INFECTION AND IMMUNITY, Sept. 2005, p. 5612–5619 Vol. 73, No. 9
0019-9567/05/$08.00+0 doi:10.1128/IAI.73.9.5612–5619.2005

Helicobacter pylori-Specific CD4$^+$ T Cells Home to and Accumulate in the Human *Helicobacter pylori*-Infected Gastric Mucosa

Anna Lundgren,[1]* Christina Trollmo,[2] Anders Edebo,[3] Ann-Mari Svennerholm,[1] and B. Samuel Lundin[1]

Department of Medical Microbiology and Immunology and Göteborg University Vaccine Research Institute, Göteborg University, Göteborg, Sweden[1]; Unit of Rheumatology, Department of Medicine, Karolinska Institutet, Stockholm, Sweden[2]; and Department of Surgery, Göteborg University, Göteborg, Sweden[3]

Received 12 January 2005/Returned for modification 23 March 2005/Accepted 11 May 2005

Helicobacter pylori **infects the stomach and duodenal mucosa. T cells are important components of the *H. pylori*-induced immune response, but little is currently known about how these cells are recruited to the infected mucosa. Here, we have characterized stomach and duodenal T cells isolated from *H. pylori*-infected and noninfected subjects with regard to subtype, expression of homing and chemokine receptors, and in vitro reactivity to *H. pylori* antigens. Higher numbers of CD4$^+$ but similar numbers of CD8$^+$ lamina propria T cells were isolated from stomach biopsies from *H. pylori*-positive compared to *H. pylori*-negative individuals. CD4$^+$ T cells from infected stomach expressed increased levels of the homing receptor L-selectin and the chemokine receptor CCR4 compared to CD4$^+$ T cells from uninfected stomach. Infected stomach mucosa also contained increased levels of the CCR4 chemokine ligand MDC/CCL22. In contrast, comparable numbers of CD4$^+$ T cells with similar receptor expression were isolated from the duodenum of *H. pylori*-positive and *H. pylori*-negative individuals. In vitro proliferation of mucosal T cells was strongly enhanced by the addition of interleukin-2 (IL-2) and IL-7 to the cell cultures. Using this approach, *H. pylori*-specific T-cell responses were detected in stomach CD4$^+$ T cells from *H. pylori*-positive but not *H. pylori*-negative individuals. Duodenal T cells from only a few individuals responded to *H. pylori* stimulation, and the responsiveness was not restricted to *H. pylori*-positive individuals, suggesting limited *H. pylori* specificity in the duodenum and possible cross-reactivity with antigens from other bacteria in this compartment. In conclusion, these results suggest that *H. pylori*-specific CD4$^+$ T cells preferentially home to and accumulate in the infected stomach and that L-selectin and CCR4/MDC are important for this recruitment.**

Helicobacter pylori colonizes the mucus layer and the epithelium in the stomach as well as areas of gastric metaplasia in the duodenum. Most infected individuals remain asymptomatic, but 10 to 15% of those infected develop peptic ulcers, and 1 to 2% develop gastric cancer (13). *H. pylori* infection induces gastritis with infiltration of neutrophils, macrophages, dendritic cells, as well as B and T cells into the stomach lamina propria (13, 38). A majority of *H. pylori*-infected individuals also develop duodenitis (16, 44), but less is known about the duodenal immune responses to the infection.

T cells seem to play an important role in the immunity against *H. pylori*. The mucosal inflammation induced by *H. pylori* is T-cell dependent, as *H. pylori* infection does not induce gastritis in T-cell-deficient mice, unless T cells are transferred to the mice (10). However, the natural T-cell responses to *H. pylori* are not protective, as the infection normally remains for life. Recent studies indicate that regulatory T cells suppress the T-cell responses to *H. pylori* infection, thereby contributing to the persistent bacterial colonization (25, 26, 36). However, vaccination can induce partial protection against *Helicobacter* infections in mice, and CD4$^+$ T cells have been shown to be important for this protection. Thus, protection cannot be achieved in mice lacking CD4$^+$ T cells, whereas protection can be induced in the absence of B cells or CD8$^+$ T cells (12, 31, 42). The natural T-cell response to *H. pylori* is of the Th1 type (23), and this type of response also seems to be involved in vaccine-induced protection against the infection (3).

To achieve protection after vaccination, T cells have to be recruited to the stomach mucosa. Thus, Michetti et al. demonstrated that immunization-induced protection against *H. felis* infection is abolished by administration of antibodies that block the interaction between the $\alpha_4\beta_7$ integrin expressed by gut-homing lymphocytes and the mucosal addressin cellular adhesion molecule 1 (MAdCAM-1) expressed on endothelial cells in intestinal as well as gastric mucosa (28). Less is known about how T cells are recruited to the gastrointestinal mucosa during natural *H. pylori* infection. However, indirect evidence suggests that the homing receptors $\alpha_4\beta_7$ and L-selectin both mediate homing of *H. pylori*-specific T cells to *H. pylori*-infected human mucosa. Thus, circulating T cells that express $\alpha_4\beta_7$ or L-selectin respond more to in vitro stimulation with *H. pylori* antigens than cells that lack expression of these receptors (34). In addition, chemokines may influence T-cell recruitment to *H. pylori*-infected mucosa. In the intestine, large proportions of T cells express the chemokine receptors CXCR3 and CCR5, which both have been associated with a Th1 cytokine profile, whereas chemokine receptors associated with Th2 polarization, such as CCR4 and CCR3, are expressed at a low extent (1). In the *H. pylori*-infected stomach, the CXCR3 ligands IP-10 (CXCL9) and MIG (CXCL10) and the CCR5 ligand RANTES (CCL5) have been detected (11, 30, 40,

* Corresponding author. Mailing address: Department of Medical Microbiology and Immunology, Göteborg University, Box 435, 405 30 Göteborg, Sweden. Phone: 46 31 7736213. Fax: 46 31 7736205. E-mail: anna.lundgren@microbio.gu.se.

46), but whether the CXCR3 and CCR5 receptors are involved in recruitment of *H. pylori*-specific T cells to *H. pylori*-infected mucosa is currently unclear.

In this study, we have analyzed the T-cell responses to *H. pylori* in the antral and duodenal mucosa and factors controlling T-cell recruitment to these sites. This has included characterization of the phenotype and specificity of antral and duodenal T cells in *H. pylori*-infected as well as uninfected individuals to determine (i) which T-cell subsets are present in antral and duodenal mucosa, (ii) what mechanisms are involved in T-cell recruitment to and retention within the antrum and duodenum, and (iii) if *H. pylori*-specific T cells are present in both the antral and duodenal mucosa of *H. pylori*-infected individuals.

MATERIALS AND METHODS

Volunteers and collection of specimens. Seventeen adult Swedes infected with *H. pylori* without subjective symptoms of the infection (median age, 54 years; age range, 24 to 61 years; four females) and 15 healthy, uninfected volunteers (median age, 32 years; age range, 22 to 63 years; six females) were included in the study. All study subjects were recruited among blood donors at the Sahlgrenska University Hospital, Göteborg, Sweden, and none of them had any previous history of gastrointestinal symptoms or illnesses or was on any medication during the preceding 3 weeks before participation. Sera from the volunteers were screened for *H. pylori*-specific immunoglobulin G antibodies using an in-house enzyme-linked immunosorbent assay (ELISA) (27), and the *H. pylori* infection status was later confirmed by culture and histology.

Biopsies were collected from the antrum and duodenum by gastroduodenal endoscopy. For flow cytometric analysis of T-cell markers, lymphocytes were isolated from 8.5 pooled biopsies, corresponding to ~16 mg tissue, from each site and individual. We have previously shown that similar numbers of $CD4^+$ and $CD8^+$ T cells are present in metaplastic and normal areas of the duodenum (41), and therefore, duodenal biopsies were collected without separation of the two types of mucosa in the present study. In addition, 1 to 2 biopsies from each individual were stored at −70°C until the biopsies were subjected to saponin extraction and chemokine analysis. Half a biopsy from the antrum and duodenum, respectively, was examined by an experienced histopathologist, and the inflammation was graded according to the Sydney system (32). All *H. pylori*-positive individuals had moderate chronic gastritis, and in a majority of the subjects, the inflammation was active, whereas no signs of gastritis were observed in the *H. pylori*-negative subjects. The majority of the *H. pylori*-positive volunteers also had mild duodenitis. Heparinized venous blood was collected at the time of endoscopy. The study was approved by the Ethical Committee for Human Research, Göteborg University, and informed consent was obtained from each volunteer before participation.

Diagnosis of *H. pylori* infection. Bacteria were cultured from the biopsies on horse blood Colombia Iso agar plates at 37°C under microaerobic conditions (10% CO_2, 5% O_2, and 85% N_2), and the plates were screened for *H. pylori* colonies after 3 days. *H. pylori* bacteria could be cultured from all *H. pylori*-positive volunteers but not from any of the *H. pylori*-negative individuals included in the study. Half a biopsy from the antrum and duodenum from each volunteer was examined by a histopathologist. *Helicobacter*-like organisms were detected in samples from all *H. pylori*-positive volunteers but not in any of the *H. pylori*-negative subjects. All *H. pylori*-positive individuals also had elevated levels of *H. pylori*-specific immunoglobulin G antibodies in serum compared to those of the *H. pylori*-negative volunteers, as determined by an in-house ELISA (27).

Isolation of lymphocytes. Lamina propria lymphocytes (LPLs) were isolated from the biopsies as previously described (25). Briefly, the epithelium was first removed by EDTA/dithiothreitol treatment, and LPLs were then isolated by collagenase-DNase digestion. Initial experiments showed that this cell isolation protocol gave a maximal yield of cells, with little of the epithelium remaining in the lamina propria fraction, and that the isolation procedure had no or only marginal effects on the expression of different cell surface markers. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood by density gradient centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden).

Flow cytometric staining and analysis. LPLs (5×10^4 to 1×10^5 cells/sample) were stained for various cell surface markers using combinations of the following antibodies: anti-CD3-fluorescein isothiocyanate (FITC), anti-CD19-FITC, anti-CD45RA-FITC (all from BD Pharmingen, San Diego, CA), anti-CXCR3-FITC (R&D systems, Minneapolis, MN), anti-CD38-phycoerythrin (PE), anti-CD62L-PE, anti-β7-integrin-PE, anti-CCR4-PE, anti-CD4-PerCP (all from BD Pharmingen), anti-CCR3-PE, anti-CCR5-PE (all from R&D systems), and anti-CD8-allophycocyanin (Diatec, Oslo, Norway). After staining, the cells were fixed in formaldehyde and analyzed by flow cytometry using a FACSCalibur equipped with blue and red lasers (BD, San Jose, CA).

The flow cytometry data were analyzed using the computer software FlowJo (Tree Star Inc.). Lymphocytes were gated via their forward and side scatter properties, and T cells were identified based on their expression of CD4 and/or CD8. Control samples showed that the contamination of $CD3^-$ cells in the CD4 and CD8 gates was negligible (<0.5%). Unstained cells and cells stained with isotype-matched control antibodies served as controls. The numbers of different lymphocyte subsets isolated from 8.5 pooled biopsies from each site and individual were estimated by multiplying the frequencies of $CD4^+$, $CD8^+$, and $CD19^+$ cells detected by flow cytometry with the total numbers of isolated LPLs.

Antigens. A membrane preparation (MP) was prepared from *H. pylori* strain Hel 305 isolated from a duodenal ulcer patient. This strain carries an intact *cag* pathogenicity island and expresses the vacuolating cytotoxin (VacA s1/m1) and $Lewis^X$ antigen. The bacteria were grown on blood agar plates under microaerobic conditions for 3 days, and the MP was prepared by sonication of the bacteria followed by differential centrifugation as previously described (5). Gel electrophoresis of the MP showed that it contained more than 20 different proteins, among which urease, the neutrophil activating protein, the *H. pylori* adhesin A, and flagellin were identified by Western blotting using monoclonal antibodies specific for the different antigens (22, 43).

T-cell stimulation and proliferation assays. LPLs were cultured in round-bottomed 96-well plates (1×10^4 cells per well) at 37°C in 5% CO_2 in Iscove's medium supplemented with 3 μg/ml L-glutamine, 50 μg/ml gentamicin, and 5% human AB^+ serum. LPLs were stimulated with MP (5 μg/ml), interleukin-2 (IL-2) (80 pg/ml; BD Pharmingen), and IL-7 (10 ng/ml; R&D Systems) in the presence of autologous, irradiated (5,000 rad) PBMCs (1.5×10^5 cells/well). After 48 h, 100 μl of the cell culture supernatant was removed from each well and replaced with fresh medium with IL-2 (160 pg/ml) and IL-7 (20 ng/ml). The cells were cultured for another 2 weeks, during which the medium was replaced twice a week, as described above. The cells were then harvested, washed, and restimulated (2.5×10^4 cells/well) with MP (5 μg/ml) in the presence of autologous, irradiated PBMCs (1.5×10^5 cells/well) but in the absence of IL-2 or IL-7. After 48 h, 100 μl of the cell culture supernatant was collected from each well, the cells were pulsed with 0.5 μCi of [^{3}H]thymidine (Amersham, Uppsala, Sweden) per well for 16 h, and the incorporated radioactivity was analyzed with a scintillation counter. All collected supernatants were stored at −70°C until they were later analyzed for their cytokine content. Control cells were stimulated with medium alone during both primary stimulation and restimulation. During primary stimulation, control cells were also stimulated with IL-2 and IL-7 alone in the absence of MP.

Chemokine and cytokine analysis. The concentrations of gamma interferon (IFN-γ), IL-4, IL-5, IL-10, and tumor necrosis factor alpha in cell culture supernatants were measured by cytometric bead array (human Th1/Th2 cytokine kit; BD Pharmingen). For analysis of mucosal chemokines, proteins were extracted from 1 to 2 biopsies from each site and individual by incubation in phosphate-buffered saline with saponin (2%), soybean trypsin inhibitor (100 μg/ml), phenylmethylsulfonyl fluoride (350 μg/ml), and bovine serum albumin (0.1%) overnight at 4°C. After centrifugation, the supernatants were collected and stored at −70°C until analyzed for chemokine and total protein content. The concentrations of the chemokines IP-10, MIG, and RANTES in the extracts were determined by cytometric bead array (human chemokine kit; BD Pharmingen). The concentration of MDC (CCL22) was measured using ELISA (R&D Systems). The total protein concentrations in the extracts were measured with the Bio-Rad (Hercules, CA) protein assay with a bovine serum albumin protein standard.

Statistical analysis. The Mann-Whitney test was used to evaluate differences. A P value of <0.05 was considered significant.

RESULTS

Increased numbers of $CD4^+$ T cells in the infected stomach. To study the mucosal T-cell responses to *H. pylori* infection, the frequencies and numbers of LPLs as well as $CD4^+$ and $CD8^+$ T cells and $CD19^+$ B cells were analyzed in antral and duodenal biopsies collected from asymptomatic *H. pylori*-pos-

TABLE 1. Numbers of lamina propria lymphocytes isolated from 8.5 pooled antral or duodenal biopsies[a]

Lymphocyte subset	Median no. (10^5) of lymphocytes (range)			
	Antrum		Duodenum	
	H. pylori positive	*H. pylori* negative	*H. pylori* positive	*H. pylori* negative
$CD4^+$	9.3* (3.0–16)	3.0 (2.0–8.4)	6.3 (3.7–12)	11 (5.6–13)
$CD8^+$	8.1 (3.3–10)	6.8 (5.7–11)	3.7 (3.3–7.7)	5.1 (4.1–9.0)
$CD19^+$	6.7* (0.9–23)	0.1 (0.1–1.3)	1.0 (0.1–3.2)	0.4 (0.1–5.0)

[a] Statistical analysis was performed using the Mann-Whitney test (*H. pylori* positive, $n = 6$; *H. pylori* negative, $n = 5$). *, P of <0.05 for comparisons between *H. pylori*-positive and *H. pylori*-negative antra.

itive subjects and *H. pylori*-negative control individuals. About twofold higher total numbers of LPLs were isolated from infected stomach biopsies compared to uninfected stomach biopsies (29×10^5 versus 17×10^5 LPLs, median values [$P = 0.04$]). Using flow cytometry, we estimated the numbers of different lymphocyte subsets isolated from the antral mucosa. About threefold higher numbers of $CD4^+$ T cells and ~70-fold more $CD19^+$ B cells but comparable numbers of $CD8^+$ T cells were isolated from infected compared to uninfected gastric mucosa (Table 1). It should be noted that the frequency data given directly by flow cytometry showed that the frequencies of $CD4^+$ T cells among LPLs were comparable in *H. pylori*-positive and *H. pylori*-negative individuals (~30%). However, since the infected stomach contained higher numbers of LPLs, this proportion of $CD4^+$ cells corresponds to higher cell numbers in *H. pylori*-positive individuals than in *H. pylori*-negative individuals. The presence of increased numbers of $CD4^+$ but not $CD8^+$ T cells in infected stomach mucosa was also supported by immunohistochemical analysis (data not shown). Although the majority of *H. pylori*-positive individuals also had mild duodenitis, the frequencies as well as numbers of $CD4^+$ and $CD8^+$ T cells as well as $CD19^+$ B cells were comparable in infected and uninfected duodenal mucosa (Table 1). The vast majority of all T cells isolated from the mucosa had a memory phenotype ($CD45RA^-$ L-$selectin^{+/-}$ or $CD45RA^+$ L-$selectin^-$ [29]), and the frequencies of memory cells were comparable in *H. pylori*-positive and *H. pylori*-negative individuals and similar in the antrum (>92%) and in the duodenum (> 98%).

Alterations in homing receptor expression in the infected stomach. Our findings of increased numbers of $CD4^+$ T cells in the infected stomach but not in the duodenum indicate that T cells may use different mechanisms for recruitment to and retention at these two sites. To investigate this possibility, the expression of homing and chemokine receptors on $CD4^+$ T cells in the antrum and duodenum of *H. pylori*-positive and *H. pylori*-negative individuals was analyzed.

First, the expression of L-selectin and the β_7 integrin was studied. L-selectin was expressed by few (<10%) $CD4^+$ T cells in uninfected stomach mucosa, whereas a subpopulation (~25%) of $CD4^+$ stomach T cells in all *H. pylori*-positive individuals expressed this receptor (Fig. 1A and B). Since the infected stomach also contained higher numbers of $CD4^+$ T cells, the increased frequencies of L-$selectin^+$ cells found in the stomach of *H. pylori*-positive individuals corresponded to a

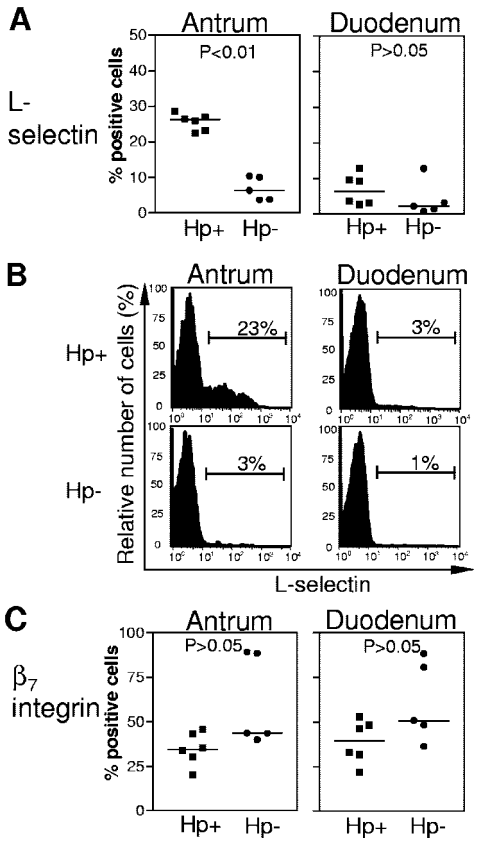

FIG. 1. Expression of L-selectin (A and B) and β_7 integrin (C) on $CD4^+$ T cells. Lymphocytes were isolated from the antral and duodenal lamina propria of *H. pylori*-positive (Hp+) ($n = 6$) and *H. pylori*-negative (Hp−) ($n = 5$) individuals, and the receptor expression was determined by flow cytometry. (A and C). Each symbol represents the frequencies of positive cells detected among cells from one individual, and median values are indicated by horizontal bars. Statistical analysis was performed using the Mann-Whitney test, and a P value of <0.05 was considered significant. (B) Histograms showing representative staining patterns for L-selectin on $CD4^+$ T cells (≥2,200 $CD4^+$ T cells were analyzed in each sample).

~10-fold increase in the total numbers of $CD4^+$ T cells that expressed this receptor in infected stomach (data not shown). In contrast, duodenal T cells expressed little L-selectin in both study groups (<13% L-$selectin^+$ cells).

The β_7 integrin was expressed by 30 to 90% of the $CD4^+$ T cells in uninfected antral and duodenal mucosa (Fig. 1C). The frequencies of $\beta_7{}^+$ $CD4^+$ T cells were slightly but not significantly decreased in both the stomach and duodenal mucosa of infected individuals. The total numbers of antral $\beta_7{}^+$ $CD4^+$ cells were similar in *H. pylori*-positive and *H. pylori*-negative individuals (data not shown).

Thus, the expression of L-selectin, but not the β_7 integrin, was increased on $CD4^+$ T cells in *H. pylori*-infected stomach mucosa.

Alterations in chemokine receptor and chemokine expression in the infected stomach. Next, the expression of chemokine receptors previously described to play a role in T-cell recruitment to intestinal mucosa (CXCR3 and CCR5) (1) and to be associated with Th1 (CXCR3 and CCR5) or Th2 (CCR4

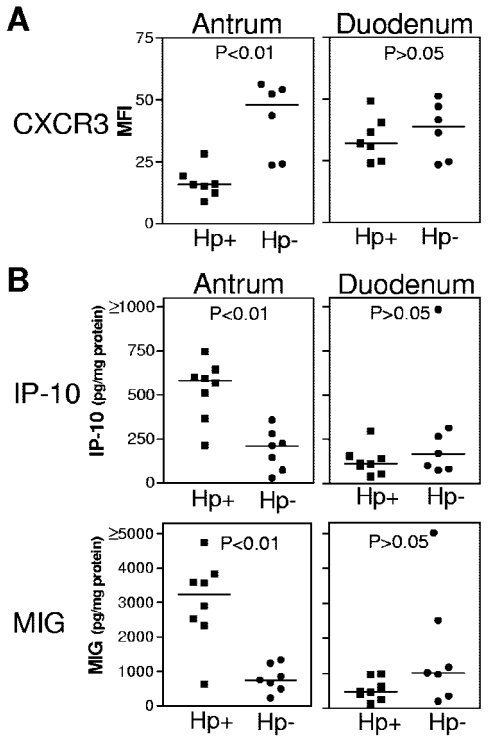

FIG. 2. Expression of CXCR3 on $CD4^+$ T cells (A) and concentrations of IP-10 and MIG in mucosal saponin extracts (B). (A) Lymphocytes were isolated from the antral and duodenal lamina propria of *H. pylori*-positive (Hp+) ($n = 7$) and *H. pylori*-negative (Hp−) ($n = 6$) individuals, and the median fluorescence intensities (MFI) of CXCR3 expression on $CD4^+$ T cells were determined by flow cytometry. (B) The concentrations of IP-10 and MIG in saponin extracts of biopsies from *H. pylori*-positive ($n = 8$) and *H. pylori*-negative ($n = 7$) subjects were analyzed by cytometric bead array and related to the total protein contents of the extracts. Statistical analysis was performed using the Mann-Whitney test, and a P value of <0.05 was considered significant.

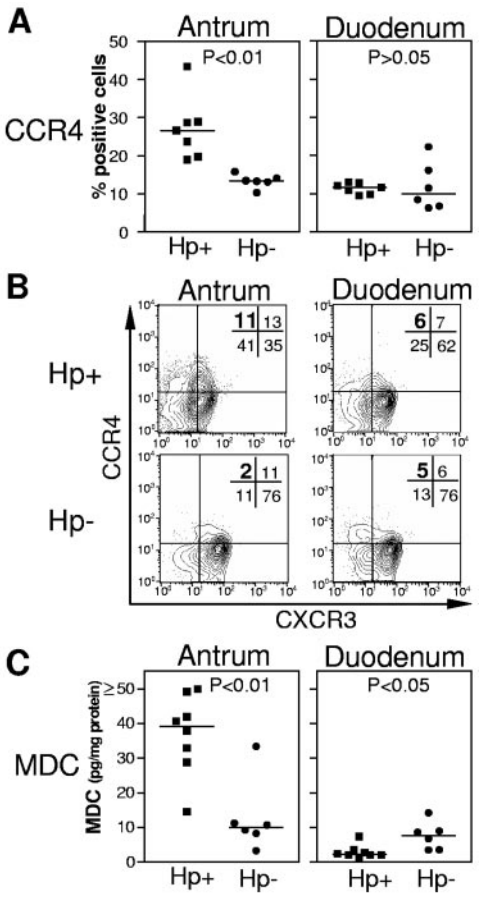

FIG. 3. Expression of CCR4 on $CD4^+$ T cells (A), coexpression of CCR4 and CXCR3 (B), and concentrations of MDC in mucosal extracts (C). (A) Lymphocytes were isolated from the antral and duodenal lamina propria of *H. pylori*-positive (Hp+) ($n = 7$) and *H. pylori*-negative (Hp−) ($n = 6$) individuals, and the frequencies of $CCR4^+$ cells were analyzed by flow cytometry. (B) Contour plots showing representative staining patterns for CCR4 and CXCR3 in $CD4^+$ T cells. Frequencies of cells expressing these receptors are indicated by the numbers within the plots; ≥2,500 $CD4^+$ T cells were analyzed in each sample. (C) The concentrations of MDC in saponin extracts of biopsies from *H. pylori*-positive ($n = 8$) and *H. pylori*-negative ($n = 7$) subjects were analyzed by ELISA and related to the total protein contents of the extracts. Statistical analysis was performed using the Mann-Whitney test, and a P value of <0.05 was considered significant.

and CCR3) cytokine production (37) was investigated on antral and duodenal T cells.

CXCR3, which was expressed at high levels by the majority of $CD4^+$ T cells in the uninfected antral as well as the duodenal mucosa, was expressed significantly less by $CD4^+$ T cells in the infected stomach mucosa (Fig. 2A). However, due to the increased numbers of $CD4^+$ T cells in infected stomach, the total numbers of CXCR3-expressing $CD4^+$ cells were comparable in *H. pylori*-positive and *H. pylori*-negative individuals (data not shown). Saponin extracts of infected antral mucosa contained significantly higher levels (~3-fold) of IP-10 and MIG (~4-fold), two of the chemokine ligands for CXCR3, than extracts of uninfected mucosa, whereas there were no major differences in CXCR3 expression or IP-10 and MIG levels in infected and uninfected duodenal mucosa (Fig. 2B).

CCR4, on the other hand, which was expressed by few cells (~10%) in uninfected stomach mucosa, was expressed in higher frequencies (~2-fold increase) (Fig. 3A) as well as numbers (fivefold increase) (data not shown) of $CD4^+$ T cells from infected mucosa. CCR4 was coexpressed with CXCR3 on a subset of $CD4^+$ T cells, but the increase in CCR4 expression in the infected antrum was mainly a result of increased frequencies of single positive $CCR4^+$ $CXCR3^-$ $CD4^+$ T cells (Fig. 3B). Saponin extracts of infected antral mucosa contained ~4-fold higher levels of MDC, one of the ligands for CCR4, than extracts of uninfected antral mucosa. The expression of CCR4 and MDC in the duodenal mucosa was low in both *H. pylori*-positive and *H. pylori*-negative individuals (Fig. 3A to C).

CCR5 was expressed by up to 60% of mucosal $CD4^+$ T cells, whereas CCR3 was only expressed by ≤15% of these cells (Table 2). The frequencies but not numbers of stomach $CD4^+$ cells expressing these receptors were slightly decreased in *H. pylori*-positive compared to *H. pylori*-negative individuals. The levels of RANTES, a ligand for both CCR3 and CCR5, were comparable in mucosal extracts from *H. pylori*-positive and *H. pylori*-negative individuals in the antrum as well as the duode-

TABLE 2. Frequencies of $CCR5^+$ and $CCR3^+$ cells among $CD4^+$ T cells from the antral and duodenal lamina propria as determined by flow cytometry[a]

Chemokine receptor	Median % frequency of lymphocytes (range)			
	Antrum		Duodenum	
	H. pylori positive	*H. pylori* negative	*H. pylori* positive	*H. pylori* negative
CCR5	24 (12–48)	46 (27–55)	33 (14–56)	48 (25–59)
CCR3	4.0 (1.1–14)	9.9 (6.5–11)	3.6 (1.9–15)	5.8 (5.0–9.9)

[a] Statistical analysis was performed using the Mann-Whitney test (*H. pylori* positive, $n = 7$; *H. pylori* negative, $n = 5$). P was >0.05 for all comparisons between *H. pylori*-positive and *H. pylori*-negative individuals.

num, respectively (antrum, 910 [460 to 3,540] pg/ml; duodenum, 1,670 [650 to 4,150] pg/ml, median values [range]).

Thus, the expression of CCR4 was increased and the expression of CXCR3 decreased on $CD4^+$ T cells in infected stomach, whereas the levels of both the CCR4 ligand MDC and the CXCR3 ligands IP-10 and MIG were elevated in infected stomach mucosa.

$CD4^+$ T cells in the infected stomach are specific for *H. pylori*. To determine if the $CD4^+$ T cells accumulating in the stomach were *H. pylori* specific, we analyzed the reactivity of LPLs from infected stomach to *H. pylori* antigens and compared it to the reactivity among cells from the duodenum and from uninfected mucosa. Stimulation of LPLs with *H. pylori* MP alone resulted in little proliferation. Addition of IL-2 to the cell cultures only marginally increased the proliferation (data not shown). However, stimulation of stomach LPLs from *H. pylori*-positive individuals with MP in combination with both IL-2 and IL-7 induced a 5- to 25-fold increase in the number of cells within 2 weeks after the initial stimulation (Fig. 4A). In contrast, stimulation of stomach LPLs from *H. pylori*-negative individuals resulted in little proliferation (one- to fivefold expansion). Stimulation of antral LPLs from both *H. pylori*-positive and *H. pylori*-negative individuals with IL-2 and IL-7 alone, in the absence of MP, resulted in only low levels of T-cell proliferation (twofold median expansion) (data not shown).

To confirm that the T-cell responses detected among antral LPLs from *H. pylori*-positive individuals were MP specific, T cells were restimulated with MP in the absence of cytokines 2 weeks after primary stimulation. Stomach T cells from *H. pylori*-positive individuals that had been expanded in response to stimulation with MP together with IL-2 and IL-7 proliferated vigorously in response to restimulation with MP, whereas corresponding cells from most *H. pylori*-negative individuals responded poorly to restimulation (Fig. 4B). Furthermore, cells cultured with IL-2 and IL-7 alone did not respond to restimulation with MP (data not shown). In contrast, duodenal cells from only a few individuals responded to MP stimulation after both primary stimulation and restimulation, and individuals with responding duodenal T cells were found in both *H. pylori*-positive and *H. pylori*-negative subjects (Fig. 4A and B). Duodenal cells expanded in response to stimulation with IL-2 and IL-7 alone did not respond to restimulation with MP.

After stimulation of stomach T cells with MP, IL-2, and IL-7, more than 90% of the T cells expressed CD4, whereas in

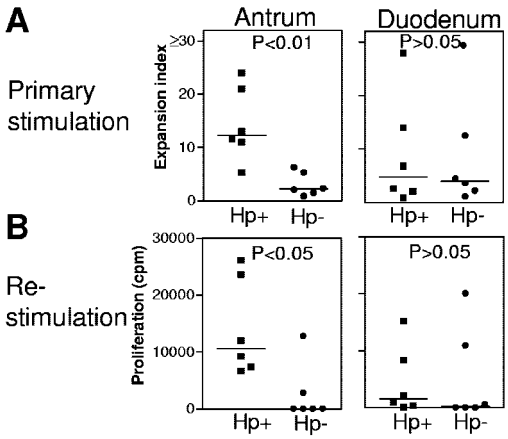

FIG. 4. MP-induced T-cell proliferation among T cells isolated from the antrum and duodenum. Mucosal LPLs from *H. pylori*-positive (Hp+) ($n = 6$) and *H. pylori*-negative (Hp−) ($n = 6$) individuals were stimulated with MP, IL-2, and IL-7. (A) After 2 weeks of culture, the cells were harvested and counted. Each symbol represents the increase in the numbers of cells harvested from each individual compared to the initial numbers of stimulated LPLs, expressed as an expansion index. Stimulation of LPLs with IL-2 and IL-7 alone, in the absence of MP, resulted in only low levels of T-cell proliferation (twofold median expansion). (B) The harvested cells from each individual were restimulated with MP in absence of cytokines, and the proliferation was determined after 48 h by incorporation of radioactive thymidine. Each symbol represents the response of T cells from one individual after subtraction of the response to medium alone. Median values are indicated by horizontal bars. Statistical analysis was performed using the Mann-Whitney test, and a P value of <0.05 was considered significant.

freshly isolated LPLs, the frequencies of $CD4^+$ and $CD8^+$ T cells were comparable (~30% of lymphocytes). Thus, cells responding to the MP/IL-2/IL-7 stimulation were preferentially $CD4^+$ T cells. MP-induced T-cell proliferation in stomach T cells was associated with production of IFN-γ (1,200 [40 to 5,460] pg/ml, median values [range]). In contrast, IL-4 or IL-5 could not be detected in any of the culture supernatants. IL-10 and tumor necrosis factor alpha were detected in some cultures after MP stimulation, but these cytokines might at least partially have been produced by antigen-presenting cells rather than T cells, as similar levels of these cytokines were also detected in cultures with antigen-presenting cells alone. In contrast, IFN-γ was only detected in cultures containing LPLs.

Thus, $CD4^+$ T cells from infected but not uninfected stomach mucosa responded to stimulation with *H. pylori* MP with proliferation and IFN-γ production.

DISCUSSION

H. pylori colonizes the gastric mucosa but also the duodenum in areas of gastric metaplasia (13, 44). T cells have been implicated to play an important role in the immune response to *H. pylori*. T cells may promote local inflammation and partially protect against the infection as well as regulate the immune response (10, 25, 26, 36). T cells are recruited from the circulation to mucosal sites by interactions between homing and chemokine receptors expressed on the surface of the T cells and the receptor ligands expressed on blood vessel endothelium and within the tissue. Several mechanisms responsible for

recruitment of T cells to intestinal mucosa have been described (1, 14), but little is currently known about how T cells are recruited to *H. pylori*-infected mucosa. In the present study, we characterized antral and duodenal T cells from *H. pylori*-positive and *H. pylori*-negative individuals with regard to subtype, expression of homing and chemokine receptors, and specificity for *H. pylori*. We found increased numbers of $CD4^+$ T cells in the antrum of *H. pylori*-positive individuals, but not in the duodenum, and comparable numbers of $CD8^+$ T cells at both sites in the two study groups. It is interesting that low numbers of both $CD4^+$ and $CD8^+$ T cells are present in uninfected stomach mucosa, in contrast to $CD19^+$ B cells, which are virtually absent from the mucosa under noninflammatory conditions. This indicates that T and B cells use different mechanisms for steady-state migration to mucosal sites. The preferential accumulation of $CD4^+$ rather than $CD8^+$ T cells in the infected stomach is in agreement with our previous immunohistochemical analysis of local T-cell responses to *H. pylori* (41), although a few other reports claim that the numbers of $CD8^+$ T cells also increase in response to infection (2, 17). *H. pylori* bacteria are rarely invasive and are therefore likely to favor activation of $CD4^+$ rather than $CD8^+$ T cells. $CD4^+$ T cells also seem to be more important than $CD8^+$ T cells for vaccine-induced protection against *H. pylori* infection in mice (12). Therefore, we focused our studies on $CD4^+$ T cells.

Since $CD4^+$ T cells accumulate in the antrum but not in the duodenum during *H. pylori* infection, different mechanisms are likely used to recruit T cells to the different sites. Interestingly, we found higher frequencies of cells expressing L-selectin in the infected compared to the uninfected stomach mucosa but similar L-selectin expression in the duodenum of the two study groups. In earlier studies, L-selectin$^+$ peripheral blood T cells from *H. pylori*-positive individuals have been found to respond more strongly to in vitro stimulation with *H. pylori* antigens than L-selectin$^-$ cells, supporting a role for this receptor in homing of *H. pylori*-specific T cells (34). L-selectin is expressed by naïve and central memory cells and mediates homing of both T-cell subsets to peripheral lymph nodes by binding to the peripheral lymph node addressin (PNAd) expressed on high endothelial venules (37). PNAd-expressing high endothelial venule-like vessels have also been detected in *H. pylori*-infected gastric mucosa but not in uninfected stomach (9, 21). PNAd seems to be preferentially expressed by vessels in the lymphoid follicles that are formed in the stomach as a consequence of *H. pylori* colonization (9). This suggests that T cells may use L-selectin to enter the lymphoid follicles and that the L-selectin$^+$ cells isolated from the infected mucosa in the present study originate from the follicles.

In contrast to the L-selectin expression, we could not detect any major differences in β_7 integrin expression on $CD4^+$ T cells from infected and uninfected mucosa. MAdCAM-1, the receptor for the $\alpha_4\beta_7$ integrin, is also expressed at similar levels in infected and uninfected mucosa (34). These results may suggest that $\alpha_4\beta_7$ integrin/MAdCAM-1 interactions are involved in the constitutive homing of T cells to both gastric and duodenal mucosa but that additional recruitment mechanisms may be responsible for the restricted recruitment of $CD4^+$ T cells to the stomach during infection. However, the β_7 integrin can also be coexpressed with the α_E integrin, and expression of $\alpha_E\beta_7$ is induced within the tissue and mediates mucosal retention of cells by binding to the receptor E-cadherin expressed on epithelial cells (14). A more detailed analysis of the coexpression of β_7 with the α_E and α_4 integrins, respectively, may thus be necessary for a more precise understanding of the importance of the β_7 integrin for homing and retention of T cells in the stomach during *H. pylori* infection.

Chemokines are also likely to influence the recruitment of immune cells to the infected mucosa. We found increased expression of the chemokine receptor CCR4 on antral $CD4^+$ T cells from infected compared to uninfected stomach mucosa. Furthermore, the levels of the CCR4 ligand MDC were increased in the infected stomach. In contrast, we only found little expression of CCR4 and MDC in duodenal mucosa from both *H. pylori*-positive and *H. pylori*-negative individuals, which is in line with previous studies of intestinal mucosa (1). CCR4 has been shown to be highly expressed by circulating $CD4^+$ $CD25^{high}$ regulatory T cells (18). We have recently demonstrated the presence of regulatory T cells in the *H. pylori*-infected stomach mucosa (25), and it is possible that CCR4 is involved in mucosal recruitment of these cells. We are currently investigating the CCR4 expression on mucosal regulatory T cells to determine the role of this receptor and its chemokine ligands in recruitment of regulatory T cells to different mucosal sites.

Intestinal T cells have previously been shown to express high levels of the CXCR3 and CCR5 receptors (1), and expression of these receptors has also recently been described on stomach lymphocytes in patients with gastric cancer (30). We found that $CD4^+$ stomach T cells from *H. pylori*-negative individuals expressed CXCR3 and CCR5 to a large extent but that the CXCR3 expression was significantly reduced and the CCR5 expression was slightly decreased in *H. pylori*-infected stomach. Both CXCR3 and CCR5 have been associated with production of Th1-type cytokines, whereas CCR4 and CCR3 have been associated with Th2 polarization (37). Decreased CXCR3 and CCR5 expression and increased CCR4 expression may thus reflect the presence of increased proportions of Th2 T cells in the *H. pylori*-infected mucosa. However, this possibility seems less likely, since previous studies have shown that the numbers of cells producing IFN-γ but not IL-4 are increased in infected mucosa (23), and we found that gastric T cells from *H. pylori*-positive individuals produced IFN-γ but not IL-4 or IL-5 upon stimulation with *H. pylori* antigens. In addition, CCR4 can be induced on Th1-type T cells after activation (6), and indeed, we found that cells coexpressed CXCR3 and CCR4 in the mucosa. Furthermore, $CXCR3^+$ and $CCR5^+$ cells have been found in diseases dominated by Th1 as well as Th2 responses (4, 33). Thus, chemokine receptors may be associated with a Th1 or Th2 cytokine profile but are not always likely to be accurate surrogate markers for polarized T-cell responses. Importantly, we found increased levels of the IFN-γ-inducible CXCR3 chemokine ligands IP-10 and MIG in the infected compared to uninfected stomach mucosa. This finding indicates that the reduced expression of CXCR3 in infected stomach may be a result of ligand-induced downregulation (24) and that CXCR3/IP-10/MIG is thus involved in the recruitment of T cells to both the infected and uninfected gastrointestinal mucosa.

When analyzing the specificity of antral and duodenal $CD4^+$ T cells for *H. pylori*, we found that stomach T cells from *H. pylori*-positive but not from *H. pylori*-negative individuals re-

spond strongly to stimulation with *H. pylori* antigens, supporting that the antral $CD4^+$ T-cell response is truly *H. pylori* specific. Previous studies have shown that $CD4^+$ T-cell clones that respond to stimulation with purified *H. pylori* antigens can be isolated from *H. pylori*-infected stomach mucosa (7). However, to appreciate these results, it is important to compare the T-cell responses in both *H. pylori*-positive and *H. pylori*-negative individuals, since circulating T cells from both *H. pylori*-positive and *H. pylori*-negative subjects have been shown to respond to stimulation with *H. pylori* antigens (19, 26, 35, 39). Our demonstration of the lack of *H. pylori* reactivity in T cells isolated from uninfected stomach mucosa thus extends and confirms previous data demonstrating *H. pylori*-specific T-cell responses in the human stomach (7). We also investigated the specificity for *H. pylori* in T cells isolated from the duodenum. Duodenal T cells from only a few individuals responded to *H. pylori* stimulation, and the duodenal responsiveness was not restricted to *H. pylori*-positive individuals. This suggests limited *H. pylori* specificity in the duodenum and possible cross-reactivity with antigens from other bacteria.

In order to detect the *H. pylori*-specific T-cell responses, the cell cultures had to be supplemented with both IL-2 and IL-7. T cells isolated from intestinal mucosa are known to be hyporesponsive to T-cell receptor triggering, compared to peripheral blood T cells (8, 20). The unresponsiveness may be a result of suppression mediated by regulatory T cells, as regulatory T cells accumulate at the site of *H. pylori* infection, and the responsiveness among circulating $CD4^+$ T cells to *H. pylori* antigens can be increased by the addition of IL-2 to the cell cultures (25, 26). The unresponsiveness may also be due to the fact the mucosal cells are highly differentiated memory cells that are prone to undergo apoptosis. IL-7 is known to inhibit apoptosis and to mediate costimulation of antigen-induced proliferation (15). Thus, IL-7 may be specifically required to promote T-cell survival and proliferation of mucosal T cells in vitro. IL-7 can be produced by epithelial cells (15), and increased levels of IL-7 mRNA have been found in the gastric mucosa of *H. pylori*-positive compared to *H. pylori*-negative individuals (45), suggesting that this cytokine may also help to sustain mucosal T-cell responses to *H. pylori* in vivo.

Collectively, the results from the present study suggest that L-selectin and CCR4/MDC are important for recruitment of *H. pylori*-specific $CD4^+$ T cells to the infected stomach. However, in the absence of infection and inflammation, T cells express similar patterns of homing and chemokine receptors in the stomach and in the duodenum, indicating that they use similar mechanisms to reach these sites under noninflammatory conditions. The increased insight into factors controlling mucosal recruitment of *H. pylori*-specific T cells may have important implications for understanding the mechanisms behind chronic *H. pylori*-induced inflammation. It also provides a basis for the identification of possible targets for treatment and prevention of *H. pylori*-induced diseases.

ACKNOWLEDGMENTS

This study was supported by grants from the Swedish Agency for Research and Economic Cooperation, the Swedish Cancer Foundation, Tore Nilsson's Foundation for Medical Research, Nanna Svartz' Foundation, Golje's Memorial Foundation, and Ragnhild and Einar Lundströms' Memorial Foundation.

We thank Gunilla Bogren for help with recruitment of volunteers and Cecilia Engström and Johanna Wennerblom for help with gastroscopies.

REFERENCES

1. **Agace, W. W., A. I. Roberts, L. Wu, C. Greineder, E. C. Ebert, and C. M. Parker.** 2000. Human intestinal lamina propria and intraepithelial lymphocytes express receptors specific for chemokines induced by inflammation. Eur. J. Immunol. **30:**819–826.
2. **Agnihotri, N., D. K. Bhasin, H. Vohra, P. Ray, K. Singh, and N. K. Ganguly.** 1998. Characterization of lymphocytic subsets and cytokine production in gastric biopsy samples from *Helicobacter pylori* patients. Scand. J. Gastroenterol. **33:**704–709.
3. **Akhiani, A. A., J. Pappo, Z. Kabok, K. Schon, W. Gao, L. E. Franzen, and N. Lycke.** 2002. Protection against *Helicobacter pylori* infection following immunization is IL-12-dependent and mediated by Th1 cells. J. Immunol. **169:**6977–6984.
4. **Annunziato, F., L. Cosmi, G. Galli, C. Beltrame, P. Romagnani, R. Manetti, S. Romagnani, and E. Maggi.** 1999. Assessment of chemokine receptor expression by human Th1 and Th2 cells in vitro and in vivo. J. Leukoc. Biol. **65:**691–699.
5. **Bolin, I., H. Lonroth, and A. M. Svennerholm.** 1995. Identification of *Helicobacter pylori* by immunological dot blot method based on reaction of a species-specific monoclonal antibody with a surface-exposed protein. J. Clin. Microbiol. **33:**381–384.
6. **D'Ambrosio, D., A. Iellem, R. Bonecchi, D. Mazzeo, S. Sozzani, A. Mantovani, and F. Sinigaglia.** 1998. Selective up-regulation of chemokine receptors CCR4 and CCR8 upon activation of polarized human type 2 Th cells. J. Immunol. **161:**5111–5115.
7. **D'Elios, M. M., M. Manghetti, F. Almerigogna, A. Amedei, F. Costa, D. Burroni, C. T. Baldari, S. Romagnani, J. L. Telford, and G. Del Prete.** 1997. Different cytokine profile and antigen-specificity repertoire in *Helicobacter pylori*-specific T cell clones from the antrum of chronic gastritis patients with or without peptic ulcer. Eur. J. Immunol. **27:**1751–1755.
8. **De Maria, R., S. Fais, M. Silvestri, L. Frati, F. Pallone, A. Santoni, and R. Testi.** 1993. Continuous in vivo activation and transient hyporesponsiveness to TcR/CD3 triggering of human gut lamina propria lymphocytes. Eur. J. Immunol. **23:**3104–3108.
9. **Dogan, A., M. Du, A. Koulis, M. J. Briskin, and P. G. Isaacson.** 1997. Expression of lymphocyte homing receptors and vascular addressins in low-grade gastric B-cell lymphomas of mucosa-associated lymphoid tissue. Am. J. Pathol. **151:**1361–1369.
10. **Eaton, K. A., M. Mefford, and T. Thevenot.** 2001. The role of T cell subsets and cytokines in the pathogenesis of *Helicobacter pylori* gastritis in mice. J. Immunol. **166:**7456–7461.
11. **Eck, M., B. Schmausser, K. Scheller, A. Toksoy, M. Kraus, T. Menzel, H. K. Muller-Hermelink, and R. Gillitzer.** 2000. CXC chemokines Groα/IL-8 and IP-10/MIG in *Helicobacter pylori* gastritis. Clin. Exp. Immunol. **122:**192–199.
12. **Ermak, T. H., P. J. Giannasca, R. Nichols, G. A. Myers, J. Nedrud, R. Weltzin, C. K. Lee, H. Kleanthous, and T. P. Monath.** 1998. Immunization of mice with urease vaccine affords protection against *Helicobacter pylori* infection in the absence of antibodies and is mediated by MHC class II-restricted responses. J. Exp. Med. **188:**2277–2288.
13. **Ernst, P. B., and B. D. Gold.** 2000. The disease spectrum of *Helicobacter pylori*: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. Annu. Rev. Microbiol. **54:**615–640.
14. **Farstad, I. N., T. S. Halstensen, B. Lien, P. J. Kilshaw, A. I. Lazarovits, P. Brandtzaeg, and A. I. Lazarovitz.** 1996. Distribution of beta 7 integrins in human intestinal mucosa and organized gut-associated lymphoid tissue. Immunology **89:**227–237.
15. **Fry, T. J., and C. L. Mackall.** 2002. Interleukin-7: from bench to clinic. Blood **99:**3892–3904.
16. **Hamlet, A., A. C. Thoreson, O. Nilsson, A. M. Svennerholm, and L. Olbe.** 1999. Duodenal *Helicobacter pylori* infection differs in *cagA* genotype between asymptomatic subjects and patients with duodenal ulcers. Gastroenterology **116:**259–268.
17. **Hatz, R. A., G. Meimarakis, E. Bayerdorffer, M. Stolte, T. Kirchner, and G. Enders.** 1996. Characterization of lymphocytic infiltrates in *Helicobacter pylori*-associated gastritis. Scand. J. Gastroenterol. **31:**222–228.
18. **Iellem, A., M. Mariani, R. Lang, H. Recalde, P. Panina-Bordignon, F. Sinigaglia, and D. D'Ambrosio.** 2001. Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR8 by $CD4^+CD25^+$ regulatory T cells. J. Exp. Med. **194:**847–853.
19. **Karttunen, R., G. Andersson, K. Poikonen, T. U. Kosunen, T. Karttunen, K. Juutinen, and S. Niemela.** 1990. *Helicobacter pylori* induces lymphocyte activation in peripheral blood cultures. Clin. Exp. Immunol. **82:**485–488.
20. **Khoo, U. Y., I. E. Proctor, and A. J. Macpherson.** 1997. $CD4^+$ T cell down-regulation in human intestinal mucosa: evidence for intestinal tolerance to luminal bacterial antigens. J. Immunol. **158:**3626–3634.
21. **Kobayashi, M., J. Mitoma, N. Nakamura, T. Katsuyama, J. Nakayama, and M. Fukuda.** 2004. Induction of peripheral lymph node addressin in human

gastric mucosa infected by *Helicobacter pylori*. Proc. Natl. Acad. Sci. USA **101:**17807–17812.
22. **Lindholm, C., J. Osek, and A. M. Svennerholm.** 1997. Quantification of conserved antigens in *Helicobacter pylori* during different culture conditions. Infect. Immun. **65:**5376–5380.
23. **Lindholm, C., M. Quiding-Jarbrink, H. Lonroth, A. Hamlet, and A. M. Svennerholm.** 1998. Local cytokine response in *Helicobacter pylori*-infected subjects. Infect. Immun. **66:**5964–5971.
24. **Loetscher, P., A. Pellegrino, J. H. Gong, I. Mattioli, M. Loetscher, G. Bardi, M. Baggiolini, and I. Clark-Lewis.** 2001. The ligands of CXC chemokine receptor 3, I-TAC, Mig, and IP10, are natural antagonists for CCR3. J. Biol. Chem. **276:**2986–2991.
25. **Lundgren, A., E. Stromberg, A. Sjoling, C. Lindholm, K. Enarsson, A. Edebo, E. Johnsson, E. Suri-Payer, P. Larsson, A. Rudin, A. M. Svennerholm, and B. S. Lundin.** 2005. Mucosal *FOXP3*-expressing $CD4^+$ $CD25^{high}$ regulatory T cells in *Helicobacter pylori*-infected patients. Infect. Immun. **73:**523–531.
26. **Lundgren, A., E. Suri-Payer, K. Enarsson, A. M. Svennerholm, and B. S. Lundin.** 2003. *Helicobacter pylori*-specific $CD4^+$ $CD25^{high}$ regulatory T cells suppress memory T-cell responses to *H. pylori* in infected individuals. Infect. Immun. **71:**1755–1762.
27. **Mattsson, A., A. Tinnert, A. Hamlet, H. Lonroth, I. Bolin, and A. M. Svennerholm.** 1998. Specific antibodies in sera and gastric aspirates of symptomatic and asymptomatic *Helicobacter pylori*-infected subjects. Clin. Diagn. Lab. Immunol. **5:**288–293.
28. **Michetti, M., C. P. Kelly, J. P. Kraehenbuhl, H. Bouzourene, and P. Michetti.** 2000. Gastric mucosal $\alpha_4\beta_7$-integrin-positive CD4 T lymphocytes and immune protection against *Helicobacter* infection in mice. Gastroenterology **119:**109–118.
29. **Mitra, D. K., S. C. De Rosa, A. Luke, A. Balamurugan, B. K. Khaitan, J. Tung, N. K. Mehra, A. I. Terr, A. O'Garra, L. A. Herzenberg, and M. Roederer.** 1999. Differential representations of memory T cell subsets are characteristic of polarized immunity in leprosy and atopic diseases. Int. Immunol. **11:**1801–1810.
30. **Ohtani, N., H. Ohtani, T. Nakayama, H. Naganuma, E. Sato, T. Imai, H. Nagura, and O. Yoshie.** 2004. Infiltration of CD8+ T cells containing RANTES/CCL5+ cytoplasmic granules in actively inflammatory lesions of human chronic gastritis. Lab. Investig. **84:**368–375.
31. **Pappo, J., D. Torrey, L. Castriotta, A. Savinainen, Z. Kabok, and A. Ibraghimov.** 1999. *Helicobacter pylori* infection in immunized mice lacking major histocompatibility complex class I and class II functions. Infect. Immun. **67:**337–341.
32. **Price, A. B.** 1991. The Sydney system: histological division. J. Gastroenterol. Hepatol. **6:**209–222.
33. **Qin, S., J. B. Rottman, P. Myers, N. Kassam, M. Weinblatt, M. Loetscher, A. E. Koch, B. Moser, and C. R. Mackay.** 1998. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. J. Clin. Investig. **101:**746–754.
34. **Quiding-Jarbrink, M., I. Ahlstedt, C. Lindholm, E. L. Johansson, and H. Lonroth.** 2001. Homing commitment of lymphocytes activated in the human gastric and intestinal mucosa. Gut **49:**519–525.
35. **Quiding-Jarbrink, M., B. S. Lundin, H. Lonroth, and A. M. Svennerholm.** 2001. $CD4^+$ and $CD8^+$ T cell responses in *Helicobacter pylori*-infected individuals. Clin. Exp. Immunol. **123:**81–87.
36. **Raghavan, S., M. Fredriksson, A. M. Svennerholm, J. Holmgren, and E. Suri-Payer.** 2003. Absence of $CD4^+CD25^+$ regulatory T cells is associated with a loss of regulation leading to increased pathology in *Helicobacter pylori*-infected mice. Clin. Exp. Immunol. **132:**393–400.
37. **Sallusto, F., C. R. Mackay, and A. Lanzavecchia.** 2000. The role of chemokine receptors in primary, effector, and memory immune responses. Annu. Rev. Immunol. **18:**593–620.
38. **Sarsfield, P., D. B. Jones, A. C. Wotherspoon, T. Harvard, and D. H. Wright.** 1996. A study of accessory cells in the acquired lymphoid tissue of helicobacter gastritis. J. Pathol. **180:**18–25.
39. **Sharma, S. A., G. G. Miller, G. I. Perez-Perez, R. S. Gupta, and M. J. Blaser.** 1994. Humoral and cellular immune recognition of *Helicobacter pylori* proteins are not concordant. Clin. Exp. Immunol. **97:**126–132.
40. **Shimoyama, T., S. M. Everett, M. F. Dixon, A. T. Axon, and J. E. Crabtree.** 1998. Chemokine mRNA expression in gastric mucosa is associated with *Helicobacter pylori* cagA positivity and severity of gastritis. J. Clin. Pathol. **51:**765–770.
41. **Stromberg, E., A. Lundgren, A. Edebo, S. Lundin, A. M. Svennerholm, and C. Lindholm.** 2003. Increased frequency of activated T-cells in the *Helicobacter pylori*-infected antrum and duodenum. FEMS Immunol. Med. Microbiol. **36:**159–168.
42. **Sutton, P., J. Wilson, T. Kosaka, I. Wolowczuk, and A. Lee.** 2000. Therapeutic immunization against *Helicobacter pylori* infection in the absence of antibodies. Immunol. Cell Biol. **78:**28–30.
43. **Thoreson, A. C., A. Hamlet, J. Celik, M. Bystrom, S. Nystrom, L. Olbe, and A. M. Svennerholm.** 2000. Differences in surface-exposed antigen expression between *Helicobacter pylori* strains isolated from duodenal ulcer patients and from asymptomatic subjects. J. Clin. Microbiol. **38:**3436–3441.
44. **Wyatt, J. I., B. J. Rathbone, M. F. Dixon, and R. V. Heatley.** 1987. *Campylobacter pyloridis* and acid induced gastric metaplasia in the pathogenesis of duodenitis. J. Clin. Pathol. **40:**841–848.
45. **Yamaoka, Y., M. Kita, T. Kodama, N. Sawai, and J. Imanishi.** 1996. *Helicobacter pylori* cagA gene and expression of cytokine messenger RNA in gastric mucosa. Gastroenterology **110:**1744–1752.
46. **Yamaoka, Y., M. Kita, T. Kodama, N. Sawai, T. Tanahashi, K. Kashima, and J. Imanishi.** 1998. Chemokines in the gastric mucosa in *Helicobacter pylori* infection. Gut **42:**609–617.

Editor: A. D. O'Brien