

Transfer of Antigen-Pulsed Dendritic Cells Induces Specific T-Cell Proliferation and a Therapeutic Effect against Long-Term *Helicobacter pylori* Infection in Mice

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Received 20 July 2005/Returned for modification 23 August 2005/Accepted 26 October 2005

***Helicobacter pylori* causes persistent infection of the stomach and results in chronic gastritis and peptic ulcers. Jaws II cells, derived from mouse bone marrow, were pulsed with live or formalin-killed or whole-cell sonicates (WCS) of *H. pylori*. Representative cell surface molecules were expressed at substantial levels on Jaws II cells, indicating that appropriate maturation of the cells was achieved with the three *H. pylori* antigens without any significant differences. *H. pylori* WCS-pulsed Jaws II cells secreted a significant amount of tumor necrosis factor alpha into the culture supernatant. The naive T cells exposed to the WCS-pulsed Jaws II cells showed significant proliferation and gamma interferon (IFN- γ) and interleukin-10 (IL-10) production in vitro. A 2-log reduction in the number of colonizing bacteria was observed in the mice treated with the WCS-pulsed Jaws II cells; however, no significant reductions were achieved in mice treated with Jaws II cells pulsed with other *H. pylori* antigens. Up-regulated production of IFN- γ and IL-10 was observed in the stomachs of the mice treated with the WCS-pulsed Jaws II cells, which is consistent with the result obtained in vitro. There were no differences in gastritis scores or *H. pylori*-specific antibody titers among the mice treated with Jaws II cells pulsed with the three different *H. pylori* antigens. The results suggest that Th1 cell-mediated immunity in combination with Th2 cell-mediated immunity plays a role in reducing colonizing bacterial numbers in mice with chronic *H. pylori* infections.**

Helicobacter pylori is a gram-negative curved bacterium that colonizes the human stomach and causes chronic gastric diseases. It is recognized as the etiologic agent of atrophic gastritis, peptic ulcers, intestinal metaplasia, mucosa-associated lymphoid tissue lymphoma, and gastric cancer.

Transmission of and infection with *H. pylori* occur mainly through female carriers via heavy contact or upon food and/or water intake during childhood before approximately 5 years of age. When an infection is established, *H. pylori* antigen-specific antibodies are developed and their production is sustained throughout the entire period of infection. Such a specific response, however, does not seem to be associated with a reduction in the number of *H. pylori* bacteria colonizing the stomach (6, 11, 37). With regard to reducing the number of colonizing *H. pylori* bacteria, cellular immunity has been reported to play the principal role where Th1-polarized rather than Th2-polarized T cells recruit mononuclear cells to the site of infection (34), resulting in elimination of the bacteria (4, 18). Meanwhile, *H. pylori* has evolved to evade not only the innate but also the adaptive immune response. For example, the antigen-dependent proliferation of T cells is specifically blocked by *H. pylori*: an intracellular T-cell mechanism is directly disrupted by secreted and internalized VacA protein. An antigenic alteration via a frameshift (28, 38) or a nucleotide transfer (36) was

reported likely to occur in an outer membrane protein which facilitates bacterial evasion of the host immune system. All this allows *H. pylori* to evade the host immune system, leading to a persistent infection in the stomach.

Triple therapy, typically consisting of two antibiotics plus a proton pump inhibitor, has become “the gold standard” for treatment to eradicate *H. pylori*; however, adverse reactions including allergies, hepatic disorders, and diarrhea remain to be overcome. Add to this the emergence of antibiotic-resistant bacteria and the situation becomes complicated.

Dendritic cells (DCs) have important functions such as antigen presentation and cytokine secretion in the innate as well as the adaptive immune system (35). They drive the host immune reaction by modulating the functions or interactions of effector cells like CD4⁺ and CD8⁺ cells, macrophages, and B cells. The immunomodulatory function of DCs has prompted researchers to work on utilizing these cells for controlling neoplastic diseases and allergic disorders (1, 26, 30). The treatment of infectious diseases is another area where DC-related immunomodulation therapy could be effective. Several DC-related works focusing on acute *Listeria monocytogenes* (32) infection and *Streptococcus pneumoniae* (3) infection have been launched; however, the number of works regarding chronic persistent infection is limited (5, 7).

In an attempt to provide an option other than triple therapy, we have studied the effectiveness of preventive or therapeutic vaccinations against *H. pylori* infection (13, 16, 22, 23), the mechanisms of which remain to be clarified. In the present study, we examined whether DCs pulsed with *H. pylori* antigens

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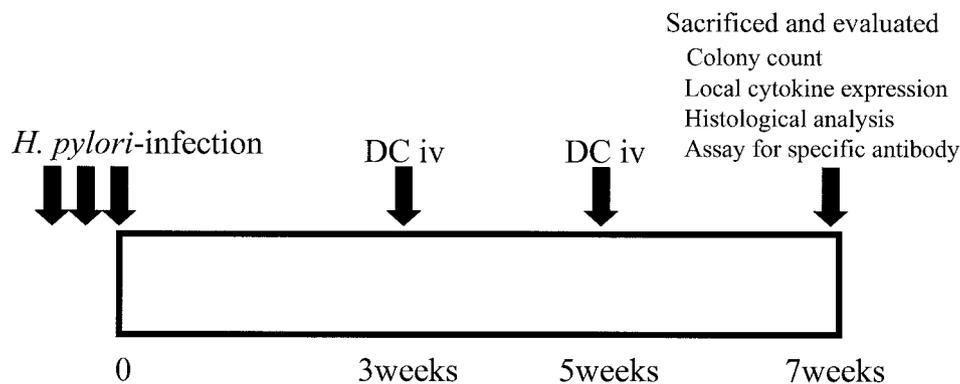


FIG. 1. Protocols for *H. pylori* infection and administration of antigen-pulsed or unpulsed Jaws II cells used in this study. Mice were infected orally with the *H. pylori* inoculum (0.5×10^8 CFU) three times and divided into five groups. The mice in each group were intravenously treated with 1.25×10^5 (100 μ l) *H. pylori* antigen (LB, FKB, or WCS)-pulsed or unpulsed Jaws II cells at 3 and 5 weeks after the last bacterial inoculation. The densities of colonizing bacteria, severities of gastritis, and levels of *H. pylori*-specific antibodies and local cytokines were compared to those in mice treated with PBS alone.

reduced bacterial loads in the stomachs of mice with chronic *H. pylori* infections and tried to clarify the mechanisms involved to test the feasibility of DC-based immunotherapy against chronic *H. pylori* infection.

MATERIALS AND METHODS

DCs. A DC line, Jaws II, was purchased from the American Type Culture Collection. Jaws II cells were originally isolated from bone marrow cultures of C57BL/6 mice deficient in p53. The cells were grown in a complete culture medium consisting of RPMI 1640 medium with glutamax (Gibco BRL, Grand Island, NY) supplemented with 10% fetal bovine serum (Equitech-Bio, Inc., Kerrville, TX), 1% penicillin-streptomycin, 50 μ M 2-mercaptoethanol, and 5 ng of recombinant mouse granulocyte-macrophage colony-stimulating factor (BD Biosciences, San Jose, CA)/ml.

Bacterial strain and *H. pylori* antigen preparations. *H. pylori* Sydney strain (SS1) stocked in our laboratory was grown in *Brucella* broth containing 10% fetal calf serum under microaerobic conditions (5% O_2 , 10% CO_2 , and 85% N_2) at 37°C for 24 h and used for the inoculation of mice or for the preparation of *H. pylori*-related antigens for pulsing of Jaws II cells. *H. pylori* sediment from ca. 25 ml of culture was suspended in 5 ml of normal saline solution (NSS) and used as the preparation containing live bacteria (LB). For the preparation containing formalin-killed bacteria (FKB), formalin was added at 0.5% to the LB preparation, and the mixture was left at room temperature for 24 h and vigorously washed. For the preparation containing whole-cell sonicates (WCS), the LB preparation was sonicated three times on ice and filtered with a 0.45- μ m-pore-sized filter.

Flow cytometry. The expression of cell surface molecules was measured by indirect immunofluorescent flow cytometric analysis using a FACSCalibur (BD Immunocytometry Systems, San Jose, CA) flow cytometer and CellQuest software. Antigen-pulsed or unpulsed Jaws II cells (10^5) were incubated on ice for 1 h with a panel of primary antibodies, rat anti-mouse CD11b (1:50), hamster anti-mouse CD11c (1:50), rat anti-mouse CD8 α (1:50), rat anti-mouse CD40 (1:50; eBioscience, San Diego, CA), mouse anti-mouse major histocompatibility complex (MHC) class I (1:50; BD Pharmingen, San Jose, CA), rat anti-mouse MHC class II (1:50; BD Biosciences), rat anti-mouse CD80 (1:50), and rat anti-mouse CD86 (1:50; provided by Hideo Yagita and Ko Okumura, Department of Immunology, University of Juntendo, Tokyo, Japan). Fluorescein isothiocyanate (FITC)-conjugated anti-rat (1:50), anti-hamster (1:50), or rat anti-mouse (1:50) monoclonal antibody was used as the secondary antibody.

Pulsing of Jaws II cells with *H. pylori*-related antigens. For the pulsing of Jaws II cells with *H. pylori*-related antigens, ca. 100 μ l (WCS preparation) to 200 μ l (LB and FKB preparations) of *H. pylori*-related antigens was added to 60 to 80% confluent Jaws II cells in a 6-well culture plate and incubated at 37°C for 48 h. The number of bacteria in 200 μ l of the LB or FKB preparation was 20-fold the number of Jaws II cells (multiplicity of infection, 20). The fluids of each culture were collected 12, 24, and 48 h after addition of the antigen, and tumor necrosis factor alpha (TNF- α) was analyzed with an enzyme-linked immunosorbent assay

(ELISA) kit (eBioscience). The *H. pylori*-related antigen-pulsed Jaws II cells were extensively washed, suspended in phosphate-buffered saline (PBS), and used for experiments with mice.

Effect of *H. pylori*-related antigen-pulsed Jaws II cells on naive T cells. The naive-T-cell-rich fraction was obtained from spleens of normal C57BL/6 mice by the magnetic cell sorting method (Miletenyi Biotec, Bergisch Gladbach, Germany). *H. pylori*-related antigen (LB, FKB, and WCS)-pulsed Jaws II cells were fixed with 0.75% formalin for 30 min on ice prior to exposure to the naive T cells. The formalin-fixed Jaws II cells (1.0×10^4) were added to ca. 2×10^5 naive T cells (1:20) cultured in a well of a 96-well flat-bottom culture plate and incubated for 72 h (8, 19). The clonal proliferation of T cells exposed to LB-, FKB-, and WCS-pulsed Jaws II cells was assayed using 5-bromo-2'-deoxyuridine (BrdU) (15) and compared to that of T cells exposed to unpulsed Jaws II cells. Briefly, 2×10^{-4} mmol of BrdU was added to each well, and the plate was incubated at 37°C for 2 h. The amount of BrdU incorporated into the T cells was measured using the anti-BrdU monoclonal antibody in the ELISA kit (Roche Switzerland).

Gamma interferon (IFN- γ) and interleukin-10 (IL-10) production was determined using the culture medium of T cells exposed to LB-, FKB-, and WCS-pulsed Jaws II cells. The culture medium was harvested at 24, 48, 72, and 96 h postexposure, and the cytokines were detected with ELISA kits (eBioscience) and compared to those in the medium of T cells exposed to unpulsed Jaws II cells.

Administration of *H. pylori*-related antigen-pulsed Jaws II cells to mice with chronic *H. pylori* infections. Specific-pathogen-free 5-week-old female C57BL/6 mice (Seac Yoshitomi, Fukuoka, Japan) that had been kept and fed appropriately were used for the experiment. Thirty-three mice were infected orally with the *H. pylori* inoculum (0.5×10^8 CFU) three times every other day and randomly divided into five groups. Each mouse was intravenously treated with 1.25×10^5 (100 μ l) Jaws II cells or PBS (100 μ l) alone at 3 and 5 weeks after the last bacterial inoculation. The groups treated with LB-pulsed, FKB-pulsed, and WCS-pulsed Jaws II cells were referred to as group 2 (G2; $n = 7$), group 3 (G3; $n = 7$), and group 4 (G4; $n = 7$), respectively. The densities of colonizing bacteria, severities of gastritis, and levels of *H. pylori*-specific antibodies and local cytokines in mice of each group were compared to those in mice treated with PBS alone (group 1 [G1; $n = 6$]) and mice treated with unpulsed Jaws II cells (group 5 [G5; $n = 6$]). At 2 weeks after the last administration of antigen-pulsed Jaws II cells (7 weeks after the initial inoculation), all mice were sacrificed and each stomach was excised, washed with NSS, and cut longitudinally into two pieces. One half was used for evaluating the density of colonizing *H. pylori* and local cytokine expression by quantitative real-time PCR analysis, and the other half was used for evaluating the severity of gastritis by histology (Fig. 1). All animal experiments were performed under the guidelines of the Ethics Committee for Animal Experiments of Oita University, Oita, Japan.

***H. pylori* colony count.** One half of the excised stomach was homogenized in 500 μ l of NSS with a glass homogenizer (IWAKI Glass Co. Ltd., Tokyo, Japan). Fifty microliters of the gastric homogenate was serially diluted with NSS and inoculated onto M-BHM pylori agar plates (Nikken Biomedical Laboratory, Kyoto, Japan) at 37°C for 4 days under microaerobic conditions. Colonies with

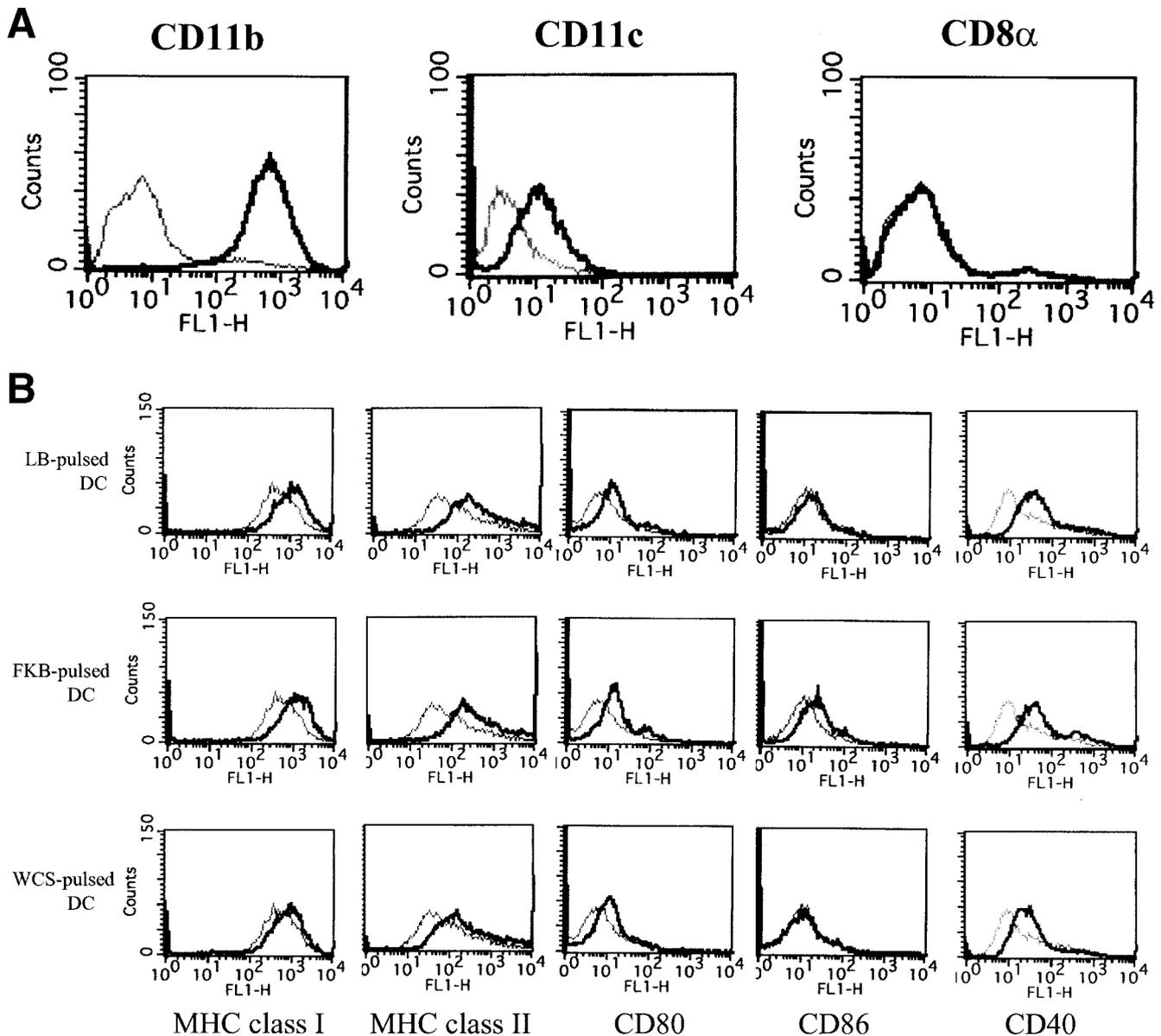


FIG. 2. Expression of cell surface molecules was measured by FACS. (A) Naïve Jaws II cells were reacted with rat anti-mouse CD11b (1:50), hamster anti-mouse CD11c (1:50), or rat anti-mouse CD8 α (1:50), followed by FITC-conjugated anti-rat (1:50) or anti-hamster (1:50) monoclonal antibody. An assay was performed to confirm that the Jaws II cells used are myeloid-type DCs. (B) Jaws II cells pulsed with LB, FKB, or WCS were reacted with mouse anti-mouse MHC class I (1:50), rat anti-mouse MHC class II (1:50), rat anti-mouse CD80 (1:50), rat anti-mouse CD86 (1:50), or rat anti-mouse CD40 (1:50) and then FITC-conjugated anti-mouse (1:50) or anti-rat (1:50) monoclonal antibody. Note that appropriate maturation of the Jaws II cells was achieved with the three antigens. Obvious differences were not observed for the three antigens.

a characteristic *H. pylori* appearance were counted, and the density of the bacteria per 1.0 g of stomach tissue was calculated.

Local cytokine expression assay by quantitative real-time PCR analysis. Total RNA was extracted from the remaining 450 μ l of the gastric homogenate by the acid guanidium thiocyanate-phenol chloroform method as described previously (13). Approximately 1.0 μ g of the total RNA was used for a reverse transcription reaction in a 40- μ l mixture containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 5 mM dithiothreitol, 500 mM deoxynucleoside triphosphates, 1 mM oligo(dT) 15 primer, 20 U of RNase inhibitor (Toyobo Biomedicals, Osaka, Japan), and 500 U of Moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA), and the mixture was incubated at 37°C for 2 h. Real-time PCR was performed using the synthesized cDNA as the template on the Smart cycler (TaKaRa, Tokyo, Japan) as described elsewhere (21). Briefly, the reaction mixture (25 μ l) contained ca. 1 ng of cDNA, 1.25 U of Ex Taq

real-time PCR version polymerase (TaKaRa), 2.5 μ l of 10 \times real-time PCR buffer, 2.5 mM MgCl₂, 10 mM (each) deoxynucleotide triphosphates, SYBR green I solution at a final concentration of 5 \times (Cambrex Bio Science, East Rutherford, NJ), and 5 μ M (each) primers as follows: β -actin, 5'-ATGGATG ACGATATCGCT-3' (sense) and 5'-ATGAGGTAGTCTGTCTGTCCAGGT-3' (antisense); IFN- γ , 5'-CCTGCAGAGCCAGATTATCTCTTCTACC-3' (sense) and 5'-CCACCCCGAATCAGCAGCGA-3' (antisense); and IL-12, 5'-TGGAAGCACGGCAGCAGAATAAAT-3' (sense) and 5'-TGCGCTGGATT CGAACAAAGAACT-3' (antisense). PCRs with 45 cycles of 95°C for 10 s, 55°C for 30 s, 72°C for 60 s, and 87°C for 6 s for β -actin; 45 cycles of 95°C for 10 s, 69°C for 10 s, and 87°C for 6 s for IFN- γ ; and 45 cycles of 95°C for 10 s, 61°C for 45 s, and 87°C for 6 s for IL-12 were carried out. Standard curves (linear regression lines) were generated using external standards (serial 10-fold dilutions of each cytokine DNA molecule with a known copy number stocked in our laboratory)

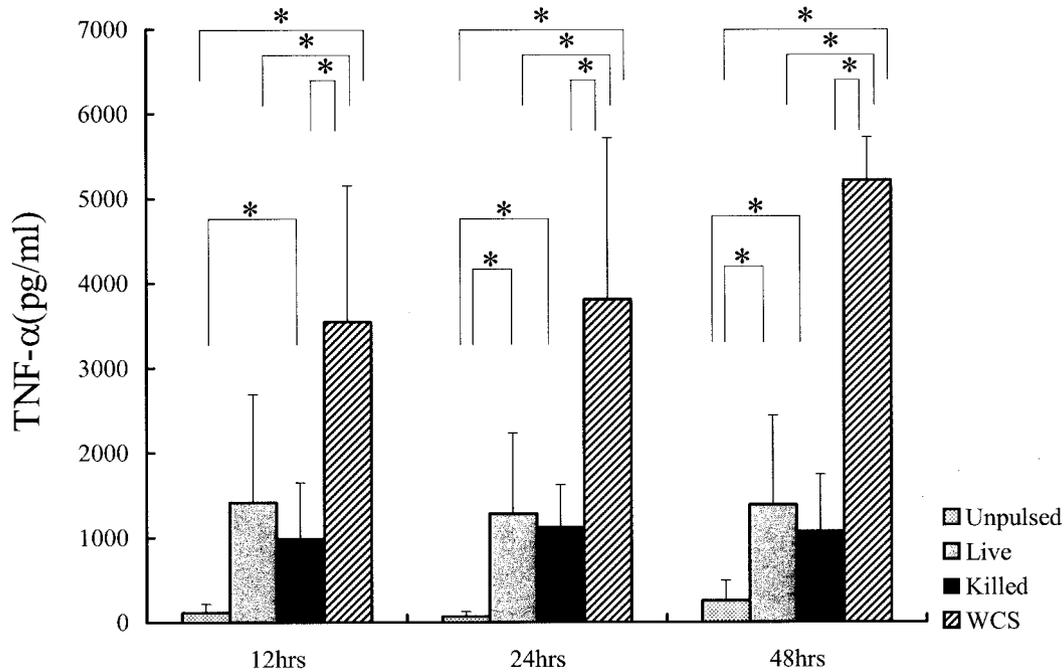


FIG. 3. TNF- α expression in Jaws II cells pulsed with LB, FKB, or WCS of *H. pylori* was analyzed. Each culture fluid was obtained at 12, 24, and 48 h after the addition of each antigen and evaluated by ELISA. Note that a significant elevation in the level of TNF- α in the supernatant of the WCS-pulsed Jaws II cells was observed at 12 h and sustained for up to 48 h postexposure. The asterisks indicate statistical significance at P of <0.05 . The results are expressed as the means \pm standard deviations of results for three independent cultures.

and used to estimate mRNA copy numbers in each sample. All data were standardized to results obtained for β -actin.

Histological analysis. The remaining half of each stomach was used for standard histological procedures and stained with hematoxylin and eosin for evaluation of the intensity of inflammation as described by Goto et al. (13). The severity of inflammation was scored as either 0 for no inflammation, 1 for mild inflammation, 2 for moderate inflammation, or 3 for severe inflammation. Each sample was examined two times, and the mean of the two scores was expressed as the gastritis score.

Assay for *H. pylori*-specific antibody. Blood samples were taken from mice in each group at the time of sacrifice, and whole immunoglobulin G (IgG), IgG1, IgG2a, and IgA titers were determined by ELISA. Briefly, a microtiter plate (Nunc, Roskilde, Denmark) coated with ca. 1.0 μ g of *H. pylori* WCS in carbonate buffer (pH 9.6) was blocked with 5% bovine serum albumin and then each serum sample was added. Peroxidase-conjugated goat anti-mouse IgG, IgG1, IgG2a, or IgA antibody was added, and the plate was incubated at 37°C for 1 h. After several washes with PBS containing 0.05% Tween 20, the conjugated peroxidase was visualized using the tetramethylbenzidine substrate reagent set (BD Pharmingen) and absorbance was determined at an optical density of 450 nm. Each sample was tested three times.

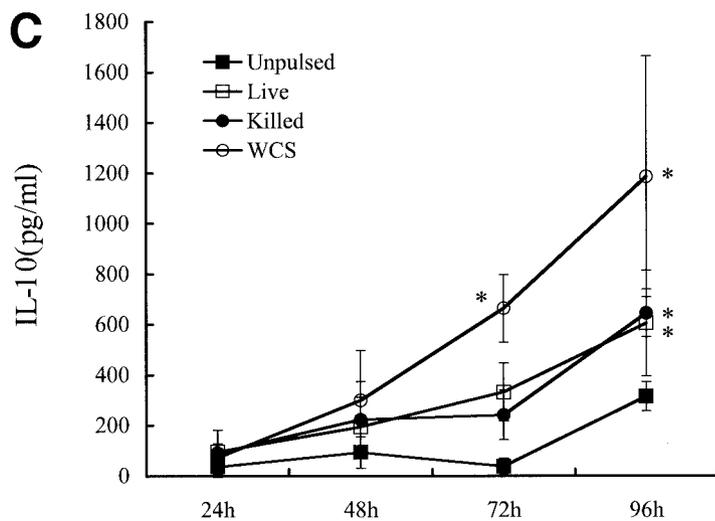
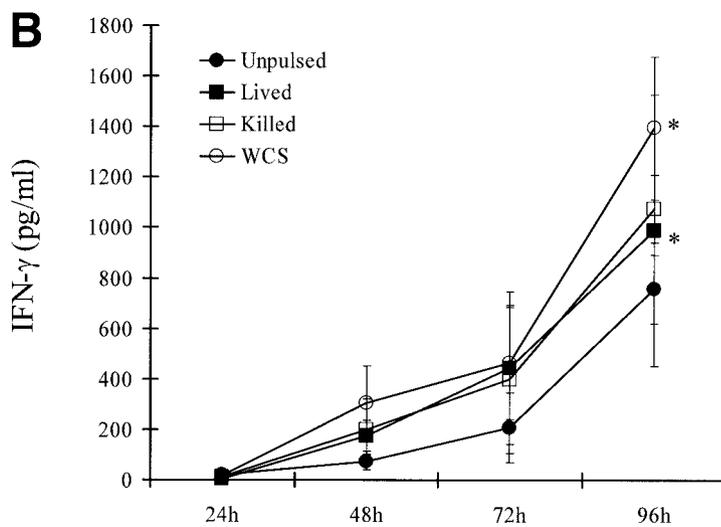
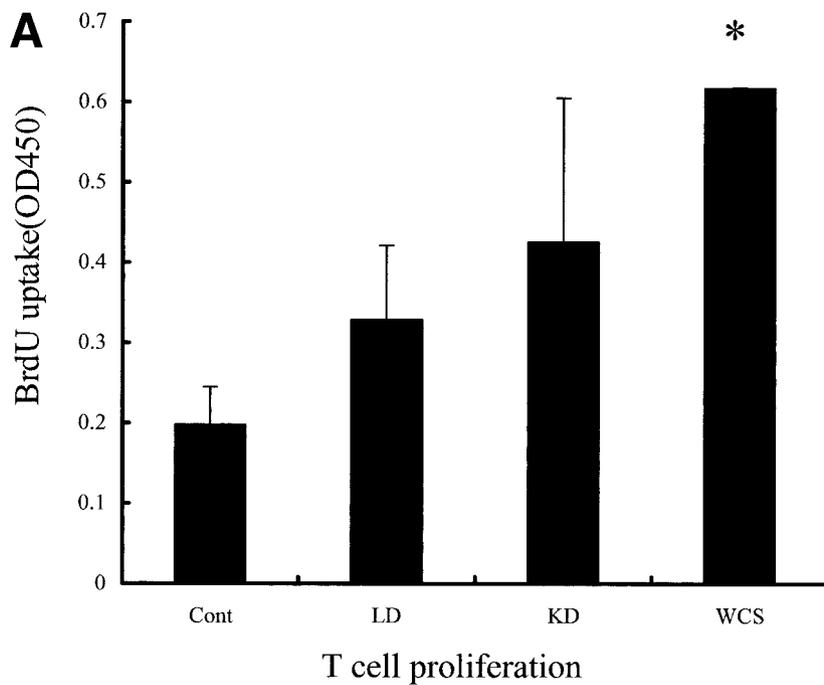
Statistical analysis. Specific antibody titers, *H. pylori* colony counts, expression levels of cytokines, and levels of naive-T-cell proliferation were analyzed and compared by the t test. Gastritis inflammation scores were compared using the Kruskal-Wallis test. P values of <0.05 were considered to indicate significant difference.

RESULTS

Confirmation of DC subtype and evaluation of surface molecule expression on Jaws II cells by pulsing with *H. pylori*-specific antigens. In the analysis by fluorescence-activated cell sorting (FACS), CD11b and CD11c were detected whereas CD8 α was not, indicating that the Jaws II cells were myeloid-type DCs (Fig. 2A) as previously reported (17, 27, 33). When Jaws II cells were pulsed with three types of *H. pylori*-derived

antigens, LB, FKB, and WCS, MHC class I antigens, MHC class II antigens, CD80, CD86, and CD40 were expressed on the cell surfaces (Fig. 2B), indicating that appropriate maturation of the Jaws II cells was achieved with those three antigens. No obvious differences in the quantities produced were observed for the three antigens used (Fig. 2B). The immature form of the DCs, with a round and nonadherent phenotype, was rendered adherent with a characteristic dendritic form as surface marker molecules were expressed (data not shown). The production of TNF- α by Jaws II cells pulsed with three different *H. pylori*-derived antigens (LB, FKB, and WCS) was analyzed by ELISA. Culture supernatants of the antigen-pulsed DCs were obtained at 12, 24, and 48 h after the addition of each antigen. A dramatic elevation in the level of TNF- α in the supernatant of the WCS-pulsed Jaws II cells was observed at 12 h. The ELISA-determined TNF- α titer for the WCS-pulsed Jaws II cells was significantly higher, exceeding 3,500 pg/ml, than those for the unpulsed Jaws II cells or the LB- or FKB-pulsed Jaws II cells. The higher TNF- α titer detected in the supernatant of WCS-pulsed Jaws II cells was sustained for up to 48 h, whereas no obvious increase was observed in the culture medium obtained from the LB- or the FKB-pulsed Jaws II cells (Fig. 3). Elevated levels of IL-10 or IL-12 in the culture medium of the LB-, FKB-, or WCS-pulsed Jaws II cells were not observed (data not shown). These results indicated that the Jaws II cells used responded like typical myeloid-type DCs.

Stimulation of naive T cells by *H. pylori* antigen-pulsed Jaws II cells. T-cell-proliferation assays were performed to evaluate whether clonal proliferation of T cells occurred after exposure



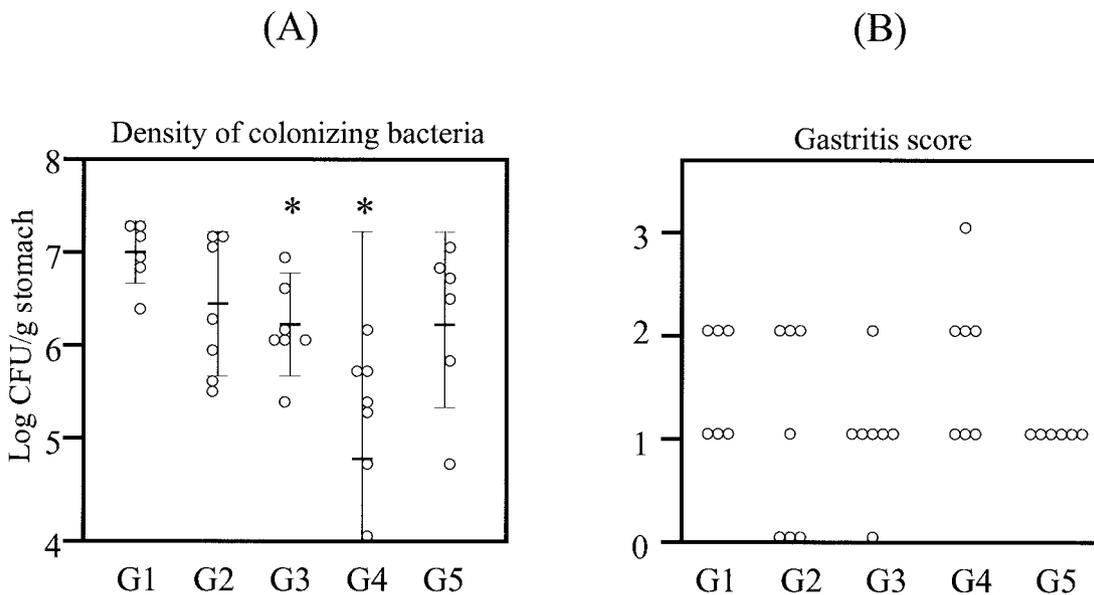


FIG. 5. C57BL/6 mice were orally inoculated with *H. pylori* strain SS1 and treated two times with the LB-pulsed (G2), the FKB-pulsed (G3), the WCS-pulsed (G4), and the unpulsed (G5) Jaws II cells, and numbers of colonizing *H. pylori* bacteria (A) and gastritis scores (B) for the mice of each group were compared to those for mice treated with PBS alone (G1). A significant reduction in the number of colonizing bacteria was observed in the G3 and G4 mice. No significant difference in gastritis scores was observed for the mice in each group. The asterisks indicate statistical significance at *P* of <0.05. The results are expressed as the geometric means (bold lines) ± standard deviations of results for each mouse.

to *H. pylori* antigen-pulsed Jaws II cells. The naïve-T-cell preparations were exposed to LB-, FKB-, or WCS-pulsed Jaws II cells for 48 h. The antigen-pulsed Jaws II cells were fixed with formalin prior to exposure in order to avoid possible direct interaction between the secreted cytokines and the T cells. The naïve T cells exposed to the WCS-pulsed Jaws II cells took up significantly more BrdU than the T cells exposed to the unpulsed Jaws II cells (Fig. 4A). The uptake by the T-cell groups exposed to the LB- or the FKB-pulsed Jaws II cells was greater than that by the negative control; however, the difference was not significant (Fig. 4A).

Culture media of the T cells exposed to the *H. pylori* antigen-pulsed and formalin-fixed Jaws II cells were used for the analysis of IFN-γ and IL-10 production. IFN-γ was detected at 48 h postexposure to the Jaws II cells, and the amount secreted gradually increased until 96 h postexposure. A level of IFN-γ that was significant compared to that in the negative control was observed in the culture medium of the T cells exposed to WCS- or LB-pulsed Jaws II cells at 96 h postexposure (Fig. 4B). A small amount of IL-10 was detected at 24 h postexposure to the Jaws II cells, and the amount secreted gradually increased with time (Fig. 4C). A significant amount of IL-10,

compared to that in the negative control, was observed in the culture medium of the T cells exposed to the WCS-pulsed Jaws II cells at 72 h postexposure. At 96 h, T cells came to produce a significant amount of IL-10 compared to the negative control, regardless of the type of *H. pylori*-derived antigen (LB, FKB, or WCS) with which Jaws II cells were pulsed (Fig. 4C).

Therapeutic DC vaccines against *H. pylori* infection. C57BL/6 mice were orally inoculated three times with 0.5×10^8 CFU of *H. pylori* strain SS1. Approximately 1.0×10^7 CFU of bacteria per 1.0 g of stomach tissue were recovered at 3 weeks after the last inoculation in the previous study (12). Those mice were used for subsequent therapeutic DC vaccine experiments.

Mice were treated two times with the LB-pulsed (G2), the FKB-pulsed (G3), the WCS-pulsed (G4), and the unpulsed (G5) Jaws II cells, and numbers of colonizing *H. pylori* bacteria were compared to those observed when PBS was administered alone (G1). A significant reduction in the number of colonizing *H. pylori* bacteria was observed in the mice treated with the FKB-pulsed (G3) and with the WCS-pulsed (G4) Jaws II cells compared to those treated with PBS (G1) (Fig. 5A). The mean bacterial number calculated for the G4 mice was 5×10^5

FIG. 4. The naïve-T-cell preparations were exposed to Jaws II cells pulsed with LB, FKB, or WCS of *H. pylori* for 48 h at 37°C. The Jaws II cells were fixed with formalin prior to exposure. The number of Jaws II cells used was 1/20 the number of naïve T cells, and cells were exposed at 37°C for several hours. (A) Clonal proliferation of T cells was assayed by measuring the uptake of BrdU with an ELISA kit (Roche Switzerland) at 72 h postexposure. The naïve T cells exposed to the WCS-pulsed Jaws II cells showed significantly greater BrdU uptake than the others. Levels of IFN-γ (B) and IL-10 (C) production were determined using culture medium harvested at 24, 48, 72, and 96 h postexposure with separate ELISA kits (eBioscience). Significant IFN-γ production was observed in T cells exposed to WCS- or LB-pulsed Jaws II cells at 96 h. Significant IL-10 production was observed in T cells exposed to the WCS-pulsed Jaws II cells at 72 h and in T cells exposed to all *H. pylori* antigens (LB, FKB, and WCS) at 96 h postexposure. The asterisk indicates statistical significance at *P* of <0.05. The results are expressed as the means ± standard deviations of results for three independent cultures.

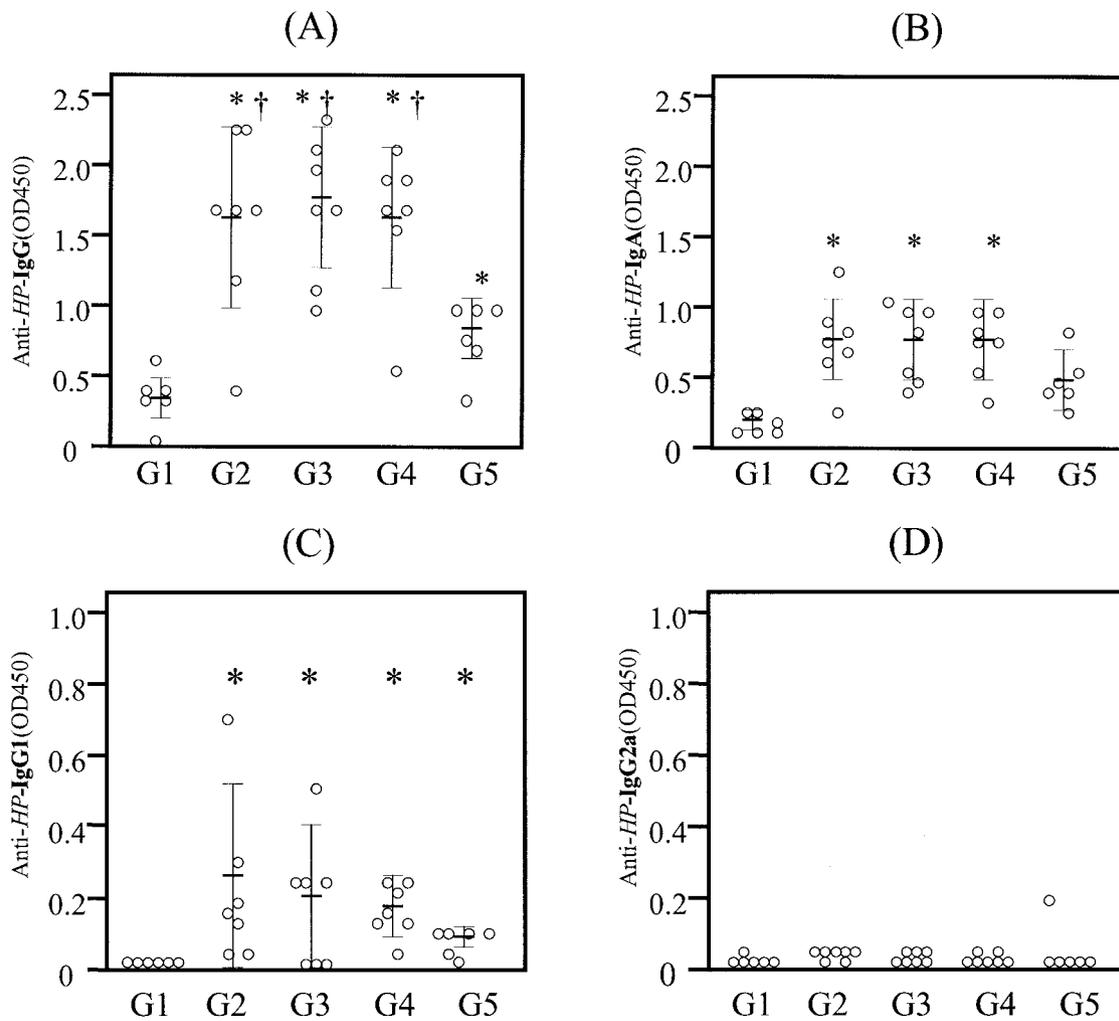


FIG. 6. Systemic humoral immune responses in mice treated with *H. pylori* antigen-pulsed Jaws II cells were evaluated. Blood samples were taken from the mice treated with the LB-pulsed (G2), the FKB-pulsed (G3), the WCS-pulsed (G4), and the unpulsed (G5) Jaws II cells, and specific antibody titers of IgG (A), IgA (B), IgG1 (C), and IgG2a (D) against *H. pylori* WCS were determined by ELISA and compared to those in mice treated with PBS (G1). Approximately 1.0 μ g of *H. pylori* WCS was used to coat each well of a microtiter plate, and bound antibodies were detected using peroxidase-conjugated goat anti-mouse IgG, IgG1, IgG2a, or IgA antibody and visualized. Significant increases in IgG levels were detected in the G2, G3, and G4 mice compared to those in the G1 and G5 mice. *H. pylori*-specific IgA titers were significantly higher in the G2, G3, and G4 mice than in the G1 mice. The titer of *H. pylori*-specific IgG1 was significantly higher in G2, G3, G4, and G5 mice than in G1 mice. The titers of IgG2a in mice of all groups were below or close to the detection limit. Each sample was tested three times. The asterisks indicate statistical significance at P of <0.05 . The results are expressed as the geometric means (bold lines) \pm standard deviations of results for each mouse.

CFU/g of stomach tissue, which is lower by 2 logs than that obtained for the G1 mice. Gastritis scores for the mice treated with the WCS-pulsed Jaws II cells (G4) tended to be higher than those for the mice treated with PBS (G1); however, the difference was not significant (Fig. 5B).

Systemic humoral immune responses in mice treated with *H. pylori* antigen-pulsed Jaws II cells. Blood samples were collected from the mice treated with the LB-pulsed (G2), the FKB-pulsed (G3), the WCS-pulsed (G4), and the unpulsed (G5) Jaws II cells, and representative titers of antibodies against *H. pylori* WCS were compared to those in mice treated with PBS alone (G1) (Fig. 6). The antibody titers in G2, G3, and G4 mice were compared to those in G5 mice to assess the effect of antigen-pulsed Jaws II cells on the rise in specific antibody titers. Significant increases were detected in the G2,

G3, G4, and G5 mice compared to the G1 mice (Fig. 6A). IgG titers were significantly higher in the G2, G3, and G4 mice than in the G5 mice (Fig. 6A). *H. pylori*-specific IgA titers were significantly higher in G2, G3, and G4 mice than in G1 mice; however, those in G5 mice were not elevated (Fig. 6B). Next, we determined titers of IgG subclasses to evaluate the status of helper-T-cell immunity. The titers of *H. pylori*-specific IgG1 were significantly higher in G2, G3, G4, and G5 mice than in G1 mice; however, the titers did not differ among the former four groups (Fig. 6C). The titers of IgG2a in mice of all groups were below or close to the detection limit (Fig. 6D).

Cytokine responses in mice treated with *H. pylori* antigen-pulsed Jaws II cells. To evaluate local cytokine responses in vivo, we determined cytokine expression levels in the gastric tissues of mice treated with the LB-pulsed (G2), the FKB-

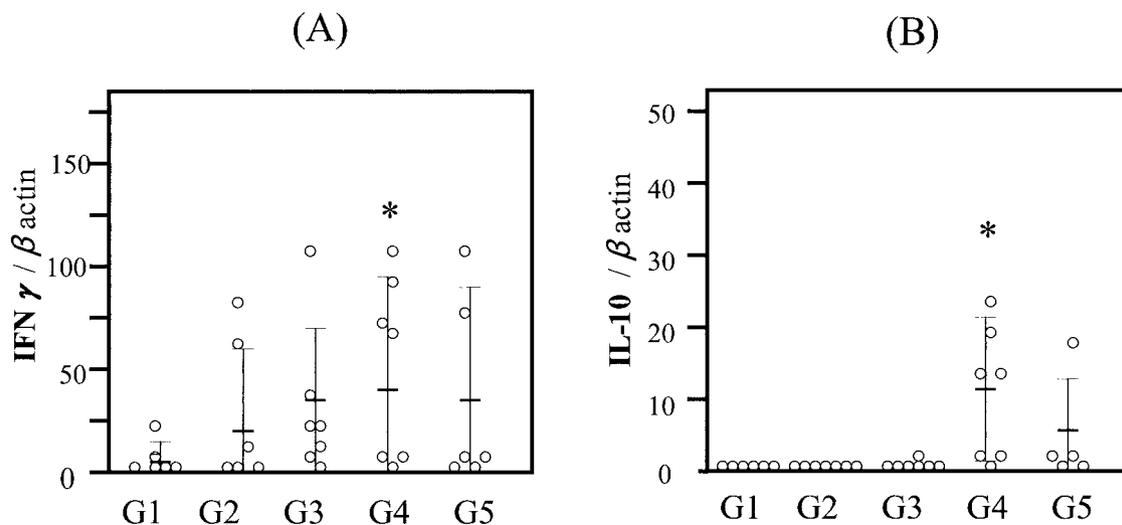


FIG. 7. Expression of IFN- γ (A) and IL-10 (B) in the gastric tissue of mice treated with the LB-pulsed (G2), the FKB-pulsed (G3), the WCS-pulsed (G4), and the unpulsed (G5) Jaws II cells was determined by real-time PCR (see Materials and Methods), and cytokine amounts were compared to those in mice treated with PBS alone (G1). Standard curves were generated using external standards for each cytokine DNA molecule with a known copy number and were used to estimate mRNA copy numbers in each sample. A significant increase in IFN- γ expression was observed in G4 mice. A significant increase in IL-10 expression was also seen in G4 mice. The asterisks indicate statistical significance at P of <0.05. The results are expressed as the geometric means (bold lines) \pm standard deviations of results for each mouse.

pulsed (G3), the WCS-pulsed (G4), and the unpulsed (G5) Jaws II cells and compared them to those in tissues of mice treated with PBS alone (G1). A significant increase in IFN- γ expression was observed only in G4 mice (Fig. 7A). A significant increase in IL-10 expression was seen only in G4 mice (Fig. 7).

DISCUSSION

DCs are categorized into two subtypes, the myeloid type and the plasmacytoid type (2, 33). The plasmacytoid-type DC is able to convert into the myeloid-type DC, responding to surrounding conditions (9, 10, 39). When cell surface markers of Jaws II cells were analyzed by FACS, CD11c and CD11b were detected whereas CD8 α was not (Fig. 2A), indicating that the Jaws II cells are myeloid-type DCs as previously reported (14, 17, 33). Many reports have described studies on DCs isolated from bone marrow, lymphoid tissues, or peripheral blood by means of the magnetic cell sorting system. In the present study, however, we have used a DC cell line, Jaws II, because a certain proportion of myeloid-type DCs are expected in a cell population.

Obvious differences in quantities of cell surface molecules (MHC class I, MHC class II, CD80, CD86, and CD40) were not observed when Jaws II cells were pulsed with LB, FKB, and WCS of *H. pylori* (Fig. 2B). Jaws II cells were pulsed with each antigen for 48 h at 37°C in the present study. Jaws II cells proliferate slowly, and MHC class II molecules were initially expressed at 12 h (data not shown); therefore, a longer incubation period, as used by another group of researchers (17), was considered to be essential for the Jaws II cells. TNF- α expression has been reported to be closely correlated with the maturation of DCs. A dramatic elevation in the level of TNF- α in the supernatant of the WCS-pulsed Jaws II cells was observed (Fig. 3); hence, WCS pulsing forced a larger proportion

of Jaws II cells to mature than pulsing with the other preparations. It is well known that the lipopolysaccharide (LPS) of gram-negative bacteria efficiently directs DCs to mature as well as inducing the expression of proinflammatory cytokines including TNF- α . *H. pylori* LPS, however, has been recognized to be less efficient in such activities (20, 25, 29, 31). In the present study, we purified *H. pylori* LPS and determined its ability to induce the expression of TNF- α and several cell surface molecules including MHC class II antigens, CD80, and CD86. Different from the LPS of other gram-negative bacteria, *H. pylori* LPS failed to induce the expression of those molecules (data not shown). Recently, Kranzer et al. reported that the level of expression of TNF- α was lower in human DC cells pulsed with *H. pylori* WCS than in those pulsed with LB or FKB (19), which contradicts our result. The difference is probably due to the fact that they used human DCs which probably comprised a heterologous DC population rather than myeloid-type DCs selected with CD11b.

H. pylori has evolved to evade not only the innate but also the adaptive immune response, which facilitates bacterial colonization of the stomach, a situation that can cause chronic gastric diseases including atrophic gastritis and peptic ulcers. Chronic persistent infections are one of the best targets of DC-related immunomodulatory therapy. DCs act to organize the host immune system by regulating the functions or interactions of effector cells like CD4⁺ or CD8⁺ T cells, macrophages, and B cells. The most significant reduction in the number of colonizing *H. pylori* bacteria was observed in the mice treated with the WCS-pulsed Jaws II cells (Fig. 5A). The 2-log reduction in this number compared to that in the control mice was one of the most impressive effects observed in vivo using C57BL/6 mice, a widely accepted animal model for *H. pylori* infection.

The ability to proliferate as well as production of IFN- γ and

IL-10 was enhanced in naïve T cells exposed to the WCS-pulsed Jaws II cells *in vitro* (Fig. 4). In addition, a significant simultaneous increase in the expression of IFN- γ and IL-10 was observed only in mice treated with the WCS-pulsed Jaws II cells (Fig. 6A). IFN- γ is a cytokine representative of Th1 cell-mediated immunity, whereas IL-10 is representative of Th2 cell-mediated immunity. Therefore, the functionally up-regulated T-cell population, with a combination of Th1 and Th2 cell-mediated immunity, might play a role in reducing the number of *H. pylori* bacteria colonizing the stomach. Significant systemic rises in IgG, IgA, and IgG1 levels were observed in all the mice treated with *H. pylori*-related antigen-pulsed Jaws II cells (Fig. 6A and B), and titers of IgG2a in mice of all groups were below or close to the detection limit, regardless of the effect on *H. pylori* (Fig. 6D). This indicates that *H. pylori*-specific antibodies were not associated with the reduction in the number of *H. pylori* bacteria colonizing the stomach, a finding which is supported by several studies (6, 11, 37). Many reports have suggested that postimmunization gastritis caused by proliferated and recruited lymphocytes may contribute to a reduction in the number of colonizing *H. pylori* bacteria (13, 24); however, the effect would be limited in the present study because gastritis scores for the mice with significant *H. pylori* reduction were not significantly higher than those for the negative control (Fig. 5B). Taken together, it could be that some unknown effects of the functionally up-regulated T-cell population, other than lymphocyte recruitment or the involvement of a specific antibody, helped to reduce the number of *H. pylori* bacteria colonizing the stomachs, probably through a combination of Th1 cell-mediated and Th2 cell-mediated immunity.

The up-regulated T-cell functions were induced only by exposure to the WCS-pulsed Jaws II cells. Potential explanations include that (i) an unknown molecule released from the cytoplasm or the periplasm of *H. pylori* is displayed by the MHC and might play a role, (ii) a larger proportion of the appropriate MHC, that is, an MHC molecule with a processed peptide, was induced on the WCS-pulsed Jaws II cells than on the other cells, (iii) an antigen-presenting molecule other than the MHC might play a role, and (iv) programmed cell death ligand, a costimulatory molecule that inhibits T-cell activities on DCs, was substantially expressed on the surface of LB- or FKB-pulsed Jaws II cells and, as a consequence, T-cell proliferation was suppressed.

The DC-related immunomodulatory therapy described here would be applicable to the eradication of microbial agents capable of evading host immunity and, consequently, causing chronic persistent infections.

ACKNOWLEDGMENTS

This work was supported by grants-in-aid no.13226104 (AN) and no. 14021102 (AN) from the Ministry of Education, Science, Sports and Culture of Japan.

We thank Kanako Yano for excellent technical assistance.

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Editor: F. C. Fang