Expression of B7-1 and B7-2 Costimulatory Molecules by Human Gastric Epithelial Cells

Potential Role in CD4$^+$ T Cell Activation during *Helicobacter pylori* Infection

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Abstract

Human gastric mucosal epithelial cells display class II MHC, the expression of which is increased during *Helicobacter pylori* infection. These observations suggest that the gastric epithelium may participate as antigen-presenting cells (APC) during local immune responses. The increase in class II MHC expression occurs in parallel with an elevation in gastric CD4$^+$ T cell numbers within and adjacent to the epithelium. Since the expression of either B7-1 (CD80) or B7-2 (CD86) on APC is required for the activation of T cells, it was important to establish human gastric epithelial cells expressed those surface ligands. The expression of B7-1 and B7-2 was detected on human gastric epithelial cell lines and freshly isolated epithelial cells from gastric biopsies with specific antibodies. B7-2 expression was higher than B7-1 at both protein and transcript levels and was increased after crosslinking class II MHC molecules on IFNγ-treated epithelial cells and in cells pretreated with the combination of IFNγ and *H. pylori*. Similarly, B7-2 expression was higher on gastric epithelial cells from *H. pylori*–infected tissues compared with those from uninfected specimens. To determine the function of these molecules on gastric epithelial cells, antibodies to B7-1 and B7-2 were shown to reduce the ability of the cells to stimulate alloreactive CD4$^+$ T cells. These observations are the first to demonstrate that B7-1 and B7-2 are expressed on mucosal epithelial cells in situ. Thus, the expression of B7-1 and B7-2 by epithelial cells may allow them to act as APC in regulating local responses such as those that occur during infection with *H. pylori*. (*J. Clin. Invest.* 1997. 99:1628–1636.) Key words: mucosal immunity • epithelial cell • antigen presentation • CD80/CD86 • costimulation pathway

Introduction

Antigen presenting cells (APC)[1] play a central role in orchestrating immune responses. Classical or "professional" APC include bone marrow–derived B cells, macrophages, and dendritic cells. One of the hallmark characteristics of APC is their expression of class II MHC molecules (1), that allows APC to bind and present antigens to CD4$^+$ T cells. The expression of class II MHC has also been noted to occur constitutively on various mucosal epithelial cells and is increased during multiple inflammatory conditions. The strategic location of gastrointestinal mucosal epithelial cells allows them to interact with both luminal antigens and resident intraepithelial and lamina propria T cells. Despite the fact that intestinal epithelial cells isolated from healthy tissue constitutively express some class II MHC molecules, they preferentially stimulate CD8$^+$ T cells (2). However, a change in the phenotype of epithelial cells during inflammation is associated with a shift from preferentially stimulating CD8$^+$ T cells to activating CD4$^+$ T cells (3). Gastric epithelial cells have also been shown to constitutively express class II MHC, that is increased during *H. pylori* infection (4, 5). Class II MHC–restricted, *H. pylori*–specific CD4$^+$ T cell clones have been derived from both peripheral blood and gastric mucosa of infected patients (6). However, the role of gastric epithelial cells in the activation of CD4$^+$ T cells is poorly understood.

Signals transduced through the T cell receptor (TCR) after antigen recognition are not sufficient for full T cell activation. Recent studies have demonstrated that at least two signals provided by APC are required to induce effective T cell activation (7). The first signal is antigen specific and occurs after T cell receptor recognition of MHC-antigen complex. The second signal, often termed the "costimulatory" signal, is produced through a set of receptor and coreceptor interactions between APC and T cells. More specifically, the interaction of B7-1 or B7-2, also known as CD80 and CD86, respectively, on APC with CD28 on T cells is required for full T cell activation and effector function (8, 9). Costimulation provided by CD80 or CD86 may influence the differentiation of Th subsets (10). The importance of these interactions is reflected in the observations that T cell receptor recognition of the MHC-peptide complex in the absence of costimulatory signals can lead to the induction of anergy (7, 11).

Although there are differences in the distribution of B7-1 and B7-2, the expression of both molecules has been documented mainly in cells of bone marrow origin, including B cells, dendritic cells, macrophages, and activated T cells (12). Interestingly, all reports to date suggest that epithelial cells in mucosal tissues lack B7 costimulatory molecules (13–17). The absence of the B7 costimulatory molecules was previously shown to restrict the ability of epithelial cells to activate CD4$^+$ T cells (13). In light of their expression of class II MHC molecules and their possible role as APC during infection with *H. pylori*, it was important to establish whether gastric epithelial cells express such costimulatory molecules and activate local CD4$^+$ T cells. Thus, in this study we examined human gastric

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1. *Abbreviations used in this paper:* APC, antigen presenting cells; G3PDH, glyceraldehyde 3-phosphate dehydrogenase; SEB, *Staphylococcal* enterotoxin B; TCR,T cell receptor.

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epithelial cells for their expression of B7-1 and B7-2 and their role in stimulating $CD4^+$ T cells.

Methods

Cell lines. The human B lymphoblastoid cell line JESTHOM was provided by Dr. Carpenter (Brigham and Women's Hospital, Boston, MA). The Kato III and AGS gastric epithelial cells, of human gastric adenocarcinoma origin, were obtained from the American Type Culture Collection (ATCC, Rockville, MD). All cells were cultured in RPMI-1640 medium supplemented with 10% FCS in a humidified 37°C, 5% CO_2 incubator.

Bacteria. H. pylori LC-11 (Cag A^+) originally isolated from the antral mucosa of a patient with duodenal ulcer as previously described (18, 19) were grown on blood-agar plates (Becton Dickinson, Mountain View, CA) at 37°C under microaerophilic conditions for 4 d before harvesting into PBS. The bacterial concentration was measured by OD at the absorbance of 530 nm using a DU-65 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA). The bacteria were then adjusted to a concentration of 2×10^8 bacteria/ml and used immediately.

Isolation of epithelial cells from gastric biopsy tissue. Biopsy specimens of the gastric antrum were obtained from consenting patients undergoing gastro-esophageal-duodenoscopy for various clinical indications in acordance with UTMB Institutional Review Board approved protocol. Patients were considered infected if *H. pylori* was detected by rapid urease testing or by histopathology on biopsies. The biopsy tissue was placed in Ca^{2+} and Mg^{2+} free HBSS, supplemented with 5% FCS, and transported immediately to the laboratory. The tissue was then placed in HBSS containing 0.1 mM EDTA and 0.1 mM DTT and agitated at 37°C for 15–30 min to remove the mucus. The biopsy tissue was placed in dispase solution (2.4 U/ml; Boehringer Mannheim, Mannheim, Germany) and agitated at 37°C for 30 min with a change of fresh dispase solution after 15 min. The supernatant was collected and the cells were pelleted by centrifugation at 200 *g* for 5 min. The cells isolated were mostly (> 90%) epithelial cells according to the morphology with May-Grunwald-Giemsa staining (Sigma Chemical Co., St. Louis, MO) and flow cytometry with immunofluorescence staining of anticytokeratin mAb (see below). The freshly isolated cells were used immediately for immunofluorescence staining as described below.

Antibodies and fusion protein. Hybridomas secreting L243 (anti–HLA-DR, mouse IgG2a), IVA12 (anti–HLA-DR,DP,DQ, mouse IgG1), and W6/32 (anti–HLA-A,B,C, mouse IgG1) were all from ATCC. Ascites was obtained by growing the cells intraperitoneally in BALB/c mice. The L307 (anti–B7-1, mouse IgG1) and IT2.1 (anti–B7-2, mouse IgG1) mAb were generous gifts from Dr. M. Azuma (Juntendo University School of Medicine, Tokyo, Japan). CTLA-4Ig, a soluble fusion protein of the T cell surface molecule CTLA-4 extracellular domain and Fc fragment of human IgG1 constant region (20), was provided by Dr. P. Linsley (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA). B9.4 (anti–human CD8, mouse IgG2b) was a gift from Dr. E. Brooks (UTMB, Galveston, TX). C-11 (anti–pan-cytokeratin, mouse IgG1) was purchased from Sigma Chemical Co. FITC-conjugated goat anti–mouse Ig $F(ab')_2$ was purchased from Jackson ImmunoResearch (West Grove, PA). FITC-conjugated avidin was acquired from Pierce (Rockford, IL).

Biotinylation of CTLA-4Ig. CTLA-4Ig was adjusted to a concentration of 1 mg/ml in PBS at a pH of 8.5. Biotin-*N*-hydroxysuccinimide-ester (Calbiochem, La Jolla, CA) was dissolved in dimethyl formamide at 5 mg/ml. CTLA-4Ig and the biotin solution were mixed at a 10:1 ratio and incubated for 1 h at room temperature. The reaction was quenched with 0.5 mg glycine. The solution was applied to a desalting column (BioRad Laboratories, Richmond, CA) to remove free biotin and then aliquoted and stored at −70°C until used.

Immunohistochemistry staining. Frozen sections from gastric biopsy specimens were processed by the immunohistochemistry service in the Department of Pathology. Subsequently, slides were stained using an automated staining process in which they were labeled with antibodies recognizing human CD3, CD4, or CD8 or an appropriate isotype control (Ventana, Tucson, AZ). Slides were then counterstained with hematoxylin and eosin (H&E). Representative frozen or formalin-fixed sections were also stained with H&E for morphological examination. After staining, tissue sections were examined by two readers who were unaware of the antibody used although the state of infection was self evident. The number of positive-labeled cells were scored as a percentage of epithelial cells.

Immunofluorescence staining and flow cytometry. Cell surface antigens were detected by routine indirect immunofluorescence staining of cytospin preparations or cells that were grown on chamber slides (Nunc Inc., Naperville, IL). In brief, the cells (5×10^5) were fixed in 3% paraformaldehyde in PBS for 20 min and washed with 2% BSA/PBS. The cells were subsequently incubated with 0.5 μg of specific antibody for 30 min, washed, and stained with FITC-conjugated goat anti–mouse Ig $F(ab')_2$ for another 30 min. The cells were then washed and visualized by a fluorescence microscope (Nikon Inc., Melville, NY). For flow cytometry, cell suspensions were stained with either anticlass II MHC, anti–B7-1, anti–B7-2, anti–CD3, anti–CD4, or isotype control antibodies for 30 min on ice. The cells were then washed with ice-cold 1% BSA/PBS followed by incubation with FITC-conjugated goat anti–mouse Ig as the secondary staining reagent. For staining with anticytokeratin mAb, the cells were fixed with 2% paraformaldehyde and permeabilized with 0.1% saponin before the incubation with the primary antibody. Cells stained with biotinylated CTLA-4Ig included FITC-avidin as the secondary reagent and biotinylated human IgG1 (Accurate Chemical & Scientific Co., Westbury, NY) as the isotypic control. After incubation with the secondary reagents, the cells were washed and fixed with 1% paraformaldehyde in PBS. The cells were then analyzed by flow cytometry using a FACScan® (Becton Dickinson).

Reverse transcription-PCR. Total RNA was extracted from Kato III cells by using TRIzol® (Life Technologies Inc., Grand Island, NY) according to the manufacturer's instructions. An aliquot (1 μl, ∼ 1 μg) of each RNA preparation was reverse-transcribed in total 20 μl reaction mixture with 2.5 μM random hexamers, 1 mM of each dNTP, and 50 U MuLV reverse transcriptase (Perkin Elmer Corp., Branchburg, NJ) and were incubated at 42°C for 1 h. The total or aliquot of resulting cDNA was then amplified by PCR in a reaction mixture with total volume of 100 μl consisting of 3 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, 0.2 mM of each dNTP, 0.1 mM of each primer and 2.5 U *AmpliTaq* DNA polymerase (Perkin Elmer Corp.). All reactions were overlaid with mineral oil and PCR was carried out in a programmed thermal cycler (Perkin-Elmer Cetus Corp., Emeryville, CA) for 35 cycles. Each cycle consisted of denaturation at 96°C for 1 min, annealing at 55°C for 1 min, and polymerization at 72°C for 1 min.

Primers were designed as described elsewhere (21). Primers for B7-1 (22) were: (sense) 5′CCTCTCCATTGTGATCCTGG3′ (corresponding to nucleotides: 611–630) and (antisense) 5′GGCGTACACTTTCCCTTCTC3′ (nucleotides: 1157–1177); and for B7-2 (9): (sense) 5′GACCTGCTCATCTATACAC3′ (nucleotides: 593–612) and (antisense) 5′CTTCATCAGATCTTTCAGG3′ (nucleotides: 1039–1057). B7-1 and B7-2 primers were synthesized locally (Dr. T. Wood, Sealy Center for Molecular Science, UTMB, Galveston, TX) using an automated DNA synthesizer (394; Applied Biosystems Inc., Foster City, CA). Primers for glyceraldehyde 3-phosphate dehydrogenase (G3PDH), (sense) 5′ACCACAGTCCATGCCATCAC3′ (nucleotides: 586–605) and (antisense) 5′TCCACCACCCTGTTGCTGTA3′ (nucleotides: 1017–1037), were purchased from Clontech Laboratories, Inc. (Palo Alto, CA).

PCR amplified products were separated by 6% polyacrylamide gel or 1.2% agarose gel electrophoresis. 100 bp ladder (GIBCO BRL, Gaithersburg, MD) was used as molecular weight markers. After electrophoresis, the gels were stained with 0.5 μg/ml ethidium bromide for 1 h and washed before exposure to an ultraviolet Foto/Prep I (Fotodyne Inc., New Berlin, WI) for visualization and photography.

In some experiments, the densitometry of each band was measured and analyzed with Sigma Gel software.

Restriction endonuclease treatment. To confirm the specificity of the PCR products, aliquots of B7-1 or B7-2 PCR amplified products were treated with restriction enzymes before electrophoresis. SspI (Promega Corp., Madison, WI), with a recognition site within the sequence of the B7-1 amplified product, cleaves the B7-1 product into two fragments of 403 and 163 bp. Similarly, digestion of B7-2 PCR product with PvuII (Promega Corp.) generates two fragments of 287 and 179 bp. The digestion was performed using 20 U of each of the enzymes for 20 μl of the corresponding PCR product during incubation at 37°C for 1 h immediately before the gel electrophoresis.

Up-regulation of B7-1 and B7-2 expression on gastric epithelial cells. To investigate the regulatory mechanisms of B7-1 and B7-2 expression, Kato III cells were incubated with either IFNγ, *H. pylori* alone, or in combination for various periods of time. IFNγ (Boehringer Mannheim) was used at 100 U/ml. *H. pylori* (LC11) were added to the cell culture at a concentration previously determined to provide optimal stimulation, which corresponded to a ratio of ~ 300 bacteria per Kato III cell. At the end of culture, the cells were washed and B7-1 and B7-2 expression were assayed by flow cytometry. Since crosslinking of surface class II MHC enhances B7 expression in B cells (23), we tried to determine whether class II MHC-bearing gastric epithelial cells respond similarly to class II MHC crosslinking. To do this, Kato III cells were first treated with IFNγ at 100 U/ml for 48 h to amplify the expression of class II MHC (24) and then further incubated either with IFNγ-containing medium (10^6 cells/ml) or in media supplemented with anticlass II MHC mAb L243 (5 μg/ml) or the superantigen *Staphylococcal* enterotoxin B (SEB, 1 μg/ml) (Sigma Chemical Co.) for 16 h. The cells were then washed and stained with anti–B7-1 mAb or CTLA-4-Ig as described above.

Enrichment of $CD4^+$ T cells. $CD4^+$ T cells were partially purified by negative selection from PBMC from normal donors. In brief, PBMC were separated by Ficoll-Hypaque gradient centrifugation. To prevent nonspecific binding during the selection process, the cells were incubated in PBS containing 0.5% human γ-globulin at room temperature for 30 min. The cells were then washed and incubated in RPMI-1640–10% FCS medium supplemented with the L243 anticlass II MHC mAb and the B9.4 anti-CD8 mAb for 30 min at room temperature. At the end of the incubation, the cells were washed and resuspended in RPMI-1640–10% FCS medium and 5 ml of the cell suspension (2×10^6 cells/ml) were added to AIS MicroCELLector flasks (Applied Immune Sciences, Inc., Santa Clara, CA), which had been coated with goat anti–mouse IgG and IgM. The flasks were incubated for 1 h at room temperature. The nonadherent cells were then recovered from the flask and their purity assessed by FACS® analysis. 96–99% of the cells obtained by this method were $CD4^+$.

Mixed epithelial cell lymphocyte culture. The allogeneic cultures were established as reported by others with some modifications (25). In brief, the gastric epithelial cells (Kato III) were irradiated with 10,000 rads to prevent their proliferation. These cells were resuspended in RPMI-1640–10% FCS medium at a concentration of 3.3×10^5 /ml. $CD4^+$ T cells were negatively selected as described above and adjusted to a concentration of 10^6 /ml. 100 μl of both Kato III and $CD4^+$ T cells were seeded into each well of a 96-well microtiter plate. Different antibodies (e.g., anti–B7-1 and B7-2) were added at a final concentration of 5 μg/ml. The plates were incubated in a humidified 37°C, 5% CO_2 incubator for 5 d. During the last 8 h of culture, the cells were pulsed with [^{3}H]thymidine (ICN Pharmaceuticals Inc., Irvine, CA) at 1 μCi/well. At the end of culture, the cells were harvested and the cell proliferation was assessed by measurement of [^{3}H]thymidine incorporation with a liquid scintillation counter (Beckman Instruments, Inc.).

Statistical analysis. Results are expressed as the mean±SD. Data were compared by the Student's *t* test and considered significant if *P* values were < 0.05.

Results

Gastric epithelial $CD4^+$ T cells are increased during H. pylori infection. As class II MHC expression increases during infection with *H. pylori*, a parallel increase in $CD4^+$ T cells would suggest a role for gastric epithelial cells in T cell activation. Although the specificity of the infiltrating T cells could not be easily documented in this study, we noted that the number of $CD4^+$ T cells were increased within the gastric epithelium of *H. pylori*–infected patients (Fig. 1). Most of the infiltrating T cells were $CD4^+$ (Table I) and were found at a 5–14 times higher frequency than in normal tissue.

Gastric epithelial cells express the costimulatory molecules B7-1 (CD80) and B7-2 (CD86). Costimulatory molecules are necessary to facilitate T cell activation. The costimulatory pathway that has received the most attention to date is that mediated via the interaction of CD28 on T cells with one of its ligands, B7-1 or B7-2, on APC. CTLA-4, a T cell expressed coreceptor related to CD28, is also a ligand for B7-1 and B7-2. CTLA-4 has a high affinity for both B7-1 and B7-2. Thus, to

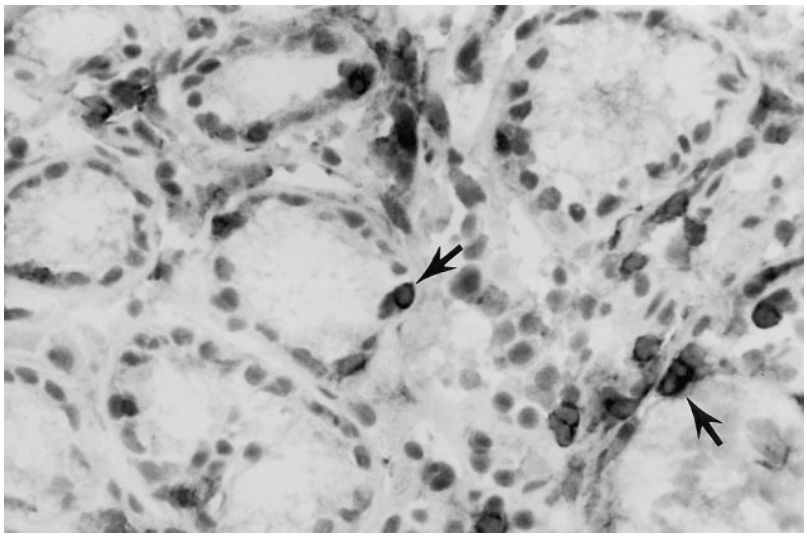

Figure 1. $CD4^+$ T cells are increased in *H. pylori*–infected gastric epithelium. $CD4^+$ T cells within a gastric biopsy section were detected by immunohistochemical staining with anti-CD4 antibodies. Representative stained T cells are indicated with the arrows.

Table I. CD4⁺ T Cells Are Increased in H. pylori–infected Gastric Epithelium

	CD4⁺ T cells/100 epithelial cells	*n*	*P* value
H. pylori −	0.6±0.5	3	< 0.005
H. pylori +	9.3±1.1	5	

CD4⁺ T cells are increased in gastric epithelium. Biopsy specimens of the gastric antral mucosa were collected from five *H. pylori* infected and three uninfected patients, processed and examined by immunohistochemistry for CD⁺ T cells. These data represent the mean±SD of the positive cells/100 epithelial cells. The results showed that intraepithelial CD4⁺ T cells were markedly increased during infection ($P < 0.005$).

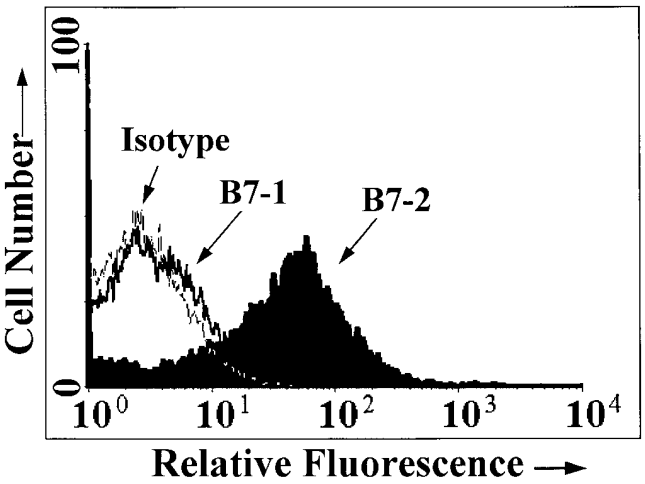

Figure 3. Expression of B7-1 and B7-2 on Kato III gastric epithelial cells. Kato III cells were stained with isotypic control mouse IgG1 (*dashed line*), L307 anti–B7-1 mAb (*solid line*), or IT2.1 anti-B7-2 mAb (*solid filled*) plus goat anti–mouse Ig-FITC. FACS® analysis showed low basal expression of B7-1 and more significant expression of B7-2 on these cells.

assess the expression of either B7-1 or B7-2 on gastric epithelial cells we used biotinylated CTLA-4Ig and specific antibodies in flow cytometry analysis. As controls, the reactivity of these reagents on conventional APC (JESTHOM B cell line) was assessed (Fig. 2). The B7-1 expression by gastric epithelial cells is at a low, but detectable level, whereas the binding of CTLA-4Ig is readily observed (Fig. 2). These observations were confirmed with at least two other gastric epithelial cell lines (data not shown). The extensive binding of CTLA-4Ig to the Kato III gastric epithelial cells was presumed largely due to B7-2. This was confirmed by staining the Kato III gastric epithelial cells with the IT2.1 anti-B7-2 mAb (Fig. 3).

To detect the expression of B7-1 and B7-2 transcripts by Kato III cells, RT-PCR analysis was performed. Both B7-1 and B7-2 mRNA were detected (Fig. 4). The amplified products for B7-1 and B7-2 had the expected length of 566 and 466 bp, respectively. After treatment with the restriction endonucleases, the B7-1 product was cleaved into two fragments of 403 and 163 bp, and the B7-2 product into two fragments of 287 and 179 bp, which confirmed the specificities of the PCR amplified products. To compare the mRNA levels of B7-1 and B7-2 in Kato III gastric epithelial cells and in JESTHOM B cells, different amounts of cDNA ranging from 20 to 0.2 or 10 to 0.1 μl were applied for PCR. The level of G3PDH was used as the internal standard in the exponential range of amplification. As shown in Fig. 5, the B7-2 mRNA level in Kato III cells was at a exponential range comparable to that in JESTHOM B cells, whereas the level of B7-1 mRNA appeared to be much lower.

Differential regulation of B7-1 and B7-2 expression by gastric epithelial cells. Although B7-1 is generally absent from unstimulated APC, B7-2 is constitutively expressed on monocytes, dendritic cells and is promptly up-regulated by crosslinking of surface receptors, i.e., Ig and class II MHC, on B cells (9, 12, 23). Cytokines have also been shown to up-regulate the expression of B7-1 and B7-2, although B7-2 expression occurred

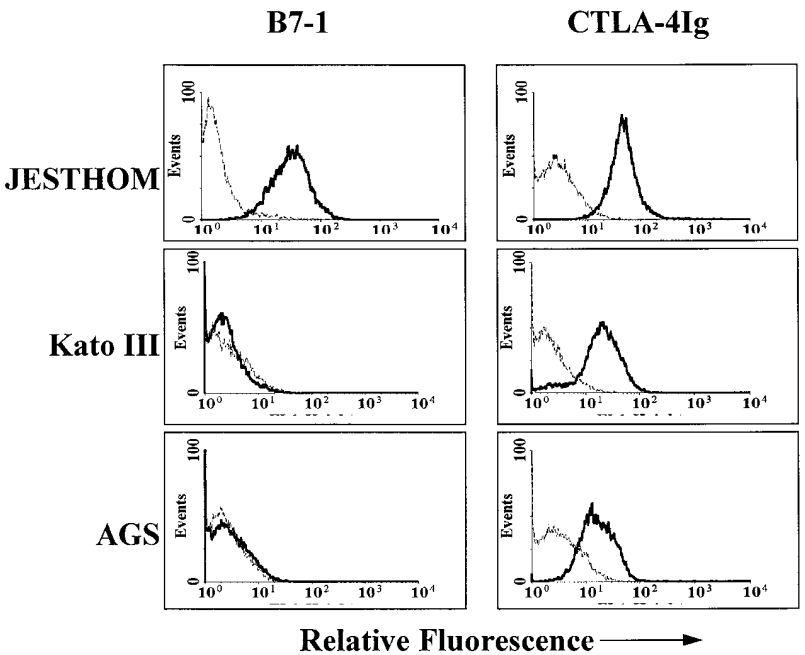

Figure 2. Reactivities of the L307 anti–B7-1 mAb and biotinylated CTLA-4Ig to B cells and gastric epithelial cells. Cells were stained by indirect immunofluorescence with L307 plus goat anti–mouse Ig-FITC or biotinylated CTLA-4Ig plus avidin-FITC and then analyzed by flow cytometry. The isotype controls for L307 and for CTLA4-Ig were mouse IgG1 and biotinylated human IgG1, respectively. In each histogram overlay, the dashed line showed the background staining with isotype controls and the solid line showed the cells stained for either B7-1 (*left*) or CTLA-4Ig (*right*). The EBV-transformed B cell line, JESTHOM cells showed high expression of B7-1 as well as the binding of CTLA-4Ig. The gastric epithelial cell lines, Kato III and AGS, showed minimal expression of B7-1 whereas the binding of CTLA4-Ig was comparable to that observed in B cells.

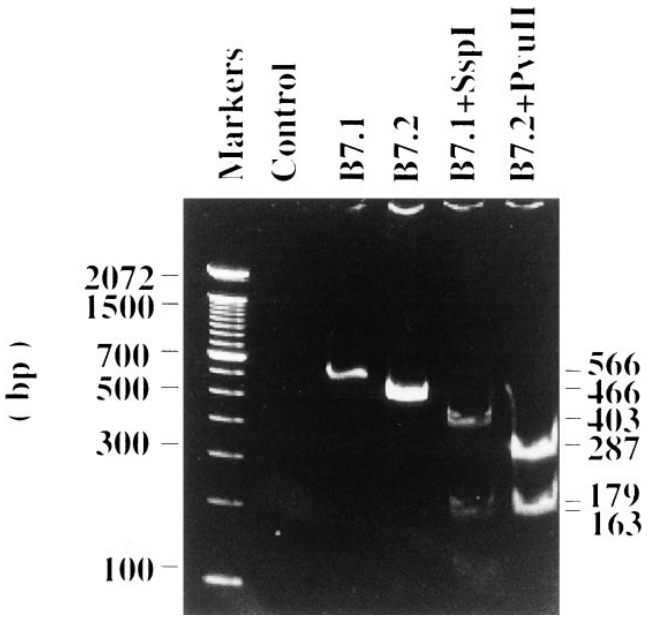

Figure 4. RT-PCR analysis of B7-1 and B7-2 mRNA in Kato III cells. The RNA extracted from Kato III cells were processed by RT-PCR with specific primers as described in Methods. After electrophoresis on a 6% polyacrylamide gel and staining with ethidium bromide, the amplified products with or without endonuclease digestion showed distinct bands of predicted molecular weights. Lanes containing the amplified B7-1 and B7-2 products displayed bands of the expected sizes at 566 and 466 bp, respectively. The treatment of B7-1 product with restriction endonuclease SspI and B7-2 product with Pvu II resulted in two bands of predicted molecular weights, which confirmed the specificity of the PCR products. The negative control was an aliquot of RNA sample that was subjected to RT-PCR under same conditions as the others except that no reverse transcriptase was applied. The molecular weight markers, 100 bp ladder, consist of 15 distinguishable fragments starting at 100 bp and progressively increasing 100 bp to 1,500 bp plus an additional band at 2,072 bp.

earlier than B7-1 (26, 27). To examine how the expression of B7-1 and B7-2 is regulated on gastric epithelial cells, Kato III cells were pretreated with either IFNγ and/or *H. pylori* or crosslinking of class II MHC by SEB or antibodies as described above. Flow cytometry analysis showed that crosslinking of class II MHC molecules with either antibody or superantigen remarkably enhanced the binding of CTLA-4Ig (Fig. 6), but not the expression of B7-1. Similarly, treatment of Kato III cells with *H. pylori* in the presence of IFNγ significantly enhanced B7-2 expression (Fig. 7) whereas *H. pylori* alone did not show any significant effect on either B7-1 or B7-2 expression. In parallel experiments, unlike what has been observed on B cells (28), monocytes (29) and keratinocytes (30), treatment of Kato III cells with PMA, or PMA plus indomethacin failed to induce up-regulation of either B7-1 or B7-2 expression (data not shown).

Expression of B7-1 and B7-2 by gastric epithelium in vivo. To confirm that the observations from the in vitro studies had relevance in vivo, epithelial cells isolated from gastric biopsy tissue were examined for the expression of B7-1 and B7-2. The isolated cells were cytokeratin$^+$ and CD45$^-$, which confirmed an epithelial cell phenotype. The freshly isolated gastric epithelial cells expressed B7-2 whereas the levels of B7-1 were minimal (Fig. 8). The expression of B7-2 was consistently higher in the epithelial cells of patients with *H. pylori* infection than those without *H. pylori* infection (Fig. 9). This strongly suggested that the above described in vitro observations reflect the conditions found in vivo. Thus, during *H. pylori* infection, gastric epithelial cells express costimulatory molecules as well as class II MHC molecules, that enable them to readily activate resident or recruited T cells.

B7-1 and B7-2 on gastric epithelial cells are necessary for activating allogeneic CD4$^+$ T cells. As B7-1 and B7-2 are also required in T cell responses to alloantigens and superantigens in an MHC-unrestricted manner (31), we examined the ability of Kato III gastric epithelial cells to stimulate alloreactive T cells in a mixed epithelial cell–lymphocyte reaction culture system.

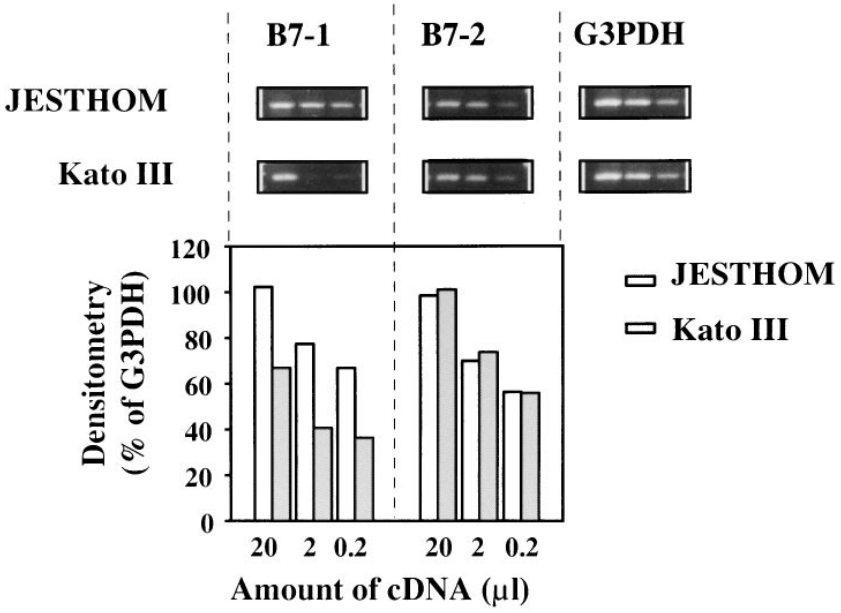

Figure 5. Comparison of densitometry of B7-1 and B7-2 mRNA in B cells and gastric epithelial cells. Similar amounts (1 μg) of each RNA extraction from EBV-transformed B cells (*JESTHOM*) and Kato III gastric epithelial cells were transcribed as mentioned earlier. 10-fold dilutions of cDNA samples (20, 2, or 0.2 μl) were applied for PCR amplification. The densitometry of each band was determined with SigmaGel software. Samples amplified for G3PDH were used as internal controls. The results showed the mRNA for B7-2 in both JESTHOM B cells and Kato III cells were at similar exponent range while the level of B7-1 mRNA was much lower in Kato III cells than that in JESTHOM cells. This is a representative of three experiments.

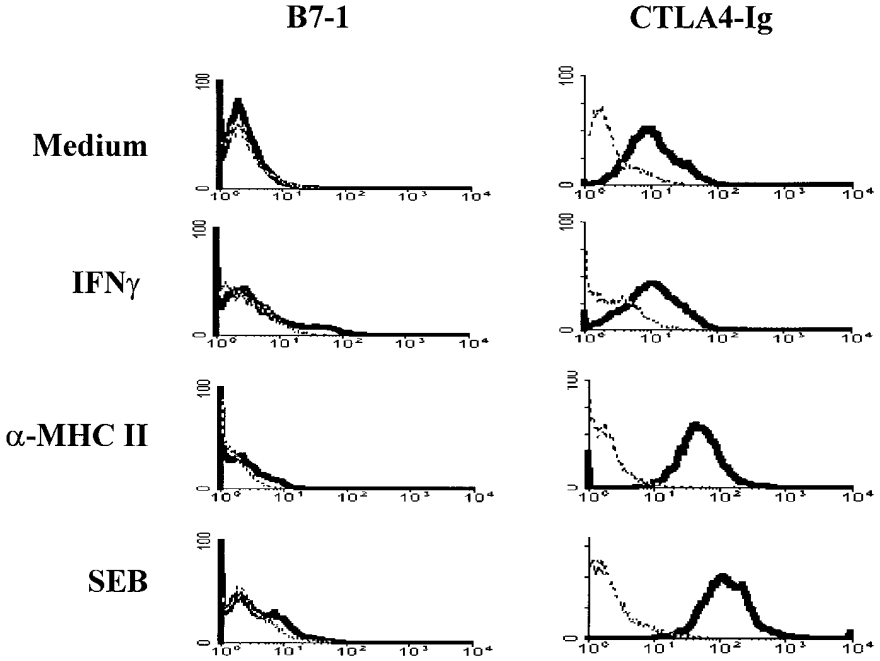

Figure 6. Upregulation of costimulatory molecule expression by crosslinking of class II MHC on gastric epithelial cells. IFNγ-pretreated Kato III cells were incubated further in IFNγ-containing medium or in media supplemented with L243 anticlass II MHC mAb or superantigen (*SEB*) for 16 h. The cells were then washed and stained for B7-1 expression (*left*) or CTLA4-Ig binding (*right*) as described. In each histogram overlay, the thin dashed line (···) represents isotypic control and the thick solid line (—) represents the specific staining. Flow cytometry analysis showed little effect on B7-1 expression but a remarkable increase of CTLA4-Ig binding in the cells incubated with IFNγ plus superantigen or L243 anti-class II MHC mAb.

As determined by [^{3}H]thymidine uptake, the irradiated Kato III gastric epithelial cells stimulated the proliferation of alloreactive CD4$^+$ T cells. Antibody blocking of B7-1 resulted in $\sim$ 16% inhibition of the T cell proliferation, whereas antibody blocking of B7-2 resulted in 59% inhibition of T cell proliferation. Blocking of both molecules led to complete inhibition of T cell proliferation (Fig. 10). This indicates that B7-2 and, to a lesser extent, B7-1 on gastric epithelial cells play an essential costimulatory role in initiating T cell proliferation. Also, the complete block by the addition of both antibodies suggests that no other costimulatory molecules related to B7 (i.e., B7-3) (32) are expressed by gastric epithelial cells.

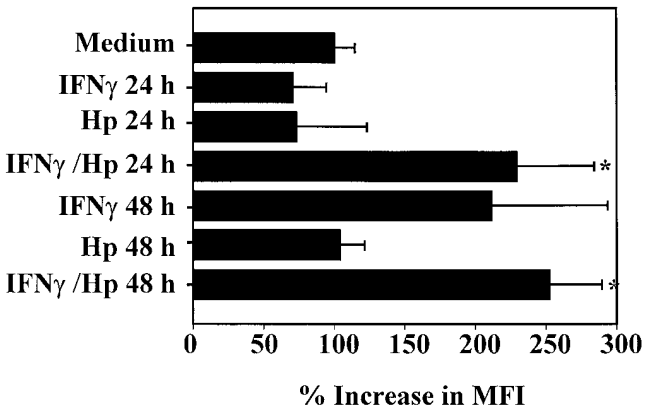

Figure 7. Effects of IFNγ and/or *H. pylori* on the expression of B7-2 by Kato III cells. Kato III cells were cultured in media alone or with IFNγ, *H. pylori* (*Hp*), or the combination of both for 24 or 48 h. The levels of B7-1 and B7-2 expression were analyzed by flow cytometry. The results of four separate experiments are presented as mean±SD of the mean fluorescence intensity values from which corresponding isotypic control fluorescence intensity levels were subtracted. *$P <$ 0.05 relative to controls.

Discussion

H. pylori is now recognized as an etiopathogenetic cause of chronic gastritis, peptic ulcers, as well as gastric adenocarcinoma and mucosal associated lymphoid tissue lymphoma of the stomach (33–35). Although microbial factors have been associated with the pathogenesis of the more severe gastric diseases associated with *H. pylori* (36, 37), family members infected with the same strain of *H. pylori* may experience different disease manifestations (38). This observation suggests that host factors may also contribute to the pathogenesis of gastric disease. This notion is supported by the observation that some of the virulence factors of *H. pylori* are linked to their ability to augment the local inflammatory response. Moreover, the gastric mononuclear cells (MNC) present during *H. pylori* infection produce IFNγ and appear to be of the Th1 type (39). As Th1 cells select for cell-mediated immunity, their presence in association with an extracellular, luminal infection may favor destruction of the epithelium and contribute to the failure of the host to clear an infection with *H. pylori.* Thus, the activation of gastric T cells may contribute to the pathogenesis of gastroduodenal disease associated with *H. pylori* infection.

It has been reported that gastric epithelial cells constitutively express class II MHC molecules that increase during infection with *H. pylori* (4, 40, 41), which we have confirmed in this study. Moreover, by immunohistochemical studies, we showed here that the number of CD4$^+$ T cells also increased within the epithelium. This further suggested an active role for gastric epithelial cells in local T cell activation. In addition to the recognition of MHC-antigenic peptide complex by TCR, T cell activation requires coreceptors on APC capable of delivering costimulatory signals. In these studies, we have demonstrated that in addition to class II MHC expression, the costimulatory molecules B7-1 and B7-2 are expressed on human gastric epithelial cell lines and freshly isolated gastric epithelial cells. The levels of B7-1 expression were low whereas B7-2 was

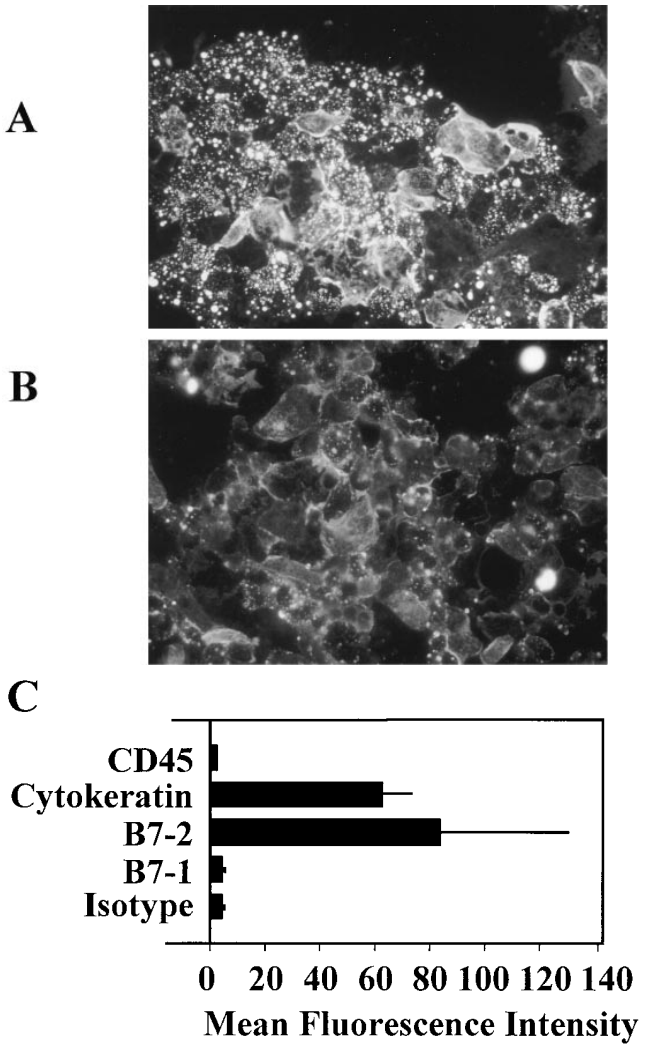

Figure 8. Immunofluorescence staining of B7 molecules on freshly isolated gastric epithelial cells. The freshly isolated cells were stained by indirect immunofluorescence on cytospin preparations. The results demonstrated immunofluorescence staining of C-11 anti–pan-cytokeratin mAb (*A*), intended to detect a marker for epithelial cells, and IT2.1 anti–B7-2 mAb (*B*) by freshly isolated gastric epithelial cells. The staining of anti–B7-1 was weak and too faint to be photographed. The relative levels of expression of the markers were determined by flow cytometry (*C*). This graph reflects a representative staining of samples from five patients with *H. pylori* infection.

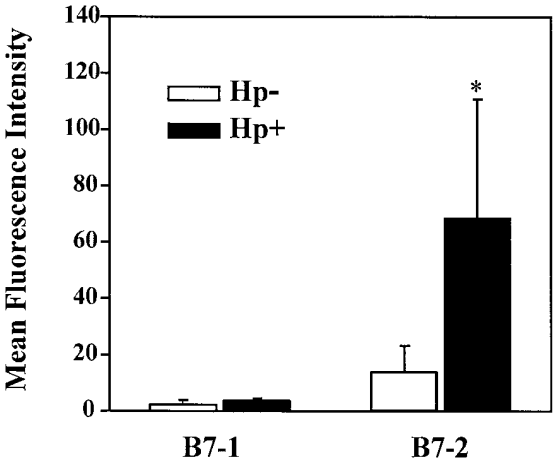

Figure 9. Differential expression of B7-1 and B7-2 on freshly isolated gastric epithelial cells from *H. pylori*–infected versus uninfected individuals. Gastric epithelial cells were isolated from endoscopic biopsy specimens from patients with or without *H. pylori* infection. B7-1 and B7-2 expression were analyzed by flow cytometry. The mean±SD of the mean fluorescence intensity values of the samples from five *H. pylori* infected and four uninfected individuals were compared for their statistical significance. The data revealed that B7-2 expression was significantly higher in cells from *H. pylori*–infected patients than that in the uninfected samples ($P < 0.05$).

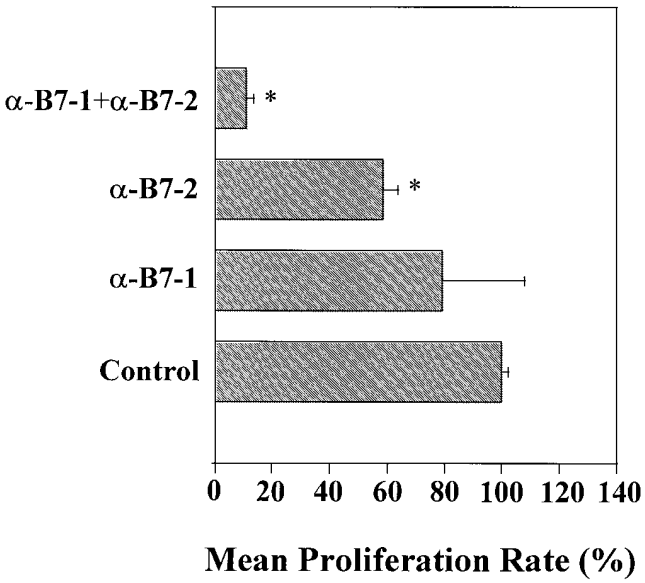

Figure 10. Costimulatory role of B7-1 and B7-2 in the Kato III gastric epithelial cell-stimulated allogeneic $CD4^+$ T cell response. $CD4^+$ T cells were negatively selected by removing class II MHC^+ as well as $CD8^+$ cells from PBMC and cultured with irradiated (10,000 rads) Kato III cells in 96-well plates for 5 d. Anti–B7-1 and anti–B7-2 mAbs were added into the culture at a final concentration of 5 μg/ml separately or in combination. Control wells received mouse IgG1 at the same concentration. The cells were pulsed with $[^3H]$thymidine (1 μCi/well) during the last 8 h of culture. At the end of culture, the cells were harvested and their proliferation was determined by $[^3H]$thymidine incorporation. The proliferation in the presence of the anti–B7-1 and anti–B7-2 antibodies was significantly inhibited ($P < 0.05$).

readily detected on gastric epithelial cells. The lower level of B7-1 transcripts in Kato III gastric epithelial cells may partly explain its marginal expression on the cell surface. However, as a possibly more sensitive readout system, the allogeneic $CD4^+$ T cell proliferation inhibited by specific antibodies further confirmed the presence of functional costimulatory molecules. Together, our observations from both in vitro and in situ studies demonstrated that gastric epithelial cells express, in addition to class II MHC, B7-1, and B7-2 costimulatory molecules and can thus effectively activate $CD4^+$ T cells.

The pattern of B7 expression by gastric epithelial cells was analogous to that of conventional APC, since B7-1 is normally expressed at low levels and B7-2 is readily detected on resting human monocytes and splenic cells (8, 9). B7-2 expression by B cells occurs rapidly upon activation, whereas B7-1 is slow to

appear (8). Memory B cells constitutively express both B7-1 and B7-2 and after stimulation through CD40 or surface immunoglobulin the expression of both molecules increases sooner and to higher levels than on naive B cells (42). Based on these observations, it has been suggested that B7-2 signals dominate during primary immune responses whereas B7-1 may act in maintaining those responses or during memory responses (43).

In spite of their similarity of receptor binding, some kinetic and molecular properties distinguishing B7-1 and B7-2 molecules had been observed, implying that these molecules have distinct interactions with CD28 and CTLA-4 on T cells (44) and subsequently affect T cell responses (45). Although intestinal epithelial cells have been shown to stimulate $CD8^+$ T cells preferentially under normal conditions, during inflammatory conditions they overexpress class II MHC and selectively activate $CD4^+$ T cells (3, 46). As mucosal or intraepithelial T cells have been shown to be phenotypically and functionally different from those in the peripheral blood (47, 48), the expression and regulation of B7-1 and B7-2 in gastric epithelial cells may reflect the distribution and status of costimulators required in their interaction with local T cell populations.

The regulation of B7-1 and B7-2 expression is controlled by cell–cell interaction and cytokines (49). Crosslinking of class II MHC on B cells deliver signals through cytoplasmic tail of class II MHC to induce B7 expression and thus result in effective antigen presentation, whereas mutant B cells with tailless class II MHC were shown to be deficient in their antigen presentation capacity, which correlated with a lack of B7 induction (23, 50). In our study, B7-2 expression was found higher in the gastric epithelial cells of *H. pylori*–infected patients than those of uninfected individuals. Similarly, the treatment of Kato III gastric epithelial cells with the combination of IFNγ and *H. pylori* (but not *H. pylori* alone) significantly up-regulated B7-2 expression. Furthermore, the expression of B7-2 on Kato III cells was markedly increased after the crosslinking of class II MHC in vitro, which mimicked recognition by the TCR in vivo. Based on these observations, it may be postulated that during *H. pylori* infection, IFNγ secreted by gastric intraepithelial and lamina propria T cells enhances the expression of class II MHC on gastric epithelial cells, that allows them to interact with local T cells. Meanwhile, signal transduction through the cytoplasmic domain of class II MHC occurs in gastric epithelial cells during their interaction with local T cells, leading to increased expression of costimulatory molecules, that could, in turn, set the conditions for complete T cell activation (23). The effect of the IFNγ plus *H. pylori* on B7-2 expression suggested that *H. pylori* per se might have a superantigen-like binding activity to the class II MHC on the surface of gastric epithelial cells and thus transduce a signal to upregulate B7 expression. Studies to examine this possibility are in progress.

To our knowledge, this is the first demonstration of either B7-1 or B7-2 expression by class II MHC^+ mucosal epithelial cells. Thus, the expression of costimulatory molecules by the gastric epithelium sets it apart from other mucosal epithelium in its potential to stimulate T cells. The absence of costimulatory molecules on other mucosal epithelial cells may explain their inability to stimulate T cell proliferation to antigens (13, 51). For instance, renal epithelial cells were shown to lack B7 expression (13). It has also been reported that no B7 was detected in the class II MHC expressing intestinal epithelial cells either in rodents (14) or in patients with inflammatory bowel diseases (15, 16). However, only B7-1 expression was examined in those studies. In one reported study, only a low level expression of B7 was induced in human intestinal epithelial cell lines cultured with GM-CSF (17).

It is important to notice that there are some essential differences between the intestine and the stomach. The primary function of the intestine is to absorb nutrients into the circulation and transport biological macromolecules while being exposed to a variety of luminal antigens derived from food and resident and invading microorganisms. The lack of B7 expression on the intestinal epithelium may be important in the induction of tolerance to luminal antigens, since T cells recognizing antigens in the absence of B7-CD28 costimulation pathway will lead to anergy (7, 11). The gastric mucosal barrier, on the other hand, consists of a relatively impermeable epithelial cell surface that transports hydrogen and bicarbonate ions and secretes mucus that prevents proteolytic or mechanical damage to the mucosal surface (52). Unlike the intestine in which a myriad of microbial antigens are present, *H. pylori* is virtually the only pathogenic microorganism associated with the gastric mucosal epithelium. Previous observations of increased expression of class II MHC in the epithelium during *H. pylori* infection suggested a role for gastric epithelial cells in local immune responses. Our present study further demonstrates that these cells express B7-1 and B7-2 costimulatory molecules and can effectively activate $CD4^+$ T. Thus, in contrast to other mucosal tissues, $CD4^+$ T cells may be activated more vigorously by gastric epithelial cells during *H. pylori* infection. Gastric MNC cells have been shown to produce predominantly IFNγ (39), which has numerous deleterious effects on local inflammation and epithelial barrier function (53, 54). Together, these findings support the notion that gastric epithelial cells may contribute to their own destruction as a consequence of their ability to activate potentially deleterious helper T cell responses. Additional studies of the regulation of local mucosal immune responses will improve our understanding of the pathogenesis of *H. pylori* infection and potentially assist in identifying individuals at the greatest risk of developing severe gastroduodenal disease as a consequence of infection.

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