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Role of Neutrophils in a Rat Model of Gastric Ulcer Recurrence Caused by Interleukin-1β

Toshio Watanabe, Tetsuo Arakawa, Takashi Fukuda, Kazuhide Higuchi, and Kenzo Kobayashi

From the Third Department of Internal Medicine, Osaka City University Medical School, Osaka, Japan

The production of several cytokines such as interleukin (IL)-1 in gastric mucosa is increased in subjects infected with* Helicobacter pylori*, a bacterium associated with ulcer recurrence. This study was performed to determine whether the administration of IL-1β can cause recurrence of gastric ulcers in rats. Rats with healed ulcers received an injection of IL-1β (0.01 to 1 μg/kg) or vehicle alone. Some rats received an injection of antiserum to rat neutrophils at the same time as 1 μg/kg IL-1β or an injection of monoclonal antibodies against adhesion molecules (anti-intercellular adhesion molecule-1, anti-CD11a, and anti-CD11b) at 0, 12, and 24 hours after the initial injection. At this dose of IL-1β, the numbers of neutrophils and monocytes/macrophages infiltrating the scarred mucosa were higher at 12 and 24 hours than without injection of IL-1β. By 48 hours, seven of the eight healed ulcers in the group treated with 1 μg/kg IL-1β had recurred, as had one of the seven healed ulcers in the group given 0.1 μg/kg IL-1β. No recurrence was found in the rats treated with 0.01 μg/kg IL-1β or vehicle alone. Treatment with antiserum to neutrophils or antibodies to adhesion molecules inhibited both neutrophil infiltration into the scarred mucosa and the ulcer recurrence caused by IL-1β. These findings suggest possible mechanisms of recurrence of human peptic ulcers. (Am J Pathol 1997, 150:971–979)

Peptic ulcers frequently recur. However, despite extensive investigation, the mechanism(s) of ulcer recurrence is not known. Treatment of rat stomachs with acetic acid causes chronic gastric ulcers that resemble human peptic ulcers macroscopically and histologically.[1] A striking feature of these experimental ulcers is that some recur spontaneously after healing.[2] Therefore, this model is useful in investigations of the process of ulcer healing[3,4] and the pathogenesis of ulcer recurrence in humans.[5,6]

Helicobacter pylori is a major cause of ulcer recurrence.[7,8] Several factors, including urease, endotoxin, platelet-activating factor, and cytokines such as interleukins (ILs) and tumor necrosis factor (TNF)-α, may be involved in mucosal injury related to *H. pylori*,[9] but to our knowledge, there is no report showing a direct relationship between these factors and ulcer recurrence. Infection with *H. pylori* often involves neutrophilic infiltration of the gastric mucosa.[10] There is evidence that inflammatory cytokines such as IL-1, IL-8, or TNF-α may be involved in such infiltration. Monocytes from healthy human donors secrete IL-1 and TNF after the cells are stimulated with soluble surface proteins from *H. pylori*,[11] and the production of IL-1β or TNF-α in gastric mucosal biopsy specimens from patients with *H. pylori* infection is greater than that in specimens from persons not so infected.[12,13]

IL-1β, which is produced mainly by inflammatory cells such as monocytes/macrophages, plays roles in many inflammatory processes.[14–16] This cytokine has a number of biological activities including promotion of T and B cell proliferation, activation of neutrophils,[17] bringing about the increased expression of endothelial adhesion molecules,[18,19] induction of prostaglandin E_2 and collagenase synthesis,[20] and stimulation of the synthesis of several cytokines including itself.[14] Adhesion molecules of both leukocytes and endothelial cells are important in the migration of leukocytes into the extravascular space and are involved in cytokine-mediated tissue injuries.[21,22] Leukocytic β_2 integrins, lymphocyte function-associated antigen (LFA-1; CD11a/CD18), and Mac-1 (CD11b/CD18), react with intercellular

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Address reprint requests to Dr. Toshio Watanabe, Third Department of Internal Medicine, Osaka City University Medical School, 1-5-7 Asahimachi, Abeno-ku, Osaka 545, Japan.

adhesion molecule (ICAM)-1 on endothelial cells.[23,24] Wallace et al demonstrated that monoclonal antibodies directed against CD18 or ICAM-1 reduced the severity of gastric mucosal damage induced by indomethacin in rabbits[25] and rats.[26] Their results suggested that β_2 integrins and ICAM-1 may play an important role in the pathogenesis of mucosal damage in the stomach.

To elucidate the role, if any, of IL-1β in the inflammatory response in gastric mucosa and in ulcer recurrence, we investigated the effects of intraperitoneal administration of IL-1β on leukocyte infiltration into scarred mucosa and on the recurrence of gastric ulcers that resulted from treatment of rats with acetic acid. We also used neutralizing monoclonal antibodies against adhesion molecules (LFA-1, Mac-1, and ICAM-1) to study the contribution of these adhesion molecules to inflammatory responses including neutrophilic infiltration after the administration of IL-1β.

Materials and Methods

Animals and Ulcer Induction

Specific-pathogen-free male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing approximately 200 g at the start of the experiment were used. Ulcers were induced by the method of Takagi et al.[1] In brief, under ether anesthesia, the abdomen was incised and the stomach was exposed. Then 0.02 ml of 20% acetic acid was injected into the submucosal layer of the antral oxyntic border of the anterior wall, and the abdomen was closed. All experimental procedures were approved by the Animal Care Committee of Osaka City University Medical School.

Endoscopy

Endoscopy was done by the method of Fukawa et al,[27] with gastric ulcers being looked for on day 90. The animals were deprived of food, but not water, for 18 to 22 hours before endoscopy. With the rats under ether anesthesia, any food residue, hair, and feces present were removed by washing of the stomach several times with saline solution warmed at 37°C and introduced through a catheter. Then a needle-type endoscope (SES-1717S, Olympus Optical Co., Tokyo, Japan) was inserted into the stomach from the mouth and the inside of the stomach was observed. Ulcers without a white coating were considered as healed ulcers.

Administration of IL-1β

Recombinant human IL-1β was purchased from Genzyme (Boston, MA); the specific activity was 2.8×10^8 U/mg and the endotoxin level was 0.056 ng/μg. The cytokine was stored at −70°C and diluted immediately before use in isotonic phosphate-buffered saline (PBS) that was pyrogen free.

Rats found to have healed ulcers on endoscopy were used for this study. Some were killed without cytokine treatment (0 hour) and others received an intraperitoneal injection of IL-1β (0.01 to 1 μg/kg) within 2 hours of endoscopy and were killed 12, 24, or 48 hours later (n = 6 to 8). In addition, nine rats received an intravenous injection of 1 μg/kg IL-1β and were killed 48 hours later. Control animals received an intraperitoneal injection of the vehicle, pyrogen-free PBS, and were killed 48 hours later. Ulcers found on endoscopy to have healed but that had a white coating again after the injection of IL-1β were considered as recurrent ulcers. Neutrophils often are responsible for tissue injury, so we also investigated the effects of IL-1β in rats with neutropenia. For preparation of such rats, some rats received an intraperitoneal injection of 1 ml/kg rabbit anti-rat neutrophil serum (ANS; Accurate Chemical Corp., Westbury, NY) at the same time as 1 μg/kg IL-1β and were killed 12, 24, or 48 hours later (each, n = 6). The specificity of this antiserum is described below.

Neutrophil Depletion

Neutrophils in rats were depleted by the intraperitoneal injection of 1 ml/kg ANS diluted to 0.5 ml with 0.9% saline. The same volume of 0.9% saline was injected into control animals. Approximately 2 ml of blood was withdrawn by cardiac puncture 24 or 48 hours later and total and differential blood counts were made. The total white blood cell counts were measured using an automated hematology analyzer (Sysmex CC-780, Toa Medical Electronics Co., Tokyo, Japan). Differential counts of leukocytes were found by the counting of 100 cells from blood smears stained with Wright-Giemsa stain. The number of eosinophils in rat blood is normally low, so it is difficult to detect a decrease in that number. This volume of ANS reduced the number of circulating neutrophils without much altering the numbers of circulating lymphocytes or monocytes (Table 1).

Table 1. *Effects of Anti-Rat Neutrophil Serum on Circulating Leukocytes*

Treatment	Hours after injection	Numbers/mm^3			
		Neutrophils	Eosinophils	Lymphocytes	Monocytes
Control	24	2392 (1234–2657)	0 (0–0)*	3871 (3349–7170)	184 (84–187)
ANS		168 (117–368)†	0 (0–0)‡	3731 (2935–3907)	41 (0–70)
Control	48	1660 (1025–1893)	0 (0–0)*	5112 (4373–5320)	142 (62–172)
ANS		255 (101–409)†	0 (0–0)‡	4306 (3598–4980)	122 (0–240)

Rats were given an intraperitoneal injection of 1 ml/kg anti-rat neutrophil serum or vehicle alone and were killed 24 or 48 hours later. Results are expressed as medians and interquartile ranges; n = 5 in each group.
†$P < 0.05$ compared with controls.
*Values for four of the five rats were zero.
‡Values for all rats were zero.

In Vivo *Blocking of Leukocyte-Endothelial Cell Adhesion*

In the second series of experiments, we also studied the effects of monoclonal antibodies against adhesion molecules in rats treated with 1 μg/kg IL-1β. All antibodies were purchased from Seikagakukogyo Co. (Tokyo, Japan). Antibody against ICAM-1 (1A29) was used as the F(ab′)$_2$ fragments of IgG$_1$. A total of 225 μg of F(ab′)$_2$ was injected intravenously into seven rats in three equal doses at 0, 12, and 24 hours after IL-1β treatment. Antibody against CD11a (WT.1, IgG2a) or antibody against CD11b (WT.5, IgA) was used as an intact antibody and was injected in a total dose of 450 μg into seven rats on the same schedule. These antibodies are characterized in detail elsewhere.[28–30] Control animals received 1 μg/kg IL-1β and were injected with a total of 450 μg irrelevant mouse IgG2a (UPC-10, Sigma Chemical Co., St. Louis, MO) into six rats at the same times. These antibodies did not induce neutropenia (data not shown). Rats were killed 48 hours after IL-1β treatment.

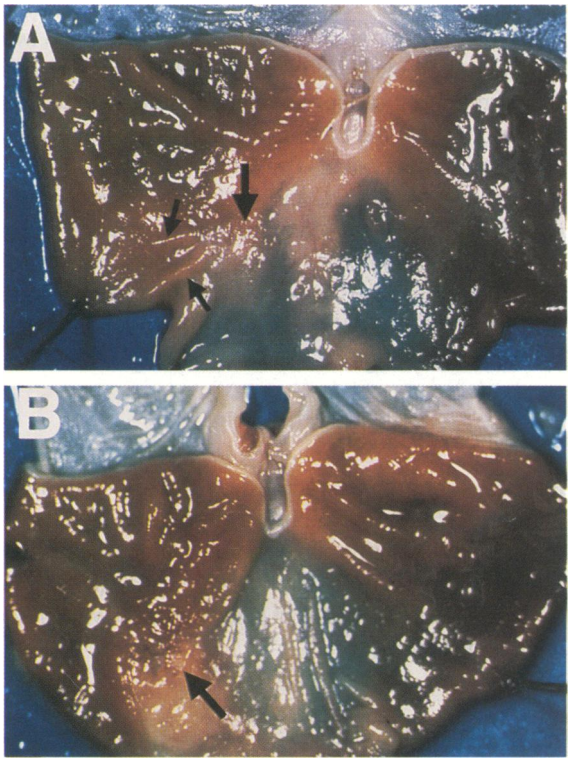

Figure 1. *Gross appearance of healed ulcers and ulcers that recurred.* A: *An ulcer that had healed by day 90 without injection of IL-1β (0 hour). Gastric folds* (small arrows) *converging to the scarred mucosa* (large arrow) *with its irregular surface are visible.* B: *An ulcer that had recurred by 48 hours after the injection of 1 μg/kg IL-1β. The recurred ulcer is on the scarred mucosa* (arrow); *the unscarred mucosa has no lesions.*

Immunohistochemical Staining

Cryostat sections cut serially at 6 μm thickness were mounted on silanized slides (Dako Japan Co., Kyoto, Japan). Mouse monoclonal antibodies against rat monocytes/macrophages (ED1, Serotec, Oxford, UK), CD4, CD8 (both from Chemicon International, Temecula, CA), and B cell leukocyte common antigen (Cedarlane Laboratories, Hornby, Ontario, Canada) were used for identification of the types of cells that had infiltrated the gastric mucosa. Immunohistochemical staining was done with a biotin-streptavidin-peroxidase kit (Histofine kit, Nichirei Corp., Tokyo, Japan). After immersion of the sections in nonimmunized rabbit serum for 30 minutes, they

Table 2. *Effects of IL-1β and Anti-Rat Neutrophil Serum on Ulcer Recurrence at 48 Hours*

Treatment (μg/kg)	Numbers recurred/treated (%)	*P*
PBS, ip	0/6 (0)	
IL-1β (0.01), ip	0/6 (0)	NS
IL-1β (0.1), ip	1/7 (14)	NS
IL-1β (1), ip	7/8 (88)	0.0012
IL-1β (1), iv	7/9 (78)	0.0031
IL-1β (1), ip + ANS	0/6 (0)	NS

Rats with healed ulcers were given IL-1β (intraperitoneally (ip) or intravenously (iv)) or vehicle (PBS) alone. Some animals received an intraperitoneal injection of 1 ml/kg anti-rat neutrophil serum at the same time as 1 μg/kg IL-1β. Rats were killed 48 hours later. NS, not significant.

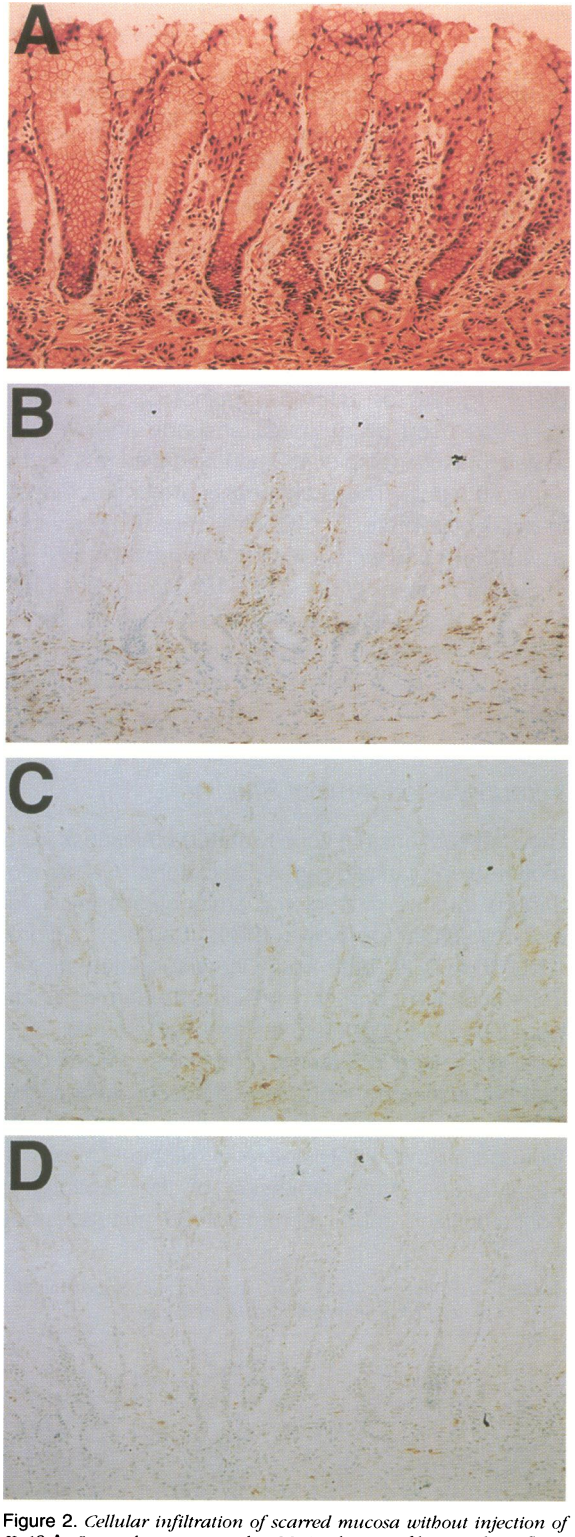

Figure 2. *Cellular infiltration of scarred mucosa without injection of IL-1β* **A**: *Scarred mucosa on day 90. Moderate infiltration by inflammatory cells, mostly mononuclear cells, is seen in the scarred mucosa.* **B** *to* **D**: *Immunohistochemical staining for monocytes/macrophages (with ED1), $CD4^+$ T cells, and $CD8^+$ T cells. There are many monocytes/macrophages in the scarred mucosa* (**B**). *$CD4^+$ T cells* (**C**) *and $CD8^+$ T cells* (**D**) *also are numerous. Original magnification, ×40.*

were incubated with an appropriately diluted specific antibody overnight at 4°C. Endogenous peroxidase was inactivated by immersion of sections in 0.3% hydrogen peroxide in methanol for 15 minutes after incubation with the first antibody. After being washed in PBS, sections were incubated with biotinylated rabbit anti-mouse IgG for 45 minutes at room temperature, washed in PBS, and incubated with peroxidase-labeled streptavidin for 30 minutes at room temperature. The sections were finally treated with 0.03% 3,3′-diaminobenzidine (Dojin, Kumamoto, Japan) containing 0.005% hydrogen peroxide and sodium azide (65 mg/100 ml) to inactivate endogenous peroxidase. Counterstaining was performed with methyl green. For negative controls, the primary antibody was replaced by isotype-matched mouse immunoglobulins (Dako Japan). Staining for morphological observations or polymorphonuclear leukocytes was with hematoxylin and eosin.

Counting of Infiltrating Leukocytes

After leukocytes were stained as described above, the infiltrating leukocytes in four randomly chosen areas of normal fundic mucosa and in four randomly chosen areas of scarred mucosa were counted. When ulcers had recurred, counting was done in two areas at each ulcer edge. The width inspected was 0.25 mm, and the depth of inspection depended on the height (from the base to the top) of the mucosa in the area of observation. The 100× objective of a light microscope was used, and regions to be inspected were measured with reference to the eyepiece of the microscope. Results are expressed as the number of cells per millimeter squared. Counting was done without knowledge of the treatment groups to which specimens belonged.

Statistical Analysis

Results other than the recurrence rate are expressed as medians and interquartile ranges and were analyzed by the Mann-Whitney U test. Differences in the recurrence rate were evaluated by the χ^2 test. A difference with a P value of less than 0.05 was taken to be significant.

Results

Ulcer Recurrence

In healed ulcers at 0 hour (no injection), scarred mucosa was detectable by the irregularity of the mucosal surface or by gastric-fold convergence

Table 3. *Infiltrating Leukocytes in Normal and Scarred Mucosa*

Mucosa	Numbers/mm^2				
	Neutrophils	Eosinophils	CD4$^+$ T cells	CD8$^+$ T cells	Monocytes/macrophages
Normal	0 (0–3.2)	84.8 (70.4–84.8)	48.0 (38.4–70.4)	22.4 (17.6–35.2)	216 (192–272)
Scarred	0 (0–4.8)	84.0 (72.0–137.6)	98.4 (91.2–219.2)*	70.4 (56.0–112.0)*	552 (528–784)*

Results are expressed as medians and interquartile ranges; n = 6.
*$P < 0.01$.

(Figure 1A). IL-1β did not induce ulcer recurrence within 24 hours of administration. At 48 hours, seven of the eight healed ulcers in rats treated with 1 μg/kg IL-1β and one of the seven healed ulcers in rats treated with 0.1 μg/kg IL-1β had recurred in the scarred mucosa (Figure 1B). The dose of 0.01 μg/kg IL-1β induced no ulcer recurrence by 48 hours. Intravenous injection of 1 μg/kg IL-1β also caused ulcer recurrence in seven of nine rats by 48 hours. In normal mucosa (at some distance from the scarring), IL-1β did not cause mucosal injury at any time. IL-1β did not cause ulcer recurrence when neutrophils were depleted with ANS. The recurrence rate by 48 hours for each group is shown in Table 2.

Histological and Immunohistochemical Results

No mucosal defect was detected on either macroscopic or histological examination in rats found to have healed ulcers on endoscopy. Mild to moderate inflammation with leukocyte infiltration was observed in scarred mucosa of healed ulcers at 0 hour (no injection). Many of the infiltrating cells were mononuclear cells, and a few were polymorphonuclear leukocytes (Figure 2A), mostly eosinophils. More than one-half of the mononuclear cells were stained by the monoclonal antibody ED1 (Figure 2B), so they were monocytes/macrophages. The other mononuclear cells were CD4$^+$ or CD8$^+$ T cells (Figure 2, C and D). B cells in the healed mucosa were few (not shown). The numbers of CD4$^+$ T cells, CD8$^+$ T cells, and monocytes/macrophages in scarred mucosa were greater than in normal mucosa, but the numbers of neutrophils and eosinophils were not (Table 3). Administration of 1 μg/kg IL-1β increased the numbers of monocytes/macrophages and neutrophils but did not increase the numbers of eosinophils, CD4$^+$ T cells, or CD8$^+$ T cells. In some specimens from rats treated with 1 μg/kg IL-1β and killed at 24 hours, the surface of the epithelium on the scarred mucosa was disrupted and had been infiltrated by neutrophils (Figure 3A). The ulcers that had recurred by 48 hours after the injection of IL-1β varied in depth; some were limited to the mucosa (Figure 3B), whereas others extended into the submucosa (Figure 3C). In such ulcers, monocytes/macrophages and neutrophils were numerous at both the margin and the base (Figure 3D). In the scarred mucosa where ulcer recurrence was not observed by 48 hours after the injection of IL-1β, there was no increase in the number of neutrophils.

In rats given ANS at the same time as 1 μg/kg IL-1β, increased leukocyte infiltration at the scarred mucosa was again observed, but mucosal injury was not detected (Figure 4A). In such animals, the number of neutrophils infiltrating the regenerated mucosa was significantly smaller than that in rats treated with IL-1β alone, but the number of monocytes/macrophages was approximately the same in the two groups (Figure 4B). Such treatment did not affect the number of CD4$^+$ T cells or CD8$^+$ T cells. The numbers of each type of leukocyte infiltrating scarred mucosa or ulcer edges after IL-1β treatment with or without ANS are shown in Table 4. In normal mucosa, treatment did not affect the number of infiltrating leukocytes (data not shown) or cause mucosal injury at any time.

Effects of Monoclonal Antibodies on Ulcer Recurrence and Neutrophilic Infiltration after IL-1β Treatment

In rats treated with irrelevant IgG2a, all six healed ulcers had recurred by 48 hours after the intraperitoneal injection of 1 μg/kg of IL-1β, but monoclonal antibodies against ICAM-1, CD11a (LFA-1), and CD11b (Mac-1) reduced the recurrence rate to three of seven rats, one of seven rats, and three of seven rats, respectively (Table 5). These monoclonal antibodies also had an inhibitory effect on neutrophil accumulation. The number of neutrophils infiltrating regenerated mucosa by 48 hours after IL-1β treatment was 253.0 (112.0 to 461.0) in control animals treated with irrelevant IgG2a. The number of neutrophils was reduced in the groups treated with monoclonal antibodies against adhesion molecules, as follows: anti-ICAM-1, 32.0 (18.0 to 110.8); anti-CD11a, 19.0 (18.5 to 79.3); anti-CD11b, 53.0 (28.3 to 124.8) (each, $P < 0.05$).

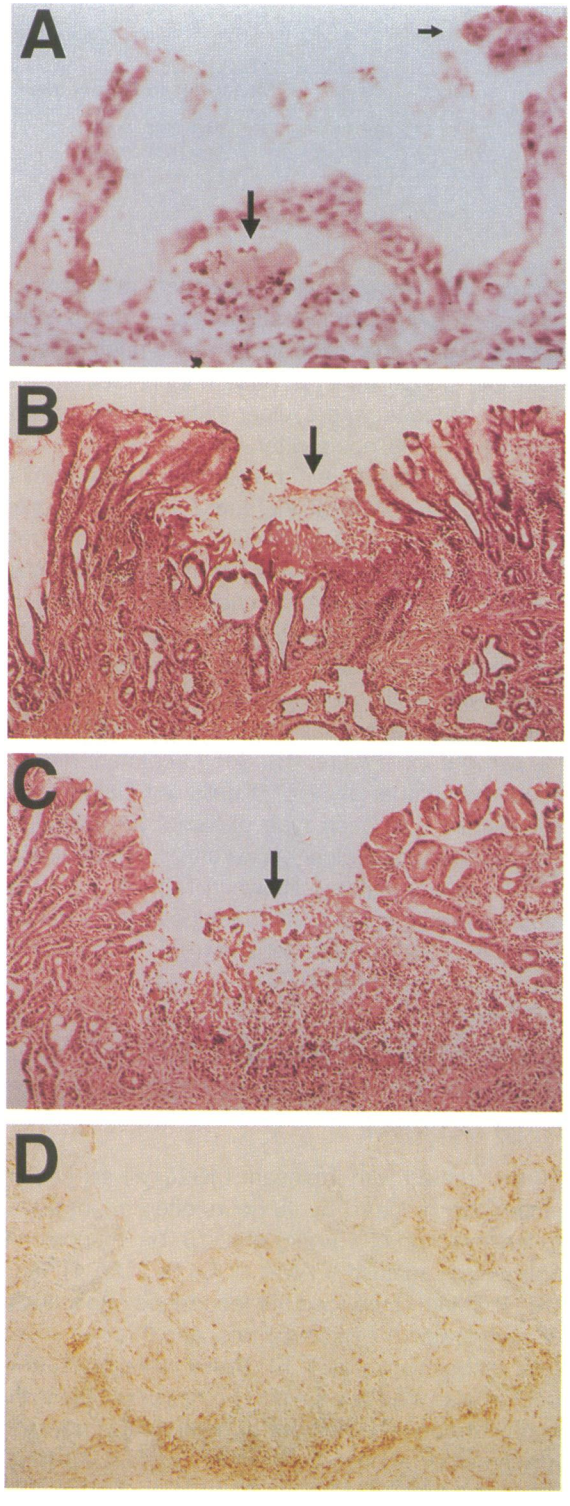

Figure 3. *Cellular infiltration and ulcer recurrence after cytokine treatment.* A: *Scarred mucosa 24 hours after the injection of 1 μg/kg IL-1β. The surface of the epithelium on the scarred mucosa is disrupted* (small arrow). *Neutrophils have accumulated, and both bleeding and focal necrosis of epithelial cells* (arrow) *can be seen.* B: *An ulcer that had recurred by 48 hours after the injection of 1 μg/kg IL-1β. This ulcer, like some others at 48 hours, is limited to the mucosa* (arrow). C: *An ulcer that had recurred by 48 hours after the injection of 1 μg/kg IL-1β. This ulcer extends into the submucosa* (arrow). D: *Immunohistochemical staining for monocytes/macrophages in an ulcer that had recurred (serial section to* C). *Infiltration by many monocytes/macrophages stained with ED1 is seen at both the margin and base of the ulcer. Original magnification, ×100* (A), *×25* (B), *and ×40* (C *and* D).

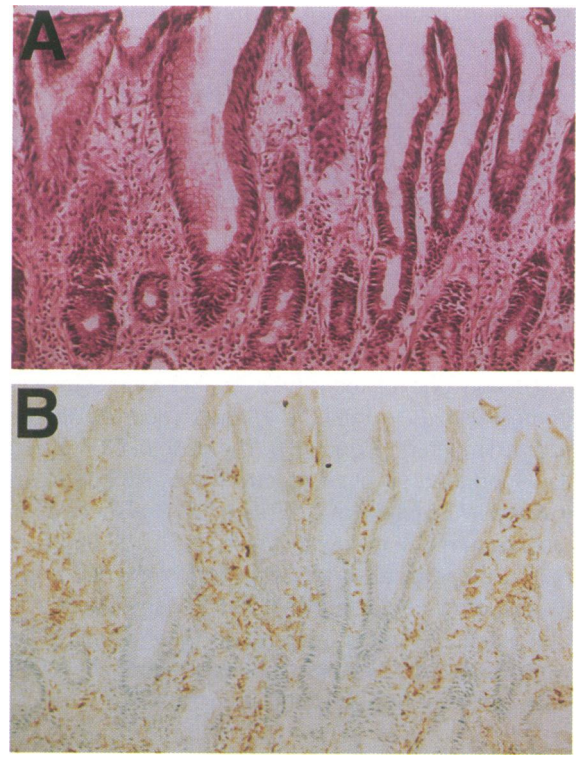

Figure 4. *Cellular infiltration by 48 hours in rats given anti-rat neutrophil serum at the same time as 1 μg/kg IL-1β.* A: *Leukocytes have infiltrated the scarred mucosa, but mucosal injury is not observed.* B: *Immunohistochemical staining for monocytes/macrophages in a serial section to* A. *Infiltration by many monocytes/macrophages is seen. Original magnification, ×50.*

Discussion

The mechanism(s) of spontaneous recurrence of some gastric ulcers induced by injections of acetic acid has not been evaluated in detail because of the difficulty in sequential observation of ulcers in individual animals and because of the difficulty in prediction of the time of ulcer recurrence. Endoscopy enabled us to undertake such an experiment. We found that intraperitoneal administration of IL-1β caused recurrence of gastric ulcers in a well characterized rat model of human chronic gastric ulcers. The effect of IL-1β administered intraperitoneally appeared to be mediated systemically rather than topically, as intravenous injection of 1 μg/kg IL-1β also caused ulcer recurrence in seven of nine rats.

Neutrophils play key roles in the development of injury and inflammation in a variety of tissues. Neutrophils are involved in the mechanism(s) of acute gastric mucosal damage caused by ethanol,[31]

Table 4. *Changes in Numbers of Leukocytes after IL-1β Treatment with or without Anti-Rat Neutrophil Serum*

Treatment	Hours after injection	n	Numbers/mm²				
			Neutrophils	Eosinophils	$CD4^+$ T cells	$CD8^+$ T cells	Monocytes/ macrophages
Control	0	6	0 (0–4.8)	84.0 (72.0–137.6)	98.4 (91.2–219.2)	70.4 (56.0–112.0)	552 (528–784)
IL-1β	12	6	89.6 (56.0–96.0)*	91.2 (57.6–235.2)	157.6 (136.0–212.8)	69.6 (43.2–72.0)	1120 (1072–1440)*
	24	6	119.2 (112.0–144.0)*	99.2 (80.0–129.6)	160.0 (112.0–198.4)	56.0 (32.0–86.4)	1112 (1088–1472)*
	48	8	244.0 (134.4–354.4)*	211.2 (88.0–214.4)	120.0 (110.4–129.6)	67.2 (65.6–68.8)	1200 (1080–1328)*
IL-1β + ANS	12	6	6.4 (6.4–8.0)†‡	176 (137.6–196.8)	154.4 (137.6–182.4)	47.2 (44.8–75.2)	1272 (960–1392)*
	24	6	0 (0–8.0)‡	52.0 (35.2–156.8)	135.2 (123.2–182.4)	104.0 (17.6–137.6)	1168 (1168–1296)*
	48	6	9.6 (0–32.0)†‡	84.8 (34.8–108.8)	132.0 (120.0–200.0)	86.4 (67.2–112.0)	880 (792–1208)*

Rats were given an intraperitoneal injection of 1 μg/kg IL-1β with or without 1 ml/kg anti-rat neutrophil serum. The numbers of each type of leukocyte infiltrating scarred mucosa or the mucosa of ulcers that had recurred were counted. Control animals had healed ulcers and had not received an IL-1β injection. Results are expressed as medians and interquartile ranges.

*$P < 0.01$ compared with controls.

†$P < 0.05$ compared with controls.

‡$P < 0.01$ compared with rats given IL-1β only at the same time.

reperfusion after ischemia,[32] and indomethacin.[33] In this study, we found that IL-1β induced acute inflammation with neutrophil accumulation in scarred mucosa in experimental gastric ulcers. Neutrophils had accumulated by 24 hours in scarred mucosa where the surface epithelium was disrupted, and the healed ulcers recurred by 48 hours after IL-1β treatment when there were many infiltrating neutrophils. Treatment with ANS prevented both neutrophil accumulation and ulcer recurrence. These findings suggest that this experimental model of ulcer recurrence caused by IL-1β is neutrophil dependent.

Adhesion molecules play important roles in the recruitment of neutrophils to sites of inflammation, leading to tissue injury, including the gastric mucosal injury induced by indomethacin.[25,26] In the present study, monoclonal antibodies against ICAM-1, CD11a (LFA-1), and CD11b (Mac-1) reduced the rate of recurrence of ulcers after IL-1β treatment and inhibited neutrophilic infiltration in scarred mucosa in response to IL-1β stimuli. These findings suggest that ICAM-1 and β_2 integrins (LFA-1 and Mac-1) are involved in neutrophil recruitment in this model of ulcer recurrence caused by IL-1β. Treatment with monoclonal antibodies did not completely prevent ulcer recurrence, although ANS did completely prevent recurrence. It is possible that both CD18 (LFA-1/Mac-1)-dependent and -independent pathways (eg, endothelial leukocyte adhesion molecule-1[34]) of neutrophil recruitment may be present in this model.

Table 5. *Effects of* in Vivo *Blocking of Adhesion Molecules on Ulcer Recurrence Caused by IL-1β*

Antibody	Numbers recurred/treated (%)	*P*
Control	6/6 (100)	
ICAM-1	3/7 (43)	0.0261
CD11a (LFA-1)	1/7 (14)	0.0020
CD11b (Mac-1)	3/7 (43)	0.0261

Rats with healed ulcers were given monoclonal antibody against ICAM-1, CD11a (LFA-1), or CD11b (Mac-1) at 0, 12, and 24 hours after the administration of 1 μg/kg IL-1β. Control animals received IL-1β and were injected with irrelevant IgG2a at the same times. All rats were killed 48 hours after IL-1β treatment.

IL-1β acts on a variety of types of cells and has multiple biological activities involved in tissue injury. IL-1β, like other cytokines such as TNF-α, up-regulates the expression of adhesion molecules on endothelial cells and leukocytes.[18,19] ICAM-1 and β_2 integrins play a role in this model of ulcer recurrence, which is neutrophil dependent. Furthermore, IL-1β induces the production of IL-8, which is a chemotactic and activating factor for neutrophils,[35,36] endothelial cells,[37] and macrophages.[38,39]

However, these direct effects alone do not explain why IL-1β administered intraperitoneally affected only scarred mucosa and not intact mucosa. We detected many monocytes/macrophages in the scarred mucosa of rats not given IL-1β, and this cytokine increased the number of infiltrating monocytes/macrophages. IL-1β induces the production of many cytokines, including itself,[14,39] by monocytes/macrophages. The effect of IL-1β given intraperitoneally on the scarred mucosa, characterized by neutrophilic infiltration, may be due in part to the smaller, direct effect of IL-1β itself plus the effect of the sustained production of cytokines by activated macrophages. Our finding of the apparently high degree of sensitivity of scarred mucosa to cytokines such as IL-1β might explain the phenomenon of ulcer recurrence at the sites of ulcer scars. Therefore, sustained production of cytokines, which can cause neutrophilic infiltration, in response to *H. pylori* may be involved in the mechanism of recurrence in human peptic ulcers.

TNF-α, which shares many biological activities with IL-1β,[17] is involved in acute gastric injury.[40,41]

IL-1β prevents acute gastric mucosal injury caused by indomethacin[42] and absolute ethanol.[43] Wallace et al[42] have reported that 1 μg/kg IL-1β given by intraperitoneal injection (the same dose and route as we used in the present study) reduced the gastric injury caused by indomethacin. In contrast, we found that IL-1β caused ulcer recurrence without the usual noxious agents used in experimental production of ulcers. These findings and our results support the hypothesis that IL-1β has dual effects on gastric mucosa that depend on local conditions of the mucosa, such as scarring.

In conclusion, IL-1β can cause recurrence of experimental gastric ulcers in rats, and neutrophilic infiltration into scarred mucosa may be responsible for this recurrence. Adhesion molecules (ICAM-1, LFA-1, and Mac-1) may play a crucial role in neutrophil recruitment in this experimental model of ulcer recurrence.

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References

1. Takagi K, Okabe S, Saziki R: A new method for the production of chronic gastric ulcer in rats and the effect of several drugs on its healing. Jpn J Pharmacol 1969, 19:418–416
2. Uchida M, Kawano O, Misaki N, Saitoh K, Irino O: Healing of acetic acid-induced gastric ulcer and gastric mucosal PGI_2 level in rats. Dig Dis Sci 1990, 35: 80–85
3. Tarnawski A, Stachura J, Durbin T, Sarfeh IJ, Gergely H: Increased expression of epidermal growth factor receptor during gastric ulcer healing in rats. Gastroenterology 1992, 102:695–698
4. Watanabe T, Arakawa T, Fukuda T, Higuchi K, Kobayashi K: Zinc deficiency delays gastric ulcer healing in rats. Dig Dis Sci 1995, 40:1340–1344
5. Tarnawski A, Hollander D, Krause WJ, Dabros W, Stachura J, Gergely H: "Healed" experimental gastric ulcers remain histologically and ultrastructurally abnormal. J Clin Gastroenterol 1990, 12:S139–S147
6. Arakawa T, Watanabe T, Fukuda T, Yamasaki K, Kobayashi K: Rebamipide, a novel prostaglandin-inducer, accelerates healing and reduces recurrence of acetic acid-induced rat gastric ulcer: comparison with cimetidine. Dig Dis Sci 1995, 40:2469–2471
7. Marshall BJ, Goodwin CS, Warren JR, Murray R, Blincow ED, Blackbourn SJ, Phillips M, Waters TE, Sanderson CR: Prospective double-blind trial of duodenal ulcer recurrence after eradication of *Campylobacter pylori*. Lancet 1988, 2:1437–1442
8. Graham DY, Lew GM, Klein PD, Evans DG, Evans DJ Jr, Saeed ZA, Malaty HM: Effect of treatment of *Helicobacter pylori* infection on the long-term recurrence of gastric or duodenal ulcer: a randomized, controlled study. Ann Intern Med 1992, 116:705–708
9. Wallace JL: Possible mechanisms and mediators of gastritis associated with *Helicobacter pylori* infection. Scand J Gastroenterol 1991, 26:65–70
10. Dixon MF: *Helicobacter pylori* and peptic ulceration: histopathological aspects. J Gastroenterol Hepatol 1991, 6:125–130
11. Mai UEH, Perez-Perez GI, Wahl LM, Wahl SM, Blaser MJ, Smith PD: Soluble surface proteins from *Helicobacter pylori* activate monocytes/macrophages by lipopolysaccharide-independent mechanism. J Clin Invest 1991, 87:894–900
12. Crabtree JE, Shallcross TM, Heatley RV, Wyatt JI: Mucosal tumour necrosis factor-α and interleukin-6 in patients with *Helicobacter pylori* associated gastritis. Gut 1991, 32:1473–1477
13. Noach LA, Bosma NB, Jansen J, Hoek FJ, van Deventer SJH, Tytgat GNJ: Mucosal tumor necrosis factor-α, interleukin-1β, and interleukin-8 production in patients with *Helicobacter pylori* infection. Scand J Gastroenterol 1994, 29:425–429
14. Dinarello CA: Biology of interleukin 1. FASEB J 1988, 2:108–115
15. Mulligan MS, Ward PA: Immune complex-induced lung and dermal vascular injury: differing requirements for tumor necrosis factor-α and IL-1. J Immunol 1992, 149: 331–339
16. Pettipher ER, Higgs GA, Henderson B: Interleukin 1 induces leukocyte infiltration and cartilage proteoglycan degradation in the synovial joint. Proc Natl Acad Sci USA 1986, 83:8749–8753
17. Le J, Vilcek J: Tumor necrosis factor and interleukin 1: cytokines with multiple overlapping biological activities. Lab Invest 1987, 56:234–248
18. Pober JS, Bevilacqua MP, Mendrick DL, Lapierre LA, Fiers W, Gimbrone MA Jr: Two distinct monokines, interleukin 1 and tumor necrosis factor, each independently induced biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. J Immunol 1986, 136:1680–1687
19. Pohlman TH, Stanness KA, Beatty PG, Ochs HD, Harlan JM: An endothelial cell surface factor(s) induced *in vitro* by lipopolysaccharide, interleukin 1, and tumor necrosis factor-α increases neutrophil adherence by a CDw18-dependent mechanism. J Immunol 1986, 136: 4548–4553
20. Dayer J-M, de Rochemonteix B, Burrus B, Demczuk S, Dinarello CA: Human recombinant interleukin 1 stimulates collagenase and prostaglandin E_2 production by human synovial cells. J Clin Invest 1986, 77:645–648
21. Mulligan MS, Johnson KJ, Todd RF, Issekutz TB, Mi-

yasaka M, Tamatani T, Smith CW, Anderson DC, Ward PA: Requirements for leukocyte adhesion molecules in nephrotoxic nephritis. J Clin Invest 1993, 91:577–587
22. Lo SK, Everitt J, Gu J, Malik AB: Tumor necrosis factor mediates experimental pulmonary edema by ICAM-1 and CD18-dependent mechanisms. J Clin Invest 1992, 89:981–988
23. Marlin SD, Springer TA: Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). Cell 1987, 51:813–819
24. Diamond MS, Staunton DE, de Fougerolles AR, Stacker SA, Garcia-Aguilar J, Hibbs ML, Springer TA: ICAM-1 (CD54): a counter-receptor for Mac-1 (CD11b/CD18). J Cell Biol 1990, 111:3129–3139
25. Wallace JL, Arfors K-E, McKnight GW: A monoclonal antibody against the CD18 leukocyte adhesion molecule prevents indomethacin-induced gastric damage in the rabbit. Gastroenterology 1991, 100:878–883
26. Wallace JL, McKnight W, Miyasaka M, Tamatani T, Paulson J, Anderson DC, Granger DN, Kubes P: Role of endothelial adhesion molecules in NSAID-induced gastric mucosal injury. Am J Physiol 1993, 265:G993–G998
27. Fukawa K, Kawano O, Misaki N, Uchida M, Irino O: Experimental studies on gastric ulcer. IV. Sequential observation and evaluation of gastric ulcers by endoscope in the rat. Jpn J Pharmacol 1983, 33:175–179
28. Tamatani T, Miyasaka M: Identification of monoclonal antibodies reactive with the rat homolog of ICAM-1 and evidence for a differential involvement of ICAM-1 in the adherence of resting *versus* activated lymphocytes to high endothelial cells. Int Immunol 1990, 2:165–171
29. Tamatani T, Kotani M, Miyasaka M: Characterization of the rat leukocyte integrin, CD11/CD18, by the use of LFA-1 subunit-specific monoclonal antibodies. Eur J Immunol 1991, 21:627–633
30. Tamatani T, Kitamura F, Kuida K, Shirao M, Mochizuki M, Suematsu M, Schmid-Schönbein GW, Watanabe K, Tsurufuji S, Miyasaka M: Characterization of rat LECAM-1 (L-selectin) by the use of monoclonal antibodies and evidence for the presence of soluble LECAM-1 in rat sera. Eur J Immunol 23:2181–2188
31. Kvietys PR, Twohig B, Danzell J, Specian RD: Ethanol-induced injury to the rat gastric mucosa: role of neutrophils and xanthine oxidase-derived radicals. Gastroenterology 1990, 98:909–920
32. Andrews FJ, Malcontenti-Wilson C, O'Brien PE: Polymorphonuclear leukocyte infiltration into gastric mucosa after ischemia-reperfusion. Am J Physiol 1994, 266:G48–G54
33. Wallace JL, Keenan CM, Granger DN: Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process. Am J Physiol 1990, 259:G462–G467
34. Mulligan MS, Varani J, Dame MK, Lane CL, Smith CW, Anderson DC, Ward PA: Role of ELAM-1 in neutrophil mediated lung injury in rats. J Clin Invest 1991, 88: 1396–1406
35. Matsushima K, Morishita K, Yoshimura T, Lavu S, Kobayashi Y, Lew W, Appella E, Kung HF, Leonard EJ, Oppenheim JJ: Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. J Exp Med 1988, 167:1883–1893
36. Lindley I, Aschauer H, Siefert J-M, Lam C, Brunowsky W, Kownatzki E, Thelen M, Peveri P, Dewald B, von Tscharner V, Walz A, Baggiolini M: Synthesis and expression in *Escherichia coli* of the gene encoding monocyte-derived neutrophil-activating factor: biological equivalence between natural and recombinant neutrophil-activating factor. Proc Natl Acad Sci USA 1988, 85:9199–9203
37. Sica A, Matsushima K, van Damme J, Wang JM, Polentarutti N, Dejana E, Colotta F, Mantovani A: IL-1 transcriptionally activates the neutrophil chemotactic factor/IL-8 gene in endothelial cells. Immunology 1990, 69:548–553
38. Strieter RM, Chensue Sw, Basha MA, Standiford TJ, Lynch JP III, Baggiolini M, Kunkel SL: Human alveolar macrophage gene expression of interleukin-8 by tumor necrosis factor-α, lipopolysaccharide, and interleukin-1β. Am J Respir Cell Mol Biol 1990, 2:321–326
39. Philip R, Epstein LB: Tumour necrosis factor as immunomodulator and mediator of monocyte cytotoxicity induced by itself, γ-interferon, and interleukin-1. Nature 1986, 323:86–89
40. Kahky MP, Daniel CO, Cruz AB, Gaskill HV III: Portal infusion of tumor necrosis factor increases mortality in rats. J Surg Res 1990, 49:138–145
41. Mahatma M, Agrawal N, Dajani EZ, Nelson S, Nakamura C, Sitton J: Misoprostol but not antacid prevents endotoxin-induced gastric mucosal injury: role of tumor necrosis factor-α. Dig Dis Sci 1991, 36:1562–1568
42. Wallace JL, Keenan CM, Cucala M, Mugridge KG, Parente L: Mechanisms underlying the protective effects of interleukin 1 in experimental nonsteroidal anti-inflammatory drug gastropathy. Gastroenterology 1992, 102:1176–1185
43. Robert A, Olafsson AS, Lancaster C, Zhang W: Interleukin-1 is cytoprotective, antisecretory, stimulates PGE_2 synthesis by the stomach, and retards gastric emptying. Life Sci 1991, 48:123–134