

The Interaction Between *Helicobacter pylori* and Atopy: Does Inverse Association Really Exist?

Sebahat Cam,* Deniz Ertem,* Nerin Bahceciler,† Tunc Akkoc,† Isil Barlan† and Ender Pehlivanoglu*

*Division of Pediatric Gastroenterology, Hepatology and Nutrition and †Division of Pediatric Allergy and Immunology, Marmara University School of Medicine, İstanbul, Turkey

Keywords

Helicobacter pylori, atopy, children, cytokine, lymphocyte culture.

Reprints request to: Deniz Ertem, MD, Associate Professor, Division of Pediatric Gastroenterology, Hepatology and Nutrition, Marmara University School of Medicine, Tophanelioglu Cd. 13-15, Altunizade, İstanbul 81190, Turkey.
E-mail: denizertem@marmara.edu.tr

Abstract

Aim: To date, cross-sectional and case-control studies suggest an inverse association between *Helicobacter pylori* infection and atopic diseases, whereas the immunologic basis has not been studied yet. In this study we investigated T helper (Th) cell function in *H. pylori*-infected children and compared cytokine responses in atopic and non-atopic groups.

Methods: The study groups was recruited from a cohort of 327 healthy children evaluated and followed-up for 6 years to assess the natural history of *H. pylori* infection. Seventy-four of 136 healthy children who underwent ¹³C urea breath test were eligible and accepted to participate. All participants were evaluated by a questionnaire, and skin-prick testing. According to the results, children were divided into four groups with respect to the presence or absence of *H. pylori* and atopy. Peripheral blood mononuclear cells isolated from 34 of 74 children were cultured with *H. pylori*, *Der p 1*, and phytohemagglutinin (PHA). Interferon-gamma (IFN- γ), interleukin (IL)-4 and IL-10, transforming growth factor-beta (TGF- β) levels were measured in supernatants.

Results: The frequency of atopy was lower in *H. pylori*-infected group (31.9% vs. 48.1, $p = .22$), while atopic symptoms were similar between infected and non-infected children. While PHA and *H. pylori* induced IFN- γ levels were significantly higher in *H. pylori*-infected children, concomitant presence of both atopy and *H. pylori* decreased the level of PHA and *H. pylori* induced IFN- γ production. PHA and *Der p 1*-induced IL-4 levels were higher in atopic children, and IL-4 production was suppressed when they were concomitantly infected with *H. pylori*. The production of TGF- β was found to be suppressed in atopic children irrespective of the presence of *H. pylori* infection.

Conclusion: The results of the current study demonstrated a counteractive Th1 and Th2 cytokine interaction between *H. pylori* infection and atopy. However, this counteractive immunologic balance did not protect against atopy.

Helicobacter pylori, which is the most common chronic infectious agent worldwide, causes chronic gastritis and peptic ulcer disease not only in adults but also in children, and is mainly acquired during childhood [1]. The relationship between atopy and *H. pylori* infection has been questioned over the past decade since it has been argued that allergic diseases are related to the genetic susceptibility of the individuals and some environmental factors such as bacterial infections [2–4]. In some studies, it was postulated that allergic diseases were less common among *H. pylori*-infected individuals, whereas other proposed a greater susceptibility to atopy in *H. pylori*-infected population [5,6]. Most of these studies

are population-based cross sectional or epidemiologic studies. On the other hand, the immunologic relationship between atopy and *H. pylori* has not been investigated yet.

Epidemiologic data provide strong evidence of a steady increase in incidence of allergic diseases over the last decades [3,7]. Concomitantly, there has been a decrease in incidence of many infectious diseases because of antibiotics, vaccination, improved hygiene, and better socioeconomic conditions [3]. While the development of allergic diseases depend on cytokines produced by Th2 cells, interferon-gamma (IFN- γ) and interleukin (IL)-2 mainly involved in mediation of infections [4]. Th2 cytokines such as IL-4 and IL-5 down-regulate Th1

immune response, while Th1 cytokines such as IFN- γ and IL-2 reciprocally inhibit Th2 immune response [8,9]. Theoretically, it can be postulated that a dominance of Th1 response may be protective against the development of allergic diseases [10,11]. There are also regulatory T cells (Treg) whose mechanism of action is still ill defined and thought to down-regulate both Th1- and Th2-mediated immune responses through inhibitory cytokines such as transforming growth factor-beta (TGF- β) and IL-10 [12]. Studies done in animal models suggested that infectious agents may stimulate the release of cytokines produced by Treg cells which can inhibit both Th1 and Th2 responses [4].

The aim of this study is to investigate the relationship between atopy/atopic diseases and infection with *H. pylori* in healthy school children who were prospectively followed-up for 6 years. Furthermore the immunologic interaction between atopy and *H. pylori* infection was evaluated by measuring the cytokines produced by Th1, Th2 and Treg cells in response to antigenic stimulation in cell cultures, obtained from *H. pylori*-infected atopic children.

Materials and Methods

Study Design and Population

Three hundred and twenty-seven healthy school children of whom *H. pylori* infection status were detected by ^{13}C -urea breath test in 1999 were prospectively followed-up for *H. pylori* status for 6 years, and the data published elsewhere [13,14]. Follow-up information was available for 136 of 327 children. Among those 136 children, 74 cases (mean age: 178 ± 25 months) whose parents gave a written consent were enrolled into the current study. The study was approved by the ethical board of the Marmara University School of Medicine. Only the ones who had either persistent positive or negative ^{13}C -UBT for *H. pylori* were included into the study. The children with converted or reverted ^{13}C -UBT were not included into the study (Fig. 1). The International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire was applied to the parents of participants through face-to-face interview [15]. All children also underwent skin-prick testing [16].

According to the results of the tests applied to the study group, children were divided into four groups as *H. pylori* (+) atopy (-), *H. pylori* (-) atopy (+), both positive, and both negative (Fig. 1).

In order to study T helper (Th) cell function and cytokine response in *H. pylori*-infected cases and to compare the cytokine responses in atopic and non-atopic groups, children who have a positive skin reaction to the commonest antigen, which was house dust mite antigen (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) (64 participants) in this study, were asked to give

a peripheral blood sample for isolation of peripheral blood mononuclear cells (PBMCs). Children with positive skin-prick result for allergens other than *Der p 1* (10 cases) were excluded from the immunologic studies in order to get a homogenous group for the analysis of cytokine levels in specific and nonspecific antigen-stimulated tissue cultures. Thirty-four of 64 parents provided consent for their children to give blood samples, and isolated PBMCs were cultured and stimulated by specific antigens for cytokine analysis (Fig. 1).

^{13}C -urea breath test

Infection status was determined by ^{13}C -UBT (INFAI, Institut for Biomedizinische, Bochum, Germany), which shows active infection with *H. pylori* [17]. Fasting children were asked to drink 100 to 200 mL of orange juice (pH 2.3–2.5) to avoid premature gastric emptying. Two baseline breath samples were collected directly into 10-mL vacutainer tubes via a straw. The tubes were recapped immediately after the breath collection. Then 75 mg nonradioactive ^{13}C -labeled urea was dissolved in 15 mL orange juice, and the children were asked to drink it. Two additional breath samples were collected 30 minutes later. All collected breath samples were stored at ambient temperature until all samples were analyzed at once. ^{13}C abundance was determined by the isotope ratio mass spectrometry (AP 2003 Analytical Precision, Manchester, UK) on breath samples. Isotope enrichment was expressed as corrected delta per mil ($\delta\text{‰}$) relative to the pee Dee belemnite limestone international standard. A change in the ^{13}C value over baseline of more than 5 ‰ was considered positive.

ISAAC questionnaire

The International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire was used to obtain data on ever and current wheezing, allergic rhinitis, doctor-diagnosed asthma, and atopic dermatitis from parents, usually the mother. Data on family history of atopy were also obtained from ISAAC questionnaire [15].

Skin-prick test

Skin-prick tests were performed with 20 common aeroallergens belonging to five groups – mites (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*), molds (*Alternaria*, *Aspergillus* mix, *Penicillium* mix, *Candida albicans*), pollens (Betulaceae, *Aesculus hippo*, *Olea europea*, *Plantago*, *Artemisia*, *Parietaria*, *Secale cereale*, *Triticum vulgare*, *Zea mays*, mixture of five grasses), animal dander (feather mixture, cat hair, dog hair), insects (cockroach), and latex

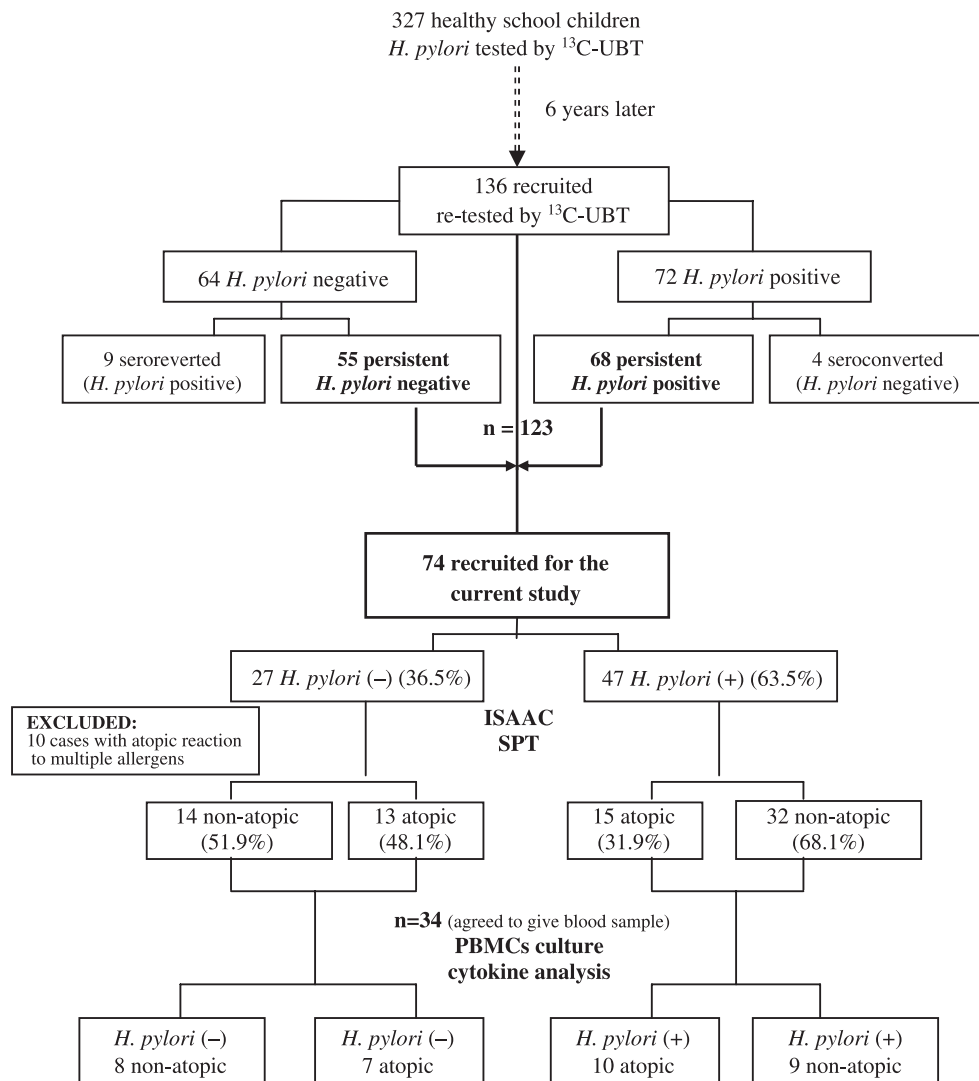


Figure 1 The organization of the study group.

(Stallergenes, Antony, France). Histamine and saline were used as positive and negative controls, respectively. A drop of each allergen extract was placed on the volar surface of the left forearm and was penetrated with a separate lancet (Mizollen, H. Herenz GmbH, Hamburg, Germany). After 15 minutes, the wheal reaction was measured as the mean of the longest diameter and the diameter perpendicular to it. A wheal diameter of at least 3 mm greater than those of the negative controls was considered as positive. Subjects who had at least one or more positive reaction to any of the tested antigens were considered atopic.

Cell culture and cytokine analysis

Ten milliliters of blood was drawn from patients in EDTA containing blood containers. Peripheral blood mononuclear

cells were isolated by Ficoll-Hypaque density gradient centrifugation and then suspended in RPMI 1640 (Sigma Chem Co., St Louis, MO, USA) with 2 mmol/L L-glutamine (Sigma Chem Co.), and 100 U/mL penicillin/streptomycin (Sigma Chem Co.) supplemented with 10% FCS (S-00016, JRH Biosciences, Lenexa, KS, USA). Lymphocytes (2×10^6 cells) were incubated with 5 µg/mL *Dermatophagoides pteronyssinus* allergen 1 (*Der p 1*, Indoor Biotechnologies, Wiltshire, UK), 20 µg/mL *H. pylori* antigen, 5 µg/mL phytohemagglutinin (PHA) (Sigma Chem Co.) and without adding any antigen in 200 µL each in quadruplets in 96-well plates (Costar Corp., Cambridge, MA, USA) at 37 °C with 5% CO₂ for 5 days. Supernatants were collected and stored at -20 °C until tested.

H. pylori antigen and recombinant *Dermatophagoides pteronyssinus* antigen (*Der p 1*) (Indoor Biotechnologies)

were used to stimulate cell cultures as specific stimulants. Phytohemagglutinin (Sigma Chem Co.) was used as nonspecific stimulant for cell cultures.

H. pylori antigen was prepared from pure cultures of *H. pylori* (National Culture Type Collection 11,637). Bacteria were cultured under microaerobic conditions on chocolate agar plates and harvested after 48 hours of incubation at 37 °C into a phosphate-buffered saline (PBS) pH 7.4 at 0.5 g/mL (approximately 10⁹ bacteria/mL) and disintegrated by sonication three times for 45 seconds at 20,000 Hz, on ice, using a Rapids 350, 19 mm probe with a 9.5-mm tip, and centrifuged at 14,000 *g* for 30 minutes at 4 °C. The supernatant protein concentration was measured by refractometry using PBS as a standard. The sonicate was stored in small aliquots at -20 °C [18].

Detection of Cytokines

IFN- γ , IL-4, IL-10, and TGF- β production in culture supernatants of PBMCs was detected by means of ELISA. The amounts of released cytokines were detected by specific solid phase sandwich ELISA (Biosource International Inc., Camarillo, CA, USA) as according to the manufacturer's instructions.

Statistical Analysis

Data were expressed as mean plus or minus the standard error of the mean. The Mann-Whitney U-test, Student's *t*-test, and Kruskal-Wallis test were used to evaluate quantitative values, while chi-square and Fisher exact chi-square tests were used to evaluate qualitative data. Differences of *p* < .05 were considered significant. Statistical analysis was performed by spss for Windows 10.0.

Results

Patients

Seventy-four school children whose parents gave consent for the study were recruited. Forty-seven (63.5%) of those children were *H. pylori* infected, while 27 (36.4%) of them were not infected depending on the results of ¹³C-UBT (Fig. 1).

Atopy was detected in 28 (39.2%) children according to results of the skin-prick test. In 18 (64.2%) of the atopic children, skin-prick test was only reactive to *Dermatophagoides pteronyssinus* (house dust mite - HDM). Five (17.9%) children had positive results for multiple allergens (HDM and *Olea europea*, cat hair, latex, mixture of grasses, *Secale cereale*) and another five (17.9%) children were detected to have skin-prick test positivity to allergens other than HDM (mixture of grasses, *Secale cereale*, *Aesculus hippo*, *Zea mays*, cockroach, *Aspergillus* mix, *Alternaria*, *Penicillium* mix).

Those children who were sensitized to HDM (*Dermatophagoides pteronyssinus*) only were chosen for further immunologic evaluation in order to exclude differences in cytokine responses to different antigenic stimuli during PBMC culture procedures. Therefore, *Der p 1* was chosen as the antigen for cultures in addition to *H. pylori* and PHA. Non-atopic patients were randomly chosen for cytokine analysis. Finally, 34 children were available for PBMC study (Fig. 1). Patients were then divided into four groups as: (1) *H. pylori* infected and sensitized to HDM, *n* = 10; (2) *H. pylori* infected and non-atopic, *n* = 9; (3) *H. pylori* non-infected and sensitized to HDM, *n* = 7; and (4) *H. pylori* non-infected and non-atopic, *n* = 8.

Although the prevalence of atopy was slightly higher in *H. pylori*-negative cases (48.1% vs. 31.9%), the difference was not statistically significant (*p* = .22).

Frequency and severity of allergic manifestations detected with ISAAC questionnaire and family history of allergy were not found significantly different among those who were infected or non-infected with *H. pylori* (*p* > .05) (Table 1).

Cytokine Analysis in Cell Cultures

IFN- γ secretion by peripheral blood mononuclear cells in response to PHA, *H. pylori* and *Der p 1* antigens were significantly higher in *H. pylori* infected cases compared to *H. pylori* non-infected controls (*p* = .004, *p* = .000, *p* = .025, respectively). When four groups were analyzed together, IFN- γ secretion was highest in *H. pylori* infected non-atopic cases (*p* = .001). The levels of IFN- γ were similar between *H. pylori* infected atopic children compared to *H. pylori* non-infected, non-atopic children (*p* > .05) (Fig. 2).

IL-4 levels in cultures stimulated by *Der p 1* and PHA were higher in atopic children than non-atopic ones (*p* = .004, *p* = .009, respectively). The level of IL-4 response to *Der p 1* and PHA stimulants was highest in *H. pylori*

Table 1 The distribution of data about allergic manifestations and skin-prick test results among children infected and non-infected with *H. pylori*

	<i>H. pylori</i> (+) <i>n</i> = 47	<i>H. pylori</i> (-) <i>n</i> = 27	<i>p</i>
Ever and current wheezing	14	7	.793
Ever and current rhinitis-conjunctivitis	12	4	.383
Ever and current eczema	8	3	.735
Doctor-diagnosed asthma	4	1	.646
Doctor-diagnosed allergic rhinitis	3	1	1.000
Doctor-diagnosed atopic eczema	3	1	1.000
Atopy history in the family	21	10	.473
Skin-prick test positivity	15	13	.215

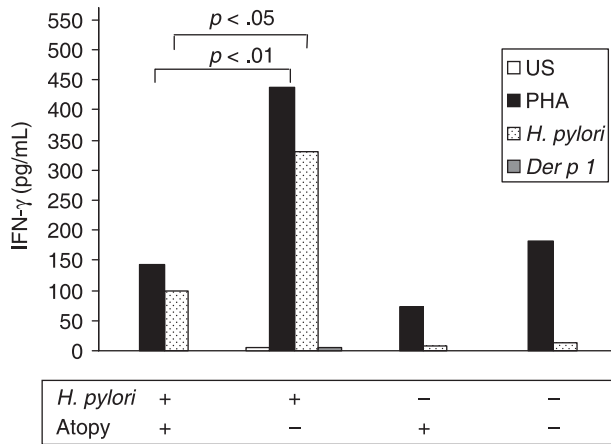


Figure 2 Interferon-gamma (IFN- γ) responses in cultured supernatants of peripheral blood mononuclear cells (PBMCs). In *H. pylori*-positive atopic subjects, IFN- γ secretion induced by phytohemagglutinin and *H. pylori* antigens was significantly lower compared to *H. pylori*-positive non-atopic children.

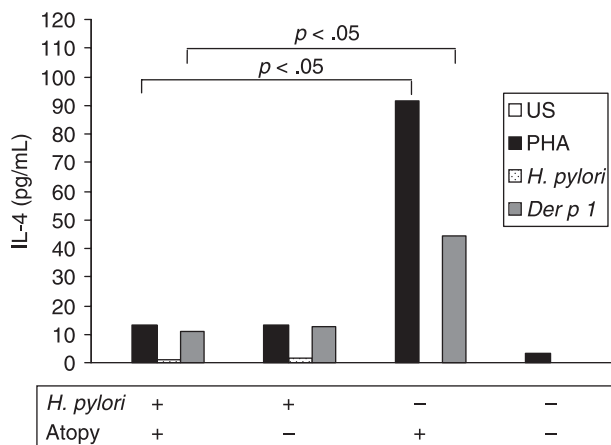


Figure 3 Interleukin (IL)-4 responses in cultured supernatants of peripheral blood mononuclear cells. Phytohemagglutinin- and *Der p 1*-induced IL-4 levels in atopic subjects were significantly lower in *H. pylori*-positive ones compared to *H. pylori*-negative subjects.

non-infected atopic cases compared to the other groups ($p < .05$, $p < .05$, and $p < .001$). Moreover, *H. pylori*-infected cases demonstrated a lower IL-4 response to PHA and *H. pylori* antigens when compared with cases not infected with *H. pylori* ($p = .035$, $p = .053$) (Fig. 3).

There was no significant difference in IL-10 secretion detected in culture supernatants stimulated by PHA, *H. pylori*, and *Der p 1* ($p > .05$) (Fig. 4).

TGF- β level in response to PHA, *H. pylori*, and *Der p 1* were not different in *H. pylori*-infected and non-infected groups. On the other hand, TGF- β secretion in response to PHA was significantly lower in atopic children compared to non-atopic ones ($p = .000$, $p = .002$ respectively) (Fig. 5).

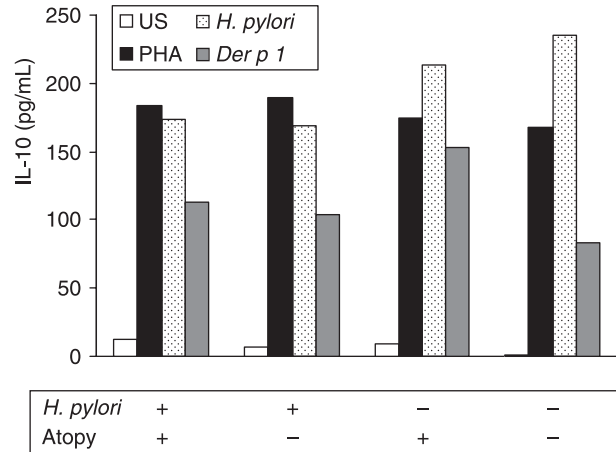


Figure 4 Interleukin-10 responses in cultured supernatants of peripheral blood mononuclear cells. There was no significant difference between the groups.

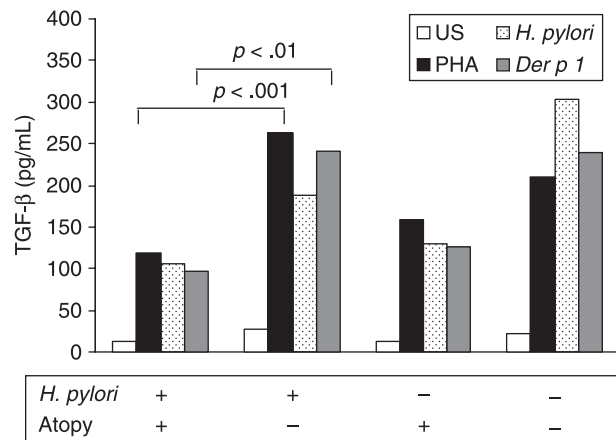


Figure 5 Transforming growth factor-beta (TGF- β) responses in cultured supernatants of peripheral blood mononuclear cells. Phytohemagglutinin- and *Der p 1*-induced TGF- β levels of *H. pylori*-infected subjects were significantly lower in atopic subjects compared to non-atopic ones.

Discussion

Interactions between allergic diseases and infections were largely investigated in the last decade since it was documented in epidemiologic studies that the prevalence of allergic diseases has been increasing significantly while exposure to infections in childhood is decreasing [2–4]. The mechanism proposed for this effect is that such infections may shift the balance of immune responses towards the Th1, thereby reducing the expression of Th2 cytokines, principally associated with allergy [4].

Today, the potential interaction between atopic diseases and *H. pylori* which is a very common infectious pathogen and mostly acquired during childhood has been interrogated

[2,5,6]. Conflicting reports have been presented on this issue. Some studies demonstrated that the prevalence of atopic diseases such as asthma and eczema were lower in *H. pylori*-infected subjects compared to non-infected ones, while others proposed a positive correlation between atopy and *H. pylori* infection [5,19–21]. Inconsistency of the results might be related to the fact that definition of atopy usually relies on subjective parameters like parents' declaration of disease and the use of serologic tests that are not very sensitive for the detection of current *H. pylori* infection in most of the studies.

A positive serologic test for *H. pylori* may be secondary to current or past infection, and the cut-off values for a positive result varies according to the manufacturer producing the test and the population investigated. On the other hand, ^{13}C UBT is the most sensitive non-invasive test to detect current *H. pylori* infection in children and adolescents [17]. In the current study, children whose *H. pylori* status had been identified by ^{13}C -UBT previously were retested and only the ones who were persistently infected or non-infected with *H. pylori* were included.

Moreover, skin-prick test was used as an objective parameter to detect atopic status. It was beyond the aim of this study but, the frequency of skin-prick test positivity, although statistically not significant, was found higher in children who were not infected with *H. pylori* (48.1% vs. 31.9%). Meanwhile, the frequency of allergic manifestations and skin-prick test positivity were not different among *H. pylori* infected and non-infected children. This finding is not in accordance with the previous data regarding *H. pylori* and atopy association.

Published studies to date investigating the relationship between atopy and *H. pylori* are mostly population-based epidemiologic studies or cross-sectional surveys examining the populations for the presence of atopy and *H. pylori* infection by serology [19–21]. The interaction between *H. pylori* infection and atopy has not been studied regarding the immunologic origin of these two counteractive conditions which may elucidate the immunologic basis and the proposed inverse relationship between infections and atopic diseases [22].

It is obvious that immune response to *H. pylori* infection involves Th1 pathway since this is a bacterial inflammatory process. Initial studies on this issue showed that lymphocytes isolated from both peripheral blood and gastrointestinal tract in adults with *H. pylori* infection had increased IFN- γ secretion representing Th1 subset dominance [23–26]. In children who were infected with *H. pylori*, an elevated level of IFN- γ was detected in gastric mucosa samples while no difference was observed in IL-4 levels, indicating a Th1 predominant cytokine profile in children as well [24]. However, even these data do not imply that *H. pylori* infection *per se* is the origin of the

elevated Th1 cytokines in the gastric tissue. Moreover, direct measurement of cytokine levels by immune-histochemistry or ELISA does not designate a specific pathogen, namely *H. pylori* inducing the production of IFN- γ . Therefore, it is more convenient to investigate cytokine responses of the lymphocytes (isolated from peripheral blood or gastric mucosa) to specific antigenic stimulants. This issue has been studied in adults, and has been demonstrated that IFN- γ secretion increased in lymphocyte cultures obtained from blood of *H. pylori*-infected patients when stimulated by *H. pylori* antigen [23,27]. The current study for the first time evaluated the production of IFN- γ in *H. pylori*-stimulated PBMCs culture supernatants in *H. pylori*-infected and non-infected children. *H. pylori*-induced IFN- γ production was found to be significantly higher in those infected with *H. pylori*. Interestingly, when atopy was simultaneously present in *H. pylori*-infected cases; *H. pylori*-induced IFN- γ production was blunted.

The main immunologic pathway concerning atopic diseases involves Th2 immune response, and IL-4 was measured representing the involvement of Th2 pathway in this study [28]. Published studies showed either no difference or a slight decrease in IL-4 level in gastric mucosa of adults infected with *H. pylori* [25,26,29,30]. It was also demonstrated that IL-4 production was not changed when the cell culture, obtained from *H. pylori*-infected adults, was stimulated by specific antigen [23]. Furthermore, the same investigators confirmed their findings by showing an increase in IL-4 level after the eradication of *H. pylori* infection. In the current study, it was demonstrated that IL-4 production was at the highest level in stimulated cell cultures isolated from non-infected atopic children. However, the production was significantly suppressed when *H. pylori* infection was concurrently present in the atopic children. Therefore, our findings indicated that an increased IFN- γ production was associated with a suppressed production of IL-4 in atopic children infected with *H. pylori*. This is the first study demonstrating the counteractive effect of Th1 and Th2 cytokines in the simultaneous presence of *H. pylori* infection and atopy.

Regulatory T-cell functions were also studied in this study by measuring TGF- β and IL-10 levels in stimulated cell cultures. Up-to-date, inconsistent data have been reported regarding the role of IL-10 in *H. pylori* infection. Some suggested no difference in IL-10 levels in *H. pylori*-infected gastric mucosa, while some others revealed an increased production [30–32]. Recently, an experiment carried out in IL-10-knockout mice demonstrated that endogenous IL-10 down-regulates *H. pylori*-induced gastric inflammatory response which may promote more effective bacterial colonization of the gastric mucosa [33]. In the current study, IL-10 production was not modified either by *H. pylori* infection or by the presence of atopy.

Regarding *H. pylori* infection and one of the other regulatory cytokine, namely TGF- β , the presence of *H. pylori* infection *per se* did not affect the production of this cytokine in stimulated cell cultures. Taken together, the results of our study could not demonstrate involvement of this regulatory cytokine in inflammatory reactions induced by *H. pylori*. On the other hand, it was found that TGF- β production in response to all antigenic stimulants was significantly decreased in lymphocyte cultures isolated from atopic children irrespective of their *H. pylori* status. Inhibitory cytokines secreted from regulatory T cells were proposed to have regulatory or suppressive properties that inhibit the effects of pathogenic autoreactive T cells but the mechanism that provides protection against the development of allergic diseases is not fully understood. Current knowledge about regulatory T-cell functions in atopy is limited to some experimental studies, indicating the presence of a defect in regulatory T-cell functions in atopic cases [34]. Our data strongly support this hypothesis and give important clues about inefficient production of regulatory T-cell cytokines in atopic children.

Although a significant difference was not detected in prevalence of atopy and allergic manifestations among *H. pylori*-infected and non-infected children, the results of the current study demonstrated the interaction between *H. pylori* infection and atopy on the basis of cytokine responses. An increased Th1 cytokine response was found in children infected with *H. pylori*, whereas Th2 immune response predominated in atopic cases. A significant decrease in the level of Th1 cytokines in the concomitant presence of *H. pylori* and atopy suggests a counteractive interaction between Th1- and Th2-dependent immunity. It can be postulated that *H. pylori* infection that primarily involves Th1 immune response may suppress Th2 pathway which is mainly associated with atopy. Therefore, long-term follow-up of the children with *H. pylori* infection may reveal a clinical relationship between these two common disorders. The current study may also represent an initial step for the development of new follow-up and treatment modalities targeting the particular cytokine responses in the management of not only atopic diseases but also *H. pylori* infection.

The authors gratefully thank Prof Dr Yakut Akyon from Hacettepe University, Department of Clinical Microbiology for the preparation and supply of the *Helicobacter pylori* antigen for this study.

This study is supported by a grant obtained from Marmara University School of Medicine (BAPKO Project No. SAG-TUS-300505-0124).

References

- Vandenplas Y. The role of *Helicobacter pylori* in pediatrics. *Curr Opin Infect Dis* 2001;14:315–21.

- Cremonini F, Gasbarrini A. Atopy, *Helicobacter pylori* and the hygiene hypothesis. *Eur J Gastroenterol Hepatol* 2003;15:635–6.
- Strachan DP. Family size, infection and atopy: the first decade of the "hygiene hypothesis". *Thorax* 2000;55:S2–S10.
- Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 2002;347:911–20.
- Corrado G, Luzzi I, Lucarelli S, et al. Positive association between *Helicobacter pylori* infection and food allergy in children. *Scand J Gastroenterol* 1998;33:1135–9.
- McCune A, Lane A, Murray L, et al. Reduced risk of atopic disorders in adults with *Helicobacter pylori* infection. *Eur J Gastroenterol Hepatol* 2003;15:637–40.
- Matricardi PM, Rosmini F, Riondino S, et al. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: an epidemiological study. *Br Med J* 2000;320:412–7.
- von Mutius E. Infection: friend or foe in the development of atopy and asthma? The epidemiological evidence. *Eur Respir J* 2001;18:872–81.
- Arshad SH. Development of allergic disease in children. *Clin Exp Allergy* 1997;27:1231–3.
- Schall TJ, Bacon KB. Chemokines, leukocyte trafficking, and inflammation. *Curr Opin Immunol* 1994;6:865–73.
- Borisch LC, Steinke JW. Cytokines and chemokines. *J Allergy Clin Immunol* 2003;111:460–75.
- D'Ambrosio D. Regulatory T cells: how do they find their space in the immunological arena? *Semin Cancer Biol* 2006;16:91–7.
- Ertem D, Harmanci H, Pehlivanoglu E. *Helicobacter pylori* infection in Turkish preschool and school children: role of socioeconomic factors and breast feeding. *Turk J Pediatr* 2003;45:114–22.
- Ozen A, Ertem D, Pehlivanoglu E. Natural history and symptomatology of *Helicobacter pylori* in childhood and factors determining the epidemiology of infection. *J Pediatr Gastroenterol Nutr* 2006;42:398–404.
- Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8:483–91.
- EAACI. Position paper: allergen standardization and skin tests. *Allergy* 1993;48:49–82.
- Mègraud F. European Paediatric Task Force on *Helicobacter pylori*. Comparison of non-invasive tests to detect *Helicobacter pylori* infection in children and adolescents: results of a multicenter European study. *J Pediatr* 2005;146:198–203.
- Akyon Y, Nielsen H, Andersen LP. Effect of four cytokines on the oxidative burst of polymorphonuclear leucocytes when stimulated with *Helicobacter pylori*. "4th International Workshop on Pathogenesis and Host Response in *Helicobacter* Infections" C2, July 6–9 2000, Helsingor, Denmark.
- Schnyder B, Helbling A, Pichler WJ. Chronic idiopathic urticaria: natural course and association with *Helicobacter pylori* infection. *Int Arch Allergy Immunol* 1999;119:60–3.
- Murakami K, Fujioka T, Nishizono A, et al. Atopic dermatitis successfully treated by eradication of *Helicobacter pylori*. *J Gastroenterol* 1996;31:77–82.
- Tsang KW, Lam WK, Chan KN, et al. *Helicobacter pylori* sero-prevalence in asthma. *Respir Med* 2000;94:756–9.
- Chen Y, Blaser MJ. Inverse associations of *Helicobacter pylori* with asthma and allergy. *Arch Intern Med* 2007;167:821–7.
- Ren Z, Pang G, Lee R, et al. Circulating T-cell response to *Helicobacter pylori* infection in chronic gastritis. *Helicobacter* 2000;5:135–41.
- Shimizu T, Haruna H, Ohtsuka Y, et al. Cytokines in the gastric mucosa of children with *Helicobacter pylori* infection. *Acta Paediatr* 2004;93:322–6.

- 25 Yamaoka Y, Kita M, Kodama T, Sawai N, Imanishi J. Expression of chemokine mRNA in gastric mucosa with *Helicobacter pylori* infection. *Gastroenterology* 1996;110:A1049.
- 26 Bamford KB, Fan XJ, Crowe SE, et al. Lymphocytes in the human gastric mucosa during *Helicobacter pylori* have a T helper cell 1 phenotype. *Gastroenterology* 1998;114:482–92.
- 27 Quiding-Järbrink M, Lundin BS, Lönröth H, Svennerholm AM. CD4+ and CD8+ T cell responses in *Helicobacter pylori*-infected individuals. *Clin Exp Immunol* 2001;123:81–7.
- 28 Magnan A, Mely L, Prato S, et al. Relationships between natural T cells, atopy, IgE levels, and IL-4 production. *Allergy* 2000;55:286–90.
- 29 D'Elia MM, Manghetti M, De Carli M, et al. T helper 1 effector cells specific for *Helicobacter pylori* in gastric antrum of patients with peptic ulcer disease. *J Immunol* 1997;158:962–7.
- 30 Bontems P, Robert F, Van Gossum A, Cadranet S, Mascart F. *Helicobacter pylori* modulation of gastric and duodenal mucosal T cell cytokine secretions in children compared with adults. *Helicobacter* 2003;8:216–26.
- 31 Meyer F, Wilson KT, James SP. Modulation of innate cytokine responses by products of *Helicobacter pylori*. *Infect Immun* 2000;68:6265–72.
- 32 Haeberle H, Kubin M, Bamford KB, et al. Differential stimulation of interleukin-12 (IL-12) and IL-10 by live and killed *Helicobacter pylori* in vitro and association of IL-12 production with gamma interferon-producing T cells in the human gastric mucosa. *Infect Immun* 1997;65:4229–35.
- 33 Chen W, Shu D, Chadwick VS. *Helicobacter pylori* infection: mechanism of colonization and functional dyspepsia reduced colonization of gastric mucosa by *Helicobacter pylori* in mice deficient in interleukin-10. *J Gastroenterol Hepatol* 2001;16:377–83.
- 34 Curotto de Lafaille MA, Lafaille JJ. The role of regulatory T cells in allergy. *Springer Semin Immunopathol* 2004;25:295–310.