# Helicobacter pylori Infection Is Associated with Increased Expression of Macrophage Migratory Inhibitory Factor—by Epithelial Cells, T Cells, and Macrophages—in Gastric Mucosa

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**The macrophage migratory inhibitory factor (MIF) plays a pivotal role in inflammatory and immune diseases; however, its role in gastrointestinal diseases has not been clarified. This study intended to determine the expression of MIF, by gastric epithelial cells, T cells, and macrophages, in *Helicobacter pylori*–induced gastritis. Sixty-four patients (30 males, 34 females; mean age, 47 years) referred for upper endoscopy were recruited. Biopsy specimens from the gastric antrum and corpus were obtained for (1) detection of *H. pylori* and histological examination, (2) single and double immunostaining to test for expression of MIF protein in epithelial cells, T cells, and macrophages, and (2) in situ hybridization for expression of MIF mRNA within the lamina propria. In mucosal specimens from each of the 2 sites, both the percentage of MIF+ epithelial cells and the numbers of MIF mRNA+ inflammatory cells, MIF+ T cells, and MIF+ macrophages were significantly higher in *H. pylori*–positive patients than in *H. pylori*–negative patients. Overall, the percentage of MIF+ epithelial cells and the numbers of MIF mRNA+ cells, MIF+ T cells, and MIF+ macrophages were higher in the antrum than in the corpus. The percentage of MIF+ epithelial cells and the numbers of MIF mRNA+ cells, MIF+ T cells, and MIF+ macrophages increased in chronic gastritis, but, in the absence of *H. pylori* infection, this increase disappeared for all except MIF+ T cells. Therefore, *H. pylori* infection is associated with increased expression of the MIF protein and MIF mRNA in gastric epithelial and inflammatory cells; along with other cytokines, MIF may play a significant role in gastric inflammation related to *H. pylori* infection.**

The macrophage migratory inhibitory factor (MIF) has been shown to play a pivotal role in inflammatory and immune-mediated diseases [1–15]; however, its role in

gastrointestinal diseases has rarely been investigated [16–19]. Our recent study using a rodent gastric-ulcer model has demonstrated that MIF is markedly up-regulated during acute ulceration, which is associated with the accumulation of macrophages at the sites of inflammation [17]. Blockade of MIF by the neutralizing anti-MIF antibody significantly inhibits both macrophage accumulation and up-regulation of MIF, as well as tumor-necrosis factor α (TNF-α), inducible nitric oxide synthase (iNOS), and intercellular adhesion molecule 1 (ICAM-1), an effect that indicates an important role for MIF in the pathogenesis of gastric ulceration [17].

*Helicobacter pylori* is a bacterium that colonizes the human stomach and causes gastric inflammation and peptic ulcer disease, and it is believed to play an im
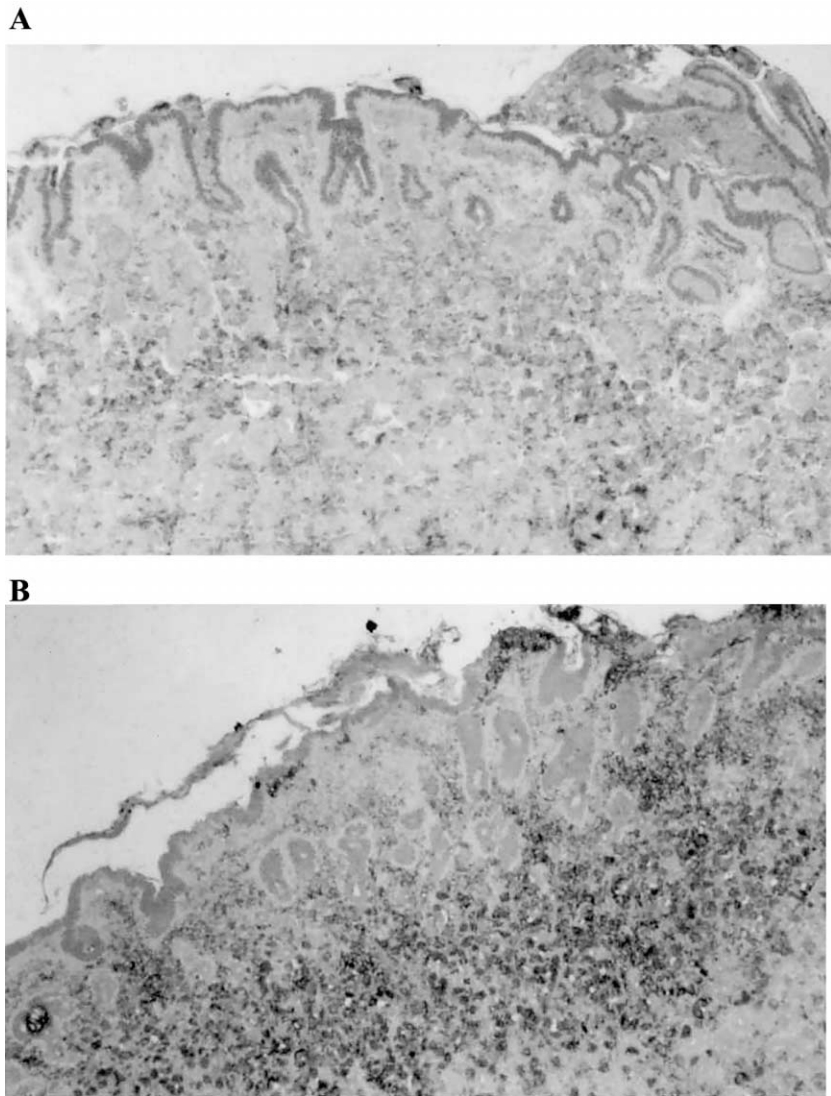
**Figure 1.** Expression of MIF mRNA in inflammatory and epithelial cells in the mucosa of the gastric antrum. In situ hybridization demonstrates weak expression in *Helicobacter pylori*–negative normal mucosa *(A)* and strong cytoplasmic or perinuclear expression *(darker areas)* in *H. pylori*–positive mucosa *(B)*. Original magnification, ×100.

portant role in the development of gastric carcinogenesis [20–24]. *H. pylori* infection causes gastric mucosal damage by producing many virulence factors, such as urease, lipopolysaccharide (LPS), a vacuolating cytotoxin (VacA), and phospholipases; by promoting inflammatory and immune responses; and by releasing large amounts of chemokines, cytokines, and reactive oxygen metabolites [18]. These changes subsequently affect the secretion of gastric acid and ascorbic acid, as well as cell apoptosis and proliferation [22, 24]. Reactive oxygen metabolites, hypochlorhydria, low levels of ascorbic acid, and imbalanced cell apoptosis and proliferation all contribute to the increased risk of the development of gastric cancer in a proportion of susceptible individuals [22, 24]. We hypothesize that *H. pylori* infection may result in gastric inflammation and ulceration, at least partly via

an MIF-mediated pathway. Therefore, the intention of this project was to determine the expression of MIF in the gastric mucosa in patients with and without *H. pylori* infection.

## PATIENTS AND METHODS

*Patients and gastric-biopsy specimens.* Sixty-four patients (30 males, 34 females; mean age ± SD, 47 ± 16 years), referred for upper endoscopy and diagnosed as having nonulcer dyspepsia, were recruited for the study. At endoscopy, 4 biopsy specimens—3 from the antrum and 1 from the corpus—were obtained; 1 of the antral-biopsy specimens was used for an in-house rapid-urease test; the other 3 biopsy specimens were fixed in formalin and embedded in paraffin, for histological examinations, im-
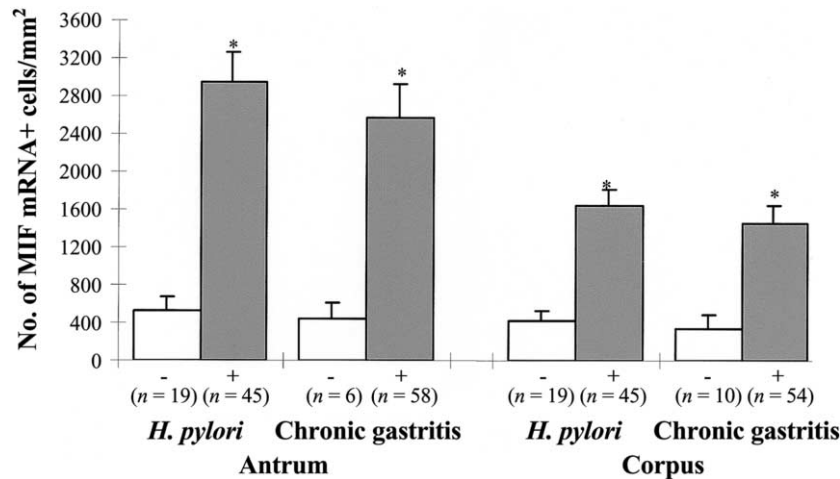
**Figure 2.** Expression of MIF mRNA in the antrum and in the corpus, with (+) or without (−) *Helicobacter pylori* infection and with (+) or without (−) histological chronic gastritis, as determined by in situ hybridization. *P < .001 , compared with patients either without *H. pylori* infection or without chronic gastritis. The vertical line extending from the top of each bar denotes the standard error of the mean.

munohistochemistry, and in situ hybridization. All patients gave informed written consents, and this project was approved by the Ethics Committee of The University of Hong Kong.

***Detection of* H. pylori *infection and histological examinations.*** The in-house rapid-urease test solution was prepared daily by dissolving 0.5 g of urea into 10 mL of distilled water and mixing the solution with 10 drops of 0.1% phenol red as a pH indicator. In the detection of *H. pylori,* this test has been shown to have a sensitivity of 99% and a specificity of 100% [25]. Sections (4 $\mu$m) of the paraffin-embedded tissue were stained with hematoxylin and eosin. Histological changes and *Helicobacter*-like organisms were examined. Patients were defined to be *H. pylori* positive if positive results were obtained by both rapid-urease test and histology (i.e., *Helicobacter*-like organisms were present) or by histology, at both the gastric antrum and the gastric corpus. Patients were defined to be *H. pylori* negative if the results of all tests were negative. Patients positive by only the rapid-urease test or by histology at only a single site were excluded from this study. The updated Sydney system was adapted to assess the severity and activity of gastric inflammation [21]. Chronic gastritis was considered to be present when there was an infiltration of chronic inflammatory cells (i.e., mononuclear cells); it was classified as grade 1 (mild), grade 2 (moderate), or grade 3 (marked), according to the number of infiltrating cells. The activity of gastritis (i.e., the presence of neutrophil polymorphs on a background of chronic inflammation) also was assessed.

***Immunohistochemistry and in situ hybridization.*** Biopsy specimens from the antrum and corpus of all patients were subjected to both a single-immunostaining (for epithelial cells) and a double-immunostaining (for activated T cells and macrophages) technique, as described elsewhere [17]. For the single immunostaining, sections were dewaxed, labeled by use of mouse anti-murine MIF monoclonal antibody (mAb)—which recognizes recombinant mouse and human MIF by Western blotting, as specified elsewhere [1, 3, 11, 17]—and developed by use of 3, 3-diaminobenzine (Sigma), which produced a brown-colored product. For the double immunostaining, sections were dewaxed, labeled by use of a mouse anti–human monocyte/macrophage (i.e., KP1) mAb or T cell (i.e., CD45RO) mAb (all from Transduction Lab), and developed by use of 3, 3-diaminobenzine, which produced a brown-colored product. After being microwaved, sections were labeled by use of mouse anti-MIF mAb that used a 3-layer mouse alkaline phosphatase/ anti–alkaline phosphatase complex and, finally, were developed by use of Fast Blue BB Base (Sigma), which produced a blue-colored product [17]. For expression of MIF in gastric epithelial cells, the percentage of the total epithelial cells (>300) that were MIF+ in 5 randomly selected fields was calculated. For the expression of MIF protein in the inflammatory cells, the numbers, per square millimeter, of T cells, MIF+ T cells, macrophages, and MIF+ macrophages in the lamina propria were counted and the percentages of T cells and macrophages that were MIF+ were calculated [17].

In situ hybridization was performed by use of a microwave-based method, as described in our previous study [17]. Any cells positively stained within the lamina propria were counted as MIF mRNA+ cells, and the number of MIF mRNA+ cells per square millimeter was calculated. These cells consisted mostly of glandular epithelial cells and infiltrated inflammatory cells.

***Statistic analysis.*** Data were expressed as mean ± SEM. The SPSS system (version 10.0) was used to perform statistical analysis. Independent-samples *t* test, the Mann-Whitney *U* test, or 1-way analysis of variance (ANOVA) was used to determine
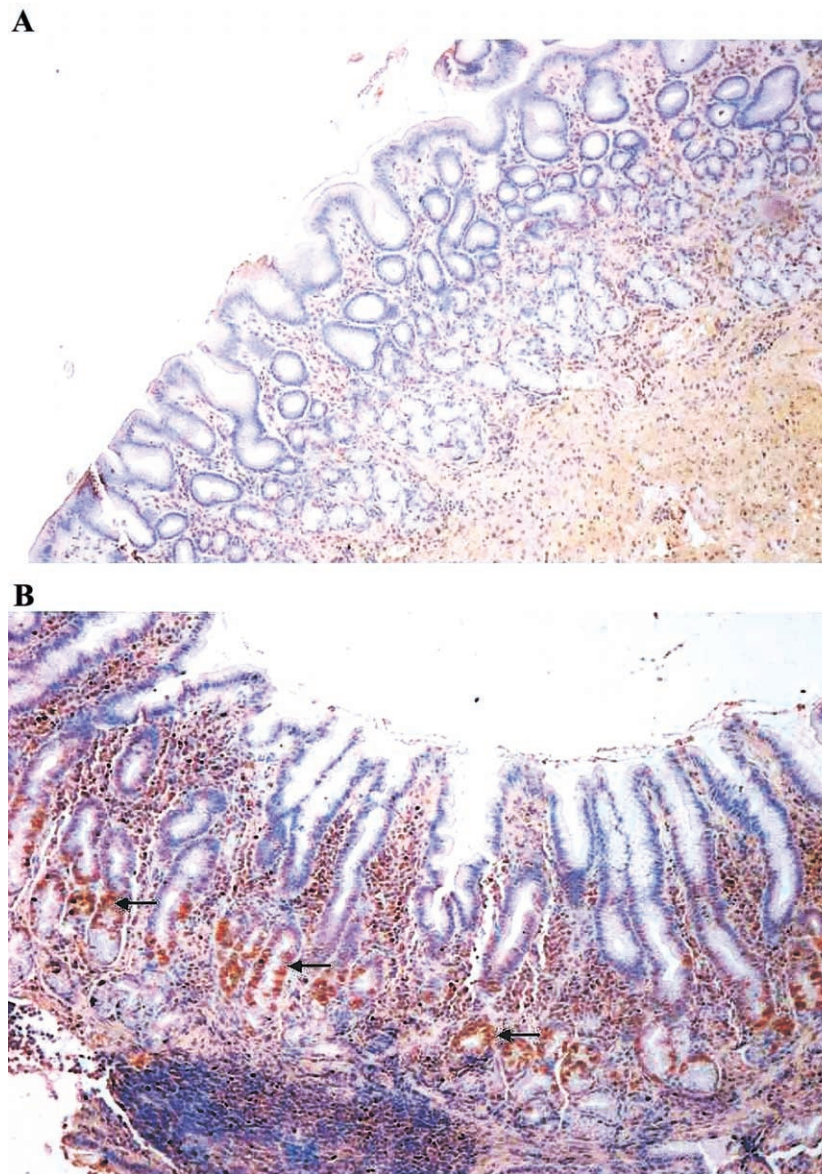
**Figure 3.** Expression of MIF in epithelial cells in the mucosa of the gastric antrum. Single immunostaining demonstrates weak expression in *Helicobacter pylori*–negative normal gastric mucosa *(A)* and strong cytoplasmic or perinuclear expression *(brown color,* denoted by *arrows)* in *H. pylori*–positive mucosa *(B)*. Original magnification, ×100.

the differences of the numeric variables. A paired-samples *t* test or a Wilcoxon signed-ranks test was used to determine the numeric-variables differences between the antrum and the corpus. All *P* values calculated were 2-tailed. The alpha level of significance was set at $P < .05$.

## RESULTS

***Expression of MIF mRNA in gastric mucosa, in relation to* H. pylori *infection and chronic gastric inflammation.***    Of the 64 patients in the present study, 45 were positive for *H. pylori* infection and 19 were negative. Significant (i.e., moderate or marked) chronic inflammation in the antrum was seen in al-most all (93.4%) of the *H. pylori*–positive patients but in none of the *H. pylori*–negative patients, and, in the corpus too it was more common in *H. pylori*–positive patients than in *H. pylori*–negative patients (53.3% vs. 2.2%).

In *H. pylori*–negative normal mucosa, the expression of MIF mRNA was weak and mostly within the glandular epithelial cells (figure 1*A*). However, *H. pylori* infection was associated with a strong cytoplasmic or perinuclear expression of MIF mRNA in both the glandular epithelial cells and the infiltrated inflammatory cells (figure 1*B*).

In general, the number of MIF mRNA+ cells in the antrum was significantly higher than that in the corpus (1814 ± 348/
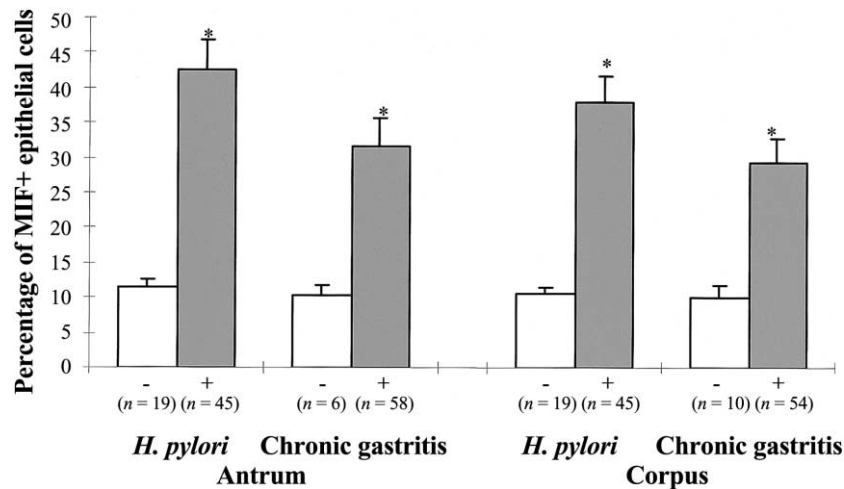
**Figure 4.** Expression of MIF in the antrum and in the corpus, with (+) or without (−) *Helicobacter pylori* infection and with (+) or without (−) histological chronic gastritis, as determined by single immunostaining. *$P < .001$, compared with patients either without *H. pylori* infection or without chronic gastritis. The vertical line extending from the top of each bar denotes the standard error of the mean.

mm$^2$ vs. 1063.9 ± 185/mm$^2$; $P < .001$). Both in the antrum and in the corpus, the number of MIF mRNA$^+$ cells was significantly higher when *H. pylori* infection and chronic gastritis were present (figure 2). Moreover, the increased expression of MIF mRNA$^+$ cells was significantly associated with severity of chronic gastritis, in both the antrum (830 ± 10/mm$^2$ in mild, 2780 ± 264/mm$^2$ in moderate, and 3084 ± 552/mm$^2$ in marked gastritis; $P < .001$, ANOVA) and the corpus (1163 ± 221/mm$^2$ in mild, 1738 ± 328/mm$^2$ in moderate, and 2080 ± 324/mm$^2$ in marked gastritis; $P < .001$, ANOVA). However, when *H. pylori*–positive patients were excluded from the analysis, the number of MIF mRNA$^+$ cells was not significantly different between patients with and patients without chronic gastritis, in both the antrum (438 ± 177/mm$^2$ vs. 830 ± 10/mm$^2$) and the corpus (344 ± 149/mm$^2$ vs. 630 ± 30/mm$^2$). Expression of MIF mRNA was not associated with the density of *H. pylori* or with the activity of chronic gastritis (data not shown).

***Expression of MIF protein in gastric epithelial cells, in relation to* H. pylori *infection and inflammation.*** MIF$^+$ gastric epithelial cells can be identified by the immunostaining technique (figure 3A and 3B). In *H. pylori*–negative patients, the percentage of MIF$^+$ epithelial cells in the antrum and in the corpus was 11.6% ± 1.0% and 10.6% ± 1.1%, respectively. When *H. pylori* infection was present, the percentage of MIF$^+$ epithelial cells was significantly higher at both sites, with the increase in the antrum (42.4% ± 4.5%) being more prominent than that in the corpus (37.9% ± 3.9%) (figure 4). The increased expression of MIF in epithelial cells was significantly associated with severity of chronic gastritis, in the antrum (12.7% ± 1.4% in mild, 34.6% ± 2.8% in moderate, and 56.3% ± 6.7% in marked gastritis; $P < .001$, ANOVA) and in the corpus (22.0% ± 4.4% in mild, 35.0% ± 6.0% in moderate, and 50.1% ± 2.0% in marked gas-

tritis; $P < .001$, ANOVA). However, when *H. pylori*–positive patients were excluded from the analysis, the percentage of MIF$^+$ epithelial cells in the antrum and the corpus was not significantly different in patients with and patients without chronic gastritis, in both the antrum (10.4% ± 1.5% vs. 12.3% ± 1.2%) and the corpus (10.0% ± 1.8% vs. 10.9% ± 1.4%). There was no association between the increased expression of MIF in epithelial cells and the density of *H. pylori* infection and the activity of gastritis (data not shown).

***Expression of MIF protein in T cells and macrophages in gastric mucosa, in relation to* H. pylori *infection and inflammation.*** MIF$^+$ activated T cells and MIF$^+$ macrophages can be identified by use of the double-immunostaining technique (figure 5A and 5B). Overall, the numbers of total T cells and of macrophages were significantly higher in the antrum than in the corpus (tables 1 and 2). The increased expression of MIF protein in T cells and macrophages when *H. pylori* infection was present correlated well with the increased expression of MIF mRNA in the gastric mucosa in both the antrum and the corpus (all $P < .001$, as determined by use of bivariate correlation).

In both the antrum and the corpus, the numbers of total T cells and total macrophages were significantly higher in *H. pylori*–positive patients than in *H. pylori*–negative patients (tables 1 and 2): in the antrum, the percentage of MIF$^+$ T cells and MIF$^+$ macrophages was significantly higher in *H. pylori*–positive patients than in *H. pylori*–negative patients (table 1); in the corpus, the percentage of MIF$^+$ macrophages was also significantly higher in *H. pylori*–positive patients than in *H. pylori*–negative patients, but the percentage of MIF$^+$ T cells was not (table 2). However, in both the antrum and the corpus, the increase in the percentages of MIF$^+$ T cells and MIF$^+$ macro-
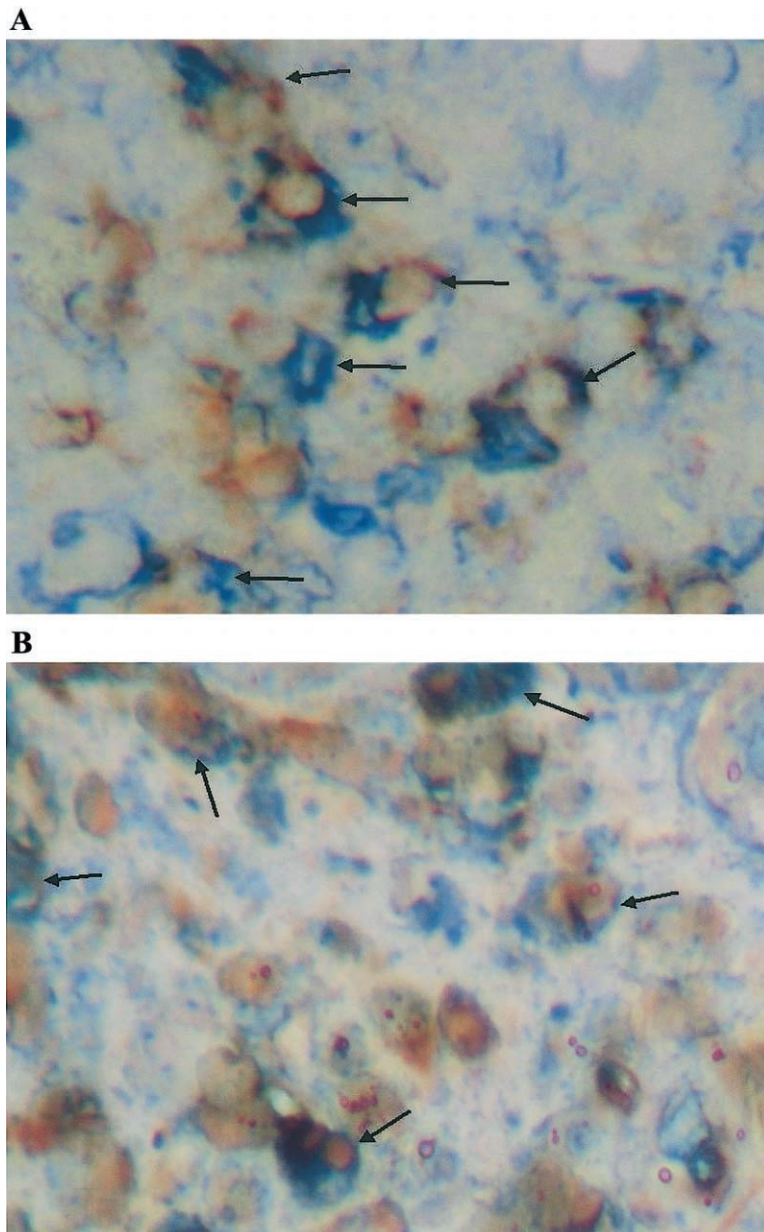
**Figure 5.** Representative examples of double immunostaining for expression of MIF protein by activated T cells and macrophages in mucosa of the gastric antrum. Strong cytoplasmic expression of MIF+ CD45RO *(brown/blue color) (A)* and of MIF+ KP1 *(brown/blue color) (B)* is seen in *Helicobacter pylori*–positive mucosa. Original magnification, ×400.

phages was independent of the density of *H. pylori* infection (tables 1 and 2).

Similarly, the numbers of total T cells, MIF+ T cells, total macrophages, and MIF+ macrophages were significantly higher in patients with chronic gastritis in the antrum and the corpus than in patients with normal gastric mucosa. In addition, the increased numbers of cells correlated well with the severity of chronic gastritis (tables 1 and 2). When chronic gastritis was present, the percentage of MIF+ macrophages, but not the percentage of MIF+ T cells, was significantly increased and correlated

with the severity (tables 1 and 2); however, when *H. pylori*–positive patients were excluded from the analysis, only MIF+ T cells in the antrum increased significantly when chronic gastritis was present, compared with what was seen for normal gastric mucosa ($432.3 \pm 39.6/\text{mm}^2$ vs. $693.2 \pm 49.5/\text{mm}^2$; $P = .03$).

## DISCUSSION

The present study clearly demonstrates that *H. pylori* infection is associated with increased expression of MIF in gastric epi-

**Table 1.** Expression of MIF protein by T cells and macrophages in the gastric antrum, in relation to *Helicobacter pylori* infection and histological chronic gastritis.

| Category (no. of patients) | T cells, no. of cells/mm² | | No. of MIF⁺ cells/total no. of T cells, % | Macrophages, no. of cells/mm² | | No. of MIF⁺ cells/total no. of macrophages, % |
|---|---|---|---|---|---|---|
| | Total | MIF⁺ | | Total | MIF⁺ | |
| Entire sample (64) | 1682 ± 131 | 1245 ± 107 | 72.1 ± 1.5 | 849 ± 97 | 479 ± 60 | 46.8 ± 2.4 |
| *H. pylori* infection at corresponding site | | | | | | |
| Positive | | | | | | |
|   Small number (10) | 1869 ± 286 | 1432 ± 266 | 74.6 ± 4.7 | 892 ± 178 | 519 ± 156 | 52.3 ± 5.3 |
|   Moderate number (8) | 1793 ± 221 | 1394 ± 217 | 76.5 ± 3.8 | 1199 ± 230 | 665 ± 110 | 56.9 ± 2.7 |
|   Large number (27) | 2220 ± 239 | 1673 ± 188 | 74.9 ± 2.0 | 1252 ± 153 | 732 ± 93 | 56.0 ± 3.2 |
|   Positive overall (45) | 2026 ± 162[a] | 1570 ± 132[a] | 75.1 ± 1.7[b] | 1167 ± 109[a] | 675 ± 68[a] | 55.4 ± 2.2[a] |
| Negative (19) | 879 ± 58 | 611 ± 45 | 68.3 ± 1.9 | 132 ± 6 | 36 ± 3 | 27.5 ± 2.5 |
| Chronic gastritis at corresponding site | | | | | | |
| Present | | | | | | |
|   Mild (16) | 1178 ± 150 | 856 ± 140 | 69.6 ± 2.4 | 228 ± 53 | 85 ± 30 | 30.9 ± 3.7 |
|   Moderate (21) | 1905 ± 152 | 1435 ± 137 | 74.8 ± 2.9 | 1091 ± 130 | 632 ± 93 | 54.7 ± 3.3 |
|   Marked (21) | 2238 ± 303[c] | 1706 ± 236[c] | 75.7 ± 2.0 | 1322 ± 188[d] | 775 ± 107[d] | 57.7 ± 3.1[d] |
|   Present overall (58) | 1824 ± 139[e] | 1373 ± 114 | 73.7 ± 1.5 | 927 ± 102[e] | 527 ± 61[e] | 49.0 ± 2.6[e] |
| Absent (6) | 639 ± 56 | 432 ± 40 | 67.7 ± 2.6 | 128 ± 6 | 34 ± 3 | 26.8 ± 2.2 |

**NOTE.** Data are mean ± standard error of the mean. MIF, macrophage migratory inhibitory factor.

[a] *P* < .001.
[b] *P* = .02, compared with *H. pylori*–negative cases.
[c] *P* < .01, as determined by use of 1-way ANOVA for cases with different severity of chronic gastritis.
[d] *P* < .001, as determined by use of 1-way ANOVA for cases with different severity of chronic gastritis.
[e] *P* < .001, compared with cases without chronic gastritis.

thelial cells. *H. pylori* infection is also associated with increased expression of MIF in T cells and in macrophages in the lamina propria.

Recent studies have shown that MIF is a proinflammatory cytokine that is constitutively expressed, stored in a preformed manner in cytoplasm, and released into circulation when stimulated [7, 8]. It is known that preformed MIF protein is present in both resting macrophages and monocytes [26], indicating that the macrophage is not only a target but also a source of MIF in vivo [1–9]. Similarly, resting T cells and epithelial cells also contain preformed MIF [1–3, 6, 11, 14, 26]. Once released, MIF functions as a cytokine to promote recruitment of neutrophils and macrophages and their migration to the site of inflammation [27–29]. MIF also causes cell activation and expression of inflammatory cytokines—including IL-1, IFN-γ, and TNF-α—and adhesion molecules—including ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1)—and plays a role in endotoxemia, septic shock, and inflammatory and immune diseases [1–17, 26, 28, 29]. Despite accumulated evidence that MIF plays an important role in inflammation and immune responses, studies of the role that it plays in the gastrointestinal diseases are rare [16–19]. Murakami et al. observed that, in the colonic mucosa, both the level of MIF in sera and the expression of MIF were higher in patients with ulcerative colitis than in normal control subjects [16]. Then they found that mature dendritic cells in colonic mucosa of patients with ulcerative colitis are a main source of MIF, which in turn induces pro-

duction of IL-1 and IL-8 by monocytes and dendritic cells [30]. We applied a double-staining technique to simultaneously detect, in gastric mucosa, activated T cells and macrophages and expression of MIF in these cells, because previous studies have shown that activated T cells and macrophages/monocytes are the major cellular sources of MIF [17, 31]. Moreover, we observed that MIF is constitutively expressed, albeit weakly, in normal gastric epithelial cells but is significantly increased in *H. pylori* infection. Similarly, our previous animal study demonstrated weak expression of MIF in normal rat gastric mucosa [17]. Recently, Maaser et al. reported that both MIF mRNA and MIF protein were abundantly expressed in human gastric epithelial cells in vivo and in gastric cancer cells in vitro, although *H. pylori* status and mucosal histology were not revealed and a gastric-cancer cell line was used in their study [18]. Therefore, we hypothesize that colonization of *H. pylori* in the stomach directly stimulates the gastric epithelial cells to release MIF, which then promotes local infiltration of inflammatory cells, including T- and B-lymphocytes and macrophages, by inducing the chemokine IL-8, as has been seen in septic-shock and inflammatory lung diseases [27–31], resulting in the production of inducible nitrite oxide synthase (iNOS) and cytotoxic cytokines such as TNF-α, IFN-γ, and ICAM-1. Consequently, activated T cells and macrophages release a large amount of MIF. In the presence of chronic *H. pylori* infection, this cycle continues, which contributes to more-severe inflammation and to gastric injury such as erosion and ulcerations.

**Table 2.** Expression of MIF protein by T cells and macrophages in the gastric corpus, in relation to *Helicobacter pylori* infection and histological chronic gastritis.

| Category (no. of patients) | T cells, no. of cells/mm$^2$ | | No. of MIF$^+$ cells/total no. of T cells, % | Macrophages, no. of cells/mm$^2$ | | No. of MIF$^+$ cells/total no. of macrophages, % |
|---|---|---|---|---|---|---|
| | Total | MIF$^+$ | | Total | MIF$^+$ | |
| Entire sample (64) | 1171 ± 98 | 786 ± 76 | 63.1 ± 1.7 | 482 ± 59 | 254 ± 39 | 40.5 ± 2.5 |
| *H. pylori* infection at corresponding site | | | | | | |
| Positive | | | | | | |
| Small number (19) | 1131 ± 127 | 756 ± 112 | 62.0 ± 3.1 | 520 ± 96 | 308 ± 81 | 48.0 ± 4.7 |
| Moderate number (14) | 1607 ± 223 | 1097 ± 162 | 66.9 ± 3.0 | 712 ± 107 | 382 ± 73 | 49.4 ± 3.8 |
| Large number (12) | 1588 ± 285 | 1090 ± 227 | 64.8 ± 3.8 | 777 ± 164 | 385 ± 89 | 47.5 ± 4.2 |
| Positive overall (45) | 1392 ± 117[a] | 944 ± 92[a] | 64.2 ± 1.9 | 647 ± 69[a] | 351 ± 47[a] | 48.3 ± 2.5[a] |
| Negative (19) | 580 ± 58 | 360 ± 44 | 60.4 ± 2.3 | 75 ± 5 | 16 ± 2 | 21.1 ± 2.7 |
| Chronic gastritis at corresponding site | | | | | | |
| Present | | | | | | |
| Mild (30) | 1075 ± 133 | 683 ± 92 | 60.5 ± 2.2 | 384 ± 80 | 186 ± 48 | 36.6 ± 3.4 |
| Moderate (18) | 1387 ± 157 | 984 ± 139 | 67.3 ± 3.2 | 668 ± 94 | 388 ± 82 | 50.8 ± 4.3 |
| Marked (6) | 1968 ± 408[b] | 1424 ± 348[b] | 70.6 ± 3.3 | 1026 ± 218[c] | 557 ± 75[c] | 57.3 ± 4.0[c] |
| Present overall (54) | 1263 ± 105[d] | 853 ± 82[d] | 63.7 ± 1.7 | 542 ± 65[d] | 290 ± 43[d] | 43.4 ± 2.7[d] |
| Absent (10) | 518 ± 80 | 311 ± 43 | 59.7 ± 2.2 | 101 ± 30 | 27 ± 12 | 22.2 ± 3.8 |

**NOTE.** Data are mean ± standard error of the mean. MIF, macrophage migratory inhibitory factor.

[a] $P < .001$, compared with *H. pylori*–negative patients.
[b] $P < .05$, as determined by use of 1-way ANOVA for cases with different severity of chronic gastritis.
[c] $P < .01$.
[d] $P < .001$, compared with cases without chronic gastritis.

Supporting this hypothesis is the recent report by de Jong et al. that MIF-deficient mice failed to develop colitis, whereas restoration of expression of MIF in MIF-deficient mice resulted in renewed colitis, indicating that the development of chronic colitis in mice is dependent on MIF [19]. Although chronic colitis and chronic gastritis are 2 different diseases, they share a similar pathogenesis—interplay between the mucosal immune system and gastrointestinal bacteria [32].

The expression of both MIF protein and MIF mRNA was significantly higher in the antrum than in the corpus, especially when *H. pylori* infection was present; moreover, when the latter was present, the percentage of MIF$^+$ T cells was significantly increased in the antrum but not in the corpus, although the percentages of MIF$^+$ gastric epithelial cells and macrophages were increased in both the antrum and the corpus. These findings imply that *H. pylori*–induced chronic inflammation is more severe in the antrum than in the corpus. Indeed, the present study and previous histological studies have observed that chronic gastritis is predominantly present in the antrum [21, 33]. More important, gastric cancer and precancerous lesions—including gastric atrophy, intestinal metaplasia, and dysplasia—occur more frequently in the antrum than in the corpus [33–36]. Therefore, it has been postulated that increased expression of MIF in the antrum, by inflammatory cells as well as by gastric epithelial cells, may be one of the factors that contribute to the progress of chronic gastritis to precancerous lesions and gastric cancer [17, 18]. In fact, studies have shown that MIF may also contribute to multiple aspects of tumor progression and neo-plasia, by modulating cell proliferation, tumor angiogenesis, and tumor-suppressor activity [37]. Recently, there has been evidence that MIF may be the factor that links inflammation to carcinogenesis, [38, 39]. We hypothesize that MIF may be one of the initiating factors that "switch" chronic inflammation to carcinogenesis in the stomach, by affecting the balance between apoptosis and cell proliferation; however, this hypothesis needs to be tested in further studies.

MIF has been shown to exhibit antiapoptotic activities in various cell lines, most likely by inhibiting the tumor-suppressor gene, *p53* [38–40]; however, it is unknown how MIF affects apoptosis in *H. pylori*–infected gastric epithelial cells. Previous studies have consistently demonstrated that *H. pylori* infection induces apoptosis, which may play an important role in *H. pylori*–induced gastric carcinogenesis [22, 41]; so MIF may have little impact on *H. pylori*–induced apoptosis in the early stage of gastric carcinogenesis. Although both p53 expression and apoptosis are increased in *H. pylori*–infected gastric epithelium [22, 42, 43], numerous studies have suggested that *H. pylori*–induced apoptosis is mediated by multiple eukaryotic signaling cascades that are not dependent on increased p53 levels [41, 44–47]. More recently, Suzuki et al. have observed that gastric epithelial apoptosis and proliferation increased in both wild-type and p53-heterozygous knockout mice 24 weeks after *H. pylori* inoculation, suggesting that both apoptosis and proliferation are independent of a deficiency of 1 p53 allele [48]. However, functional studies are necessary to elucidate the potential role that MIF plays in the regulation of

*H. pylori*–induced apoptosis and proliferation, especially in the late stage of gastric carcinogenesis.

In conclusion, *H. pylori* infection stimulates the expression of both MIF protein and MIF mRNA, in both gastric epithelial and inflammatory cells. Thus, MIF, along with other cytokines, may play a significant role in gastric inflammation related to *H. pylori* infection.

## Acknowledgment

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