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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

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The objective of these experiments was to examine the ability of *Helicobacter pylori* to stimulate interleukin-10 (IL-10) or IL-12 and select for either Th1 or Th2 cells. Gastric biopsy specimens were collected from patients who were categorized with respect to the presence of *H. pylori* and gastric disease as well as their age, gender, medications, and other factors. As Th1 and Th2 cells are selected by IL-12 and IL-10, respectively, biopsy specimens were screened for mRNA and protein for these cytokines. Although mRNA for IL-12 and IL-10 was detected in biopsy specimens obtained from both infected and uninfected patients, IL-12 protein predominated. Levels of IL-10 and IL-12 in gastric tissue did not change in response to infection. Moreover, gamma interferon (IFN-γ)-producing T cells were found in both the infected and the uninfected gastric mucosa. Stimulation of peripheral blood leukocytes from either infected or uninfected donors with various concentrations of live or killed *H. pylori* induced immunoreactive IL-12 and IL-10. After stimulation with live *H. pylori*, IL-12 levels increased more than 30-fold, whereas IL-10 levels increased only 2- to 5-fold, compared to cells stimulated with medium alone. Interestingly, killed *H. pylori* induced significantly more IL-10 ($P < 0.05$) than live *H. pylori*, while recombinant urease only induced IL-10. These results demonstrate that live *H. pylori* selectively stimulates the induction of IL-12 and Th1 cells that produce IFN-γ, whereas preparations used in oral vaccines induce more IL-10 and may favor Th2 cell responses.

Helicobacter pylori infects a substantial proportion of the population, and this infection leads to the development of chronic, active gastritis (16, 20, 26). In a relatively small portion of infected individuals, *H. pylori* contributes to the development of more severe gastric pathology including peptic ulceration and gastric cancer (3, 7). Investigations have shown that family members can express different manifestations of gastric disease even though they are infected with genetically similar strains of *H. pylori* (1a, 2). This, and other evidence, suggests that virulence factors of *H. pylori* interact with other elements within the gastric milieu to cause the more severe gastric diseases observed in some infected patients. Since the gastric mucosa is infiltrated with immune and inflammatory cells during *H. pylori* infection, the host response may represent one element that contributes to the pathogenesis of disease associated with *H. pylori* infection.

It is generally believed that immunoglobulin A (IgA) contributes substantially to effective immunity in the mucosal tissues, including the gastrointestinal tract. Moreover, this isotype is selected by cytokines secreted by the Th2 subset of helper T cells including interleukin-4 (IL-4), IL-5, IL-6, and transforming growth factor β (35, 37, 45, 55, 59, 62). The inability of the host to clear the *H. pylori* infection suggests that the local immune responses are not quantitatively or qualitatively adequate. Thus, one might predict that Th1 responses predominate during persistent infection with *H. pylori*. In fact, *H. pylori* has been shown to stimulate the production of gamma interferon (IFN-γ) by peripheral blood mononuclear cells (PBMC) (56). Other studies have shown that gastric mononuclear cells isolated from infected patients produce IFN-γ (21, 34) while IL-4-producing cells are relatively infrequent in these preparations (34). More recently, T-cell clones have been derived from infected patients, and most are characterized as being Th1-like (15). These observations support the notion that *H. pylori* preferentially induces the expansion of Th1 cells.

Helper T-cell subset selection is mediated at least in part by cytokines. In particular, IL-12 and IL-10 production have been implicated in the selection of Th1 and Th2 cells, respectively (24, 43, 52). Thus, the purpose of this study was to evaluate the relative expression of IL-12 and IL-10 in gastric mucosa and to determine the relative numbers of IFN-γ- and IL-4-producing T cells in this tissue. The data presented suggest that IL-12 does predominate in the gastric tissue and that stimulation with live *H. pylori* leads to IL-12 production and selection of Th1 cells. The implications for this concerning gastric immunity to *H. pylori* are discussed.

MATERIALS AND METHODS

Patient population. Tissue and blood used for these studies were obtained from consenting adults (20 to 55 years old) as approved by the institutional review boards at Baylor College of Medicine and the University of Texas Medical Branch. Biopsy specimens of the gastric antrum were obtained from consenting

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patients undergoing esophago-gastro-duodenoscopy for various clinical indications. These subjects were considered to be infected if *H. pylori* was detected by either a rapid urease test or histopathology of the biopsy specimens. Individuals regularly using nonsteroidal anti-inflammatory drugs or antisecretory drugs were excluded from the donor population. All of the infected patients included as a source of biopsy specimens were diagnosed with gastritis that was not complicated with peptic ulcer or gastric cancer. Control tissue from uninfected patients did not have any evidence of gastritis or ulceration. PBMC were isolated from a separate group of infected (but asymptomatic) and uninfected volunteer donors. The status of infection with *H. pylori* in these donors was determined by serology, with ELISA kits kindly provided by BioWhitacre (Walkersville, Md.), as described previously (53).

Cell preparation. PBMC were isolated from heparinized venous blood of *H. pylori*-infected and uninfected donors with Ficoll-Hypaque density gradients by standard techniques (13, 14). Cells were washed in RPMI 1640 (GIBCO, Grand Island, N.Y.) without antibiotics but containing L-glutamine and 10% heat-inactivated fetal calf serum (FCS) (Intergen, Purchase, N.Y.) and were counted, and viability was ascertained by trypan blue exclusion.

Gastric T cells were isolated by a modification of previously described techniques (2a, 21). Briefly, biopsy specimens were collected into collection medium at 4°C (calcium- and magnesium-free Hanks balanced salt solution with 5% FCS and penicillin plus streptomycin). The biopsy specimens were stored at 4°C for up to 18 h prior to processing, this having previously been shown not to alter T-cell function. All manipulations were carried out by using aseptic techniques. Biopsy specimens were rinsed with aqueous betadine and then immediately rinsed four times in collection medium and placed in collection medium containing 1 mM dithiothreitol and 1 mM EDTA (Sigma Chemical, St. Louis, Mo.). The specimens were agitated for 1 h at 37°C to remove intraepithelial lymphocytes and epithelial cells. The biopsy specimens were removed, placed in complete RPMI medium (RPMI 1640, 10% FCS, penicillin plus streptomycin), and washed three times with this medium. Subsequently, lamina propria T cells were liberated by treatment with collagenase (30 U/ml; Worthington Biochemical Corp., Freehold, N.J.) in complete RPMI medium for 3 h. Undigested material from the tissue sample was removed, and the cells were washed three times with complete RPMI medium. The resulting cell suspensions were washed, and the viability of the mononuclear cells was determined by trypan blue exclusion. Cells were not used if viability did not exceed 90%.

Cell culture conditions. (i) Bacterial stimuli. *H. pylori* LC-11, first isolated from the antral mucosa of a child with primary gastritis and associated duodenal ulcer, was stored at −70°C as described previously (19). The organisms were cultured by inoculation into 10 ml of brucella broth (GIBCO) supplemented with 10% FCS, 10 mg of vancomycin (Sigma)/liter, and 5 mg of trimethoprim (Sigma)/liter and incubated overnight at 37°C under microaerobic conditions (i.e., 10% CO_2, 5% O_2 and 85% N_2). The concentration of the bacteria was estimated by measuring the absorbance of the suspension and comparing the value to a standard curve. The standard curve was generated by measuring the absorbance of an array of serially diluted samples before quantifying the number of viable bacteria in each sample by a colony assay. After centrifugation at 2,500 × *g* for 15 min, bacteria were resuspended in sterile phosphate-buffered saline (PBS) (pH 7.4) to a range of concentrations from 10 to 10^9 bacteria per ml, diluted in PBS. The motility of the organisms was confirmed by phase-contrast microscopy prior to use. For the experiments with killed bacteria, *H. pylori* was treated with gentamicin (4 mg/ml; Solo Park, Elk Grove Village, Ill.) for 45 min at 4°C, washed, and diluted in PBS to the same concentrations as the live bacteria. All procedures were done with the approval of the above-mentioned institutional biosafety review committees and in compliance with their guidelines for biohazards.

Recombinant urease was kindly provided by C. Lee and T. Monath of Oravax (Cambridge, Mass.) and was prepared as described previously (40). Briefly, urease was expressed in *Escherichia coli* as a holoenzyme. Cells were lysed, and urease was purified by chromatography. Urease purity was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and specific identification of the material was confirmed by Western blotting with antisera recognizing the 65- and 30-kDa polypeptides. Subsequently, the material was stored at −20°C in 50% glycerol.

(ii) Stimulation of cytokine responses by *H. pylori*. Approximately 5×10^6 PBMC were cultured in 1 ml of RPMI 1640 containing 10% FCS. Cells were grown in 24-well plates and were stimulated with medium alone or with various doses of live or killed *H. pylori*, *E. coli* lipopolysaccharide (LPS) (Sigma), *Staphylococcus aureus* Cowan strain 1 (SAC) (Pansorbin; Calbiochem-Behring Corp., La Jolla, Calif.) (36), or various doses of urease (0.1 to 10 μg/ml [31]). After incubation (37°C, 5% CO_2) for 18 h, supernatants were collected, filtered, and frozen at −20°C until being analyzed.

In order to compare the results obtained in vitro with the expression of cytokines in the gastric mucosa, IL-10 and IL-12 were measured in preparations of solubilized tissues by a modification of a previously described technique (29). Briefly, samples of standards or biopsy specimens collected from patients as described above were suspended in 1 ml of RPMI 1640 with 10% FCS and a mixture of proteinase inhibitors consisting of leupeptin (100 μM), pepstatin A (10 μM), phenylmethanesulfonyl fluoride (1.0 mM), *N*-ethylmaleimide (100 μM), and bovine serum albumin (BSA) (1%) (all purchased from Sigma). The protein concentrations of the solubilized biopsy specimens were estimated by measuring optical density at 280-nm-wavelength light with a DU-65 spectrophotometer (Beckman, Fullerton, Calif.) and correcting for the extinction coefficient (estimated as 12). Samples were then frozen at −20°C until being analyzed, and the data are expressed as amounts of cytokine per milligram of protein.

Cytokine assays. (i) Immunoreactive protein. IL-12 p40 was measured by radioimmunoassay as previously described (13, 14) with the monoclonal antibody (MAb) pair C11.79-C8.6. Briefly, flat-bottom 96-well vinyl plates (Dynatech, Chantilly, Va.) were coated with C11.79 MAb, followed by incubation with the samples overnight. Subsequently, ^{125}I-labeled C8.6 MAb was added to detect the captured IL-12. Finally, the levels of IL-12 p40 were estimated by counting the ^{125}I-labeled C8.6 MAb with a gamma counter. The sensitivity of this assay was approximately 12 to 15 pg/ml.

The sandwich enzyme-linked immunosorbent assay for IL-10 was performed as described elsewhere (27). Briefly, 96-well plates (Immunolon IV; Dynatech) were coated with a rat anti-human IL-10 MAb (IgG1 isotype, clone JES3-9D7; Pharmingen, San Diego, Calif.), and samples or standards were added. The IL-10 in the samples was then detected following the addition of a biotinylated rat anti-human IL-10 (JES3-12G8 IgG2a; Pharmingen). Subsequently, horseradish peroxidase (Zymed, San Francisco, Calif.) and the substrate *O*-phenylenediamine dihydrochloride (Sigma) were added, and the absorbance was read at 492 nm and compared to a standard curve derived with recombinant IL-10. The sensitivity of this assay was approximately 80 pg/ml.

(ii) Detection of mRNA. Total cellular RNA from biopsy specimens of gastric antrum (five infected and three uninfected patients) was extracted by a modification of previously described techniques (10) with the Ultraspec II RNA kit (Biotecx Laboratories Inc., Friendswood, Tex.). The amount of RNA was estimated by the Invitrogen Dipstick method (Invitrogen Corp., San Diego, Calif.). RNA was reverse transcribed, and the cDNA product was used as the template for PCR. The cDNA was amplified with primers for IL-12 p40, IL-10, or β-actin. The sequence for the IL-12 5′ primer was CCT GCT GGT GGC TGA CGA CAA T (+435 to +456), and the sequence for the 3′ primer was CTT CAG CTG CAA GTT GTT GGG T (+745 to +724). For IL-10, the sequence for the 5′ primer was ATG CCC CAA GCT GAG AAC CAA GAC CCA (+313 to +339), and the sequence for the 3′ primer was TCT CAA GGG GCT GGG TCA GCT ATC CCA (+664 to +638). PCR was carried out for 35 cycles of 1 min at 94°C and 2 min at 60°C. Specificity of the product was confirmed by Southern blotting after the PCR product was separated on a 2% agarose gel, transferred to a nylon membrane (Hybond-N+; Amersham, Arlington, Ill.), and hybridized with a digoxigenin-labeled probe with the Genius kit according to the manufacturer's instructions (Boehringer Mannheim, Indianapolis, Ind.).

(iii) Cytoplasmic cytokines in gastric T cells. Isolated gastric mononuclear cells were activated with a brief stimulation with phorbol myristate acetate (Sigma) and ionomycin (Calbiochem) for 4 h as described elsewhere (2a). Briefly, isolated gastric mononuclear cells were stimulated by adding an appropriate amount of a stock solution of phorbol myristate acetate and ionomycin to give final concentrations of 50 and 500 ng/ml, respectively. Monensin (Calbiochem) was added from a stock solution to stimulated cells to give a final concentration of 1 M. Cells were incubated at 37°C in 5% CO_2 for 4 h. Subsequently, the cells were labeled with antibodies to CD3 (Pharmingen) and immediately postfixed with 1% paraformaldehyde in PBS by standard techniques. The staining technique for the cytoplasmic cytokines was a modification of the antibody supplier's instructions and a previously described procedure (49). Briefly, after staining for CD3, as described above, cells were fixed in 4% paraformaldehyde in Dulbecco's PBS for 20 min at room temperature, thoroughly washed, resuspended in blocking buffer (1% skim milk, 1% BSA, 0.1% sodium azide [pH 7.4]), and incubated at room temperature for 15 min. Following centrifugation (500 × *g*, 4°C, 6 min) the supernatant was discarded, and cells were resuspended in permeabilization buffer (Dulbecco's minimal essential medium containing 1% BSA, 1% [wt/vol] skim milk, 0.1% [wt/vol] saponin, and 0.1% [wt/vol] sodium azide) and incubated at room temperature for 15 min. Cells were then resuspended in 50 μl of permeabilization buffer, optimal amounts of fluorochrome-conjugated antibodies recognizing IFN-γ and IL-4 (Pharmingen) were added, and the mixture was incubated at 4°C for 30 min. Finally, cells were washed in permeabilization buffer, pelleted, resuspended in Dulbecco's minimal essential medium containing 1% BSA and 0.1% (wt/vol) sodium azide, and analyzed on a FACS Vantage flow cytometer (Becton Dickinson, San Jose, Calif.) as previously described (10).

Statistical analysis. Results are expressed as means ± standard errors of the mean (SEM). Data were compared by the Student's *t* test or the Wilcoxon rank sum test and are considered significant if *P* values were <0.05.

RESULTS

Detection of immunoreactive IL-12 and IL-10 in gastric biopsy specimens. As previous reports have suggested that *H. pylori* stimulates IFN-γ production in vitro (56) as well as in gastric cells isolated from infected patients (21, 34), we evaluated the relative expression of immunoreactive IL-12 and IL-10 in gastric mucosal biopsy specimens obtained from patients that were either infected with *H. pylori* or uninfected and

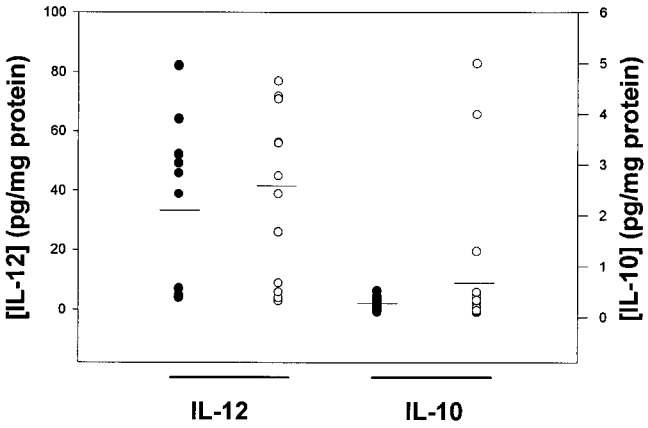

FIG. 1. Detection of immunoreactive IL-12 and IL-10 in gastric biopsy specimens. Gastric biopsy specimens were collected from 16 infected (solid circle) and 18 uninfected (open circle) patients, sonicated, and assayed for IL-12 p40 and IL-10 as described in Materials and Methods. Relatively more IL-12 than IL-10 protein was detected in the gastric biopsy specimens. However, no statistically significant differences in the concentrations of the cytokines were detected between the two groups. (The mean value is indicated as a horizontal line.)

free of microscopic gastritis. The tissue was sonicated in the presence of buffers and protease inhibitors before being assayed for IL-12 and IL-10 by radioimmunoassay or enzyme-linked immunosorbent assay. Whereas IL-10 was virtually undetectable, IL-12 was detected in samples from both infected and uninfected tissue (Fig. 1). No statistically significant difference between the levels of either cytokine was observed in the tissues obtained from the infected and uninfected donors. The low levels of IL-10 were unlikely to be due to adsorption or degradation of the IL-10 as IL-10 could be detected in biopsy material after spiking the samples with exogenous, recombinant human IL-10.

Expression of IL-12 and IL-10 mRNA in gastric biopsy specimens. To evaluate the association between mRNA and protein in the gastric tissue, RNA was extracted from gastric biopsy specimens, reverse transcribed, and amplified by PCR with primers for IL-10, IL-12, and β-actin. After performing reverse transcription-PCR, the PCR products were transferred to membranes and assayed by Southern blotting. The presence of β-actin product in all samples verified the integrity of the mRNA (data not shown). In general, the amount of mRNA for IL-12 p40 varied although it was more easily detected in the infected specimens (Fig. 2). In general, mRNA levels for IL-10 were lower in infected than in uninfected patients. The range

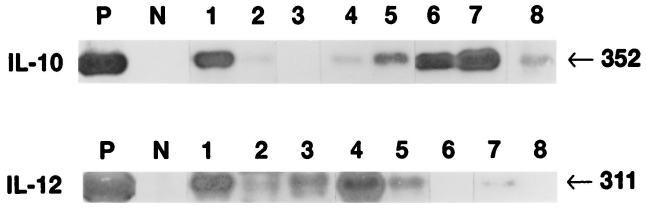

FIG. 2. Expression of IL-12 and IL-10 mRNA in gastric biopsy specimens. Biopsy specimens were collected from infected and uninfected patients as described in the text. Subsequently, RNA was extracted and reverse transcribed, and the resulting cDNA was amplified by PCR with primers for IL-12 p40 and IL-10. A Southern blot of the PCR products with probes specific for IL-10 and IL-12 p40 is shown. P, positive-control template mRNA; N, no template mRNA. Lanes 1 to 5, specimens from infected patients; lanes 6 to 8, specimens from uninfected patients. These results show that mRNA for these cytokines was present in gastric tissue.

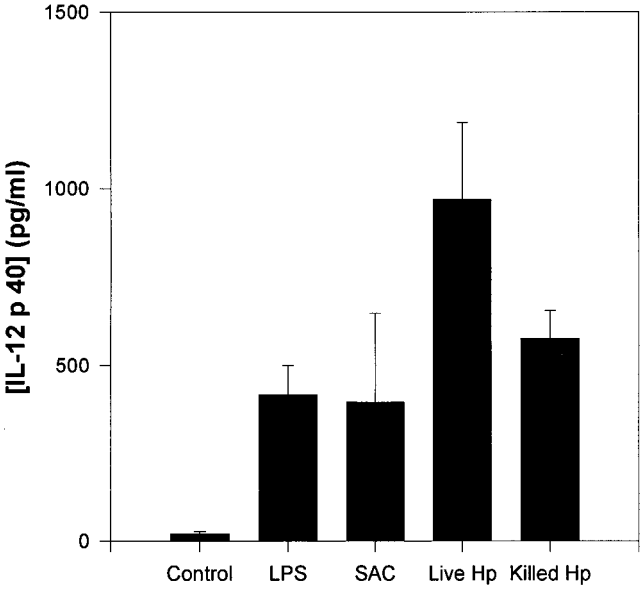

FIG. 3. Induction of IL-12 by *H. pylori*. PBMC obtained from seropositive or seronegative donors were purified and stimulated with SAC, LPS, or live or killed *H. pylori* (10^6/ml). Either live or killed *H. pylori* was capable of inducing an IL-12 response that was significantly greater ($P < 0.05$) than that of the control cells grown in medium alone. The results are means (± SEM) for five separate experiments.

of expression of mRNA resembled the variation in our ability to detect immunoreactive IL-10 and IL-12 proteins as shown in Fig. 1.

Preferential production of IFN-γ by gastric T cells. In view of the evidence that IL-12 was detected in gastric biopsy specimens at significantly higher levels than IL-10, mononuclear cells were isolated from biopsy specimens, activated for 4 h, stained for CD3 to identify them as T cells, and then examined for the expression of intracellular IFN-γ or IL-4. IFN-γ could be detected in $CD3^+$ T cells isolated from the gastric mucosa of both infected (mean [± SEM] percent positive for four subjects, 85.6% ± 12.7%) and uninfected subjects (mean [± SEM] percent positive for four subjects, 63.8% ± 21.5%). In contrast, less than 5% of the $CD3^+$ T cells were shown to produce IL-4 (data not shown). These results suggest that the relative predominance of IL-12 in both infected and uninfected gastric tissue is associated with Th1-like cells.

Induction of IL-12 by *H. pylori*. Since Th1 cells predominate in both uninfected and infected gastric tissue, the ability of *H. pylori* to induce IL-12 was examined. Given the difficulty of isolating sufficient numbers of mononuclear cells from biopsy specimens for in vitro culture, PBMC from infected or uninfected donors were stimulated with 10^6 bacteria/ml of live or killed *H. pylori* for 18 h. This concentration was previously determined to induce both IL-10 and IL-12. SAC (1:10,000 [vol:vol]) and LPS (1 μg/ml) were used as positive controls (36). Subsequently, supernatants were collected and assayed for immunoreactive IL-12 by radioimmunoassay. Live *H. pylori* induced a greater IL-12 response ($P < 0.05$) than the positive-control cultures or the cultures stimulated with killed bacteria (Fig. 3).

The effect of *H. pylori* concentration on cytokine secretion. PBMC from infected or uninfected donors were stimulated with increasing numbers of live *H. pylori* ranging from 10 to 1×10^7 bacteria/ml. After 18 h, the supernatant was collected, and the concentrations of IL-10 and IL-12 were determined. As

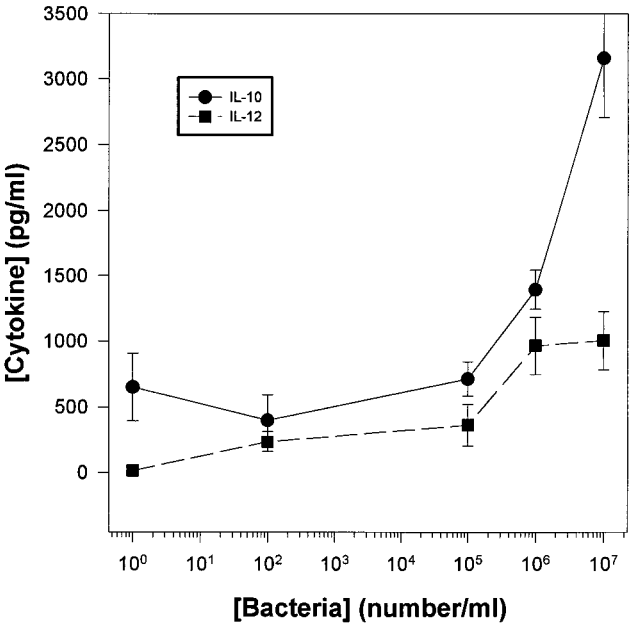

FIG. 4. The effect of *H. pylori* concentration on IL-12 and IL-10 production. Various doses of live *H. pylori* were used to stimulate PBMC obtained from seropositive or seronegative donors. Subsequently, supernatants from stimulated cells were collected and assayed for immunoreactive IL-12 and IL-10. The induction of both cytokines was dependent on the dose of *H. pylori*. The results are means (± SEM) for six to eight separate donors.

shown in Fig. 4, the concentrations of both cytokines increased as the concentration of *H. pylori* increased. Whereas the increase in IL-10 was approximately 5-fold more than the level in control supernatants from cells in medium alone, the concentration of IL-12 increased over 30-fold, from a mean of 30 pg/ml in control supernatants to 1,007 pg/ml, in response to stimulation with 10^7 organisms/ml.

Influence of the immune status on the cytokine response. In order to determine if prior natural exposure to *H. pylori* altered the immune status of the host and affected the detection of IL-12 and IL-10, the cytokine responses of PBMC from seropositive and seronegative donors were compared for a range of doses of *H. pylori*. PBMC from uninfected ($n = 6$) and infected patients ($n = 6$) were exposed to 10^6 bacteria/ml, and the supernatants were collected and assayed. No statistically significant differences in IL-12 or IL-10 production were observed when the seropositive and seronegative groups were compared (Fig. 5).

Comparison of different preparations of *H. pylori* on cytokine production. Previous studies have suggested that persistent infection of mice with *Helicobacter* spp. is associated with Th1-cell responses (47), while mice can be made immune by oral vaccine preparations that favor the induction of a Th2-cell response (5, 12). These vaccines have employed killed, whole-cell preparations of *H. pylori* (38) or recombinant *H. pylori* urease (11, 22, 40, 46, 50). As live bacteria, killed bacteria, and bacterial subunits are known to vary in their ability to direct the differentiation of T cells, we compared the relative production of IL-12 and IL-10 using killed *H. pylori*, live *H. pylori*, and 1.0 μg of recombinant urease/ml. All three stimuli differed significantly in their ability to induce IL-12 and IL-10. Both live and killed *H. pylori* caused a statistically significant increase in the IL-10 concentration over the basal level. In addition, killed *H. pylori* induced a fourfold increase in IL-10 that was significantly greater than the twofold increase induced by live organisms ($P < 0.05$) (Fig. 6). In contrast, live *H. pylori* induced significantly more IL-12 compared to killed organisms ($P < 0.05$). The differential abilities of the live and killed bacterial preparations to induce either cytokine were also evident when the relative ratios of IL-12 and IL-10 were compared. The ratio of IL-10 to IL-12 was 1.4:1 after stimulation with live *H. pylori* in contrast to a ratio of 3.9:1 after stimulation with killed bacteria. Urease alone induced a significant IL-10 response but did not induce an IL-12 response.

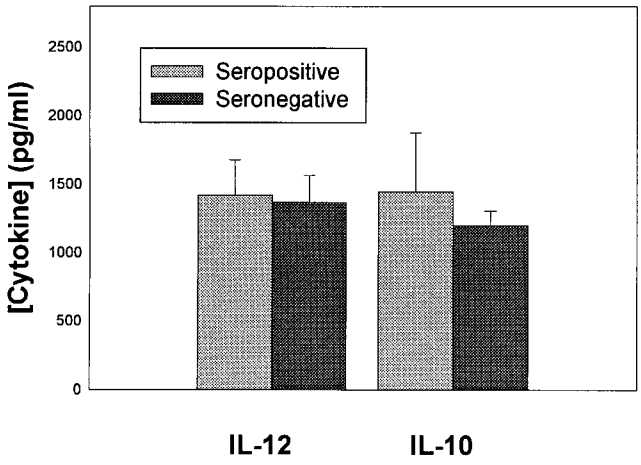

FIG. 5. Influence of the immune status on the cytokine response. As the levels of IL-10 and IL-12 could be modified by prior infection, the cytokine responses of PBMC from seropositive and seronegative donors were compared. Comparable responses were observed in both groups. The results are means (± SEM) of duplicate samples from six separate seropositive and six seronegative donors.

DISCUSSION

Previous studies have shown that *H. pylori* is capable of inducing the production of IFN-γ (56). Recent reports have

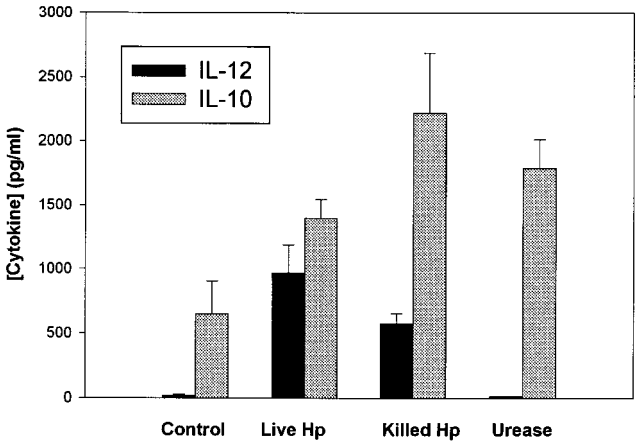

FIG. 6. Effect of the viability of the bacteria on cytokine production. Live or killed *H. pylori* (10^6/ml) or 1.0 μg of *H. pylori* urease/ml was used to stimulate PBMC from either infected or uninfected subjects. Subsequently, supernatants were collected and assayed for immunoreactive IL-12 and IL-10. The results are means (± SEM) for five separate experiments. IL-10 levels were significantly higher after stimulation with killed *H. pylori* compared to those of controls or cells stimulated with live bacteria ($P < 0.05$), while IL-12 levels were greater after stimulation with live *H. pylori* than with killed organisms ($P < 0.05$). In contrast, recombinant urease only induced IL-10.

also shown that the number of IFN-γ-producing cells is increased to a greater degree than that of IL-4-producing cells in the stomach during gastritis (34). However, these reports have not established that the gastric T cells were the source of IFN-γ. The differentiation of helper T cells into Th1 and Th2 cells is mediated in part by IL-12 and IL-10, respectively. In order to evaluate the control of helper T-cell selection by *H. pylori*, this study has examined the relative expression of IL-12 and IL-10 in response to *H. pylori* infection in vivo and in vitro and related these responses to the cytokine production by freshly isolated gastric T cells.

The presence of mRNA and immunoreactive protein for IL-12 in both infected and uninfected gastric tissue suggests that the stomach favors the selection of the Th1 subset of T cells. This notion is supported by the observation that IFN-γ-producing T cells predominated in the gastric mucosa of both infected and uninfected subjects. However, the total number of the Th1-like cells increases during infection (2a). Moreover, a marked increase in the absolute number of IFN-γ-producing cells has been described in both bacterial and noninfectious gastritis (34). These data suggest that Th1 cells predominate in the normal gastric mucosa as well as during inflammation including gastritis associated with *H. pylori* infection.

The variation in the expression of mRNA for IL-12 and IL-10 in gastric tissue was substantial and may have contributed to our inability to identify a statistically significant difference between the levels of immunoreactive protein in infected and uninfected individuals. Moreover, it is possible that higher levels of these cytokines will be detected in patients infected with specific strains of *H. pylori* or in patients with different gastric diseases when these parameters are compared to the results from tissue in which gastritis was the only clinical manifestation of the infection. Despite the variation in the levels of IL-10 mRNA, the gastric tissue had relatively low levels of IL-10. Biologically significant changes in the expression of this cytokine may have been lost by the dilution of the biopsy specimens. Alternatively, selective sampling may obscure important changes in expression associated with infection that may be detectable by histochemical techniques. However, levels of IL-10 and IL-12 in the corpus of the stomach correlated well to those in the antrum of the same patient although the levels in the corpus were generally lower (unpublished observations). Thus, the results suggest that IL-12 does indeed predominate in gastric tissue and may correlate to the predominance of Th1 cells.

Since gastric mononuclear cells are not easily isolated in numbers that permit extensive manipulation in vitro, PBMC were used to examine the ability of *H. pylori* to stimulate cytokine responses. Live *H. pylori* was shown to induce a substantial production of the p40 chain of IL-12 by PBMC. In fact, the response to a range of doses of *H. pylori* was equal to or greater than the response to the positive controls, LPS and SAC. Since these studies were performed using PBMC, it is possible that the response differs from what might be observed in the gastric mucosa. This is evidenced by the fact that *H. pylori* induced IL-10 in PBMC but the concentration of this cytokine was extremely low in gastric tissue. Moreover, *H. pylori* is noninvasive so most responses by cells in the gastric lamina propria are probably driven by soluble material that has crossed the epithelial barrier. Thus, it will be important to characterize the cells responsible for the production of IL-12 in gastric mucosa as well as the response of these cells to soluble factors released by *H. pylori*. These difficulties notwithstanding, it appears as if IL-12 is present in both infected and uninfected tissue and can play a role in regulating the gastric immune response.

Biologically active IL-12 is a heterodimer of 70 kDa composed of p35 and p40 subunits (57). The p40 subunit is virtually always produced in levels that exceed those of p70, and therefore, measuring levels of p40 does not directly predict the induction of IL-12 bioactivity. It has been suggested that monomers and homodimers of p40 may act as antagonists for IL-12 in vitro with murine (44) and human systems (41). However, the role of IL-12 p40 as a significant antagonist in vivo is unlikely as the p35 subunit is essential for optimal receptor binding (41).

Whereas stimulation of monocytes by LPS alone preferentially induces IL-12 p40, priming of monocytes and macrophages with IFN-γ and/or tumor necrosis factor alpha (TNF-α) can stimulate the transcription of both p40 and p35 (42), leading to the production of biologically active IL-12 after exposure to LPS (4, 32) or mycobacterial infection (25). Moreover, IFN-γ has stronger effects on the production of biologically active IL-12 than on the induction of p40 due to its ability to preferentially enhance the production of IL-12 p35 (54). Since TNF-α expression is increased during infection (9) and gastric T cells appear to be of the Th1 type, inflammatory material from *H. pylori* as well as TNF-α and IFN-γ are usually present together and would facilitate the production of biologically active IL-12. This is supported by an earlier study by Tarkkanen et al. showing that *H. pylori* is capable of inducing IFN-γ and enhancing NK cell activity in PBMC (56). As biologically active IL-12 was previously referred to as natural killer cell stimulatory factor (43), it is likely that the results presented by Tarkkanen and colleagues reflect the ability of *H. pylori* to stimulate the production of biologically active IL-12, which ability led to their observed increase in IFN-γ and NK cell activity.

These models assume that IL-12 selects for Th1 cells, but recent studies show that IL-12 can induce both IL-4 and IL-10 production (28, 33, 61). This suggests that IL-12 may combine with other stimuli to select for a particular T-cell phenotype. However, in some of these systems, IL-12 was working directly on T-cell clones and in others it was working in the presence of other modulators such as anti-IL-4 (61). Moreover, one of the major *H. pylori* cellular proteins, urease, induced IL-10 independent of IL-12. Thus, while IL-12 may have contributed to IL-10 production in some studies, in the system described in this report, IL-12 levels were inversely correlated to those of IL-10 in both the in vitro cultures and the gastric tissue.

Further evidence that persistent infection with *Helicobacter* spp. is associated with a Th1 response is found in animal models. Several investigators have shown that *Helicobacter felis* can persistently infect the stomachs of mice (39), and this is associated with a Th1 response (47). The presence of Th1 cells and IFN-γ would be consistent with the marked increase in major histocompatibility complex class II molecules that are expressed on gastric epithelial cells in the inflamed stomach during *H. pylori* infection (6, 58). These gastric Th1 cells may favor the destruction of tissue through cell-mediated immune responses rather than the eradication of this extracellular pathogen (20). The absence of potentially anti-inflammatory cytokines from Th2 cells may prevent the Th1 responses from being restrained. In addition, Th1 cells could enhance the production of complement-fixing antibodies in the gastric tissue. In view of the reports that antibodies produced in response to *H. pylori* can bind to cells within the gastric mucosa and contribute to gastric inflammation (1, 30, 48, 60), gastric damage may be enhanced further by the inappropriate regulation of local B-cell responses by Th1 cells.

In contrast to natural infection, administration of oral vaccines and adjuvants, such as cholera toxin or *E. coli* labile toxin,

that select for a relatively greater Th2 response can clear and prevent infection with *H. felis* (5, 8, 11, 12, 18, 23, 38, 51). Other investigators have shown that these vaccine preparations are also effective at clearing an existing infection (17), possibly by inducing additional, complementary Th2-cell responses. Thus, the selection of the appropriate antigen and adjuvant may facilitate the induction of a response that differs substantially from that induced by natural infection with live *H. pylori*. The fact that killed *H. pylori* and urease alone induced relatively more IL-10 than live *H. pylori* supports the notion that vaccines, including whole-cell vaccines and recombinant urease, may be effective due to their ability to alter the selection of T-cell subsets.

In summary, the data presented in this report support the view that the relative expression of IL-10 and IL-12 in the human stomach favors the development of a subset of helper T cells that are biased to IFN-γ production. In turn, this response is associated with Th1 cells and enhanced cell-mediated immunity. Additional studies are in progress to further define the functional properties of gastric T cells in response to *H. pylori* infections.

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