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Mucosal Immunity Related to CD8+ T Lymphocytes in Children with *Helicobacter pylori* Gastritis

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ABSTRACT

Purpose: We investigated the role of CD8+T cells as host immune factors in pediatric patients with *Helicobacter pylori* gastritis.
Methods: Gastric mucosal tissue and blood samples were collected from 39 children, including 11 children with *H. pylori* infection and 28 children as controls. Anti-CD8 and anti-T-bet antibodies were used for immunohistochemistry of the gastric mucosa. For the cell surface and intracellular staining, peripheral blood mononuclear cells were stained with anti-IL7Rα, anti-CX3CR1, anti-CD8, anti-T-bet, and anti-IFN-γ antibodies. Cytokines of sera such as tumor necrosis factor alpha (TNF-α) and CX3CL1 were analyzed using enzyme- linked immunosorbent assay (ELISA).
Results: In the immunohistochemistry of gastric mucosa, the frequency of $CD8^+$ and $T\text{-}bet^+$ T cells cells was higher in the *H. pylori*-positive group than in the control group (26.9± 7.8% vs. 16.9±3.3%, $p<0.001$; 5.0±2.5% vs. 2.2±0.7%, $p=0.001$). Between the control and *H. pylori*-positive groups, the frequency of $IL\text{-}7R\alpha^{low}CX3CR1^+$ $CD8^+$ and $T\text{-}bet^+$ $INF\text{-}\gamma^+$ $CD8^+$ T cells were not significantly different between surface and intracellular staining, respectively (40.4±24.0% vs. 38.2±17.8%, $p=0.914$; 40.4±24.0% vs. 38.2±17.8%, $p=0.914$). In the ELISA, no significant differences in TNF-α and CX3CL1 concentrations were observed between the control and *H. pylori*-positive groups (34.3±12.1 pg/mL vs. 47.0±22.6 pg/mL, $p=0.114$/0.5± 0.1 pg/mL vs. 0.5±0.1 pg/mL, $p=0.188$).
Conclusion: $CD8^+$ T and Th1 cells, which secrete IFN-γ, might play important roles in the mucosal immunity of the stomach in children with *H. pylori* infection.

Keywords: *Helicobacter pylori*; Gastritis; Child; T-lymphocyte

INTRODUCTION

Helicobacter pylori is a gram-negative, spiral-shaped, microaerobic bacterium. It is an important causal factor for chronic gastritis, peptic ulcers, gastric mucosal and lymphoid tissue-associated lymphoma, and gastric cancer. Over 50% of the world's population is chronically infected with *H. pylori*. Early *H. pylori* infections can occur during infancy and remain chronic if left untreated. Although the *H. pylori* infection rate in Korean children and adolescents is decreasing, 33% of asymptomatic or healthy children are currently infected [1-3].

Conflict of Interest
The authors have no financial conflicts of interest.

In the gastric mucosal immune response by *H. pylori*, the role of Th1 cells in CD4+ T cells is well known to be mainly involved in the gastric mucosal immune response to *H. pylori*; however, the role of CD8+ T cells in the gastric mucosal immune response to *H. pylori* is unclear [4].

Therefore, this study aimed to investigate the role of CD8+T cells as host immune factors in pediatric patients with *H. pylori* gastritis.

MATERIALS AND METHODS

Children with abdominal pain who visited or were hospitalized in the Department of Pediatrics at Jeju National University between July 2019 and May 2022 were included in this study. Gastric mucosal tissue and blood samples were collected from 39 patients, including 11 patients who were positive for one of two tests (campylobacter-like organism [CLO] test/rapid urease test, or Giemsa stain) to confirm *H. pylori* infection, and 28 controls who were negative. Four gastric mucosal tissue samples were collected from the control and *H. pylori*-positive groups after gastroscopy. Two biopsies were performed for the CLO test and for histology with Giemsa staining at the great or lesser curvature of the pre-pyloric antrum. Two biopsies were performed for the same tests at the great and lesser curvatures of the gastric midbody. Two biopsies were also performed at preparation, and blood samples were collected by extracting 7 mL of blood from the control and *H. pylori*-positive groups into heparin-treated tubes.

Immunohistochemical staining

Gastric mucosal tissue samples were prepared using anti-CD8 (VENTANA) and anti-T-bet (Thermo Fisher Scientific) antibodies. Immunohistochemical staining was performed according to manufacturer's instructions. Antibody incubation and detection were performed using an autostainer (Ventana Bench Mark ULTRA, OptiView DAB IHC Detection kit; Ventana Medical Systems). Immunohistochemical staining involves the visualization of antigens through sequential reactions with specific antibodies (primary antibodies) against the antigen, followed by a secondary antibody attached to the primary antibody, an enzyme complex, a chromogenic substrate, and a washing step. Enzyme activation by a chromogenic substrate generates a visual reaction product at the antigen–antibody binding site.

Finally, the numbers of CD8+ and T-bet+ T cells were analyzed using an optical microscope (Leica DM LB2; Leica) and the ImageJ software (National Institute of Health), respectively.

Anti-CD8 staining procedure

Unstained slides were prepared by cutting 4-μm-thick sections. Antigens were retrieved using ULTRA Cell Conditioning Solution (Ventana Medical Systems). The sections were incubated with the anti-CD8 primary antibody for 20 minutes at 36°C. The slides were visualized using the OptiView DAB IHC Detection Kit, followed by hematoxylin II counterstaining, which was performed using the bluing reagent.

Anti-T-bet staining

The antigen retrieval procedure was the same as in the anti-CD8 staining procedure and reacted with the primary antibody. Subsequently, the antigen was attached to the primary antibody using a secondary antibody and horseradish peroxidase enzyme, and a color

reaction was induced using H_2O_2 and DAB chromogen. For the controls, nuclei were stained using hematoxylin II, the bluing reaction was induced using the bluing reagent, and the sample slides were stored at room temperature and covered with a coverslip liquid.

Flowcytometry analysis

The peripheral blood mononuclear cell (PBMC) layer was extracted after placing the whole blood sample in Ficoll–Paque Premium (GE Healthcare) and centrifuging (700 g, 20 minutes, break zero).

For the surface staining of PBMC 1×10^6 cells, anti-APC-Cy7-CD3, anti-Pacific Blue-CD8, anti-PE-Cy7-CCR7, anti-PE-Cy5-CD45RA (BD Biosciences), anti-FITC-interleukin-7 receptor alpha (IL-7Rα) (R&D Systems), and anti-PE-CX3CR1 (BioLegend) antibody complexes were used. The results of cell surface staining for PBMCs was analyzed by flowcytometry, LSRFortessa® flow cytometer (BD Biosciences) and FlowJo software (Tree Star). First, the lymphocyte zone was gated, followed by sequential gating for $CD3^+$ and $CD8^+$cell zones, CD45RA/CCR7 positive/negative zones to establish effector memory $CD8^+$ T cells. For the intracellular stimulation of PBMC 1×10^6 cells, phorbol myristate acetate (acetate, 50 ng/mL; SigmaAldrich), ionomycin (1 μg/mL; SigmaAldrich), and GolgiPlug (BD Biosciences) were added in the *H. pylori*-positive group, whereas only GolgiPlug was added to the PBMC 1×10^6 cells in the control group. Each mixture was combined with Roswell Park Memorial Institute Medium and placed in a CO2 incubator for 4 hours. Cell fixation and permeabilization were performed using FOXP3 (forkhead box P3) FIX/Perm Buffer (BioLegend). Anti-APC-Cy7-CD3, anti-Pacific Blue-CD8, anti-PerCP/cyanine5.5-T-bet (BioLegend), and anti-PE cy7-INF-γ (BD Biosciences) antibody complexes were used for staining. After the intracellular staining of PBMC, the flow cytometry analysis was performed for the gating of CD8+ T cell zones, followed by $T\text{-}bet^+$ and INF-γ fractions of CD8+ T cells.

Cytokine analysis with enzyme-linked immunosorbent assay (ELISA) assay

Serum preparation was performed 1 day before the analysis. Serum samples of the control and *H. pylori*-positive groups were stored in −80.0°C, thawed at room temperature, and then centrifuged (4.0°C, 15,000 rpm, 15 minutes). Subsequently, each 150 μL supernatant of samples was placed into 96 well polystyrene microplates and stored in a refrigerator at 4.0°C overnight. Based on protocols by manufacturers of the ELISA kit, two cytokines, including TNF-α (Invitrogen) and CX3CL1 (R&D), were analyzed. Finally, the concentrations of the two cytokines were measured using an immunosorbent spectrophotometer (SUNRISE), and the results were analyzed using GainData (Arigobio, www.arigobio.com/elisa-analysis).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Co.). Results are presented as means±standard deviations (SDs) for the control and *H. pylori* positive groups. The two groups were compared using the Mann–Whitney U-test. Statistical significance was set at $p<0.05$.

Ethics statement

Before sample collection, the study was approved by the Institutional Review Board of the Jeju National University Hospital (JNUH 2019-02-001). Informed consent was obtained from all the children and their parents.

RESULTS

Demographic and clinical characteristics

Among the 39 children, the sex distribution (male/female) was 16/12 in the control group and 7/4 in the *H. pylori*-positive group. Of the *H. pylori*-positive group, 81.8% and 100.0% were positive in the rapid urease test (CLO test) and Giemsa staining, respectively.

The mean age (mean±SD) was 12.4±2.6 years in control group and 12.9±3.2 years in the *H. pylori*-positive group, without any significant difference between the two groups (p=0.724). In addition, the two groups did not demonstrate a significant difference in height (151.3±15.7 cm vs. 151.9±15.7 cm, p=0.949), weight (46.5±12.0 kg vs. 50.6±18.5 kg, p=0.638), and body mass index (20.3±3.5 kg/m^2 vs. 21.3±4.4 kg/m^2, p=0.434). According to the blood test, C-reactive protein (0.2±0.6 mg/dL vs. 0.5±1.1 mg/dL, p=0.009) levels were significantly different between the two groups, whereas white blood cell count (6,461.5±1,359.7/mm^3 vs. 7,154.5±3,176.6/mm^3, p=0.857) and hemoglobin (13.9±1.4 g/dL vs. 12.8±1.9 g/dL, p=0.087) levels were not significantly different. These results indicate that the control and *H. pylori*-positive groups were appropriately set for each other (**Table 1**).

Immunohistochemical staining

In the immunohistochemistry for gastric mucosal tissues of 39 children (high magnification, ×200), the frequency of CD8+ T cells was higher in the *H. pylori*-positive group than in the control group (26.9±7.8% vs. 16.9%±3.3%, p<0.001) (**Fig. 1**). When the frequency is analyzed according to the biopsy site such as the antrum and body, the frequency was also higher in the *H. pylori*-positive group than in the control group (antrum, 26.53±10.34% vs. 17.24±4.66%, p=0.001; body, 22.63±11.25% vs. 16.87±5.10%, p=0.04). Likewise, the frequency of T-bet$^+$ T cells was also significantly higher in the *H. pylori*-positive group than in the control group (5.0±2.5% vs. 2.2±0.7%, p=0.001) (**Fig. 1**). When the frequency was analyzed according to the biopsy site such as the antrum and body, the frequency was also higher in the *H. pylori*-positive group than in the control group (antrum, 5.0±2.68% vs. 2.4±1.17%, p<0.001; body, 5.07±3.08% vs. 2.42±1.23%, p=0.001).

Endoscopic findings were almost exclusively lymphofollicular gastritis, except in one case of lymphofollicular gastritis and a duodenal ulcer. Therefore, we did not analyze the differences in CD8+ T cell counts based on endoscopic findings.

Table 1. Demographic and clinical characteristics of the control (*Helicobacter pylori*-negative) and *H. pylori*-positive groups

Variable	Control (n=18)	*H. pylori* + (n=11)	p-value*
Sex (n=39), male/female	16/12	7/4	
Age (yr)	12.4±2.6 (n=28)	12.9±3.2 (n=11)	0.724
Height (cm)	151.3±15.7 (n=27)	151.9±15.7 (n=11)	0.949
Weight (kg)	46.5±12.0 (n=22)	50.6±18.5 (n=11)	0.638
BMI (kg/m^2)	20.3±3.5 (n=21)	21.3±4.4 (n=11)	0.434
White blood cell (/mm^3)	6,461.5±1,359.7 (n=26)	7,154.5±3,176.6 (n=11)	0.857
Hemoglobin (g/dL)	13.9±1.4 (n=26)	12.8±1.9 (n=11)	0.087
CRP (mg/dL)	0.2±0.6 (n=24)	0.5±1.1 (n=11)	0.009
Endoscopic finding (n)	Within normal limit (18)	Lympho-follicular gastritis (11) Duodenal ulcer (1)	
CLO test +, A/B (%)	0	81.8/45.5	
Giemsa staining+ (%)	0	100	

Values are presented as number only or mean±standard deviation.
BMI: body mass index, CRP: C-reactive protein.
*Mann–Whitney U-test.

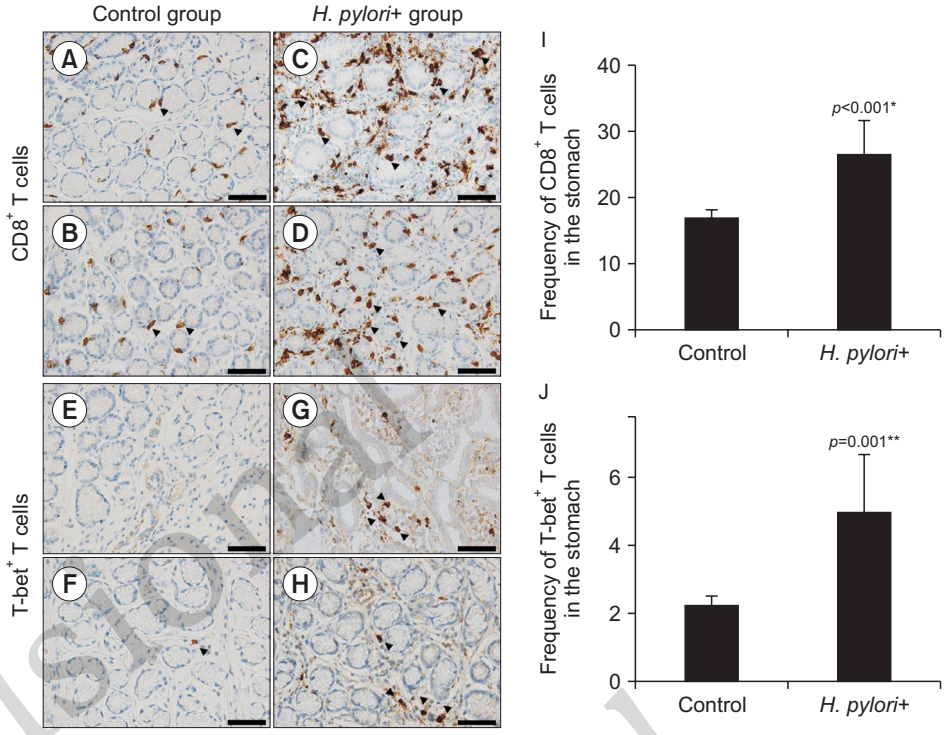

Fig. 1. The immunohistochemical staining with anti-CD8 and anti-T-bet antibody against the tissue of gastric mucosa of control (*H. pylori* negative) and *H. pylori* positive group. Scale bars=100 μm (A-H). Compared with the control group, the frequency of CD8⁺ T cells was higher in the *H. pylori*-positive group (16.9±3.3% vs. 26.9±7.8%, **p*<0.001) (I). The frequency of T-bet⁺ T cells was also higher in the *H. pylori*-positive group (2.2±0.7% vs. 5.0±2.5%, ***p*=0.001) (J).
H. pylori: *Helicobacter pylori*.

Flowcytometry analysis

In the cell surface staining of the PBMCs, no significant difference in the frequency of IL-7Rα^{low}CX3CR1⁺CD8⁺T cells was observed between the control and *H. pylori*-positive groups (40.4±24.0% vs. 38.2±17.8%, *p*=0.914) (**Table 2**, **Fig. 2**). The frequency of IL-7Rα^{low}CX3CR1⁻CD8⁺T cells was higher in the *H. pylori*-positive group than in the control group without clinical significance (8.5±3.4% vs. 5.2±2.1%, *p*=0.003). In the intracellular staining of PBMCs, no significant difference in the frequency of T-bet⁺INF-γ⁺ CD8⁺ T cells was observed between the control and *H. pylori*-positive groups (16.8±10.1% vs. 14.0±9.7%, *p*=0.549). Further, no significant difference in the frequencies of T-bet⁺CD8⁺T cells (28.2±14.3% vs. 28.1±16.0%, *p*=0.905) and INF-γ⁺ CD8⁺ T cells (20.4±10.5% vs.19.7±12.1%, *p*=0.905) was identified between the two groups (**Table 3**, **Fig. 3**).

Table 2. Frequency of IL-7Rα$^{high/low}$CX3CR1$^{+/-}$ (effector memory) CD8⁺ T cells

Variable	Control	*H. pylori* +	*p*-value*
IL-7Rα^{low}CX3CR1⁻ (%)	5.2±2.1	8.5±3.4	0.003
IL-7Rα^{low}CX3CR1⁺ (%)	40.4±24.0	38.2±17.8	0.914
IL-7Rα^{high}CX3CR1⁻ (%)	49.1±22.8	47.9±16.3	0.866
IL-7Rα^{high}CX3CR1⁺ (%)	5.3±3.0	5.4±3.9	0.842

Values are presented as mean±standard deviation.
IL-7Rα: interleukin-7 receptor-alpha, *H. pylori: Helicobacter pylori*.
Surface staining of peripheral blood mononuclear cells in the control (*H. pylori*-negative) and *H. pylori*-positive groups. CD8⁺ T cells are cytotoxic T cells that include IL-7Rα^{low}CX3CR1⁻/IL-7Rα^{low}CX3CR1⁺/IL-7Rα^{high}CX3CR1⁻/IL-7Rα^{high} CX3CR1⁺effector memory T cells.
*Mann–Whitney U-test.

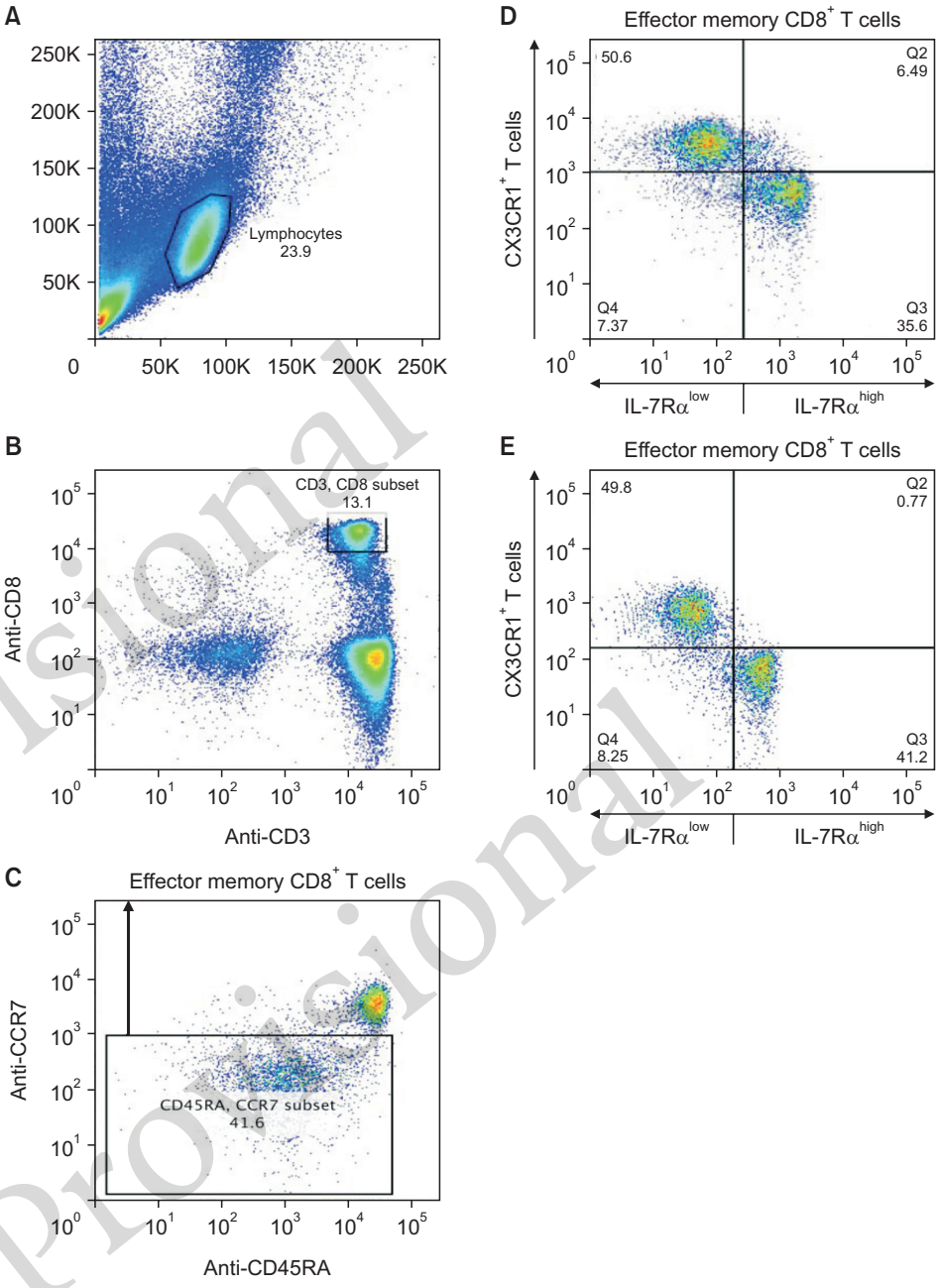

Fig. 2. An example of surface staining with anti-IL-7Rα and anti-CX3CR1 antibody against effector memory CD8+ T cells in the peripheral blood mononuclear cell of control (*H. pylori* negative; A-D) and *H. pylori* positive group (A-C, E). As shown in Table 2, the parameters related to effector memory CD8+ T cells did not show a significant difference between the two groups.
IL-7Rα: interleukin-7 receptor-alpha, *H. pylori: Helicobacter pylori*.

Table 3. Frequency of T-bet+ and/or IFN-γ+ CD8+ T cells in the intracellular staining for peripheral blood mononuclear cells of the control (*H. pylori*-negative) and *H. pylori*-positive groups

Variable	Control	*H. pylori* +	*p*-value*
T-bet+ (%)	28.2±14.3	28.1±16.0	0.905
INF-γ (%)	20.4±10.5	19.7±12.1	0.905
T-bet+ INF-γ+ (%)	16.8±10.1	14.0±9.7	0.549

Values are presented as mean±standard deviation.
IFN-γ: interferon-gamma, *H. pylori: Helicobacter pylori*.
*Mann–Whitney U-test.

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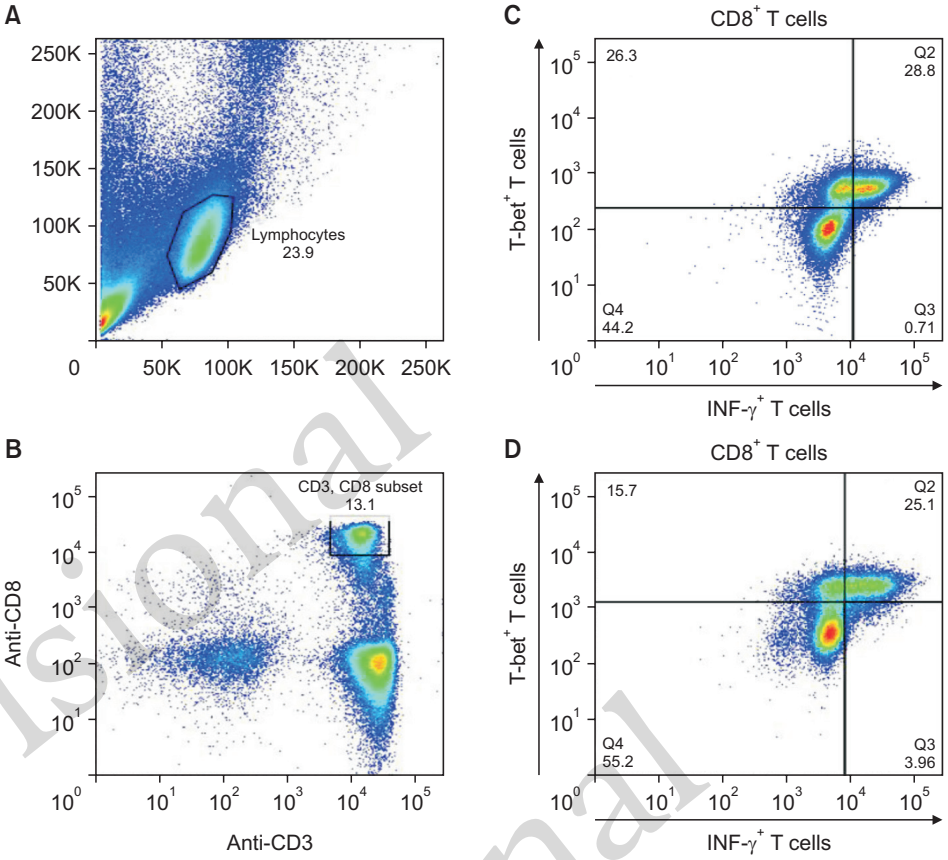

Fig. 3. An example of intracellular staining with anti-T-bet and anti-IFN-γ antibody against CD8+ T cells in the peripheral blood mononuclear cell of control (*H. pylori* negative) and *H. pylori* positive group. As shown in **Table 3**, the parameters related to CD8+ T cells did not show a significant difference between the two groups.
IFN-γ: interferon-gamma, *H. pylori: Helicobacter pylori*.

Table 4. Concentrations of cytokine TNF-α and CX3CL1 analyzed by enzyme-linked immunosorbent assay in the sera of control (*H. pylori*-negative) and *H. pylori*-positive groups

Variable	Control	*H. pylori* +	*p*-value*
TNF-α (pg/mL)	34.3±12.1	47.0±22.6	0.114
CX3CL1 (pg/mL)	0.5±0.1	0.6±0.1	0.188

Values are presented as mean±standard deviation.
TNF-α: tumor necrosis factor alpha, *H. pylori: Helicobacter pylori*.
*Mann–Whitney U-test.

Cytokine analysis with ELISA assay

In the cytokine analysis of serum samples, no significant differences in the mean concentrations of TNF-α and CX3CL1 were observed between the control and *H. pylori*-positive groups (34.3±12.1 pg/mL vs. 47.0±22.6 pg/mL, p=0.114; 0.5±0.1 pg/mL vs. 0.6±0.1 pg/mL, p=0.188) (**Table 4**).

DISCUSSION

In our study, immunohistochemical staining of gastric mucosal tissue revealed that the frequency of CD8+ T cells was higher in the *H. pylori*-positive group than in the control group (26.9±7.8% vs. 16.9±3.3%, p<0.001) (**Fig. 2**). Thus, CD8+ T cells may play an important role in

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the mucosal immune response in the stomachs of children with *H. pylori* infections, which is consistent with the hypotheses in previous studies.

In a previous study, Maciorkowska et al. [5] performed immunohistochemical staining of the anterior region of the stomach in children and compared the frequency of CD8+ T cells in three groups, a normal control group and two *H. pylori*-infected groups before and after eradication therapy. Unlike in our study, the normal control group tended to have a higher frequency of CD8+ T cells than the *H. pylori*-infected group, and a significant difference was observed between the two groups before and after eradication therapy. Although the reason for this difference is unknown, it conflicts with our previous hypothesis that CD8+ T cells are largely involved in gastric mucosal tissue immunity in *H. pylori*-infected groups. In addition, these results differ significantly from ours. Bamford et al. [6] stated that both CD8+ and CD4+ T cells were increased in the *H. pylori*-infected group than in those in the adult control group, and the ratio of CD4+ T cells to CD8+ T cells was higher in the adults with *H. pylori* infection who underwent gastric antral immunohistochemistry. Furthermore, they have reported that both CD4+ and CD8+ T cells demonstrated INF-γ expression in flowcytometry performed for the isolated T cells from gastric mucosal tissue with *H. pylori* infection and that CD8+ T cells secreted IFN-γ more predominantly than CD4+ T cells.

These results suggest that the Th1 cell-type immune response is dominant in the immune response of gastric mucosal tissue infected with *H. pylori*. Unfortunately, in our study, CD4+T cell staining and IFN-γ expression of T cells in gastric mucosal tissues were not investigated. Moreover, Bamford et al. [6] have suggested that unlike the Th2 cell-driven immune response in the colon, a Th1 cell phenotype-dominant immune response in the gastric mucosa could contribute to the chronicity of *H. pylori* infection. Considering the characteristics of gastric mucosal infection with *H. pylori*, further studies on the association between the IFN-γ secretion-driven immune response of CD4+ T cells and CD8+ T cells may be necessary. Further research is necessary to investigate why the host cannot stop or overcome chronic *H. pylori* infection.

In this study, the frequency of T-bet+ T cells was also higher in the *H. pylori*-positive group than in the control group (5.0±2.5% vs. 2.2±0.7%, p=0.001) in the immunohistochemical staining of gastric mucosal tissue (**Fig. 2**). Thus, the roles of Th1 cells and CD8+ T cells which secrete INF-γ may be greatly increased.

T-bet+ (encoded byTbx21) T cells are immune cell-specific members of the T-box transcription factor family. Together with INF-γ, it is expressed in several cells of innate immunity (natural killer cells, natural killer T cells, innate lymphoid cells, and acquired immune cells (CD4+T cells, CD8+T cells) [7]. T-bet T cells are essential for the development of Th1 cells, together with signal transducers and transcriptional activators (STAT4) [8].

Th1 cells expressed by T-bet T cells produce cytokines, including INF-γ and TNF-α [9], which play an important role in protecting against intracellular infection by inducing a cell-mediated immune response [10]. In addition, the T-bet transcription factor plays an important role in the formation and function of effector and memory CD8+ T cells, which induces the expression of INF-γ, granzyme B, perforin, CXCR1, and CXCR4 to generate CTLs. Further, it is well known to play a role in maintaining homeostasis of memory CD8+ T cells by inducing the expression of IL-2Rβ (=CD122) [11].

In a previous study, according to Bagheri et al. [12], the adult patient group infected with *H. pylori* exhibited a higher frequency of T-bet+ T cells and INF-γ than those who were not infected in the immunohistochemical staining of gastric mucosal tissue. Moreover, among the *H. pylori*-infected group infected, adult patients with peptic ulcer disease (PUD) demonstrated increased T-bet+ T cell and INF-γ expressions compared to those without PUD. However, how the correlation between T-bet+ and CD8+ T cells affects the mucosal immune response of stomach infected by *H. pylori* remains unknown.

Effector memory CD8+ T cells can be classified into subtypes by expression of receoptors such as IL-7Rα and CX3CR. They express higher levels of CX3CR1 in IL-7Rα^{low} CD8+ T cells than in IL-7Rα^{high}. CD8+ T cells [13]. In this study, no significant difference in the frequency of IL-7Rα^{low} CX3CR1+CD8+T in PBMCs was observed between the control and *H. pylori*-positive groups (40.4±24.0% vs. 38.2±17.8%, p=0.914) (**Table 2**). Therefore, the lack of significant differences in the immune response between the two groups cell surface staining may be caused by the major changes in the immune response being confined to the gastric mucosal tissue and not reflected in the blood. Memory CD8+ T cells can be classified into subtypes of IL-7Rα and CX3CR1, and memory CD8+ T cells have been reported to express higher CX3CR1 levels in IL-7Rα$^{low\ cells}$ than in inIL-7Rα^{high13} cells.

In a previous study, Sun et al. [14] used flow cytometry to demonstrate that more CD4+ Tem cells (CD44+CD69-CCR7- effector memory T cells) were recruited via the CX3CL1/CX3CR1 pathway in the gastric mucosal tissue of *Cag* A- *H. pylori*-infected mice than in *Cag* A+ *H. pylori*-infected mice. However, in patients with *H. pylori* infections, memoryCD8+ T-cell subtypes need to be elucidated regarding the mechanism of the immune response. Moreover, the role of CD8+T cells in the blood of patients with *H. pylori* infections is less clear than that of CD4+ T cells [15].

In this study, the frequency of T-bet+INF-γ+CD8+ T cells was not significantly different between the control and *H. pylori*-positive groups (16.8±10.1% vs.14.0±9.7%, p=0.549) (**Table 3**). Therefore, the major changes in the immune response may be possibly limited to the gastric mucosal tissue and were not reflected in the blood.

In previous studies, *H. pylori* infection has been demonstrated to be associated with Th1 immune responses, mainly focusing on CD4+T cells, whereas the type of Th1 immune response in CD8+T cells is less clear [4,16]. Recently, Kronsteiner et al. [16] have reported that the number of T-bet+ CD8+ T cells increased 42 days after *H. pylori* infection in a pig model. Previous studies have demonstrated that although the progression of acute reactions in children and adults with *H. pylori* infection is similar, the immune responses in gastric mucosal immunity differ. Moreover, in children infected with *H. pylori*, TGF-β1 and IL-10 associated with Treg T cells are increased rather than the Th1 immune response [17-19].

In the serum cytokine analysis, TNF-α and CX3CL1 concentrations were not significantly different between the control and *H. pylori*-positive groups (34.3±12.1 pg/mL vs. 47.0±22.6 pg/mL, p=0.114/0.5±0.1 pg/mL vs. 0.5±0.1 pg/mL, p=0.188) (**Table 4**). As such, TNF-α and CX3CL1 concentrations in serum samples did not significantly differ in the immune response between the control and H pylori-positive groups, and the main changes in the immune response were confined to the gastric mucosal tissue and not reflected in the blood. This study has some limitations. First, the number of patient groups was very small despite enrollment for 2 years. This may be attributed to the low incidence of complications in

children with *H. pylori* infections. Second, we did not investigate the infiltration of CD4+ T cells or subsets of CD8+ T cells into the gastric mucosa. Lastly, we did not check for changes in CD8+ T cell frequency after *H. pylori* eradication.

In conclusion, immunohistochemical staining of gastric mucosal tissue demonstrated that the frequency of CD8+ T cells was higher in the *H. pylori*-positive group than in the control group, suggesting that CD8+ T cells play an important role in the gastric mucosal immune response in children with *H. pylori* infection. Moreover, the frequency of T-bet+ T cells was also higher in the *H. pylori*-positive group than in the control group in the immunohistochemical staining of gastric mucosal tissue, and the role of Th1 cells or CD8+ T cells that secrete INF-γ in the gastric mucosal immune response of children infected with *H. pylori* may be increased.

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