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Chemokine Receptor 5 Expression in Gastric Mucosa of *Helicobacter pylori*-Infected and Noninfected Children

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Experimental data from human adults or animal models indicate that the *Helicobacter pylori*-specific immune response is dominated by inflammatory T cells of the Th1 type. To investigate whether a Th1 immune response is established in early *H. pylori* infection, gastric biopsy samples from 70 children were subjected to immunohistochemical analysis. To this end, T cells, B cells, monocytes, neutrophils, and chemokine receptor 5 (CCR5)-expressing ($CCR5^+$) cells, which are associated with Th1 immune responses, were quantified. Children were classified according to *H. pylori* status and clinical, laboratory, and macroscopic (during endoscopy) findings, without knowledge of histological findings. Group 1 included 31 *H. pylori*-infected children, group 2 contained 24 children with other conditions possibly affecting the stomach, and group 3 contained 15 children without verifiable pathological findings in the stomach. Lymphoid follicles were present in 90% of biopsy samples from group 1 and 48% of those from group 2 but absent in group 3 biopsy samples. Intraepithelial T cells and $CCR5^+$ cells were regularly detected in all groups without significant differences. B cells, monocytes, and neutrophils were not found. In contrast, the numbers of lamina propria T cells ($P < 0.003$) and $CCR5^+$ cells ($P < 0.001$) were increased significantly in *H. pylori*-infected children. B cells (in 13 of 66 children) were detected in children with active ($n = 11$) or previously cleared ($n = 2$) *H. pylori* infections but were absent in healthy children. The numbers of monocytes (in 10 of 67 children) did not differ among the groups. Calculations indicated that the majority of gastric T cells express CCR5; this finding is in contrast to the low percentage of $CCR5^+$ T cells in the peripheral circulation. Thus, an increase in the numbers of $CCR5^+$ cells in *H. pylori*-infected stomach mucosa suggests that this molecule may play an important role in gastric immune responses.

An infection with *Helicobacter pylori* is one of the most prevalent infectious diseases in the world, affecting over half of the world population. It is acquired during early childhood (50), and without specific therapy, the bacterium usually persists throughout life. The infection of children differs in several aspects from the infection of adults: while spontaneous clearance of the pathogen is a rare event in adults, it seems to be quite common during childhood (17, 47). The degree and activity of gastritis, the extent of *H. pylori* colonization, and the degree of atrophic gastritis with intestinal metaplasia differ significantly between children and adults (30). In addition, the antral mucosa of symptomatic and asymptomatic *H. pylori*-infected children shows nodularity, which is the macroscopic correlate of newly formed organized lymphoid tissue (16). This nodularity is noticeably less common in adults. These clinical observations lend support to the hypothesis that the *H. pylori*-specific immune response may change during progression from the earlier stages of the infection in childhood to the later stages in adulthood. It is generally accepted that infection with *H. pylori* leads to a predominant Th1 response in the gastric mucosa, which may promote local inflammation (7, 14, 21, 33, 44). Data from *H. pylori*-infected mice support this contention (14, 33). Previous studies of *H. pylori* responses in humans investigated adult peripheral blood lymphocytes (25) or used experimental systems that analyzed only gastric T-cell clones (12), single cells isolated from the stomachs of infected adults (44), or cultured stomach explants that were infected in vitro (26).

Chemokines are chemoattractant cytokines that coordinate the migration of leukocytes to sites of inflammation (28). Chemokines are classified into four families (CC, CXC, CX_3, and C) based on the positioning of amino acids between the two N-terminal cysteine residues (35). CX_3 and C chemokines are each represented by single members. While the group of CXC chemokines acts preferentially on neutrophils, the CC chemokine group is mainly involved in the attraction of lymphocytes (5). The corresponding chemokine receptors are grouped according to the structures of their chemokine ligands. Due to their distinct effector functions, Th1 and Th2 lymphocyte subsets travel to different tissues in a process guided by chemokines and adhesion molecules. Accordingly, they show selective expression of chemokine receptors: human interleukin 4 (IL-4)-producing cells have been shown to express chemokine receptor 3 (CCR3) (36), suggesting that at least a subset of Th2 cells expresses this marker. Other chemokine receptors, including CCR4 and CCR8, are also highly selective for Th2 cells (10, 52). On the other hand, CXCR3 and CCR5 are predom-

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TABLE 1. Monoclonal antibodies used for immunohistochemical analysis

Antibody	Specificity	Dilution	Source
M-T910	CD2	1:3	E. P. Rieber, Dresden, Germany
M-T310	CD4	1:3	E. P. Rieber
M-T811	CD8	1:9	E. P. Rieber
M-M42	CD14	None (undiluted)	E. P. Rieber
M5E2	CD14	1:50	BD Pharmingen
HIB19	CD19	1:80	BD Pharmingen
MC5	CCR5	1:5	M. Mack, Munich, Germany
Isotype control	IgG2a and IgG2b	1:100	BD Pharmingen
Isotype control	IgG1	1:20	Sigma, Taufkirchen, Germany
P 260	Mouse IgG	1:50	Dako

inantly expressed by Th1 cells (9). Increased numbers of CCR5-expressing ($CCR5^+$) cells have been observed in a number of Th1-dominated diseases, such as rheumatoid arthritis (29, 49), inflammatory kidney disease (39), multiple sclerosis (6), hepatitis C (41), and inflammatory bowel disease (4). In contrast, CCR5 knockout mice have impaired production of the Th1 cytokine gamma interferon (3). Patients lacking $CCR5^+$ cells due to a homozygous deletion within the CCR5 gene show prolonged renal transplant survival (15), indicating a diminished Th1 response.

In the study presented here, we investigated whether a Th1 response could be demonstrated in situ in the gastric mucosa of children, in whom an undisturbed cellular microenvironment was preserved. For this purpose, we compared the levels of expression of CCR5 as a surrogate marker of Th1 cells in gastric biopsy samples from *H. pylori*-infected and noninfected children.

MATERIALS AND METHODS

Patients and tissue sampling. Children and adolescents younger than 18 years of age and requiring an upper endoscopy for diagnostic purposes and clinical indications were considered for inclusion in the study. At the time of endoscopy, the following information was obtained by using a standardized questionnaire: age; sex; nationality; presence of chronic disease, particularly inflammatory bowel disease, celiac disease, and suspected or proven food allergy; and intake of medication during the preceding 4 weeks. Within 2 days of the upper endoscopy, a ^{13}C-urea breath test (^{13}C-UBT) was performed as described previously (23). Upper endoscopy was performed by the same investigator (S.K.) using a pediatric gastroscope (Olympus PQ20) with a 2.8-mm working channel. Macroscopic findings for the esophagus, stomach, and duodenum were reported in a standardized fashion. Forceps biopsy samples were obtained from different sites for the following purposes: one each from the antrum and corpus for histological analysis and two additional antrum samples for rapid urease testing and culturing of *H. pylori*. In addition to these routine biopsy samples, one or two biopsy samples of the antrum were obtained for immunohistochemical analysis. After mucus was removed by swabbing the surface of the biopsy samples, the specimens were snap-frozen on dry ice, embedded in Tissue-Tek (Sakura Finetek Europe B.V., Zoeterwoude, The Netherlands), and stored at −80°C until processing.

A child was considered infected with *H. pylori* when the ^{13}C-UBT and the rapid urease test and/or cultures gave positive results and as noninfected when all three tests gave concordant negative results. Children not fulfilling these criteria were excluded from the study. *H. pylori*-infected patients with a known immunological disorder (e.g., food allergy, acquired or congenital immunodeficiency, celiac disease, or inflammatory bowel disease) or with intake of antibiotics, acid-suppressing agents, or anti-inflammatory drugs within 4 weeks prior to endoscopy were excluded from the study.

The children were divided into three groups according to *H. pylori* status, clinical and laboratory findings, history, and macroscopic findings during upper endoscopy. However, classification was made without knowledge of the results of histological and immunohistochemical analyses. Group 1 included all children with proven *H. pylori* infection. Group 2 included noninfected patients with suspected or proven gastric disease, defined as an abnormal macroscopic appearance of the gastric mucosa (erythema, erosion, ulceration, or nodularity), a known underlying disease, or intake of drugs that may affect the gastric mucosa. This group was considered a disease control group. Group 3 included the remaining *H. pylori*-negative children, who underwent gastric endoscopy, had no intake of drugs during the preceding 4 weeks, and had no known condition that would affect the stomach. Although patients in group 3 were not all healthy children, they were considered a healthy control group because they had no evidence of gastric disease. Written informed consent was obtained prior to endoscopy. The study protocol was approved by the Ethics Committee of Ludwig-Maximilians University.

Blood sampling. Prior to endoscopy, peripheral venous blood was drawn from each child for a blood count to exclude anemia. For nine children, residual material from the blood count was used for flow cytometry.

Histological analysis. Histopathological classification of gastritis was performed by one pathologist (D.A.) using paraffin-embedded sections with hematoxylin-eosin and modified Giemsa staining. The pathologist was blinded for clinical symptoms, diagnoses, and medications. The upgraded Sydney classification (13) was applied and included grading of chronicity, activity, atrophy, intestinal metaplasia, and bacterial density on a scale from 0 to 3 (none, mild, moderate, and severe, respectively).

Immunohistochemical analysis. Immunohistochemical analysis was performed with air-dried 5-μm cryostat sections of antral mucosa biopsy samples by the indirect immunoperoxidase method. In brief, sections were incubated for 35 min with 40 μl of a primary mouse monoclonal antibody. Optimal dilutions were determined with human tonsil samples (Table 1). Endogenous peroxidase was blocked with blocking reagent (Dako, Hamburg, Germany). After washing, a secondary peroxidase-labeled rabbit anti-mouse immunoglobulin G (IgG) antibody was applied for an additional 30 min. Sections were developed with diaminobenzole under visual control until brownish staining developed. Counterstaining was done with Mayer's Haemalaun (Merck, Darmstadt, Germany). Isotype controls were included. Granulocytes were detected in unblocked sections by the activity of endogenous peroxidase. All biopsy samples were analyzed with the aid of image-processing software (dhs database, version 4.0) by digitizing three nonoverlapping areas (×20 objective; digital 0.5-in.-chip-size TK-C1380 camera [JVC]).

Intraepithelial cells were quantified as cells per 100 epithelial cells. For the lamina propria, a grid with a side length of 40 μm was used. The lamina propria area was calculated by subtracting the number of intercept points falling into the lamina propria from the number of total intercept points. A total area of 0.625 mm^2 was analyzed. Results are expressed as cells per square millimeter.

Flow cytometry. Three-color flow cytometry was performed with freshly drawn whole-blood samples treated with the anticoagulant EDTA as described elsewhere (18). The following primary antibodies were used: for CD4, allophycocyanin-mouse IgG1 (BD Pharmingen, Heidelberg, Germany); for CD8, phycoerythrin (PE)-cyanin-5–mouse IgG1 (Immunotech, Marseille, France); for CD19, PE-mouse IgG1 (Immunotech); and for CCR5, PE-mouse IgG2a (R&D Systems, Wiesbaden, Germany). As isotype controls, mouse IgG1-fluorescein isothiocyanate (Immunotech) and mouse IgG2a-PE (Immunotech) were used. Cells were immediately analyzed by flow cytometry (Calibur; Becton Dickinson, Heidelberg, Germany), and calculations were performed with CellQuest analysis software. To determine the percentage of chemokine receptor-positive T cells, lymphocytes were first gated according to their forward-scatter and side-scatter characteristics. A second gate was set for $CD4^+$ or $CD8^+$ cells, and the number of chemokine receptor-positive cells was calculated after defining a cutoff value by using an isotype control.

Determination of CCR5 genotype. CCR5 genotype analysis was performed by PCR with genomic DNA isolated from gastric biopsy samples. Biopsy samples were placed in 1 ml of PCR buffer with nonionic detergents (50 mM KCl, 10 mM Tris-HCl [pH 8.3], 2.5 mM $MgCl_2$, 0.01% [wt/vol] gelatin, 0.45% [vol/vol] Nonidet P-40, 0.45% [vol/vol] Tween 20) and proteinase K (5 μl of a 20-mg/ml stock solution in water). Samples were incubated overnight at 56°C to achieve complete lysis. Proteinase K was inactivated by heating (10 min at 95°C), and 1 μl of the lysate was used directly for PCR analysis. A genomic fragment of the human CCR5 gene spanning a region with a 32-bp deletion was amplified by PCR with primers and conditions described previously (15).

Determination of CagA tyrosine phosphorylation and IL-8 production. For measurement of CagA tyrosine phosphorylation, AGS cells (ATCC CRL-1739) were grown in six-well plates (9.5 cm^2) and infected with *H. pylori* at a multiplicity of infection of 100. Adherent bacteria were removed by washing five times in phosphate-buffered saline (PBS). Cells were suspended in 1 ml of ice-cold PBS* (PBS, 1 mM EDTA, 1 mM *o*-vanadate, 1 mM phenylmethylsulfonyl fluoride, 1 μM leupeptin, 1 μM pepstatin), collected by centrifugation, and resuspended in 3 μl of PBS*. Lysates of infected cells were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis by using a minigel apparatus (Bio-Rad) and were blotted onto a polyvinylidene difluoride membrane at 1 mA/cm^2 by using a semidry blot system (Biotec Fischer). For detection of CagA, the filters were blocked with 3% bovine serum albumin in 50 mM Tris-HCl (pH 7.5)–150 mM NaCl and incubated with antiserum AK257 (anti-CagA) (34). Alkaline phosphatase-coupled protein A was used to visualize the antibody bound by decomposition of nitroblue tetrazolium. For detection of tyrosine phosphorylation of proteins, a blot was blocked overnight in 50 mM Tris (pH 7.4)–200 mM NaCl–0.1% Tween 20– 5% milk powder. For detection of tyrosine phosphorylation of CagA, phosphotyrosine-specific monoclonal antibody PY99 was applied (Santa Cruz Biotechnology, Inc.).

A sandwich enzyme-linked immunosorbent assay (38) was used for the quantitative determination of IL-8. The anti-IL-8 capture antibody was bound to a 96-well microtiter plate. After four washing steps and blocking of nonspecific binding sites, 100 μl of cell culture supernatant was tested per well. Each sample was tested in duplicate, and the assay was standardized with 0 to 800 pg of recombinant human IL-8/ml. After overnight incubation (4°C) and four washing steps, the wells were incubated with biotinylated secondary anti-IL-8 antibody G265-8 (0.5 μg/ml in blocking buffer–10% PBS) for 1 to 2 h at room temperature. A streptavidin-alkaline phosphatase conjugate was added, and the mixture was incubated for 1 h at 37°C. After four washing steps, the substrate was added, and the mixture was incubated for 20 min at 37°C in the dark before the absorbance at 405 nm was measured with an enzyme-linked immunosorbent assay reader.

Statistical analysis. The nonparametric Kruskal-Wallis test was applied to calculate differences among the three groups. The Mann-Whitney U test was used to test differences between two groups. A probability of ≤ 0.05 was regarded as statistically significant. The Spearman rho test was performed to test the correlation among scores of Sydney classification parameters, CagA expression, and cell subsets. A correlation was considered positive when correlation coefficient *r* was greater than 0.3. Statistical analysis was performed with the SPSS statistical program (version 10; SPSS Inc., Chicago, Ill.) and with the expert advice of a statistician (P. Dirschedl, Institute of Biometry and Epidemiology, Ludwig-Maximilians University).

RESULTS

Patient characteristics. A total of 70 children were included in this study: 38 males and 32 females with a median age of 9.5 years and a range of 0.9 to 17.5 years. Thirty-one patients (17 females) were infected with *H. pylori* and formed group 1. All patients in group 1 had positive results for the ^{13}C-UBT and the rapid urease test; all but three had positive cultures. Their median age was 10.7 years, with a range of 3.5 to 17 years. The remaining 39 children were not infected with *H. pylori*, based on concordant negative results in all diagnostic tests. Twenty-four of these noninfected patients were considered disease controls and formed group 2 (12 females; median age, 7.2 years, and range, 0.9 to 17.5 years). Sixteen of the 24 children had a chronic inflammatory disease of the digestive tract: 4 children had Crohn's disease or ulcerative colitis, 6 patients had proven or highly suspected food allergy, 5 patients had celiac disease with flat duodenal mucosa, and 1 child had had chronic varioliform gastritis with associated autoimmune thyroiditis for 2 years. Five children had been successfully treated for *H. pylori* infection within the preceding 12 months. The remaining three children had gastric erythema in the antrum: one patient had *H. heilmannii* infection, and the two remaining patients had severe reflux esophagitis, delayed gastric emptying, and gastric erythema due to biliary reflux. Fifteen noninfected children (9 females; median age, 6.2 years, and range, 1.6 to 15.7 years) had no indication of gastric involvement and were assigned to group 3. Seven of them had clinical symptoms suggestive of reflux esophagitis; however, their esophagus was without pathological macroscopic findings. Three children with previous reflux esophagitis underwent control endoscopy after weaning from medication. These children had neither clinical nor endoscopic signs of esophagitis. One child was investigated because of refusal to eat, but esophagitis or other pathological findings could not be demonstrated. One child had achalasia, and one had suspected celiac disease, which was not confirmed with duodenal biopsy samples. One severely mentally retarded child underwent endoscopy to rule out reflux disease and received a percutanous endoscopic gastrostoma because of malnutrition.

Histopathological analysis. The results of the histopathological grading for activity and chronicity of gastritis and bacterial density are summarized in Fig. 1. Higher grades of activity and chronicity were found only in *H. pylori*-infected children. Mild atrophy (grade 1) was observed in four, two, and three patients in groups 1, 2, and 3, respectively. Intestinal metaplasia was not observed in any of the patients. Lymphoid follicles were present in 28 *H. pylori*-infected children and in 10 patients in group 2, whereas no follicles were seen in children without gastric disease.

Intraepithelial lymphocytes. The numbers of intraepithelial T cells were not significantly different among the groups. $CD8^+$ cells (median values, 6.2, 6.3, and 7.0% in groups 1, 2, and 3, respectively) clearly predominated over $CD4^+$ T cells (median values, 2.2, 0.9, and 1.7% in groups 1, 2, and 3, respectively). The CD4/CD8 ratio was slightly, but not significantly, higher in the *H. pylori*-infected group than in the other two groups (0.44 versus 0.29). B cells, defined as CD19-staining cells, monocytes, and neutrophils, were not observed within the epithelium. $CCR5^+$ cells were demonstrated in the epithelium of 64 of 70 children. No differences were observed among the groups (median values, 3.2, 2.5, and 3.1% of cells in groups 1, 2, and 3, respectively, with a range of 0 to 10.3% of cells). The children lacking $CCR5^+$ cells belonged to groups 2 and 3. In four of these children, $CCR5^+$ cells were also not detected in the lamina propria.

Lamina propria lymphocytes. All patient biopsy samples had T cells in the lamina propria, with a predominance of $CD4^+$ cells. The numbers of $CD4^+$ and $CD8^+$ cells were significantly higher in *H. pylori*-infected children (Fig. 2). However, the CD4/CD8 ratio was not altered among the groups. No significant differences were found between disease controls and healthy children. B cells were present in 13 of 66 children. Eleven of these children were infected with *H. pylori*. The two *H. pylori*-negative children whose biopsy samples stained for B cells in the lamina propria had been successfully treated for an

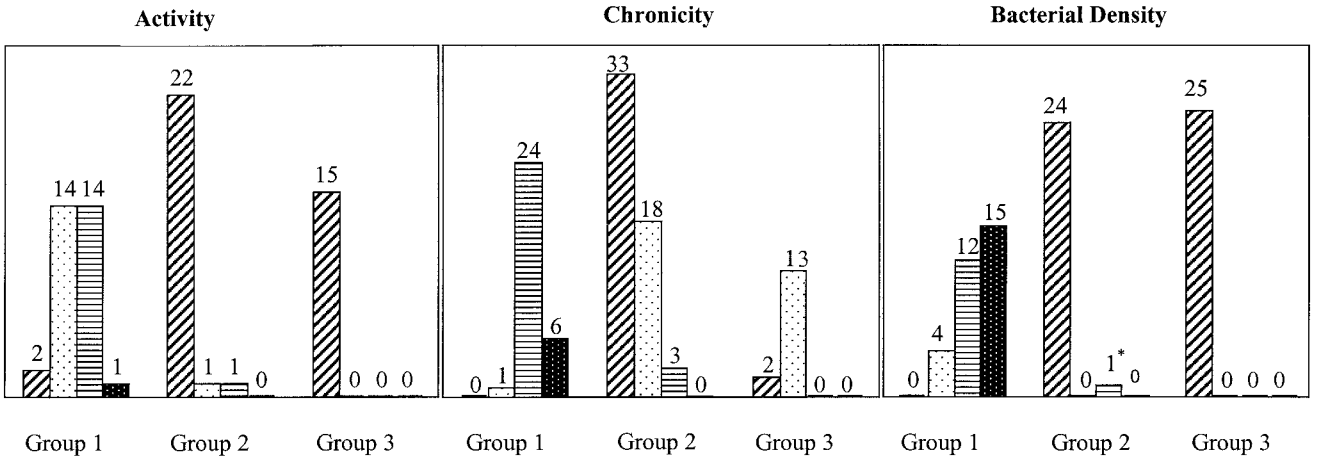

FIG. 1. Sydney classification for the three patient groups. Bars for each group indicate, from left to right, grades 0, 1, 2, and 3, respectively. Numbers above bars indicate numbers of patients in groups. The asterisk indicates one child who was infected with *H. heilmannii* but not with *H. pylori*.

H. pylori infection within the preceding 12 months. Most biopsy samples had very low numbers of B cells (median, 0%; range, 0 to 117%). Three children with unusually high numbers of B cells in their biopsy samples were siblings from an Afghani family. $CD14^+$ cells were present in only 10 of 67 children and were distributed in all groups without apparent differences. In contrast, $CCR5^+$ cells could be demonstrated in 66 of 70 children, and there was a highly significant difference between *H. pylori*-positive and -negative individuals (Fig. 2). The four children lacking $CCR5^+$ cells in the lamina propria also lacked intraepithelial $CCR5^+$ cells. Two children each belonged to group 2 and group 3. Individuals homozygous for the 32-bp deletion in the CCR5 gene express a truncated protein that cannot be expressed at the cellular surface (11, 37). Therefore, CCR5 genotyping was performed for these four children to determine whether this deletion could have accounted for the lack of $CCR5^+$ cells. This analysis revealed that one individual in each group was heterozygous for the deletion. Therefore, the lack of $CCR5^+$ cells was not due to the presence of homozygous mutant alleles.

To determine whether the increase in the numbers of $CCR5^+$ cells was specific for *H. pylori* infection, we calculated the ratios of $CCR5^+$ cells to T cells for the different groups. No significant changes in the $CCR5^+$ cell/T-cell ratios were observed. As would be expected, neutrophil numbers differed highly significantly between infected and noninfected children (Fig. 2).

Association of $CCR5^+$ cells with lymphocyte subsets. We further analyzed whether $CCR5^+$ cells correlated with the presence of other cells. No significant correlations were found within the epithelium. In contrast, $CCR5^+$ cells showed highly significant correlations with $CD4^+$ cells ($r = 0.63$; $P < 0.001$) and $CD8^+$ cells ($r = 0.48$; $P < 0.001$) in the lamina propria. A lower correlation was also found for B cells ($r = 0.31$; $P = 0.01$). To exclude bias due simply to higher cell numbers in *H. pylori*-infected individuals, we analyzed the association of $CCR5^+$ populations with other lymphocyte populations in individual groups (Fig. 3). A strong correlation was still found for T cells in groups 1 and 2. No correlation was found in the healthy children.

Correlation of CCR5 expression between lamina propria and peripheral blood. The expression of CCR5 on peripheral blood lymphocytes was compared with the expression in the lamina propria for nine patients. Six, two, and one patients

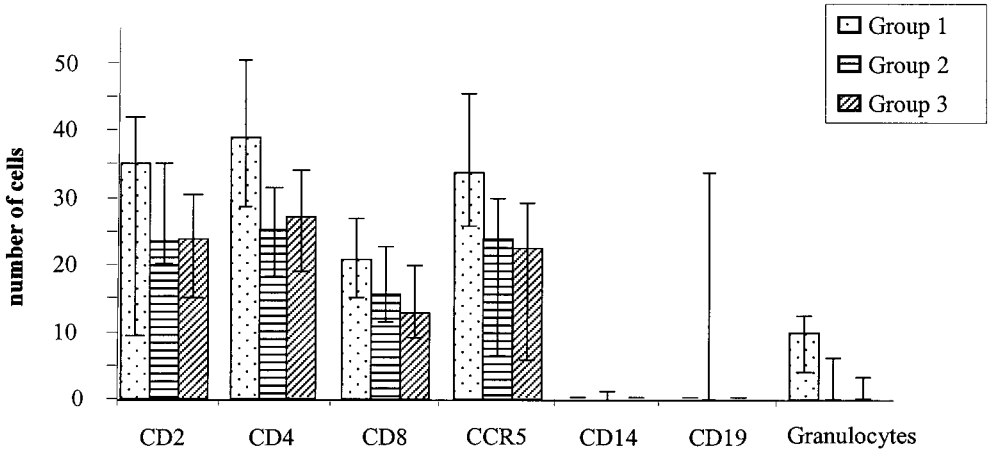

FIG. 2. Cells per square millimeter in the lamina propria. Bars indicate interquartile ranges.

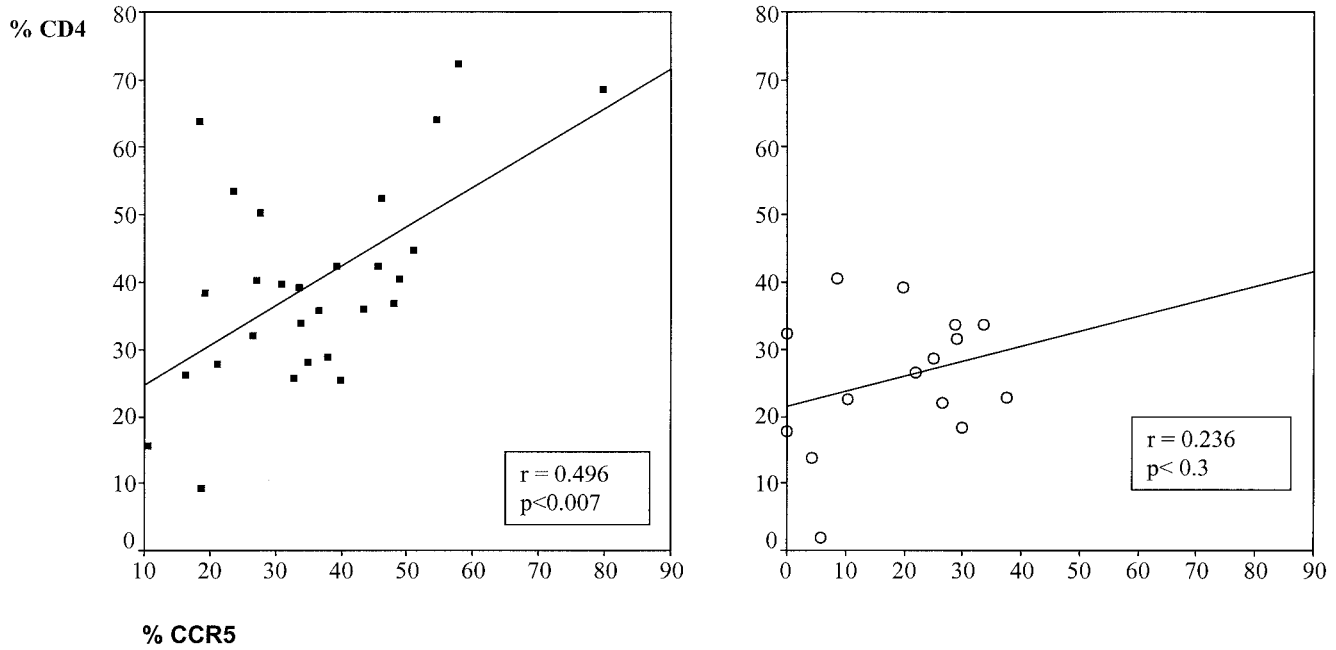

FIG. 3. Correlation of $CCR5^+$ and $CD4^+$ cells in the lamina propria. (Left) Group 1. (Right) Group 3. The Spearman rho test was used.

belonged to groups 1, 2, and 3, respectively. In peripheral blood, the percentage of $CCR5^+$ T cells ranged from 1.2 to 28.2% (median, 6.55%). Since monocytes were very rare or absent in the lamina propria, it was presumed that CCR5 expression in the gastric mucosa was essentially due to T cells. The biopsy samples with numbers of $CCR5^+$ cells higher than the sum of $CD4^+$ and $CD8^+$ cells had higher numbers of B cells, a fact which most likely accounted for the additional $CCR5^+$ cells. A comparison of the values for peripheral T cells with those for lamina propria T cells revealed much higher numbers of $CCR5^+$ T cells in the lamina propria (Fig. 4), suggesting specific accumulation or selective expansion of these cells in the lamina propria.

Correlation with CagA status. From 24 *H. pylori*-infected patients, the respective *H. pylori* strain was isolated and analyzed for its capacity to induce IL-8 secretion and to translocate and phosphorylate the CagA protein in AGS epithelial cells. Both phenotypes are dependent on an intact *cag* pathogenicity island (PAI), which encodes a functional type IV secretion system. Fifteen isolated strains were able to induce IL-8 secretion and to translocate and tyrosine phosphorylate CagA. Both markers showed a positive and very strong correlation with the numbers of neutrophils in the lamina propria (for IL-8, r was 0.562 and P was <0.005; for Tyr-P, r was 0.589 and P was <0.002). In contrast, the numbers of $CCR5^+$ cells and all other lymphocyte subsets did not correlate significantly with the presence or absence of a functional *cag* PAI.

DISCUSSION

The present work demonstrates that $CCR5^+$ cells and T cells are regularly present in gastric mucosa and that their levels increase noticeably in inflamed gastric mucosa. Previous studies investigating the immune response in *H. pylori*-infected gastric mucosa in humans were performed mainly with adults. However, the gastric immune response of adults can be altered by factors not related to the infection, including exposure to alcohol, tobacco, or pharmaceutical agents (43). Therefore, we investigated the gastric mucosa of children, in the majority of whom these factors could be largely excluded. Some studies have classified patients according to histological criteria only (40, 48). This procedure might introduce selection bias, since higher numbers of immunohistochemically stained cells are expected in tissues that are rich in inflammatory cells. For this reason, we based our patient classification on the results of

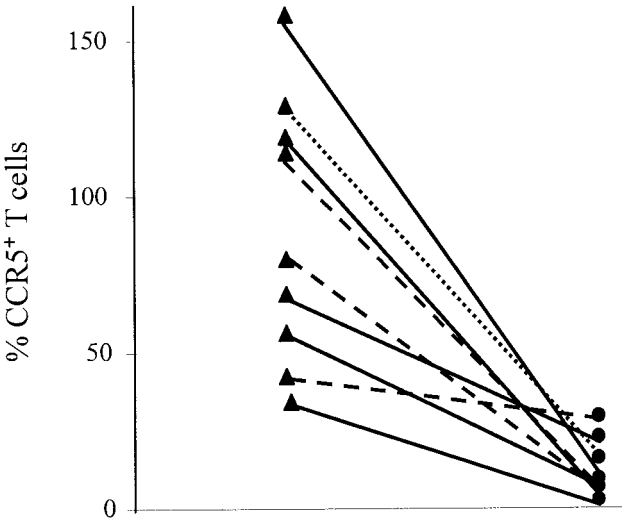

FIG. 4. $CCR5^+$ T cells in lamina propria (triangles) and peripheral blood (circles) for group 1 (solid line), group 2 (dashed line), and group 3 (dotted line).

three diagnostic tests. Because we wanted to exclusively assess the immune response to *H. pylori*, we excluded all infected children with an additional underlying condition that may have influenced gastric inflammation. The division of noninfected children into disease controls and healthy controls was based on the clinical history and endoscopic findings, but we did not consider the results of histological analyses. The finding of lymphoid follicles, which clearly represent a pathological finding in the stomach, in 90% of the *H. pylori*-infected children and in 42% of the disease controls but in none of the healthy controls confirmed, retrospectively, the assignment of the patients to the three groups. Group 2 included five children with recently eradicated *H. pylori* infection. It seemed most reasonable to include these children in group 2, since cellular infiltrates may persist for several months after successful treatment of the infection. Exclusion of these children from the calculation, however, did not alter the results (data not shown). *H. pylori* infection is mainly acquired in early childhood, but symptoms develop only after a longer period of infection. This explains why the children in group 1 were significantly older ($P \leq 0.04$) than the noninfected children. However, a relationship between age and numbers of $CCR5^+$ cells seems unlikely, since CCR5 expression within each group did not correlate with age. There were also differences in nationality, since group 1 included more children of non-German origin than the other two groups. However, analysis within the groups revealed no correlation of CCR5 expression with nationality.

Intraepithelial T cells were regularly detected, with a predominance of $CD8^+$ cells. A slight but nonsignificant increase in the CD4/CD8 ratio was observed in infected gastric mucosa. This finding is in line with the findings of Hatz et al. (19) but contradicts those of other studies in which significant increases in the numbers of T cells (24) or $CD8^+$ cells (20) were found in *H. pylori*-infected mucosa. In contrast, the numbers of lamina propria $CD4^+$ and $CD8^+$ T cells increased significantly during *H. pylori* infection, with a consistent predominance of $CD4^+$ cells, corresponding to the findings of Hatz et al. (19). The CD4/CD8 ratio was not significantly different among the groups, in contrast to studies that reported a selective enrichment of $CD4^+$ cells (40) or $CD8^+$ cells (20) in infected individuals. These cited studies were performed exclusively with adults and had substantial methodological differences that may have contributed to the divergent results. B cells were rarely detected in the biopsy samples from the children. However, when B cells were present, they were almost exclusively found in children with active or recent *H. pylori* infection.

Detailed studies of the local and systemic immune responses against *H. pylori* have led to the concept that infection with *H. pylori* elicits a predominant Th1 immune response (21, 33, 44). Both in vitro and in vivo studies have shown that CCR5 expression is associated with a Th1 response (4, 9, 27, 32, 45). Recent studies revealed CCR5 expression on isolated lymphocytes of the human jejunum (1, 31). Nevertheless, only indirect evidence suggests that $CCR5^+$ cells may be also involved in gastric inflammatory disease, on the basis of the finding that the levels of the CCR5 ligands MIP-1α (2, 42) and RANTES (22, 43, 51) increase during *H. pylori* infection. However, the binding of MIP-1α or RANTES is not restricted to $CCR5^+$ cells. Furthermore, monocytes and macrophages express CCR5 in addition to T cells. In our study, high numbers of $CCR5^+$ cells were present in inflamed gastric mucosa, with a strong statistical correlation between T cells and CCR5 expression in the lamina propria of *H. pylori*-infected children and those with suspected gastric disease. In healthy children, this correlation did not exist, a result which is possibly due to the overall lower cell numbers in healthy gastric mucosa. Taking into account that macrophages were absent or very rare, we assume that CCR5 expression was primarily restricted to T cells; differentiation between $CD4^+$ and $CD8^+$ cells could not be made. In four *H. pylori*-negative children, $CCR5^+$ cells were absent in both the epithelium and the lamina propria. The two children carrying one allele with a CCR5 mutation had average numbers of $CD2^+$ T cells in both tissue compartments (intraepithelial lymphocytes, 4 to 8%; lamina propria, 19 to 23 cells/mm^2). Thus, the absence of $CCR5^+$ cells in these children could not be explained by an overall absence of infiltrating lymphocytes in the tissues. Other polymorphisms in the CCR5 gene that lead to reduced expression of the protein were not examined in these children, since they are very rare mutations (8, 46).

The expression of CCR5 on high numbers of cells in the gastric mucosa contrasted with the considerably lower numbers of peripheral $CCR5^+$ T cells. A high percentage of organ-infiltrating $CCR5^+$ T cells has also been described for Th1-associated diseases, such as hepatitis C (41) and different forms of arthritis (29). Whether this finding represents selective recruitment of $CCR5^+$ memory T cells, local upregulation of CCR5 expression on T cells, or local proliferation of such T cells in this microenvironment is unknown at present. The *cagA* PAI, which consists of multiple genes, seems not to be involved in the regulation of CCR5 expression. An intact PAI induces gastric expression of the CXC chemokines IL-8, ENA-78, and GRO-α, while that of the CCR5 ligand RANTES appears to be independent of the PAI (43).

In conclusion, the present study shows that *H. pylori* leads to major changes in cellular compositions in the lamina propria but not in the gastric epithelium. Furthermore, it provides evidence that $CCR5^+$ cells are constitutively present in healthy gastric mucosa. The increased numbers of $CCR5^+$ cells in *H. pylori*-infected stomach mucosa indicate that this molecule may play an important role in gastric mucosal immune responses. Taking into consideration that CCR5 is a Th1-associated molecule, we speculate that the gastric mucosa is primarily capable of exerting Th1 immune responses irrespective of the nature of the intruding pathogen.

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