

Increased Interleukin-10 in *Helicobacter pylori* Infection Could Be Involved in the Mechanism Protecting From Allergy

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ABSTRACT

Background: A protective effect of *Helicobacter pylori* infection against allergic diseases has been reported. The increasing incidence of childhood allergy in developed countries may be a result of reduced stimulation of the immune system by early chronic infections, with the protective effect of gastrointestinal microbes being mediated by regulatory T lymphocytes and production of interleukin (IL)-10. To elucidate a possible mechanism involved in protecting against the development of atopy, we measured expression of IL-10 in gastric mucosa of children with *H pylori* gastritis.

Patients and Methods: Gastric biopsies were performed during endoscopy in 48 children (median age, 9 years), 32 of whom had *H pylori* gastritis and 16 of whom served as controls. Interferon- γ (IFN- γ), interleukin-1 β (IL-1 β), and IL-10 were measured in tissue homogenate by quantitative reverse-transcriptase polymerase chain reaction (RT-PCR). The amounts of IFN- γ , IL-1 β , and IL-10 transcripts were quantified via competitive RT-PCR with use of dilution series of specific competitors.

Results: Expression of IFN- γ and IL-10 were significantly higher in *H pylori*-infected children. No direct correlation with age was found, but a further increase in IL-10 expression

was found in *H pylori*-infected children older than 4 years, whereas in control subjects, IL-10 expression tended to be lower in older children. IL-1 β expression was similar in infected children and control subjects. In *H pylori*-infected children, the prevalence of allergy was significantly higher in children with lower cytokine expression in gastric mucosa.

Conclusions: In children, *H pylori*-induced inflammatory response is associated with development of cell-mediated immunity of T-helper 1 type, as demonstrated by increased IFN- γ expression. The significantly increased expression of gastric IL-10 in *H pylori*-infected children and its further increase in older children suggest that this chronic infection may influence IL-10 production even beyond the age of 4 years. *H pylori* may be one of the infections with the potential to modulate immune responses. *JPGN* 45:301–305, 2007.

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INTRODUCTION

Evidence of a possible protective effect of *Helicobacter pylori* infection against allergic diseases has been provided by epidemiological studies (1–3). Moreover, in the first

half of the 20th century, the prevalence of *H pylori* infection in Western countries approached 80% to 90% and then rapidly decreased in the second half of the century. It is still high in developing countries, with an inverse relationship with the prevalence of atopic disorders, making this infection a good candidate as a modulator of cell-mediated protective immunity.

According to the hygiene hypothesis (4), the increasing prevalence of atopy could be caused by ameliorated hygienic conditions: less infections in early age and less stimulation of the regulatory T cells—a T cell subset producing a large amount of interleukin-10 (IL-10) (5) and transforming growth factor- β —and IL-10 has been proposed to mediate their protective effect against atopy (6). Intermittent or chronic stimulation of the immune system by a variety of infectious agents early in life results in development of a robust regulatory T cell

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repertoire, which provides negative feedback to the immune system, suppressing unwanted responses via a mechanism mainly dependent on production of the anti-inflammatory cytokine IL-10, which seems to hold the key to counterregulate the immune deviation responsible of atopy (7). As a result, T-helper 1 cells (Th1) and Th2 responses are inhibited and expansion of naïve T cells is prevented (8–10).

Several infections have been suggested to be involved in causing the immune deviance proposed by the hygiene hypothesis. To be a good candidate for the hygiene hypothesis, an infectious agent should have always been present throughout evolution of the mammalian immune system, and should have been progressively depleted from the environment of industrialized countries during recent decades (10,11). *Helicobacter* species have coevolved in many mammals, and early transmission of *H pylori* is likely to occur in large sibships with young children and/or with low socioeconomic status or in developing countries where atopic diseases are less frequent. In addition, to be protective against atopy, a chronic infection should be associated with increased IL-10 production (12).

Our hypothesis was that a chronic infection like *H pylori* acquired in early childhood (13), by continuing to cause mucosa inflammation and production of proinflammatory cytokines, could induce IL-10 stimulation and therefore protect against the development of atopy. We measured the expression of interferon- γ (IFN- γ) interleukin-1 β (IL-1 β), and IL-10 in the gastric mucosa of children with *H pylori* infection and compared it with the findings in a group of children matched for age, sex, and socioeconomic status with a normal gastric mucosa without infection.

PATIENTS AND METHODS

The present study was conducted according to good clinical practice and was approved by our hospital ethical committee. After informed consent was given by parents, biopsy specimens from gastric antrum were taken from 48 children (27 female; median age, 9.2 years; age range, 1–17 years) undergoing gastroscopy for dyspeptic symptoms. Children with any other acute or chronic inflammation or any treatments in the previous 2 months were excluded.

In 32 of the patients (18 female; median age, 9.5 years) rapid urease test and histological examination showed *H pylori* gastritis, which was scored according to the updated Sydney System (14) after hematoxylin and eosin staining, and *H pylori* was microscopically identified after Giemsa staining. The other 16 children with dyspepsia (9 female; median age, 8.9 years) matched for age, sex, and socioeconomic status—as assessed by parental education and household density—had a normal gastric mucosa without *H pylori* infection and were considered to be control subjects (1 control subject per 2 patients).

Further gastric antrum biopsy specimens (1 for each of the 48 children) were snap-frozen and stored in liquid nitrogen until expression of IFN- γ , IL-1 β , and IL-10 were measured in tissue homogenate by quantitative reverse-transcriptase polymerase

chain reaction (RT-PCR). Total RNA was extracted with an RNeasy kit (Qiagen, Hilden, Germany) and complementary DNA was prepared from 600 ng of RNA in 25 μ L of reverse transcriptase reaction mixture with use of a RetroScript kit (Ambion, Austin, TX). The amount of IFN- γ , IL-1 β , and IL-10 transcripts in gastric biopsy specimens were quantified via competitive RT-PCR with use of dilution series of a competitor specific for each cytokine. Transcript concentrations in complementary DNA samples were deduced by comparison between intensities of sample amplicon and competitor amplicons (Competitive Quantitative RT-PCR kit; Ambion).

Statistical Analysis

Medians and interquartile ranges (IQRs) were calculated. Comparison between groups was performed using the Mann-Whitney *U* test. Correlation between tested cytokine expression and other parameters, such as age and severity of gastric inflammation, were analyzed by Spearman rank-sum test.

RESULTS

Expression of IL-10 was significantly higher in *H pylori*-infected children compared with controls ($P = 0.00002$; Table 1). In *H pylori*-infected children, there was no direct correlation with severity of gastric inflammation, as scored according to the updated Sydney System. IL-10 expression tended to be lower in 21 infected children with active gastritis—ie, with polymorphonuclear infiltration (median, 2000; IQR, 240–6000)—compared with 11 children with superficial gastritis only (median, 2500; IQR, 200–6000), and tended to be higher in 9 children with associated esophagitis (median, 2800; IQR, 600–4800) compared with 23 children without it (median, 2000; IQR, 400–4000), but these differences were not significant. No direct correlation with age was found, but IL-10 expression was significantly higher in *H pylori*-infected children older than 4 years than in *H pylori*-infected children younger than 4 years of age (Table 2), whereas in controls, IL-10 expression tended to be lower in older children, although not significantly. In *H pylori*-infected children prevalence of allergy was higher in those with IL-10 expression levels lower than 3000 (6 of 19) than in those with IL-10 expression levels higher than 3000 (0 of 13; $P = 0.02$).

TABLE 1. Quantitative analysis of cytokine transcripts of gastric biopsies by competitive RT-PCR in *H pylori*-infected children and *H pylori*-negative controls

Cytokine	<i>H pylori</i> -infected		Controls		<i>P</i> *
	Median	IQR	Median	IQR	
IL-10	2250	400–4000	180	70–500	0.00002
IFN- γ	850	368–2000	400	180–600	0.002
IL-1 β	3000	1900–6000	3250	800–4000	NS

Values are copy numbers of complementary DNA where applicable.

*Mann-Whitney *U* test.

TABLE 2. Quantitative analysis of cytokine transcripts of gastric biopsies by competitive RT-PCR *H pylori*-infected children younger or older than 4 years old

	Age <4 y		Age >4 y		<i>P</i> *
	Median	IQR	Median	IQR	
IL-10	500	300–1800	3000	1000–4000	0.02
IFN- γ	1110	300–3000	850	400–2000	NS
IL-1 β	2300	1200–4200	3500	2000–6000	NS

Values are copy numbers of complementary DNA where applicable.
* Mann-Whitney *U* test.

Expression of IFN- γ was significantly higher in *H pylori*-infected children compared with controls ($P=0.002$; Table 1). In 21 *H pylori*-infected children with active gastritis, IFN- γ expression tended to be higher than in 21 children with active gastritis (median, 1500; IQR, 480–2300 vs median, 600; IQR 250–1500 in 11 children without active gastritis; $P=0.07$), and was significantly higher in 9 children with esophagitis (median, 2000; IQR, 1000–2500 vs median, 600; IQR, 300–1800 in 23 children without esophagitis; $P=0.03$). Expression of IFN- γ tended to be lower in older children (Table 2), although not significantly. In *H pylori*-infected children prevalence of allergy was higher in those with IFN- γ expression levels lower than 700 (6 of 15) than in those with IFN- γ expression levels higher than 700 (0 of 17; $P=0.005$).

Expression of IL-1 β was similar in *H pylori*-infected children and control subjects (Table 1) and was not increased in *H pylori*-infected older children compared with younger ones (Table 2). In *H pylori*-infected children, IL-1 β expression tended to be lower in children with active gastritis (median, 3000; IQR, 1600–6000 vs median, 4000; IQR, 2000–6000 in children without active gastritis) and was similar in those with and without esophagitis. In *H pylori*-infected children prevalence of allergy was higher in those with IL-1 β expression levels lower than 3000 (6 of 19) than in those with IL-1 β expression levels higher than 3000 (0 of 13; $P=0.02$).

Expression of all 3 cytokines tended to be lower in 15 *H pylori*-infected children with duodenitis, compared with 17 *H pylori*-infected children with a normal duodenal mucosa, but differences failed to achieve statistical significance. No correlation was found between inflammation score defined by the Sydney system and any of the cytokines. A significant correlation was found among all tested cytokines by Spearman rank-sum test (IFN- γ and IL-10, $r=0.51$; $P=0.0002$; IFN- γ and IL-1 β , $r=0.37$; $P=0.01$; IL-10 and IL-1 β , $r=0.50$; $P=0.003$).

Prevalence of allergy was similar in *H pylori*-infected and noninfected children (6 of 32 vs 3 of 16 in controls), even though in 3 *H pylori*-infected children, atopic dermatitis in the first 2 years of age had disappeared by the age of 1 to 5 years.

DISCUSSION

Our study was aimed at elucidating the mechanism involved in protecting against development of atopy in *H pylori*-infected subjects as reported by epidemiological studies. Our findings confirm that in children *H pylori*-induced inflammatory response is associated with high proinflammatory cytokine expression in the gastric mucosa and development of cell-mediated immunity of Th1 type, as demonstrated by the significant increase of IFN- γ and already reported in other series of children (15,16), in which increased TGF- β expression has also been found (17). In addition, we found that gastric inflammation is associated with higher levels of IL-10 in gastric mucosa, as suggested by some reports in adults (18). However, in children, only a mild and nonsignificant increase of IL-10 expression has been reported in the gastric mucosa in a small series of *H pylori*-infected Italian children (15), and a significant increase of IL-10 expression in the duodenal mucosa only was found in *H pylori*-infected Belgian children (16). The different results we had in regard to the increased IL-10 expression in gastric mucosa of infected children may be a result of the different method we used to measure IL-10 expression or the larger series of patients we studied. In our children, IL-10 expression was not correlated with the severity of gastric inflammation as scored according to the Sydney System, and was similar in children with active and nonactive gastritis. These findings suggest that IL-10 expression, known to be influenced by genetic variation in the IL-10 promoter haplotypes, does not influence the severity of gastric inflammation, which is similar to reports in adults (18,19). Our most interesting finding was the further significant increase in *H pylori*-infected children older than 4 years. These data suggest that this chronic infection, by continuing to stimulate inflammation via the production of a proinflammatory cytokines, may influence IL-10 production even beyond the age of 4 years until adolescence.

There is evidence that secretion of IL-10 has a direct inhibitory effect on the function of Th2 cells (20,21), and IL-10-secreting cells can also inhibit inflammation in asthma (22). Recently, in different animal models, IL-10 was shown to control Th2-mediated inflammatory processes and airway eosinophilia (23). Increased levels of IL-10 and TGF- β produced by regulatory T cells were shown to potently suppress immunoglobulin (Ig) E production while simultaneously increasing production of noninflammatory isotypes IgG4 and IgA (24), and IL-10 was higher in nonatopic children and adult control subjects compared with subjects with allergic diseases (7). The relevance of the regulatory role of IL-10 has been outlined in studies of IL-10 upregulatory therapy (25).

In noninfected children we found a tendency for IL-10 expression to be lower in older children, but not significantly and without any age correlation, in accordance

with the findings of a previous report (26) that showed that levels of IL-10 protein expression tend to be regulated on an individual basis during infancy and early childhood.

Interleukin-1 β was not significantly increased in *H pylori*-infected compared with control subjects, probably because its expression is affected more by IL-1 polymorphisms than *H pylori* infection (27).

Exposure to food and orofecal pathogens such as hepatitis A virus, *Toxoplasma gondii*, and *H pylori* was associated with reduced risk of allergic asthma or rhinitis by more than 60% in Italian military cadets (1), but these infections were interpreted merely as markers of poor hygienic conditions in childhood. However, a 30% reduction in the prevalence of asthma, eczema, and allergic rhinitis was found in people from Bristol, UK, who had active *H pylori* infection and it was concluded that "*H pylori* infection is associated with a substantially reduced risk of 3 common atopic disorders" (2). Similarly, a study in a Finnish population (3) found a 3.5-fold increase of allergen-specific IgE prevalence rates and IgE antibody levels from 1973 to 1994, but prevalence of IgE-positive subjects had increased mainly in the subgroup without *H pylori* antibodies, and the authors supported the hypothesis that *H pylori* could be one of the microbes that counteracts atopy. Our study was not aimed at assessing the prevalence of atopy in *H pylori*-infected children because our series was too small, and indeed there was a similar prevalence of atopy in infected and control children. However, in *H pylori*-infected children, prevalence of atopy was significantly higher in those with lower IL-10, IFN- γ , and IL-1 β expression, thereby strengthening our hypothesis.

Although we demonstrated an association only between *H pylori* infection and increased IL-10 expression, and this is not a demonstration of causality, our findings could suggest some interesting hypotheses: if chronic *H pylori* infection in children—by stimulating IL-10 in gastric mucosa in addition to the reported increase in duodenal mucosa (16)—may be protective against development of atopy, and infection of longer duration is associated with higher IL-10 production, childhood *H pylori* infection could be hypothesized to have a behavior similar to helminth infestation (28,29), in which continuous antigenic challenge results in an anti-inflammatory response mediated by IL-10 to restrict tissue damage. In addition, childhood *H pylori* infection could exhibit behavior similar to curative immunotherapy, in which allergen tolerance is induced by increasing IL-10 (12,20).

Our findings need to be further studied in larger patient groups enrolled in prospective studies, and, if confirmed, may have some relevant and intriguing clinical implications: this could be another reason to avoid screening with noninvasive tests in asymptomatic children or children with mild nonspecific symptoms (30) and to postpone treatment in these children until a later age, like young

adulthood, to allow the infection to exercise its protective effect against development of atopy for a longer time. In children, eradication treatment is less effective (31) and more severe complications like atrophy and intestinal metaplasia of gastric mucosa leading to gastric cancer usually develop at a later age.

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