, using an olive-tip 20-gauge feeding needle (Fine Science Tools). Control animals received 0.2 mL of H. felis–free carrier fluid in the same fashion [10]. After inoculation with either H. felis or placebo, all animals were kept for 6 weeks and then sacrificed by carbon dioxide asphyxiation.

HbA1c levels. HbA1c was measured in all animals before STZ administration and at the end of the observation period. Five drops of whole blood collected from the tail were placed in 1 mL of saline and incubated at 37°C for 4 h to remove the labile fraction. A hemolysate was then prepared by the addition of 10 μL of packed erythrocytes to 200 μL of hemolyzing reagent (10 mM KCN and 5 mM EDTA). All hemolysates were stored at −70°C before analysis. The proportion of HbA1c in mouse blood was determined by capillary isoelectric focusing, using a mixture of ampholytes (pH 6–8 and pH 3–10), as described elsewhere for analysis of human blood [11]. Mouse blood contained 2 major hemoglobin peaks (figure 1) that were equivalent to adult hemoglobin and HbA1c levels in humans. Identification of the mouse HbA1c peak as HbA1c was confirmed by isoelectric focusing, using a mixture of ampholytes (pH 3–10), as described elsewhere for analysis of human blood [11]. Mouse blood contained 2 major hemoglobin peaks (figure 1) that were equivalent to adult hemoglobin and HbA1c levels in humans. Identification of the mouse HbA1c peak as HbA1c was confirmed by the increase in the area of this peak when mouse erythrocytes were incubated in PBS containing 100 mM glucose for 1 h. Mouse HbA1c results are expressed as a percentage of adult hemoglobin.

Histopathology. The stomach of each mouse was removed, fixed in formalin, and stained with hematoxylin-eosin to determine the degree of gastritis. Gastric inflammation was graded by a single pathologist (H.C.), who was unaware of the inoculation status of the animals, using the updated Sydney score [12]. This score uses a visual-analogue scale to classify the degree of polymorphonuclear cell (PMNC) and mononuclear cell (MNC) infiltration as normal, mild, moderate, or marked; for the purpose of statistical analysis, these categories were assigned values of 0, 1, 2, and 3, respectively. The gastritis score was the average of the scores assigned to the stomach antrum and corpus. Next, specimens were stained with Warthin-Starry stain to investigate the presence of H. felis organisms, which were identified as small, curved bacilli overlying the stomach mucosa (figure 2).

Statistical analyses. The main outcome variable was the HbA1c value at the end of the study. The value distributions between the different groups were compared using the nonparametric Mann-Whitney U test. The association between continuous variables (i.e., degree of gastritis and HbA1c level) was determined with simple linear regression analysis. Confidence intervals and P values for 2-tailed tests were calculated using SPSS version 8.0. P < .05 was considered to be significant.

Results

Animal model. Thirty female C57BL/6 mice were injected with STZ. Two of these mice died within 48 h (likely as a result of STZ toxicity), 2 mice maintained normal blood glucose values, and 26 mice (87%) developed diabetes. Subsequently, 5 of those 26 mice died of unknown causes (also likely as a result of STZ toxicity). Of the remaining 21 mice, 11 were inoculated with H. felis. Infection was confirmed in 10 of those 11 mice on postmortem examination. Ten mice received sham inoculations, and postmortem examination confirmed that none of those mice were infected. Therefore, 10 infected and 10 uninfected mice with diabetes were included in the trial.

Gastric histopathology. Table 1 shows the gastritis scores for infected and uninfected mice, as measured in the 2 main cell types: PMNCs and MNCs. Because gastritis was characterized mainly by PMNC, rather than MNC, infiltration, for the rest of the present analysis, gastritis refers to PMNC infiltration. As expected, H. felis–infected mice had significantly more severe gastritis than did uninfected animals. All uninfected animals had no or minimal gastritis (gastritis score of 0 or 1). On the other hand, the level of gastritis was variable among infected animals; some mice had no or minimal gastritis ($n = 4$), and others had moderate or marked gastritis (gastritis score of $\geq 2$; $n = 6$). Because we hypothesized that the effect of H. felis infection was mediated by the associated gastric inflammation, we compared the outcome variables (table 2) for the 3 groups of mice: uninfected mice (group A), infected mice that had no gastric inflammation (group B), and infected mice that had gastric inflammation (group C).

Blood glucose levels. Before STZ injection, baseline blood glucose levels were higher in animals that had been assigned to the sham-inoculation group, a finding unlikely to be of significance, because all values were within the normal range. After diabetes had been induced, blood glucose values significantly increased in all mice; the mean blood glucose values for the 6-week observation period were higher in group C than in groups A and B.

Figure 1. Capillary isoelectric focusing of hemoglobin from a C57BL/6 mouse before (at baseline) and 6 weeks after induction of diabetes by administration of streptozotocin. As is seen in blood from humans with diabetes, the minor peak anodal to the major hemoglobin variant (adult hemoglobin: HbA) is higher and is identified as glycated hemoglobin (HbA1c) in subjects with diabetes. A415, absorbance at 415 nm.
HbA1c values also were similar among the 3 groups at baseline measurement but were significantly higher at the end of the study for group C mice, compared with values for mice in the other 2 groups (table 2). In addition, the increase in HbA1c levels after STZ injection was more pronounced in group C than in groups A and B (median increase, 5.5% vs. 2.5%; \( P = .02 \)). Finally, simple linear regression found a positive (\( r = +0.68 \)) and significant (\( P = .03 \)) correlation between gastritis score and amount of increase in HbA1c levels in infected animals, which suggests that a dose-response effect exists. Because of the minimal gastric inflammation found in uninfected mice, similar analysis for that group showed no correlation between increases in HbA1c levels and gastritis (\( P = .58 \)).

Discussion

We observed a significant association between gastric inflammation and HbA1c levels in a mouse model of STZ-induced diabetes and \( H. \) felis infection. The association was made evident by the finding that infected animals with gastritis had higher HbA1c values and by the demonstration of a dose-effect relationship between the degree of gastric inflammation and the level of HbA1c. These results are consistent with our previous findings from studies involving children with type 1 diabetes, which showed that children infected by \( H. \) pylori had higher HbA1c values and that these values decreased after eradication of the infection [7, 8]. These findings may have important implications for management of diabetes, especially because increasing HbA1c levels are associated logarithmically with long-term microvascular complications of diabetes, such as retinopathy, nephropathy, and neuropathy [13].

There are many mechanisms by which gastric inflammation may correlate with increased levels of HbA1c. Most simply, increases in HbA1c levels reflect increased mean blood glucose levels. Mean blood glucose levels were higher in infected mice with gastritis than in mice without gastric inflammation. HbA1c levels may also be increased by increased glycation of HbA, and other authors have shown that the oxidative status, which is increased during infections, affects HbA1c biosynthesis [14].

Table 1. Gastritis scores among \( H. \) felis–infected and uninfected mice.

<table>
<thead>
<tr>
<th>Infiltration</th>
<th>Among infected mice (( n = 10 ))</th>
<th>Among uninfected mice (( n = 10 ))</th>
<th>( p^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMNCs</td>
<td>2.5 (0.5–3.0)</td>
<td>1.0 (0.5–1.5)</td>
<td>.01</td>
</tr>
<tr>
<td>MNCs</td>
<td>0.0 (0.0–0.5)</td>
<td>0.0 (0.0–0.5)</td>
<td>.34</td>
</tr>
</tbody>
</table>

\( ^a \) See Methods for a description of the gastritis scoring system.

\( ^b \) Nonparametric, 2-tailed Mann-Whitney \( U \) test was used for comparisons.
other than \textit{H. felis} or by noninfectious agents could have a similar effect would be of interest. Finally, an alternative explanation for our findings is that subjects with more-severe hyperglycemia may become more severely infected. If so, it is possible that the animals in which HbA\textsubscript{1c} levels increased the most after STZ administration may have developed the most-pronounced gastritis. In other words, we cannot exclude the possibility that high HbA\textsubscript{1c} levels might be the cause and not the consequence of increased gastric inflammation. Our study design does not allow us to differentiate between these possibilities.

As expected, gastritis was more pronounced in infected than in uninfected mice, which was demonstrated by the predominance of PMNC, rather than MNC, infiltration. This cellular infiltration pattern differs from that commonly reported in humans. For example, in another study, which included 52 \textit{H. pylori}–infected children in Peru [15], the same pathologist (H.C.) who was involved in our study described a mean score for PMNC infiltration of 1.9 (similar to what is described in the present study) and for MNC of 1.3 (higher than what is described in the present study), which implies that the mouse model of \textit{H. felis} infection is not entirely analogous to \textit{H. pylori} infection in humans. One reason for this difference could be the length of the observation period; maximum MNC infiltration may require > 6 weeks. Another reason might be the infecting strain itself. \textit{H. felis} does not have some of the important virulence factors carried by \textit{H. pylori}, such as the protein of the \textit{cagA} gene [16], which has been associated with the production of gastritis [17, 18]. Because our study results suggest that the elevation of HbA\textsubscript{1c} correlates with the degree of gastritis, we would predict that \textit{Helicobacter} species capable of inducing the most gastric inflammation (such as \textit{cagA}\textsuperscript{+} \textit{H. pylori} strains) would be associated with the greatest increase in HbA\textsubscript{1c} levels.

In our experimental design, we chose to use STZ to induce diabetes. STZ has a direct toxic effect on the \(\beta\) cells of the pancreatic islets, which renders the recipient insulin deficient [19]. This is a well-accepted model of type 1 diabetes that has high efficiency and a rapid effect. It should be noted, though, that this is a chemical, not an autoimmune, phenomenon. We chose to observe the effect of infection on HbA\textsubscript{1c} over the course of 6 weeks, to replicate clinical practice. The life span of human red blood cells is ~120 days, and HbA\textsubscript{1c} levels are measured in our patients every 12 weeks. Because the life span of mouse red blood cells is 40–50 days [20], we chose a follow-up period of 6 weeks. It would be of interest to repeat our study, using genetic mouse models of type 1 diabetes (e.g., the inbred nonobese diabetic mouse) and longer observation periods (e.g., ≥ 12 weeks).

In conclusion, we confirmed that an association exists between \textit{H. felis} infection and gastritis in mice, as has been reported elsewhere [10]. In addition, we found that \textit{H. felis}–infected mice with STZ-induced diabetes had elevated HbA\textsubscript{1c} levels that correlated with the degree of gastritis. The association between infection, gastritis, and metabolic derangement in animal models and human subjects with diabetes needs further scrutiny.

References

5. Genta RM. The immunobiology of \textit{Helicobacter pylori} gastritis [review]. Semin Gastrointest Dis 1997;8:2–11.


