CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, July 1997, p. 440–446 Vol. 4, No. 4
1071-412X/97/$04.00+0

T-Cell, Antibody, and Cytokine Responses to Homologs of the 60-Kilodalton Heat Shock Protein in *Helicobacter pylori* Infection

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Received 6 January 1997/Returned for modification 20 February 1997/Accepted 14 April 1997

For *Helicobacter pylori*, the hsp60 heat shock protein encoded by *hspB* is being considered as a potential candidate for subunit vaccines. We investigated the humoral and cellular responses to *H. pylori* hsp60 and its cross-reactivity with the homologous *Mycobacterium bovis* p65 protein and autologous human hsp60 protein. *H. pylori*-infected persons had significantly higher levels than uninfected persons of serum immunoglobulin G antibodies recognizing *H. pylori* hsp60, but not *M. bovis* p65 or human hsp60, as determined by enzyme-linked immunosorbent assay. In contrast, immunoblotting demonstrated cross-reactivity of *H. pylori* hsp60 with human hsp60. T-cell recognition of *H. pylori* hsp60 was found in both infected and uninfected subjects, and there was no recognition of human hsp60. T cells from infected and uninfected subjects that had been activated in response to *H. pylori* hsp60 or *M. bovis* p65 were phenotypically similar but appeared to secrete different levels of gamma interferon and interleukin-10. These results demonstrate an apparent difference in the epitopes recognized by the T and B cells responding to *H. pylori* hsp60 in *H. pylori*-infected persons. In contrast to the T-cell responses, which were highly variable in all subjects and showed no recognition of autologous proteins, a specific B-cell response that may have cross-reactivity to human hsp60 is evident in some infected subjects.

Infection with the gram-negative bacterium *Helicobacter pylori* has been recognized as an important public health problem worldwide (5, 16, 31). Essentially all *H. pylori*-infected persons develop chronic superficial gastritis, while a few may develop peptic ulceration or gastric neoplasia after years of infection (9, 33, 37). The pathogenic mechanisms that lead to these diverse outcomes remain to be elucidated (3, 4, 24).

The *H. pylori* 60-kDa heat shock protein (hsp60; also called HspB) (a GroEL homolog) and the 13-kDa HspA protein (a GroES homolog) are being considered as potential candidates for subunit vaccines (15, 19). *H. pylori* hsp60 has many similarities in primary structure to hsp60 homologs (11, 26) and is one of the dominant proteins recognized by most *H. pylori*-infected persons (26, 39). In other infections, stress proteins, including hsp60 homologs, appear to be major targets of host humoral and cellular responses (48). Consequently, they have been considered as potential vaccine candidates that might be capable of eliciting cross-protective responses to a variety of organisms or even as vaccine adjuvants (2, 12).

However, there is evidence that the immune response to the family of 60-kDa heat shock proteins may play a role in the pathogenesis of chronic inflammatory diseases (48). Since heat shock proteins are highly conserved from bacteria through eukaryotes, it has been hypothesized that antibodies or T cells reactive to autologous heat shock proteins may contribute to autoimmune diseases (22, 46, 48). It is important to define the immune responsiveness to these stress proteins in humans, especially since they are being considered as vaccines against *H. pylori* infection (15, 19). In a preliminary investigation, we observed a dichotomy in the T- and B-cell recognition of *H. pylori* antigens, including hsp60, among *H. pylori*-infected persons (39). Although humoral responses to *H. pylori* antigens were restricted to *H. pylori*-infected persons, T-cell recognition, as measured by proliferation to *H. pylori* antigens, was found in infected and uninfected persons to similar degrees.

In the present study, we attempted to characterize in greater detail the cellular and humoral responses to *H. pylori* hsp60 homologs and their cross-reactivity with human and mycobacterial heat shock proteins and to further identify the components of the cellular response that might distinguish between *H. pylori*-infected and uninfected persons. Since we had observed antigenic cross-reactivity of the *H. pylori* and human hsp60 proteins in immunoblots when rabbit hyperimmune sera were used, and because earlier reports had indicated the existence of antibodies cross-reactive between *H. pylori* and host gastric cells (29, 30), we examined human humoral responses by both enzyme-linked immunosorbent assay (ELISA) and immunoblotting.

To test the premise that *H. pylori* infection may lead to polarized immune responses that mediate distinct cellular functions that could lead to either protection or susceptibility as reported for other infections (28, 45), we assessed the levels of type 1- and type 2-associated cytokines (25) in response to *H. pylori* hsp60. Type 1 cells are known to produce interleukin-2 (IL-2) and gamma interferon (IFN-γ) and are involved in the activation of macrophages, cytotoxic T cells, and cell-mediated immunity (25, 28). Type 2 lymphocytes produce IL-4, IL-5, and IL-10 and are responsible for B-cell activation and differentiation; they also have been shown to down-regulate

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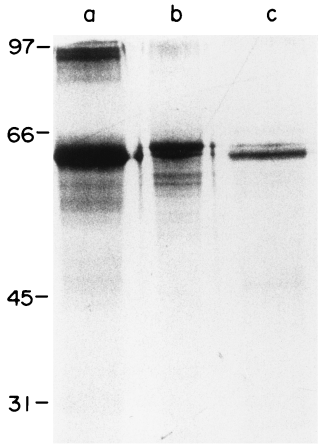

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% acrylamide) of *H. pylori* hsp60 and hsp60 homologs. Lane a, recombinant 60-kDa human heat shock protein (P1); lane b, recombinant 65-kDa protein of *M. bovis* BCG; lane c, purified *H. pylori* hsp60. Protein bands were visualized by silver staining. Molecular masses (in kilodaltons) are on the left.

the inflammatory response to persistently infecting agents (27, 28, 44).

MATERIALS AND METHODS

Subjects. All subjects were adults and gave informed consent prior to participation. These studies were approved by the Vanderbilt University Institutional Review Board. A total of 46 subjects participated in this study. Eighteen persons were not infected with *H. pylori* (mean age, 44.6 years; 72% Caucasian; 5.6% with peptic ulcer disease), and 28 were infected with *H. pylori* (mean age, 51.3 years; 75% Caucasian; 21.4% with peptic ulcer disease). *H. pylori* infection was determined by ELISA with a pool of *H. pylori* whole-cell sonicates, as previously described (34). Of the 28 infected subjects, 7 were asymptomatic and were diagnosed on the basis of positive serology (referred to as seropositive subjects) and 21 had undergone gastroduodenal endoscopy at the Nashville Veterans Affairs Medical Center because of dyspeptic symptoms. For these subjects, *H. pylori* infection was confirmed by culture and histologic examination of gastric biopsy samples and by serology, as described previously (10, 41).

Preparation of antigens. A pooled *H. pylori* sonicate extract was prepared from whole cells as described previously (35). *H. pylori* hsp60 was purified from concentrated culture supernatants from strain 88-23 by fast protein liquid chromatography (Pharmacia, Uppsala, Sweden), as previously described (11). Briefly, concentrated culture supernatants were prepared by centrifuging 48-h broth cultures, concentrating the cell-free supernatant with a Centriprep 30 apparatus (Amicon, Beverly, Mass.), and precipitating it with 60% ammonium sulfate. The precipitate was then analyzed by anion-exchange chromatography on a MonoQ column for purification to homogeneity (11).

Immunoaffinity-purified recombinant *Mycobacterium bovis* p65 was provided by J. D. A. van Embden (National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands) under the auspices of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. Recombinant P1 protein, a 63-kDa heat shock protein of human origin, was provided by R. Gupta (40). The homogeneity of the antigens, as assessed by silver staining of preparations resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (34), is shown in Fig. 1. Tetanus toxoid was obtained from the Massachusetts Institute of Biologics (Boston, Mass.).

ELISA and Western blot procedures for detection of serum antibodies. Serum antibodies directed against hsp60 proteins were assayed by ELISA, as previously described (39), by using 60 ng of antigen/well and serum diluted 1:100 for the assays with the purified hsp60 proteins. Each serum sample was run in duplicate wells and assayed on two or more days. Immunoblotting of human hsp60 (P1 protein) (15 μg/gel) was performed as previously described (34). The human serum samples tested were diluted 1:800 and the rabbit antisera against hsp60 homologs were diluted 1:500 for both *H. pylori* hsp60 and human hsp60 (P1). The preparation and characteristics of polyclonal rabbit antisera to P1 have been described (40). Antiserum to *H. pylori* hsp60 was produced in rabbits, as described previously (11).

Lymphocyte proliferation assay. Heparinized peripheral blood (25 ml) was collected from each subject. The peripheral blood mononuclear cells (PBMC) were separated on a Ficoll-Hypaque gradient (Lymphoprep; Accurate Chemical & Scientific Corp., Westbury, N.Y.), as previously described (39). A 100-μl suspension of 2×10^6 cells/ml in RPMI 1640 supplemented with 10 mM HEPES buffer, 2 mM glutamine, and 20 μg of gentamicin (Whittaker) per ml plus 5% heat-inactivated human AB serum (Advanced Biotechnologies, Inc., Columbia, Md.) was added to each well of flat-bottomed 96-well microtiter plates (Falcon; Becton Dickinson, Lincoln Park, N.J.). The hsp60 homolog antigen preparations were added at concentrations as indicated below for each experiment, and proliferation assays were performed as described previously (39). Results are reported as the mean net counts per minute (Δcpm) representing the difference between mean counts per minute in the presence and absence of antigen. Proliferation was considered positive when the stimulation index was ≥5 and the Δcpm was ≥2,000.

Flow cytometry and cytokine measurement. From a random subset of the patients, in parallel with proliferation experiments, cell cultures also were established for flow cytometry and cytokine analysis. T-cell surface markers were examined on days 2, 4, 6, and 8 of culture with the antigen preparations. In the majority of cases, peak expression of the activation markers HLA-DR and IL-2 receptor (IL-2R) on both $CD4^+$ and $CD8^+$ T cells was observed on day 6 after antigenic stimulation. Supernatants collected from these cultures were analyzed for IL-2, IL-4, IL-10, or IFN-γ levels with commercial ELISA kits (Biosource International, Camarillo, Calif.). Briefly, 2×10^6 PBMC in RPMI 1640 supplemented with 10 mM HEPES buffer, 2 mM glutamine, and 20 μg of gentamicin (Whittaker) per ml plus 5% heat-inactivated human AB serum (Advanced Biotechnologies, Inc.) were added to each well of a flat-bottomed 24-well plate (Falcon; Becton Dickinson) with or without the bacterial hsp60 homologs or crude *H. pylori* cell extract preparation (1 μg/ml). Tetanus toxoid (2 μg/ml) served as a positive control. T cells were phenotypically characterized by dual immunostaining with fluorescein-conjugated anti-CD25 (IL-2R) or anti-HLA-DR and with phycoerythrin-conjugated anti-CD4 or anti-CD8 (all from Becton Dickinson, San Jose, Calif.). Briefly, 10^6 cells were incubated in 100 μl of phosphate-buffered saline with each antibody for 30 min at 4°C, washed twice in cold phosphate-buffered saline containing 0.1% azide and 0.2% bovine serum albumin, and fixed in 1% paraformaldehyde. Five thousand events were analyzed on a FACScan (Becton Dickinson).

Statistical analysis. Values derived for infected and uninfected persons were compared by using Student's *t* test (two-tailed). Differences were considered significant when the *P* value was ≤0.05.

RESULTS

Purified *H. pylori* hsp60 cross-reacts with human hsp60 (P1). In immunoblots, rabbit antiserum against human hsp60 recognized a single band at 54 kDa in whole-cell lysates of *H. pylori* 88-23 in the ammonium sulfate precipitates of culture supernatants and in the fast protein liquid chromatography-purified *H. pylori* hsp60 preparation (Fig. 2). Similarly, purified human hsp60 was recognized as a single band by rabbit antiserum against purified *H. pylori* hsp60 (data not shown). These data indicate the antigenic cross-reactivity of the two related proteins when hyperimmune sera were used.

Characterization of antibody recognition of hsp60 homologs. Next, we assessed the antigenic relatedness of the heat shock protein homologs by analyzing the serum immunoglobulin G (IgG) responses to *H. pylori* and human hsp60 proteins by ELISA and immunoblotting of samples from 32 representative subjects. The 19 *H. pylori*-infected subjects (12 symptomatic and 7 asymptomatic) had significantly higher levels of serum IgG antibodies recognizing *H. pylori* hsp60 than did the 13 uninfected persons (Table 1). The higher response was observed in both symptomatic and asymptomatic infected persons. In contrast, there was essentially no antibody recognition of human hsp60 or *M. bovis* p65 by *H. pylori*-infected or uninfected persons. In the *H. pylori*-infected group, there was no association between patient age and ELISA optical density values obtained with the *H. pylori* hsp60, *M. bovis* p65, or human hsp60 (P1) antigen. Among the 19 *H. pylori*-infected persons, we next assessed whether the levels of antibodies to *H. pylori* hsp60 were related to the levels of antibody to *H. pylori* cellular antigens used in the clinically diagnostic IgG ELISA. Levels of serum IgG to the cellular antigens significantly correlated with the levels of antibody to *H. pylori* hsp60 ($r = 0.47$, $P = 0.04$). In contrast to the data obtained by ELISA, sera from 18 (95%) of 19 *H. pylori*-infected subjects showed recognition of both *H. pylori* hsp60 and human hsp60 in immunoblot analysis. In comparison, sera from only 2 (15%) of 13 unin-

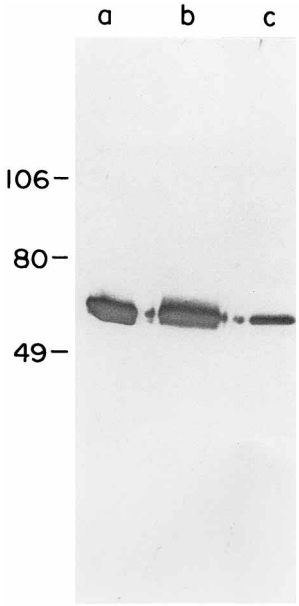

FIG. 2. Immunoblot analysis of *H. pylori* preparations with rabbit polyclonal antisera against human hsp60 (P1). Antigens (2 μg of protein) were as follows: lane a, *H. pylori* 88-23 whole cells; lane b, 60% ammonium sulfate precipitate from culture supernatant; lane c, purified *H. pylori* hsp60. Molecular masses (in kilodaltons) are on the left.

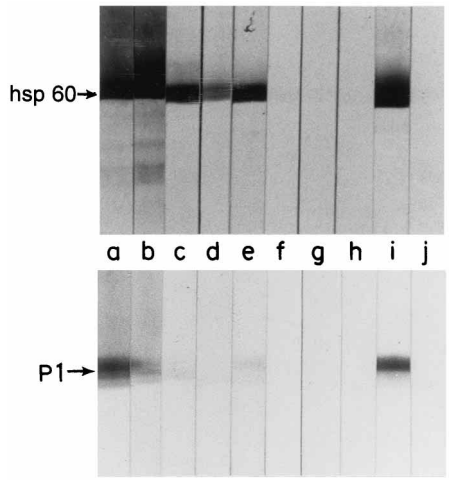

FIG. 3. Immunoblot of *H. pylori* hsp60 and human hsp60 (P1) with human sera. Each lane contained 2 μg of protein. The sera (1:800 dilution) tested were from five representative persons infected with *H. pylori* (lanes a to e) and from five uninfected persons (lanes f to j).

fected persons recognized *H. pylori* hsp60 and human hsp60. Representative data for five infected and five uninfected persons are shown in Fig. 3; the intensity of binding varied among the infected persons examined. These studies demonstrate that detection of cross-reacting antibodies to human hsp60 among *H. pylori*-infected subjects is dependent on the analytical technique employed; cross-reactivity is shown by immunoblotting with denatured antigens but not by ELISA with native antigens.

Peripheral blood lymphoproliferative responses to *H. pylori* hsp60 and its homologs. To characterize the T-cell response to the hsp60 homologs, lymphoproliferative responses to these molecules in 13 infected and 9 uninfected subjects were compared. All subjects responded to tetanus toxoid (positive control) and to a crude *H. pylori* extract preparation, as observed previously (data not shown). Marked heterogeneity in proliferative responses to *H. pylori* hsp60 was observed in both *H. pylori*-infected and uninfected persons, but responses were substantially lower than that to tetanus toxoid (Fig. 4). Lymphocytes from 4 of the 13 infected subjects studied responded to *H. pylori* hsp60, and lymphocytes from 5 responded to *M. bovis* p65. Of the nine uninfected persons studied, five responded to *H. pylori* hsp60 and six responded to *M. bovis* p65. None of the infected or uninfected subjects responded to human hsp60 (data not shown). There was no significant difference in the responses to *H. pylori* hsp60, *M. bovis* p65, or tetanus toxoid that distinguished infected from uninfected persons. The similarity between T-cell responses to *H. pylori* hsp60 in *H. pylori*-infected and uninfected persons is in marked contrast to the specific B-cell response observed in infected patients (Table 1) and confirms our earlier findings (39).

TABLE 1. Serum IgG responses to hsp60 homologs as measured by antigen-specific ELISA

Antigen[b]	Optical density[a]		*P* value[e]
	H. pylori infected[c] (n = 19)	Uninfected[d] (n = 13)	
H. pylori hsp60	0.42 ± 0.07	0.06 ± 0.03	<0.0002
Human p60	0.02 ± 0.005	0.04 ± 0.01	NS
M. bovis p65	0.08 ± 0.02	0.08 ± 0.05	NS

[a] Values are means ± standard errors of the means.
[b] Each antigen was used at 60 ng/well.
[c] Twelve were positive by examination of endoscopic biopsy samples; seven were seropositive asymptomatic persons.
[d] Negative by examination of endoscopic biopsy samples.
[e] As determined by independent *t* test, two-tailed. NS, not significant.

Phenotypic expression of the activation markers IL-2R and HLA-DR on $CD4^+$ and $CD8^+$ T cells after PBMC stimulation with *H. pylori* hsp60 and its homologs. Since there was no overall difference in the T-cell reactivity to *H. pylori* hsp60 among *H. pylori*-infected and uninfected persons, we next sought to determine whether we could identify a component(s) of the cellular response that might distinguish between these groups. To this end, using flow cytometry, we monitored the expression of the activation markers IL-2R and HLA-DR on $CD4^+$ and $CD8^+$ T cells in PBMC cultures (Fig. 5). Among four infected and four uninfected persons whose cells showed proliferative responses to *H. pylori* hsp60, we observed a two- to threefold increase in the percentage of activated $CD4^+$ and $CD8^+$ T cells in cells cultured with *H. pylori* hsp60. Stimulation with *M. bovis* p65 yielded similar results for the infected and uninfected groups. There was no difference between the *H. pylori*-infected and uninfected persons in the percent positivity for IL-2R or HLA-DR expression for the $CD4^+$ and $CD8^+$ T-cell populations after stimulation with *H. pylori* hsp60, *M. bovis* p65, or tetanus toxoid. For tetanus toxoid-stimulated cultures, however, the increase in activated $CD4^+$ IL-$2R^+$ and $CD8^+$ IL-$2R^+$ populations was much greater than for cultures stimulated with the hsp60 preparations, results that parallel the lymphoproliferative responses to these antigens. Thus, even among the subset of subjects who show proliferative responses to the hsp60 homologs, we found no difference between infected and uninfected persons in the expression of these particular T-cell activation markers.

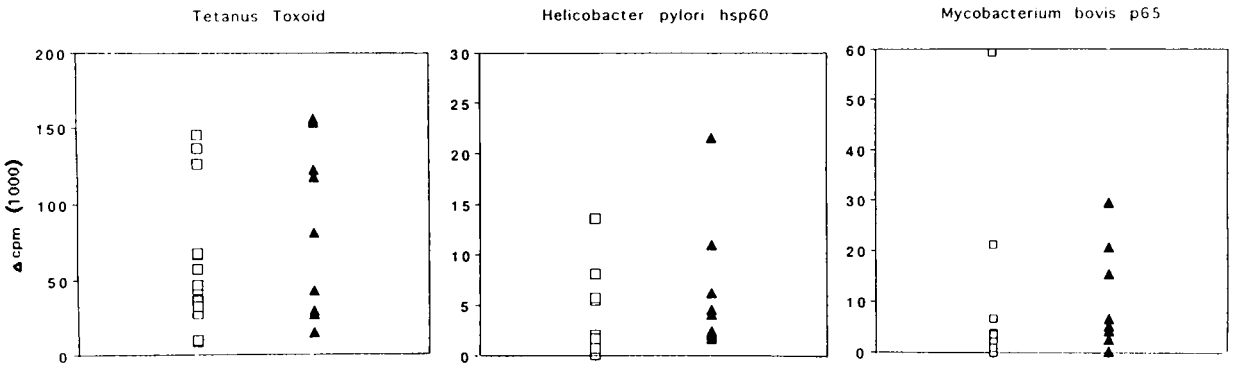

FIG. 4. PBMC proliferation after 8 days of stimulation with *H. pylori* hsp60, *M. bovis* 65-kDa protein, and tetanus toxoid. Antigens were added at a final concentration of 5 μg/ml (0.1 μM) to cells from *H. pylori*-infected (squares) or uninfected (triangles) persons. Data are expressed as the mean net [^{3}H]thymidine incorporation for triplicate cultures. Each symbol represents the results obtained for an individual subject. The results (means ± standard deviations) for unstimulated cultures were 610 ± 293 and 851 ± 599 cpm for infected and uninfected persons, respectively.

PBMC cytokine secretion after stimulation with *H. pylori* hsp60 and its homologs. Next, using ELISA, we analyzed the supernatants from antigen-stimulated PBMC cultures for type 1 (IL-2 and IFN-γ) and type 2 (IL-4 and IL-10) cytokine production. For both *H. pylori*-infected and uninfected persons, the levels of IL-2 and IL-4 were negligible in supernatants from cultures stimulated with an *H. pylori* sonicate extract or hsp60 (data not shown). IFN-γ and IL-10 production in hsp60-stimulated PBMC cultures generally peaked on days 6 and 1, respectively, for both *H. pylori*-infected and uninfected

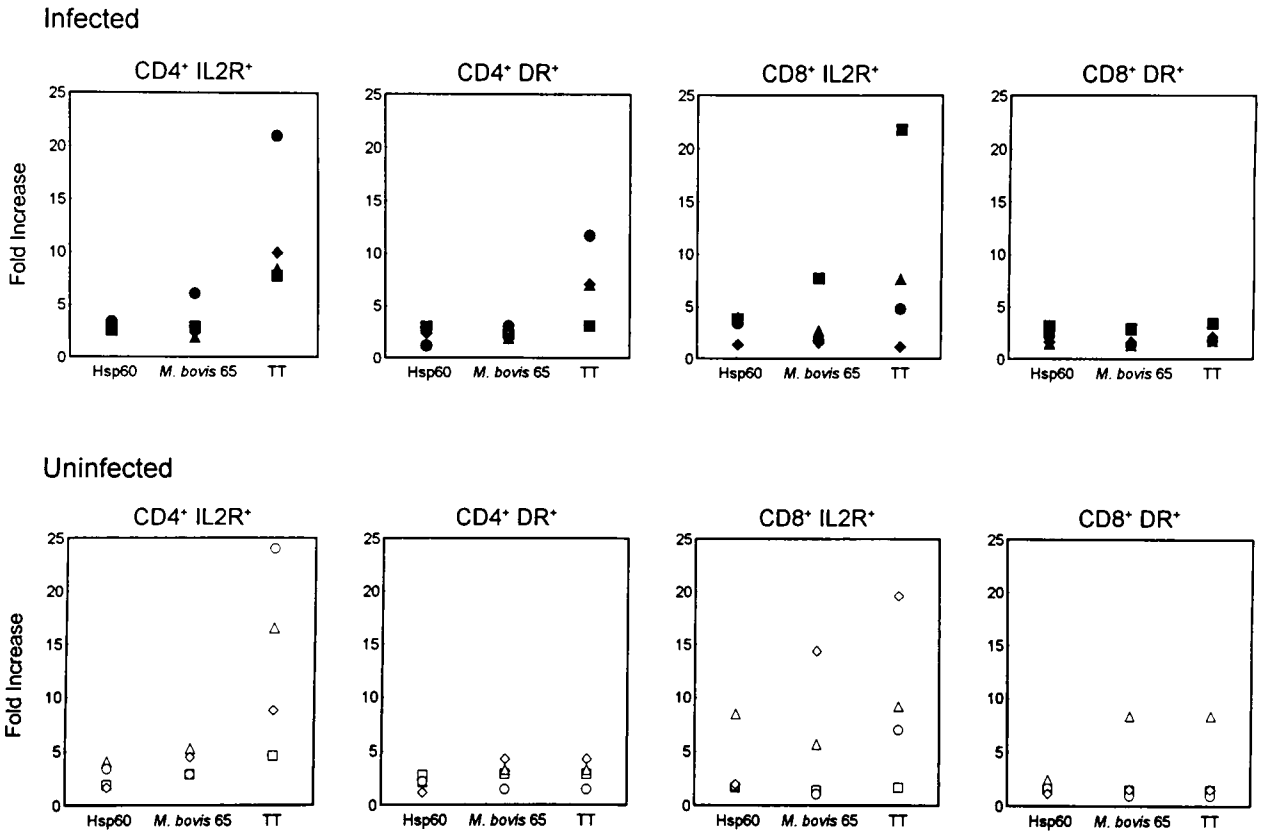

FIG. 5. Expression of the activation markers IL-2R and HLA-DR on $CD4^+$ and $CD8^+$ lymphocyte subpopulations, from *H. pylori*-infected (closed symbols) and uninfected (open symbols) persons, stimulated with *H. pylori* hsp60, *M. bovis* p65 (65), or tetanus toxoid (TT). The increase in expression of IL-2R and HLA-DR on lymphocyte subpopulations in day 6 PBMC cultures was determined by dividing the percentage of positive cells in antigen-stimulated cultures by the percentage of positive cells in unstimulated cultures. Expression of the activation markers IL-2R (CD25) and HLA-DR on $CD4^+$ and $CD8^+$ cells was detected by dual staining with monoclonal antibodies and analyzed by flow cytometry as described in Materials and Methods. Each symbol represents an individual subject. No statistically significant difference between the groups was observed for any antigen.

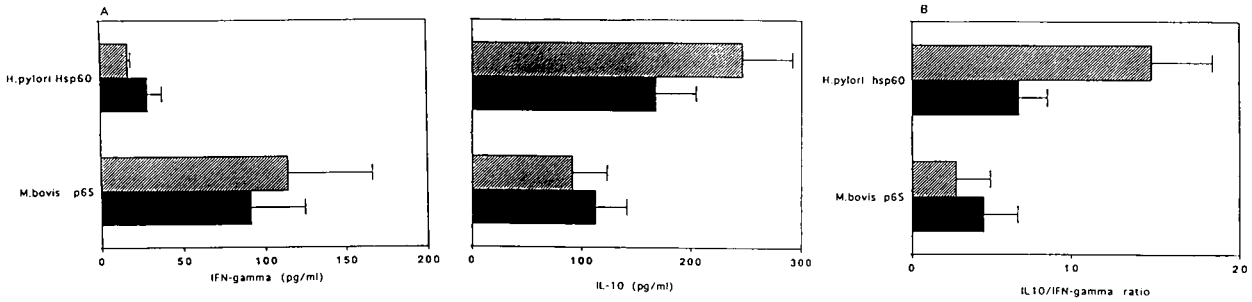

FIG. 6. Production of IFN-γ and IL-10 by antigen-stimulated PBMC cultures from *H. pylori*-infected (gray bars; $n = 12$ for hsp60 and 13 for p65) or uninfected (black bars; $n = 7$ for hsp60 and 8 for p65) persons. Error bars indicate standard deviations. (A) Concentrations of IFN-γ and IL-10 were detected by ELISA in supernatants from day 6 and day 1 stimulated cultures, respectively. (B) Ratio of day 1 IL-10 to day 6 IFN-γ in supernatants of PBMC cultures stimulated with *H. pylori* hsp60 and *M. bovis* p65 from *H. pylori*-infected or uninfected persons.

persons (data not shown). Levels of IFN-γ in cultures stimulated with *M. bovis* p65 were three- to fourfold higher than those induced by *H. pylori* hsp60 ($P < 0.04$) (Fig. 6A). While *M. bovis* p65-stimulated PBMC cultures from both *H. pylori*-infected and uninfected persons produced comparable levels of IFN-γ and IL-10, *H. pylori* hsp60-stimulated cultures produced more IL-10 than IFN-γ. The ratio of day 1 IL-10 levels to day 6 IFN-γ levels in cultures stimulated with *H. pylori* hsp60 was significantly greater ($P < 0.03$) among uninfected persons than in the infected group (Fig. 6B). Thus, although the magnitudes of the T-cell proliferative responses to *H. pylori* hsp60 and *M. bovis* p65 were similar, there were significant differences in the cytokine IL-10/IFN-γ ratios on activation with the hsp60 homologs.

DISCUSSION

Although *H. pylori* infection invokes a vigorous immune response consisting of local and systemic antibody release concomitant with the generation of a dense local cellular infiltrate with neutrophils, lymphocytes, and macrophages, this response is not effective in eliminating the infection and could play a role in tissue injury (3, 4, 47). Antibodies cross-reactive between *H. pylori* and host gastric epithelial cells have been reported (29), and the intensity of this cross-reactivity may correlate with the severity of the injury (30). If *H. pylori* expression of conserved and immunodominant heat shock proteins induces an autoimmune response (13, 29), such host injury could be a problem for vaccines, especially those based on whole cells.

Our analysis of the systemic response to hsp60, measured by serum antibody production, PBMC proliferation, and cytokine production, may not reflect the local B- and T-cell response or cytokine patterns generated with *H. pylori* antigens in the gastric mucosa, but the data provide insight into the immunogenicity and degree of cross-reactivity between *H. pylori* hsp60 and its mycobacterial and human homologs. Since rabbit antibodies against human hsp60 demonstrated cross-reactivity with *H. pylori* hsp60, we sought to further investigate the immune response to *H. pylori* hsp60 and its homologs in *H. pylori*-infected and uninfected subjects. One finding of our study is that cross-reactivity of *H. pylori* hsp60 with human hsp60, while not shown by ELISA, was demonstrated by immunoblotting, especially with sera from infected persons. However, unlike antibodies, T-cell proliferative responses to *H. pylori* hsp60, but not to the autologous human hsp60 protein, were evident in both *H. pylori*-infected and uninfected persons.

The possibility that cross-reactive serum IgG antibodies recognizing human hsp60 may be induced by *H. pylori* hsp60 during infection warrants further study. Self-reactive B-cell immunity to hsp60 appears to contribute to pathology in chlamydial infection (46). Similar cross-reactivity could play a role in *H. pylori*-induced gastric injury and is consistent with the observation that in *H. pylori*-infected persons, the magnitude of IgG responses to hsp60 is significantly associated with the severity of mucosal inflammation (36).

Priming of the immune system to the hsp60 family, whose members are the immunodominant proteins of many bacteria, is a common phenomenon occurring early in life (7). Subclinical infections by a variety of microbes boosting the immune response to heat shock proteins (48) could explain the T-cell responses to hsp60 that we observed in some of the uninfected persons. The heterogeneity in the individual T-cell responsiveness to *H. pylori* hsp60 and its homologs confirmed our earlier observations (39) and may reflect underlying differences in host recognition of hsp60 molecules in general. Although heterogeneity in T-cell recognition of *Mycobacterium leprae* hsp60 has been correlated with the polar types of leprosy (18, 38), we found no such correlation between the T-cell reactivity to hsp60 and the disease outcome (peptic ulcer disease versus no symptoms) in *H. pylori*-infected subjects (data not shown), although our sample was small. That both $CD4^+$ and $CD8^+$ T-cell populations were activated by *H. pylori* hsp60 and mycobacterial hsp65 in both *H. pylori*-infected and uninfected persons confirms observations of Kartunnen, who used a formalin-inactivated *H. pylori* preparation and purified protein derivative (20). Since *H. pylori* persists in the gastric mucosa, inducing increased expression of hsp60 by stressed gastric cells (13), and *H. pylori*-specific $CD4^+$ T cells are present in the mucosa (8), a study of the responses to hsp60 homologs by cells present at the site of infection may be more relevant.

In a mouse model of arthritis, mycobacterial hsp65 stimulates either a type 1- or a type 2-like response, depending on the experimental conditions and mode of priming (1, 12). In both *H. pylori*-infected and uninfected subjects, PBMC activated in response to *H. pylori* hsp60 or mycobacterial hsp65 secreted the lymphokines IFN-γ and IL-10. It is likely that in addition to $CD4^+$ T cells, IL-10 may have been produced by other cell types, including monocytes, B cells, and $CD8^+$ T cells, whereas IFN-γ may have been derived from NK cells and $CD8^+$ T cells.

Although the number of subjects studied was small, we included representative asymptomatic and symptomatic persons to avoid selection bias. We used total PBMC cultures rather than a selective population of T cells or T-cell clones, which would have required at least 100 ml of blood for simultaneous

analysis of proliferation and cytokines and for flow cytometry studies. One goal of the present study was to define parameters that could form the basis for future studies. The lack of detection of IL-2 and IL-4 secretion in PBMC supernatants after stimulation with *H. pylori* hsp60 or a crude extract of the whole bacterium confirms observations made by Kartunnen (20), who used a whole-bacterium preparation. Recent reports have questioned the type 1-type 2 paradigm, since in both mice and humans, T-cell populations that display restricted cytokine profiles that are neither type 0-, type 1-, nor type 2-like appear to be present (21).

IL-10 is an essential immunoregulator in the intestinal tract, and IL-10-deficient mice develop chronic intestinal inflammation (23). The significantly greater IL-10/IFN-γ ratios observed in *H. pylori* hsp60-stimulated PBMC cultures from uninfected than from *H. pylori*-infected persons suggest that an *H. pylori*-mediated down-regulation of type 2 anti-inflammatory reactions may occur. Such a phenomenon could counterbalance the effects of the proinflammatory cytokines tumor necrosis factor, IL-6, and IL-8, the levels of which are elevated in *H. pylori*-infected persons (6, 14, 17, 32). This difference in responses between infected and uninfected subjects could be the basis of future studies using, for example, IL-2-primed PBMC. Although colonization with *H. pylori* does not appear to increase γσ T-cell receptor intraepithelial lymphocytes (43), contact of lymphocytes with glutaraldehyde-fixed *H. pylori* cells augments NK cell activity and induces IFN-γ production (42). The preponderant IL-10 response to hsp60 that we report, which suggests a type 2 response, may reflect methodological differences between these studies.

In summary, the results of this study demonstrate an apparent difference in the epitopes seen by the T and B cells responding to *H. pylori* hsp60 in *H. pylori*-infected persons. In contrast to the T-cell responses, which were highly variable in all subjects and showed no recognition of autologous proteins, a specific B-cell response which shows cross-reactivity to denatured human hsp60 antigens may occur in infected subjects. Whether or not this finding has biological significance in relation to natural infection or vaccination strategies remains to be determined.

ACKNOWLEDGMENTS

This study was supported in part by R01 DK 50837 from the National Institutes of Health and the Medical Research Service of the Department of Veterans Affairs.

We thank J. D. A. van Embden and R. Gupta for reagents used in this study.

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