Tumor Necrosis Factor Alpha and Interleukin 1β Up-Regulate Gastric Mucosal Fas Antigen Expression in Helicobacter pylori Infection

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Fas-mediated gastric mucosal apoptosis is gaining attention as a cause of tissue damage due to Helicobacter pylori infection. We explored the effects of H. pylori directly, and the effects of the inflammatory environment established subsequent to H. pylori infection, on Fas-mediated apoptosis in a nontransformed gastric mucosal cell line (RGM-1). Exposure to H. pylori-activated peripheral blood mononuclear cells (PBMCs), but not H. pylori itself, induced Fas antigen (Fas Ag) expression, indicating a Fas-regulatory role for inflammatory cytokines in this system. Of various inflammatory cytokines tested, only interleukin 1β and tumor necrosis factor alpha induced Fas Ag expression, and removal of either of these from the conditioned medium abrogated the response. When exposed to Fas ligand, RGM-1 cells treated with PBMC-conditioned medium underwent massive and rapid cell death, interestingly, with a minimal effect on total cell numbers early on. Cell cycle analysis revealed a substantial increase in S phase cells among cells exposed to Fas ligand, suggesting an increase in their proliferative response. Taken together, these data indicate that the immune environment secondary to H. pylori infection plays a critical role in priming gastric mucosal cells to undergo apoptosis or to proliferate based upon their Fas Ag status.

Helicobacter pylori, first isolated from gastric biopsies in 1982 by Marshall and Warren (19), has become well recognized as the major etiologic factor in ulcer disease, chronic atrophic gastritis (19), and gastric lymphoma and carcinoma (24). The bacterium attaches, colonizes the gastric mucosa, and incites both cellular and humoral immune responses (5). Histological examination of infected tissue reveals acute and chronic inflammatory cells throughout the mucosa, with the spiral bacterium in the overlying mucus layer. The organism is not invasive, although rarely it can be found deep in crypts.

H. pylori infection is associated with elevated levels of both mucosal apoptosis (12, 15, 21, 31) and proliferation (3, 8, 15). The initiation and the regulation of the pathways that promote these paradoxical cellular responses are still unclear. Using H. pylori-infected human biopsy specimens, we previously demonstrated concomitant Fas antigen (Fas Ag) expression and gastric mucosal apoptosis, suggesting a role for Fas signaling in H. pylori-associated apoptosis (12). Fas Ag is a transmembrane receptor, which when bound specifically to its ligand (Fas L) trimerizes and initiates a cascade of events resulting in apoptosis in a variety of cell settings (2, 9, 10, 16). Interestingly, the pathway can be modulated at various points throughout, including regulation of the number of Fas receptors on the cell membrane as well as regulation of the availability of Fas L (9, 16). Fas Ag and Fas L expression have been shown to be regulated at the mRNA level in various cell types by inflammatory cytokines such as interleukin 1β (IL-1β), IL-2, tumor necrosis factor alpha (TNF-α), and gamma interferon (IFN-γ) (10, 12, 16, 22, 23). Although the Fas pathway has been well characterized in the immune system, less is known about the role this pathway plays in nonlymphoid tissue. Not surprisingly, H. pylori infection is also associated with increased mucosal inflammatory cytokines, including IL-1β, IL-6, IL-8, and TNF-α (6) and IFN-γ (6, 11). Production of IL-1β, IL-6, IL-8, and TNF-α has been demonstrated in H. pylori-stimulated peripheral blood mononuclear cell (PBMC) cultures, suggesting that immune cells may be the source of the mucosal cytokines found clinically (11, 18). Previously, we demonstrated the responsiveness of both gastric (KATO III) and small bowel (IEC-6) cell lines to exogenous cytokine-mediated regulation of Fas Ag mRNA (12). Furthermore, upon exposure to Fas L, these cells undergo apoptosis, confirming that the Fas pathway is intact and functional. Since cytokines have shown the capability to induce Fas Ag expression in malignant gastric cell lines (12), we postulated that the cytokines generated during the immune response to H. pylori could prime nonmalignant gastric tissue for apoptosis by increasing mucosal expression of Fas Ag. In this scenario, Fas L, which is expressed on lymphocytes present in infected gastric tissue (12), could trigger Fas-mediated apoptosis. This study was undertaken to specifically test this prediction and to further characterize the immune regulation of Fas Ag expression in H. pylori infection. Because malignant gastric cell lines already possess a growth advantage as well as altered apoptotic sensitivity, we chose to use a nontransformed gastric cell line to address these issues. Using the RGM-1 cells as a tissue culture model, we examined individual selected components of the immune response to H. pylori for their ability to regulate gastric mucosal cell proliferation and apoptosis through Fas signaling.
MATERIALS AND METHODS

Bacterial culture and CFU determination. *H. pylori* strain 43504 was obtained from the American Type Culture Collection and grown as recommended. After 4 days of culture, single colonies were picked, resuspended in 2 ml of tryptic soy broth (TSB) containing 5% fetal calf serum (FCS) in 15-ml conical tubes, and grown with mild agitation under microaerophilic conditions. Optical densities at 600 nm were measured, and CFU were determined by serial dilution plating on TSB-5% sheep blood plates. A standard curve was established based on triplicate optical density readings. Bacteria were diluted with TSB-5% FCS to a final concentration of 10^8 CFU/ml for use in cell culture.

Isolation and activation of Wistar rat PBMCs. Ten-week-old male Wistar rats were housed in a controlled environment with intraperitoneal injection of Ketamine (60 mg/kg) and Xylazine (7.5 mg/kg). Using a laparotomy approach, 10 ml of blood was obtained by direct aortic puncture. PBMCs were harvested using Optiprep (Nycomed Pharma, Oslo, Norway) according to the manufacturer’s protocol. Cells were washed twice in phosphate-buffered saline, resuspended in RPMI medium (Gibco BRL, Rockville, Md.)–20% FCS, and counted using a Royco automated cell counter, and 1 million cells per 100-mm-diameter dish were plated and incubated at 37°C in 5% CO_2_. After 4 h, 100 μl of *H. pylori* bacterial culture (final concentration of 10^6 CFU/ml as outlined above) or 100 μl or sterile bacterial medium was added to 5 ml of culture medium, and culture was continued for another 15 h. Medium (conditioned medium from dishes containing PBMCs and *H. pylori* or PBMC control medium from dishes cultured without *H. pylori*) was collected, pooled, filter sterilized, and used in tissue culture experiments.

Tissue culture. RGM-1 cells, a nontransformed rat gastric cell line, were obtained from the RIKEN cell bank, Tsukuba Science City, Japan, and grown in 100-mm-diameter dishes to 70% confluence in Dulbecco’s modified eagle medium-Ham’s F12 nutrient medium (DME/F12) (Gibco BRL) containing 20% FCS in 5% CO_2 at 37°C. Cells monolayers were exposed to the following experimental conditions (5 ml total volume).

(i) Controls. Controls included RPMI containing 20% FCS, DME/F12 containing 20% FCS and 100 μl of *H. pylori* growth medium, and PBMC control medium (as described above).

(ii) Bacteria. Monolayers were exposed to 100 μl of *H. pylori* (in 20 μl of TSB containing 5% FCS) per ml of culture medium for 2, 4, 6, 8, 12, 24, 48, 72, or 96 h.

(iii) PBMC-conditioned medium. PBMC-conditioned medium was as described above and was used undiluted or diluted to 50%, 25%, and 10% (by volume) in RPMI containing 20% FCS. Monolayers were exposed to 2, 4, 6, 8, or 24 h.

(iv) Cytokines. Monolayers were exposed to recombinant mouse IL-1β (1 ng/ml), recombinant rat IL-2 (20 μl/ml), recombinant human IL-8 (50 ng/ml), recombinant human TNF-α (300 U/ml), or recombinant IFN-γ (300 U/ml) (Genzyme, Cambridge, Mass.) for 2, 4, 8, 16, and 24 h. All cytokines were certified by the manufacturer to be active in the rat system.

Neutralizing antibody. Activated PBMC medium (25% by volume) was preincubated for 1 h with 24 μg of normal or purified monoclonal IgG (control) or with anti-IL-1β (2 or 4 μg/ml) or anti-TNF-α (12 or 24 μg/ml) neutralizing antibodies (R&D Systems, Minneapolis, Minn.) and applied to cell monolayers for 2 h. Dilation and exposure times were derived from prior experiments and are indicated in the figure legend.

ELISA analysis of IL-1β and TNF-α concentrations. Rat TNF-α and IL-1β enzyme-linked immunosorbent assay (ELISA) detection kits (Cytoscreen; Bio-source International, Camarillo, Calif.) were used according to the manufacturer’s instructions. Mouse ELISA concentrations in the undiluted conditioned medium; 50%, 25%, and 10% diluted conditioned medium; and control medium were determined and plotted against a standard curve. All samples were assayed a minimum of twice each.

Fas L treatment: measurement of apoptosis and proliferation. RGM-1 cells were plated in 60-mm-diameter dishes to achieve 20% confluence, washed after 4 h, and cultured with 1 ml of one of the following: DME/F12 containing 20% FCS (control), the control supplemented with 100 or 500 ng of Fas L (Alexis Biochemicals, San Diego, Calif.) per ml of 25% conditioned medium, or 25% conditioned medium plus 100 or 500 ng of Fas L per ml. Total cell counts were taken using the Royco cell counter, and 1 million cells per 100-mm-diameter dish were plated and incubated for 45 min at room temperature. After the membrane was washed twice in phosphate-buffered saline, resuspended in RPMI medium (Gibco BRL, Rockville, Md.)–20% FCS, and counted using a Royco automated cell counter, and 1 million cells per 100-mm-diameter dish were plated and incubated at 37°C in 5% CO_2_. After 4 h, 100 μl of *H. pylori* bacterial culture (final concentration of 10^6 CFU/ml as outlined above) or 100 μl of sterile bacterial medium was added to 5 ml of culture medium, and culture was continued for another 15 h. Medium (conditioned medium from dishes containing PBMCs and *H. pylori* or PBMC control medium from dishes cultured without *H. pylori*) was collected, pooled, filter sterilized, and used in tissue culture experiments.

Results

In order to determine the regulation of *H. pylori*-associated gastric mucosal Fas expression, we first examined individual components of the infected mucosal milieu. Under routine culture conditions (control medium), RGM-1 cells express low, but consistently detectable, levels of Fas Ag mRNA as determined by RT-PCR. Addition of 100 μl of TBS medium alone to the normal RGM-1 culture did not alter this (Fig. 1A, panel 1). *H. pylori* applied to RGM-1 cells also did not change the level of Fas Ag expression at 2, 4, 6, 8, 12, 24, 48, 72, or 96 h postexposure (the 6-h point is shown in Fig. 1A, panel 2), suggesting that neither direct bacterial contact nor a secreted bacterial factor directly induces Fas Ag mRNA expression in the RGM-1 cells. However, the cell-free conditioned medium from PBMCs cocultured with *H. pylori* for 15 h markedly upregulated Fas Ag expression by 2 h (Fig. 1A, panel 3), with declining levels at 4 h (Fig. 1A, panel 4) and a return to basal levels at 24 h (data not shown), suggesting that a factor or factors secreted by *H. pylori*-activated PBMCs, and not direct cell contact, were responsible for the up-regulation of Fas Ag in this cell line. Because *H. pylori* has been shown to modulate expression of a variety of cytokines in PBMCs in vitro and in *H. pylori*-infected tissue in vivo, we examined selected individual components (IL-1β, IL-2, IL-8, TNF-α, and IFN-γ) of the inflammatory response for their ability to up-regulate Fas Ag mRNA expression in our system. Addition of IL-1β or TNF-α resulted in a substantial increase in Fas Ag expression at 2 h (Fig. 1B, panels 2 and 5, respectively), which paralleled the
increase seen with conditioned medium (Fig. 1B, panel 1). Addition of IL-2 (Fig. 1B, panel 3), IL-8 (Fig. 1B, panel 4), or IFN-γ (Fig. 1B, panel 6) alone at levels shown to induce Fas Ag expression in other systems (10, 22, 23) did not result in a significant change in Fas Ag expression in RGM-1 cells at 2 h (Fig. 1B) or at 4, 8, 16, or 24 h (data not shown).

Next, we determined the effect of conditioned medium on Fas Ag up-regulation over a range of dilutions to verify whether the cytokine levels reached in our experimental cul-

FIG. 1. RT-PCR analysis of Fas Ag and Fas L mRNA expression in RGM-1 cells. RT-PCR data for GAPDH are shown for each experimental condition and are used to standardize the lanes. (A) Levels of Fas Ag expression in RGM-1 cells grown in control medium plus 100 μl of TSB containing 5% FCS for 4 h (panel 1), 10² H. pylori (HP) bacteria/ml for 2 h (panel 2), or filter-sterilized medium from PBMCs cocultured with 10² H. pylori (HP) bacteria/ml (conditioned medium) for 2 or 4 h (panels 3 and 4, respectively). (B) Fas Ag mRNA induction in response to conditioned medium (panel 1) or treatment with IL-1β (1 ng/ml) (panel 2), IL-2 (20 U/ml) (panel 3). IL-8 (50 ng/ml) (panel 4), TNF-α (300 U/ml) (panel 5), or IFN-γ (300 U/ml) (panel 6) in control medium for 2 h. (C) Expression patterns of Fas Ag and Fas L mRNAs in control medium (RPMI containing 5% FCS) (panel 1) or with decreasing concentrations of conditioned medium for 2 h. (D) Efforts of neutralizing antibodies on Fas Ag expression in RGM-1 cells. Levels of Fas Ag and Fas L mRNAs in cells grown in control medium 1 (DME/F12 containing 20% FCS) (panel 1), control medium 2 (RPMI containing 20% FCS) (panel 2), conditioned medium (panel 3), and conditioned medium preincubated with one or two times the ND₅₀ of anti-TNF-α (panels 4 and 5, respectively) or anti-IL-1β (panels 6 and 7, respectively), with two times the ND₅₀ of both antibodies together (panel 8), or with a goat IgG control (panel 9) for 2 h are shown. (Bottom panel) Densitometry values for panels A, B, C, and D. All values are corrected for background signal and standardized to those for GAPDH. Values are reported in comparison to the normal control [control or (−) control] for samples run in the same RT-PCR and visualized on the same gel. The normal control is not shown for panel B.
experimental conditions. We tested low and high doses of Fas L (values were chosen based on the manufacturer’s recommendations) to test for the effect on RGM-1 cells expressing Fas Ag. Control cell populations showed normal monolayer morphology, without floating cells. (Fig. 3A, top panel). Addition of Fas L induced minimal but progressive changes, with a small fraction of adherent cells rounding up, followed by the appearance of increasing numbers of floating cells. These changes were both time and dose dependent. Addition of 100 ng or Fas L per ml resulted in minimal changes, with the appearance of <2% floating cells after 16 h. When the Fas L concentration was increased to 500 ng/ml, the number of floating cells approached 12% at 16 h. We believe that addition of ligand to the control culture resulted in a small but measurable increase in cell death because of activation through basolateral levels of Fas receptor. Monolayers exposed to conditioned medium alone also showed morphologic changes comparable to those of the control cells treated with Fas L. Although we did not measure this, we speculate that the conditioned medium contains low levels of soluble Fas L (shown to be released from PBMCs in response to activation [30]) and would be expected to induce the observed changes. In contrast, Fas L added to monolayers previously exposed to PBMC-conditioned medium, and expressing the most Fas Ag, showed dramatic changes (Fig. 3A, bottom panel). Rounding up of adherent cells, along with detachment and blebbing, occurred as early as 3 h after the addition of ligand, with maximal changes noted at 20 h (500 ng/ml) and 36 h (100 ng/ml), respectively. Upon addition of 500 ng of ligand per ml, fewer than 10% of the monolayer cells remained adherent at 20 h. Total cell counts with the percentage of viable cells determined by trypan blue exclusion assay confirmed the extent of cell death in cells exposed to 500 ng of Fas L per ml (Fig. 3B).

To verify whether the observed loss of viability was due to apoptosis, we employed two assays that detect different but definite stages of apoptosis. Acridine orange nuclear staining, which allows morphologic determination of apoptosis, was performed on aliquots of cultures treated with 500 ng of ligand per ml. Clumping, margination, and fragmentation of chromatin, as determined by fluorescence microscopy, were considered evidence for apoptotic cell death. Control cultures had <0.2% apoptotic cells detected at 4, 16, or 20 h. With the addition of ligand control cultures had 2.9% (4 h), 7.2% (16 h), and 10.5% (20 h) apoptotic cells. Cells preincubated with conditioned medium prior to the addition of ligand showed 16.8% (4 h), 24.6% (16 h), and 42% (20 h) apoptotic cells. To verify the disparity between the quantitation of loss of viability and acridine orange staining (which distinguishes later stages of apoptosis), we used annexin staining, a technique widely used to detect both early and late changes of apoptosis. Early in apoptosis, phosphatidylserine is translocated from the inner to the outer leaf of the cell membrane. Annexin V shows high-affinity binding to phosphatidylserine in the presence of Ca2+ and is useful for detecting early stages of apoptosis, prior to nuclear condensation or morphologic changes. Quantitation of apoptosis by using annexin staining revealed a close correlation with the trypan blue viability results (Fig. 3c). A progressive increase in the rate of apoptosis was observed in RGM-1 cells treated with Fas L (Fig. 3C, column b) or conditioned medium with exogenous Fas L (Fig. 3C, column b), while virtually no increase was observed in control cells (Fig. 3C, column a). Comparison of low-abundance Fas Ag (control medium [Fig. 3C, column b]) to high-abundance Fas Ag (conditioned medium [Fig. 3C, column d]) showed increased susceptibility to Fas L-induced apoptosis related to the quantity of surface receptor. A difference in the baseline cell viabilities determined with trypan blue and annexin was probably because of membrane damage to the cells during harvesting, which may have partially permeabilized a percentage of cells, resulting in overestimation of cell death with annexin staining.

A comparison of growth properties of cells under different treatment conditions led to an interesting observation. Cells treated with Fas L consistently showed a proliferative advantage over cells grown in control medium alone, at all time points. Even in cells treated with both conditioned medium and Fas L, where more than 90% cell death was observed by multiple assays, the total cell number as determined after excluding fragmented cells was maintained with minimal effect (Fig. 4A). Taking into account the presence of more than 40% acridine-orange positive apoptotic cells at 20 h after Fas L treatment, most of which are excluded by Royco cell counts, the maintenance of a stable cell number is not plausible without a compensatory increase in cell proliferation. The growth advantage of cells exposed to control medium plus ligand, compared to those exposed to control medium alone, was modest but consistent and reproducible (Fig. 4A). [3H]thymidine studies were performed to confirm the observed increase in proliferation as a result of ligand exposure. RGM-1 cells grown in control medium showed a marked increase (three times the control value) in [3H]thymidine incorporation at 30 h, verifying the findings from standard cell counts (data not shown). These observations thus suggest a simultaneous activation of cell death and proliferation pathways under the conditions tested. To determine whether the treatment conditions that induced Fas-mediated apoptosis also enhanced the cycling of the cells, we examined the cell cycle status of RGM-1 cells treated with control medium alone, control medium with the
addition of Fas L, and conditioned medium with Fas L (Fig. 4B) by using FACS techniques. The addition of ligand resulted in a substantial increase (compared to control cells without ligand) in the percentage of cells proliferating, represented by an increase in cells in the S phase of the cell cycle. A similar increase was seen in cells exposed to conditioned medium prior to addition of ligand (Fig. 4B); however, this was also accompanied by massive concomitant cell death. This also suggests that the quantity of Fas Ag protein expression plays an important role in the commitment of mucosal cells towards choosing between a proliferative response or apoptotic cell death.

DISCUSSION
Apoptotic cell death has been increasingly recognized as a factor in *H. pylori*-related mucosal injury (12, 15, 21, 28, 31), with several lines of evidence supporting the involvement of the Fas Ag-Fas L pathway (12, 14, 25). Using biopsy specimens from *H. pylori*-infected gastric and duodenal ulcers, we have shown that Fas Ag mRNA is up-regulated concomitant with elevated mucosal apoptosis (12). We also demonstrated sensitivity of gastric (KATO III) and small bowel (IEC-6) cell lines to cytokine-mediated up-regulation of Fas Ag mRNA and subsequent increased susceptibility to ligand-induced apoptosis, suggesting a role for the Fas pathway in the pathogenesis of *H. pylori*.
pylori-related ulcer disease. On the other hand, Jones et al. (14) have recently shown that *H. pylori* is capable of directly inducing death in gastric cell culture in the absence of immune cells or their products. KATO III cells underwent necrotic cell death following prolonged culture with the bacterium, whereas AGS cells, another malignant gastric cell line, underwent apoptosis, in association with increased Fas Ag receptor expression (14). However, the factor(s) responsible for regulation of the Fas pathway and its potential role in cell death in gastric epithelial cells is largely unknown. Addressing these problems with malignant cell lines is problematic. Malignant cells often have dysregulated apoptotic, proliferative, and intracellular signaling pathways, which makes them less-than-ideal model systems to study in vivo disease progression. In order to circumvent these problems, we chose to study RGM-1 cells, a nontransformed rat gastric cell line which represents normal gastric epithelium.

In contrast to the case for AGS cells, the presence of *H. pylori* alone did not regulate Fas expression in RGM-1 cells; therefore, endotoxin or other secreted or surface components of the bacteria are unlikely candidates for the Fas-activating factor in this system. On the other hand, conditioned medium from *H. pylori*-activated PBMCs markedly increased Fas Ag expression in RGM-1 cells, prompting us to explore the possible involvement of inflammatory cytokines as an activating factor(s). The cytokines we chose to examine were those shown to be increased in clinical *H. pylori* infection and known to up-regulate Fas expression in other systems. *H. pylori*-activated PBMCs behave like “generically” activated PBMCs and produce IL-1β, IL-2, IL-6, IL-8, TNF-α, IFN-γ, and surface Fas L in addition to other immune mediators (5, 6, 11). IL-1β, IL-2, TNF-α, and IFN-γ are known inducers of Fas Ag expression in a variety of cell types and were previously shown by us to up-regulate Fas Ag expression in the KATO III and IEC-6 cell lines (12). In the RGM-1 cells, however, only the addition of IL-1β or TNF-α increased Fas Ag expression levels, which were comparable to levels induced with PBMC-conditioned medium, suggesting that these cytokines, alone or in combination, may act to regulate Fas Ag expression in *H. pylori* disease. Of particular interest also for us to investigate was IL-8, the cytokine which has been shown to most closely correlate with *H. pylori* disease severity in vivo (26, 27). Addition of IL-8 showed no effect on Fas Ag expression in the present experimental setup. Of note is that both IL-1β and TNF-α regulate IL-8 production through the JNK and mitogen-activated protein kinase pathways as well as through NF-κB (20), suggesting that IL-8 may be a marker for the presence of these cytokines rather than causing mucosal injury itself. *H. pylori* has also been shown to activate NF-κB directly (17). We also confirmed by ELISA that the levels of IL-1β and TNF-α present in conditioned medium represented levels that could be found in vivo (32). Neutralizing antibody experiments suggested that TNF-α and IL-1β are the predominant activators, because neutralizing either of them from the medium decreased Fas Ag mRNA expression to basal levels.

Clinical *H. pylori* infection is associated with both apoptosis (ulcer disease) and increased proliferation (predisposition to malignancy). *H. pylori* infection activates PBMCs that contribute to a local cytokine environment capable of affecting regulation of the Fas signaling pathway. Activated lymphocytes may supply the necessary Fas L required to activate the pathway and lead to mucosal cell apoptosis. Based on these data, the Fas pathway may be responsible for (or at least contribute to) the apoptosis that accompanies *H. pylori* ulcer disease. Interestingly, proliferation and apoptosis coexist in *H. pylori* infection. It has been suggested that the proliferation seen with mucosal *H. pylori* infection may be a result of apoptotic tissue damage and comprises a normal healing response, while another school of thought suggests that the bacteria or bacterial products directly enhance cell growth (3, 8, 15). We present evidence in this communication to suggest, for the first time, that Fas signaling may be involved in simultaneous activation of dysregulated cell death and proliferation of RGM-1 cells. Much work, however, is needed to define the pathways that lead to these paradoxical cellular responses in *H. pylori*-infected gastric mucosal cells. In RGM-1 cells, *H. pylori* did not directly alter proliferation (J. Houghton, unpublished data). However, addition of Fas L to RGM-1 cells grown in control or PBMC-conditioned medium led to substantial increases in proliferative capacity, as demonstrated by a marked increase in the percentage of cells in the S phase of the cell cycle. Cells grown in control medium expressed this proliferative advantage as an increase in total cell numbers. Cells grown in conditioned medium (which also promoted Fas Ag induction), on the other hand, revealed both an increase in proliferation and a marked increase in cell death. These paradoxical cell responses as a result of Fas activation were confirmed by cell cycle analysis and apoptosis assays and were instrumental in...
maintaining the total cell number without major fluctuations over a period of time. Ligand binding to the Fas Ag receptor may initiate dual signaling programs, and the decision to pursue apoptosis or proliferation may depend upon the cell type and the magnitude of receptor aggregation (1, 9). In addition, reverse signaling through Fas L has recently been shown to induce proliferation (29). Fas L was not detected by either RT-PCR or Western blot analysis in RGM-I cells exposed to PBMC-conditioned medium; therefore, autocrine Fas L signaling is unlikely to cause proliferation in RGM-I cells.

H. pylori infection is almost always associated with inflammation; however, ulcer disease and gastric carcinoma occur only in a subset of patients. Within the subset of patients with disease, the natural history is one of recurrences interspersed with disease-free periods. Interindividual cytokine variations are felt to be related to polymorphisms within the cytokine genes themselves and have been demonstrated for TNF-α and IL-1β, as well as other cytokines. In addition, there appear to be genetic differences in the Fas Ag gene (4, 13) which may introduce another level of differential regulation, further complicating the issue. Little is known specifically about these genetic variations within the cytokine genes or the Fas promoter in populations at risk for different aspects of H. pylori disease. However, it is tempting to speculate that genetically determined differential responses of the Fas promoter to H. pylori infection may dictate some of the differences in disease susceptibility and presentations. In addition to or in combination with these differences, variations in the cytokine response of the individual (secondary to concomitant disease, smoking, stress, etc.) may affect the regulation of Fas Ag expression. Understanding the complex interplay of H. pylori and the immune system may help target patients at greater risk for complications of disease and enable physicians to focus intervention and therapy more specifically with these patients.

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REFERENCES