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Ethanol-Induced Acute Gastric Injury in Mast Cell-Deficient and Congenic Normal Mice

Evidence That Mast Cells Can Augment the Area of Damage

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The authors used stereomicroscopy and planimetry to measure the area of glandular stomach mucosa acutely injured by oral ethanol in mast cell-deficient and congenic normal (+/+) mice, and examined the damaged areas in 1-μ sections. Ethanol caused degranulation and/or disruption of gastric mucosal mast cells, and, at certain concentrations of ethanol, mast cell-deficient WBB6F$_1$-*W/W^v* or WCB6F$_1$-*Sl/Sl^d* mice developed significantly less (43–90% less) acute gastric injury than either congenic +/+ mice or WBB6F$_1$-*W/W^v* mice whose mast cells were restored by bone marrow transplantation from WBB6F$_1$-+/+ mice. Nevertheless, ethanol produced detectable, and in some cases substantial, gastric injury even in the complete absence of mast cells. Thus, ethanol can produce some damage to the gastric mucosa independently of mast cells. But these data suggest that under certain circumstances mast cells can augment the area of acute gastric injury induced by ethanol. (Am J Pathol 1987, 128:131–140)

SEVERAL lines of evidence have implicated mast cells in the pathogenesis of acute gastric injury. Histamine has long been regarded as a critical mediator in the development of acute gastric mucosal damage,[1-3] and studies in genetically mast cell-deficient WBB6F$_1$-*W/W^v* mice and their normal (+/+) littermates indicate that ~ 50% of the histamine in the mouse glandular stomach mucosa is associated with mast cells.[4,5] In rats, restraint stress causes both gastric mast cell degranulation and acute gastric ulceration,[6] and oral administration of compound 48/80, which provokes mast cell degranulation, is followed by the development of gastric edema and ulceration.[7] Increased numbers of mast cells may be found in the vicinity of human gastroduodenal ulcers.[8,9] Finally, dogs with mastocytomas may have a high incidence of gastroduodenal ulcers.[10] Yet the importance and nature of the mast cell's contribution to acute gastric injury has been difficult to define, in part because until recently no model systems were available which permitted such processes to be studied both in the presence and in the complete absence of mast cells.

In the present study, we used genetically mast cell-deficient WBB6F$_1$-*W/W^{v11}* and WCB6F$_1$-*Sl/Sl^{d12}* mice, which totally lack identifiable gastric mast cells,[11-13] and their congenic normal (+/+) littermates to determine whether gastric mucosal mast cells contribute to ethanol-induced acute gastric damage. We chose this approach for several reasons: analysis of the expression of biologic responses in genetically mast cell-deficient mice and the congenic normal mice represents the only animal model currently available for defining the unique role of mast cells in these responses[14,15]; the administration of oral ethanol represents an established model for the analysis of acute gastric injury due to the administration of a exogenous toxin[16-18]; and the extent of gastric damage induced by ethanol can be assessed quantitatively in a

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"blinded" fashion.[17] Furthermore, augmentation of microvascular permeability, a response which can be induced by several mast cell-derived mediators,[19–21] represents an early event in the pathogenesis of ethanol-induced acute gastric injury.[18] Our results show that ethanol can produce detectable acute damage of the gastric mucosa in the complete absence of mast cells, but that at certain concentrations of ethanol, two- to tenfold more gastric injury occurs in the presence of mast cells than in their absence. Some of these findings have been reported in abstract form.[22]

Materials and Methods

Mice

All mice were obtained from the Jackson Laboratory, Bar Harbor, Maine, and were used at 8–12 weeks of age unless otherwise specified. All mice were males housed in polycarbonate cages with pine shaving bedding, which was changed twice weekly; they were given Purina Rodent Chow (Formula 5008) and tap water *ad libitum* unless otherwise specified. Mice of the following genotypes were used: mast cell-deficient WBB6F$_1$-*W/W^v*([WB-*W*/+ x C57BL/6-*W^v*/+]F$_1$-*W/W^v* mice) and pooled normal (WBB6F$_1$-+/+) littermates[11]; mast cell-deficient WCB6F$_1$-*Sl/Sl^d* mice ([WC-*Sl*/+ x C57BL/6-*Sl^d*/+]F$_1$-*Sl/Sl^d* mice) and pooled normal (WCB6F$_1$-+/+) littermates[12]; anemic WBB6F$_1$-*an/an* mice and pooled normal (WBB6F$_1$-*an*/+) littermates.[23,24]

Induction of Ethanol-Induced Acute Gastric Injury

Procedures for the elicitation and quantitation of ethanol-induced acute gastric damage have been described in detail.[17,18] Briefly, the mice were given no food and were placed in hanging steel wire cages, for the 24 hours before receiving 0.2 ml 100% ethanol or 0.2 ml 75% ethanol in 0.9% NaCl, by gavage.[18] The mice were killed by cervical dislocation. The stomachs were removed, opened along the greater curvature, and fixed flat on a corkboard either in 10% buffered formalin[18] or (for 1-μ Epon-embedded, Giemsa-stained sections[25]) in 2.0% paraformaldehyde, 2.5% glutaraldehyde, and 0.025% $CaCl_2$ in 0.1 M sodium cacodylate buffer, pH 7.3. The fixed stomachs were coded and examined under a stereomicroscope by a single observer unaware of the identity of individual stomachs. Areas of mucosal damage were identified as regions exhibiting superficial erosion, often accompanied by hemorrhage, and were measured by planimetry of the projected image.[17,18] the results were expressed as the percentage of the total glandular stomach involved by damage.[17,18] The technique results in highly reproducible measurements of areas of mucosal injury. When four individual specimens in one experiment were analyzed on three different occasions, the maximum variation of individual measurements from the mean of the three values was 6% ± 2% (mean ± SD). After completion of the planimetric measurements, we prepared representative paraffin- or Epon-embedded sections of stomachs obtained 1 hour after ethanol challenge to examine the histologic features of the areas of mucosal injury that had been quantitated by planimetry.

As will be discussed in greater detail below, ~ 10% of the WBB6F$_1$-*W/W^v* or WCB6F$_1$-*Sl/Sl^d* mice raised at the Jackson Laboratory spontaneously develop chronic gastric antral ulcers and/or forestomach papillomas.[4,26] These mice generally appear to be runts and can be discriminated from their littermates by their low body weight. Such mice were not included in our experiments. A few of the *W/W^v* or *Sl/Sl^d* mice of normal body weight which were given ethanol were found to have small antral ulcers and/or forestomach papillomas at autopsy; these mice were not included in the results reported here.

Quantitation of Gastric Mast Cells

Mast cells were counted as previously described in 1-μ, Epon-embedded, Giemsa-stained sections examined at ×400 in a light microscope equipped with an ocular grid.[27] *Mast cells were classified according to anatomic location:* mast cells superficial to the deep border of the muscularis mucosae were classified as "mucosal"; mast cells occurring below the deep border of the muscularis mucosae but above the superficial border of the muscularis propria were classified as "submucosal." The counts were performed by a single observer on coded sections and are expressed as number of mast cells per 0.01 sq mm of mucosa or submucosa (mean ± SD).

It should be noted that many authors have used the term "mucosal mast cell" to refer to a population of murine mast cells expressing a constellation of morphologic, histochemical, and functional characteristics distinct from those of the "connective tissue type" mast cells which occur in the skin and serosal cavities (reviews[13,14,19–21,28–32]). But this use of the term "mucosal mast cell" can be misleading. While the mouse and rat gastrointestinal mucosa represents the anatomic site where such "mucosal mast cells" have been investigated most extensively, mast cells with characteristics similar to those of "mucosal mast cells" can be observed in other sites as well (reviews[28–30]) and can even develop from hematopoietic precursors *in vitro*

(reviews[14,30-32]). Moreover, recent evidence indicates that cultured mast cells with phenotypic features of "mucosal mast cells," when introduced into suitable microenvironments *in vivo*[13,34] or *in vitro*,[33] may give rise to cells whose phenotype resembles that of "connective tissue mast cells."[13,33,34] One reasonable explanation for these findings is that mast cells with phenotypic features of "mucosal mast cells" can acquire the features of "connective tissue mast cells" as a result of a maturational process. But whatever the precise relationship between the "mucosal mast cells" and "connective tissue mast cells" which are defined by histochemical criteria, for the purposes of this study, we classified all of the gastric mast cells simply according to their anatomic location.

Results

Ethanol-Induced Gastric Injury in Mast Cell-Deficient and Congenic Normal (+/+) Mice

We first tested WBB6F$_1$-*W/W^v* mice and their normal (WBB6F$_1$-+/+) littermates. As shown in Table 1, the kinetics of development of hemorrhagic erosions after ethanol treatment in WBB6F$_1$-+/+ mice was similar to that previously reported in the rat,[17,18] with grossly evident lesions reaching maximal size by 1 hour. WBB6F$_1$-*W/W^v* mice also developed glandular stomach erosions, but at all intervals tested the mast cell-deficient mice exhibited less gastric injury than did the normal (+/+) littermates. The differences between the mast cell-deficient and congenic normal animals were greatest at 1 and 2 hours, at which times the aggregate areas of the lesions in *W/W^v* mice were less than those in +/+ mice by 67% and 75%, respectively. In another experiment in which *W/W^v* and congenic +/+ mice were examined 1 hour after challenge with 100% ethanol (Figure 1a), lesions occupied 6.5% ± 1.3% (n = 10) of the gastric mucosal surface area in the +/+ mice and 3.7% ± 0.8% (n = 11) of the area in the *W/W^v* mice ($P < 0.001$). The reason for the variability of the response of the different groups of +/+ mice used in these two experiments is not clear. On the other hand, the observation underlines the value of using groups of pooled littermate mice for comparative studies of biologic responses elicited in mutant mice and the congenic normal animals. When challenged with 75% ethanol, both WBB6F$_1$-*W/W^v* and -+/+ mice developed small areas of injury (~ 2.0%) which did not differ significantly in extent in the *W/W^v* and +/+ animals (data not shown).

We next tested WCB6F$_1$-*Sl/Sl^d* mice and their +/+ littermates. Although *Sl/Sl^d* mice resemble *W/W^v* mice by phenotype (both are anemic, virtually lack mast cells and melanocytes, and are sterile), these abnormalities reflect distinct mutations involving different chromosomes.[12,15,35] When challenged with 100% ethanol (Figure 1b), *Sl/Sl^d* mice developed more extensive glandular mucosal injury than did similarly treated *W/W^v* mice (Table 1, Figure 1a), and the extent of injury did not differ from that observed in the congenic +/+ mice. But when challenged with 75% ethanol (Figure 1b), *Sl/Sl^d* mice developed only about one-third as much acute gastric damage as did the congenic normal controls ($P < 0.005$).

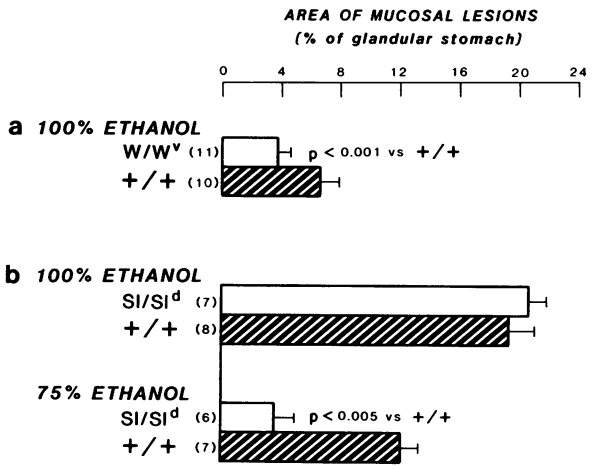

Figure 1—Area of ethanol-induced erosions involving the glandular stomach mucosa in mast cell-deficient and congenic normal (+/+) mice. **a**—100% ethanol in WBB6F$_1$-*W/W^v* (W/W^v) or WBB6F$_1$-+/+ (+/+) mice. **b**—100% or 75% ethanol in WCB6F$_1$-*Sl/Sl^d* (Sl/Sl^d) or WCB6F$_1$-+/+ mice. The values shown are the mean ± SEM (number of mice). The significance of differences between groups in individual experiments was determined by means of the Mann–Whitney U test (two-tailed). NS, not significant ($P > 0.05$).

Table 1—Area of Ethanol-Induced Gastric Mucosal Injury in Mast Cell-Deficient WBB6F$_1$-*W/W^v* Mice and Normal Littermate (+/+) Controls at Various Intervals After Challange With 100% Ethanol

Interval after challenge	Mice	
	A: +/+	B: *W/W^v*
1 minute	8.0 ± 2.0 (8)	3.6 ± 1.1 (5) $P < 0.17$ vs A
1 hour	11.1 ± 3.0 (6)	3.7 ± 0.8 (7) $P < 0.03$ vs A
2 hours	12.1 ± 1.5 (8)	3.0 ± 1.1 (6) $P < 0.002$ vs A
6 hours	7.7 ± 2.7 (7)	4.0 ± 0.4 (5) $P < 0.24$ vs A

The procedures for inducing gastric mucosal lesions with oral ethanol, and for quantitating the lesions by stereomicroscopy and planimetry, are described in Materials and Methods. The values are mean ± SEM; tests for statistical significance of differences in values were by the Mann–Whitney U test (two-tailed).

Ethanol-Induced Gastric Injury in Bone Marrow-Reconstituted WBB6F$_1$-*W/W^v* Mice and WBB6F$_1$-*an/an* Mice

The gene products affected by the mutations in *W/W^v* and *Sl/Sl^d* mice have not been characterized,[15,35] and we were concerned that these mutations might influence the susceptibility of the gastric fundus to ethanol-induced injury by an effect independent of mast cell deficiency. To evaluate this possibility, we tested the response of *W/W^v* mice whose mast cell populations had been restored by transplantation of bone marrow cells from congenic +/+ mice.

W/W^v mice received WBB6F$_1$-+/+ bone marrow cells (2.0×10^7 cells/recipient, intravenously[11,30]) at 6–8 weeks of age; the success of bone marrow reconstitution was confirmed 12 weeks later by demonstrating correction of the hematocrit of the recipient +/+ → *W/W^v* mice.[11,36] When +/+ → *W/W^v* mice were challenged with 100% ethanol 2 weeks later (ie, 14 weeks after bone marrow transplantation), the extent of gastric damage produced was statistically indistinguishable from that observed in the congenic +/+ controls (Figure 2). By contrast, untreated *W/W^v* mice from the same group of pooled littermates developed significantly less injury than either +/+ controls ($P < 0.002$) or +/+ → *W/W^v* mice ($P < 0.02$). Histologic examination using 1-μ Epon-embedded, Giemsa-stained sections confirmed previous work showing that transplantation of +/+ bone marrow cells restored the gastric mast cell population of +/+ → *W/W^v* mice.[26,36,37]

Transplantation of congenic +/+ bone marrow cells corrects both the mast cell deficiency and the anemia of *W/W^v* recipients.[15,36,37] Because stereomicroscopic identification of ethanol-induced gastric lesions depends in part on recognition of areas of mucosal hemorrhage, we wondered whether correction of their anemia might be sufficient to account for the increased areas of ethanol-induced gastric injury in +/+ → *W/W^v* mice. We evaluated this issue by two different approaches. First, we tested WBB6F$_1$-*an/an* anemic mice and their normal (*an*/+) littermates. The genetic background of *an/an* mice is similar to that of WBB6F$_1$-*W/W^v* mice, and the two mutants are reported to express similar mild macrocytic anemias,[23,24] a finding we confirmed (Table 2). But *an/an* mice are not mast cell-deficient (Y. Kitamura, personal communication, a finding we confirmed histologically). When challenged with 100% ethanol, *an/an* mice and littermate *an*/+ controls developed the same amount of acute gastric injury (Table 2).

We then took a more direct approach, testing WBB6F$_1$-*W/W^v* mice at two different intervals after transplantation with congenic +/+ bone marrow cells. In accord with previous work by Kitamura et al,[11] we found that the hematocrit of WBB6F$_1$-*W/W^v* mice transplanted with WBB6F$_1$-+/+ bone marrow became normal about 2 weeks after transplantation, whereas maximal reconstitution of the recipients' tissues with mast cells occurred much later (about 10 weeks). The different kinetics of correction of the anemia and the mast cell deficiency of WBB6F$_1$-*W/W^v* mice thus provided an opportunity to compare the amount of ethanol-induced gastric injury produced in WBB6F$_1$-*W/W^v* mice with normal hematocrits but no identifiable mast cells with the amount of injury produced in recipients that had undergone repair of both their hematocrit and mast cell deficiency.

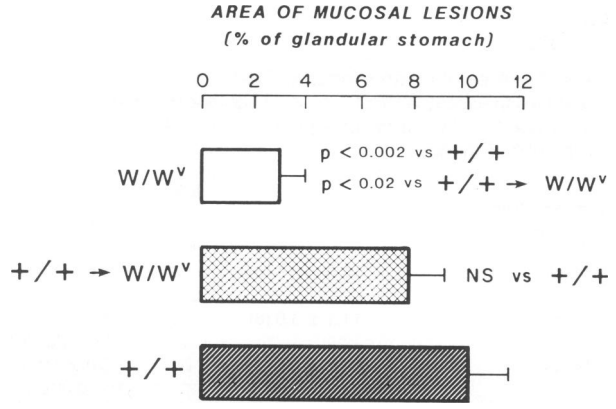

Figure 2—Area of ethanol-induced erosions involving the glandular stomach mucosa in WBB6F$_1$-*W/W^v* (W/W^v) or WBB6F$_1$-+/+ (+/+) mice, or in *W/W^v* mice reconstituted with congenic +/+ bone marrow cells 14 weeks earlier (+/+ → W/W^v). Two separate experiments gave similar results, and the data were pooled for presentation as mean ± SEM. A total of 12 *W/W^v* mice, 11 +/+ mice, and 13 +/+ → *W/W^v* mice were studied. The significance of differences between the groups was determined by means of the Mann–Whitney U test (two-tailed). NS, not significant ($P > 0.05$).

Table 2—Hematocrit and Area of Ethanol-Induced Gastric Mucosal Injury in WBB6F$_1$-*an/an* Anemic Mice and Normal Littermate (*an*/+) Controls

	Mice	
	A: *an/an*	B: *an*/+
Hematocrit (%) (number of mice)	41.0 ± 1.0 (7) $P < 0.02$ vs B	51.0 ± 0.7 (8)
Area of mucosal lesions after 100% ethanol (% of glandular stomach)	14.0 ± 3.3 NS* vs B	14.5 ± 3.7

The procedures for inducing gastric mucosal lesions with oral ethanol, and for quantitating the lesions by stereomicroscopy and planimetry, are described in the Materials and Methods. The values are mean ± SEM (number of mice); tests for statistical significance of differences in values were by the Mann–Whitney U test (two-tailed).

*NS, not significant ($P > 0.05$).

For this experiment, WBB6F$_1$-*W/W^v* mice were tested 17 or 70 days after intravenous transplantation of WBB6F$_1$-+/+ bone marrow cells. Some mice were sacrificed for quantitation of gastric mast cell populations, others were given 100% ethanol for measurement of area of gastric injury. As shown in Table 3, the hematocrit of +/+ → *W/W^v* mice was statistically indistinguishable from that of pooled littermate WBB6F$_1$-+/+ mice 17 days after bone marrow transplantation. By contrast, virtually no mast cells were identifiable in the stomach of the same +/+ → *W/W^v* mice. At 70 days after transplantation, the number of mast cells in the glandular stomach mucosa of +/+ → *W/W^v* mice was statistically indistinguishable from that in the corresponding +/+ mice, whereas the number of mast cells in the glandular stomach submucosa of +/+ → *W/W^v* mice actually exceeded that in the corresponding +/+ mouse tissues.

The hematocrit values of the mice used for measurements of ethanol-induced injury (Table 3) were similar to those of the mice used for quantitation of gastric mast cells (Table 3). Seventeen days after bone marrow transplantation, the amount of ethanol-induced gastric injury in +/+ → *W/W^v* mice with a normal hematocrit was identical to that in anemic *W/W^v* mice (2.7 ± 0.8%, ~ 29% the value obtained in +/+ mice, $P < 0.001$). By contrast, at 70 days after transplantation, +/+ → *W/W^v* mice developed about tenfold more gastric injury than pooled littermate *W/W^v* mice tested at the same time (Table 3). The amount of gastric injury in +/+ → *W/W^v* mice tested 70 days after bone marrow transplantation also was significantly ($P < 0.01$) greater than that observed in +/+ → *W/W^v* mice tested 17 days after bone marrow transplantation, but was not statistically different from that observed in +/+ mice.

Histologic Studies

The histologic analysis established two findings of interest. First, the gastric damage produced by ethanol in the presence of mast cells and that in their absence appeared qualitatively similar by morphologic study. The grossly evident areas of mucosal damage which were quantitated by stereomicro-

Table 3—Hematocrit, Gastric Mast Cell Counts, and Areas of Ethanol-Induced Gastric Injury in WBB6F$_1$-*W/W^v* Mice 17 or 70 Days After Intravenous Transplantation of WBB6F$_1$-+/+ Bone Marrow Cells

	Interval after bone marrow transplantation (days)	Mice		
		A: +/+	B: *W/W^v*	C: +/+ → *W/W^v*
Mice used for mast cell counts				
Hematocrit (%) (number of mice)	17	48.0 ± 0.9 (6)	39.8 ± 1.1 (6) $P < 0.02$ vs A, C	47.5 ± 0.8 (6) NS vs A
	70	48.5 ± 0.6 (6)	40.0 ± 0.6 (6) $P < 0.02$ vs A, C	48.6 ± 0.3 (6) NS* vs A
Mast cells (number per square millimeter)				
Glandular stomach, mucosa	17	40 ± 22	0	0.6 ± 0.7
	70	71 ± 33	0	67 ± 23 NS vs A
Glandular stomach, submucosa	17	64 ± 33	0	0 ± 0
	70	47 ± 19	0	87 ± 29 $P < 0.04$ vs A
Mice given oral ethanol				
Hematocrit (%)	17	48.8 ± 0.3 (10)	38.9 ± 0.8 (8) $P < 0.02$ vs A, C	47.2 ± 0.5 (7) NS vs A
	70	ND†	37.6 ± 0.6 (6) $P < 0.02$ vs A, C NS vs 17-day value	46.8 ± 0.9 (6) NS vs 17-day value
Area of mucosal lesions after 100% ethanol (% of glandular stomach)	17	9.2 ± 1.2	2.7 ± 0.8 $P < 0.001$ vs A	2.7 ± 0.8 $P < 0.001$ vs A, NS vs B
	70	ND	1.2 ± 0.8	11.7 ± 3.4 $P < 0.01$ vs B $P < 0.01$ vs 17-day value (C) NS vs 17-day value (A)

Pooled littermate WBB6F$_1$-+/+ mice were left untreated (group A) or were used as donors for bone marrow cells, which were administered intravenously (2.0×10^7 cells/mouse) to pooled littermate WBB6F$_1$-*W/W^v* mice (group C). Other pooled littermate *W/W^v* mice were left untreated (group B). Mice in groups A–C were sacrificed 17 or 70 days after transplantation of group C mice; some of the mice did not receive ethanol and were used for determination of hematocrit and quantitation of gastric mast cells; others were used for determination of hematocrit and quantitation of areas of ethanol-induced gastric mucosal injury (see Materials and Methods). The values are mean ± SEM or, for mast cell counts, mean ± SD; tests for statistical significance of differences in values were by the Mann–Whitney U test (two-tailed).

*NS, not significant ($P > 0.05$).

†ND, not done.

scopy/planimetry consisted of regions of epithelial necrosis extending focally to the muscularis mucosae, with associated vascular dilatation and stasis, hemorrhage, and edema (Figure 3). Occasional areas of injury also exhibited focal infiltration of neutrophils. Administration of ethanol also caused injury of virtually all of the most superficial glandular stomach surface epithelial cells, a change not detected by stereomicroscopy but evident upon microscopic examination of the grossly unremarkable areas of the ethanol-treated stomachs (eg, Figure 3c). All of these changes have been described in studies of ethanol-induced gastric damage in rats.[16,38]

But in addition to confirming in the mouse features of ethanol-induced gastric injury previously recognized in the rat, 1-μ sections of stomachs from mice

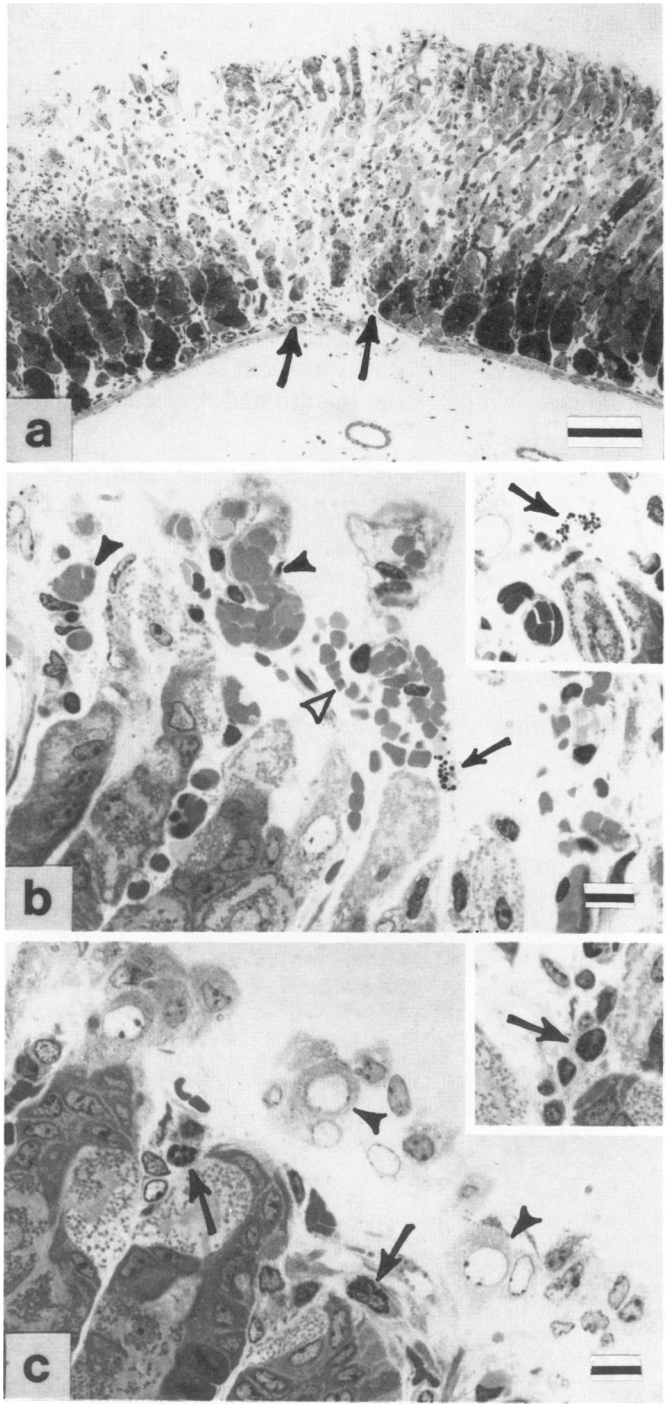

Figure 3—Photomicrographs of the glandular stomach of a WCB6F$_1$-+/+ mouse that received 100% ethanol 1 hour before sacrifice. **a**—A well-circumscribed region of mucosal injury which was evident upon gross examination of the fixed stomach. The area of damage, indicated by the loss of staining intensity of the affected epithelial cells, extends focally to the muscularis mucosae (*arrows*). (Bar = 100 μ) **b**—Higher magnification of the superficial mucosa at the periphery of an area of damage similar to that shown in **a**. There is compaction of erythrocytes within superficial mucosal vessels (*solid arrowheads*), evidence of vascular stasis, and focal hemorrhage (*open arrowhead*). A mast cell (*arrow*) exhibits disruption, with release of cytoplasmic granules and loss of nuclear staining. **Inset**—Another disrupted mast cell (*arrow*) in a field adjacent to that in **b**. (Bar = 10 μ) **c**—Grossly normal-appearing area of superficial mucosa from the same stomach shown in **b**. There are a few desquamated necrotic cells (*arrowheads*), but no other evidence of tissue injury. Two mast cells (*arrows*) near the mucosal surface appear intact. **Inset**—Another intact mast cell (*arrow*) in a field adjacent to that in **c**. (**a–c**, 1-μ-thick, Giemsa-stained, Epon-embedded sections. (Bar = 10μ)

containing mast cells demonstrated extensive degranulation and/or disruption of mucosal mast cells in areas of deep mucosal injury (Figure 3b). Many affected mast cells exhibited loss of nuclear staining (Figure 3b), suggesting that at least some of the mast cell activation/disruption induced by ethanol was the result of a cytotoxic mechanism. In contrast to mucosal mast cells in areas of injury, mucosal mast cells in grossly unaffected areas (Figure 3c), and the mast cells in the submucosa and deeper layers of the stomach, appeared normal by morphology. In accord with previous reports,[11-13] we found that the stomachs of W/W^v and Sl/Sl^d mice totally lacked mast cells by histologic criteria.

Discussion

Our experiments were designed to evaluate three issues: 1) Is there morphologic evidence that mast cells might have a role in ethanol-induced acute gastric injury? 2) Are gastric mast cells *required* for the elicitation of acute gastric mucosal injury by oral ethanol? 3) Can gastric mast cells *modulate* the extent of ethanol-induced injury? The answer to the first question clearly is yes. In stomachs of mice containing mast cells, areas of ethanol-induced injury were associated with extensive changes in gastric mucosal mast cells. Some mast cells exhibited loss of cytoplasmic granules; others exhibited diminished staining of the nucleus as well. The latter finding indicates that oral administration of 75% or 100% ethanol resulted in a toxic effect on gastric mucosal mast cells. This is not surprising, because many other cellular elements of the gastric mucosa in the areas of ethanol-induced injury also exhibited evidence of necrosis by histologic study.

Although it is likely that ethanol caused release of mediators stored in mast cell granules at least in part by damaging or killing mast cells in areas of mucosal damage, we cannot rule out involvement of other mechanisms of mast cell mediator release as well. A wide variety of stimuli can induce mast cells to release stored and newly formed mediators in the absence of cytotoxic effects on the mast cells.[19-21] Some of these secretogogues, such as the anaphylatoxins formed as a result of complement activation and certain basic substances derived from circulating granulocytes, might well be generated locally in areas of ethanol-induced gastric mucosal damage.

Although extensive mast cell disruption/degranulation occurred in areas of ethanol-induced injury, as has been reported in other forms of acute gastric damage,[6,7,39] our data clearly show that mast cell-dependent mechanisms are *not required* for the development of ethanol-induced lesions. When tested at either 75% or 100%, oral ethanol caused detectable areas of acute gastric injury in both WBB6F$_1$-W/W^v and WCB6F$_1$-Sl/Sl^d mice. Because each of these two different mutants totally lacks morphologically identifiable gastric mast cells, and because the lesions in mast cell-deficient and +/+ mice were qualitatively similar in both gross and histologic features, it follows that gastric mast cells are not essential for the development of the acute gastric injury induced by oral ethanol.

On the other hand, several findings are consistent with the possibility that mast cells may augment the area of gastric mucosa damaged by ethanol. The most convincing evidence is derived from the experiments with W/W^v mice. WBB6F$_1$-W/W^v mice have a defect in hematopoietic stem cells which results in anemia and a marked deficiency of mast cells: the skin of adult WBB6F$_1$-W/W^v mice contains $<0.3\%$ the number of mast cells present in congenic +/+ mice; and no mast cells whatsoever, of either the "connective tissue" or "mucosal" type, have been observed in WBB6F$_1$-W/W^v mouse forestomach, glandular stomach, or a variety of other anatomic sites.[11,13,15,27,40] By contrast, WBB6F$_1$-W/W^v and -+/+ mice do not differ in numbers of gastric parietal cells, chief cells, surface, mucous neck, or pyloric mucous cells, basal output of gastric HCl or pepsinogen, or gastric mucosal PGE_2 content.[4,26,37] WBB6F$_1$-W/W^v mice developed 43–71% less mucosal injury 1 hour after 100% ethanol than did the congenic normal mice. Moreover, we found that W/W^v mice tested ~10–14 weeks after +/+ bone marrow transplantation, a time sufficient to permit both correction of their anemia and reconstitution of their gastric mast cell populations, developed amounts of ethanol-induced injury which were statistically indistinguishable from those produced in the congenic +/+ mice.

We also tested some W/W^v mice 17 days after transplantation of congenic +/+ bone marrow cells. In accordance with previous work,[11] we found that this interval was sufficient to permit correction of the recipients' anemia, but too soon to permit restoration of morphologically identifiable gastric mast cell populations. In these mice, the amount of ethanol-induced injury was statistically indistinguishable from that produced in anemic W/W^v mice that had not been transplanted with +/+ bone marrow cells. This result showed that correction of the anemia of W/W^v mice was not sufficient to normalize the mutant's response to the injurious effects of oral ethanol. The hypothesis that the increased susceptibility of $+/+ \rightarrow W/W^v$ mice to the toxic effects of oral ethanol was not due simply to correction of the recipients'

anemia was also supported by an experiment with WBB6F$_1$-*an/an* mice. The WBB6F$_1$-*an/an* mice, which are not mast cell-deficient but which express a macrocytic anemia similar to that of the genetically related WBB6F$_1$-*W/W^v* mice,[23,24] developed the same amount of gastric injury after 100% ethanol as did their normal (*an*/+) littermates.

Our experiments with mast cell-deficient WCB6F$_1$-*Sl/Sl^d* mice also gave interesting results. When challenged with 100% ethanol, *Sl/Sl^d* mice and their congenic normal (+/+) littermates developed essentially the same amounts of gastric mucosal injury. This was true when the lesions were quantitated 1 hour after challenge (Figure 1b) and also when the extent of injury was measured 1 minute or 2 hour after challenge (data not shown). However, when challenged with 75% ethanol, *Sl/Sl^d* mice developed significantly less (~70% less) gastric glandular mucosal injury than did the +/+ littermates.

One possible interpretation of the experiments with WCB6F$_1$ mice is that, in this strain, mast cells make little or no contribution to the extent of acute gastric injury induced by 100% ethanol. This conclusion would be particularly attractive if it were known that the presence or absence of mast cells represented the only difference between the stomachs of *Sl/Sl^d* mice and congenic +/+ mice. But we believe that the latter possibility is unlikely. For example, there is evidence that genetically mast cell-deficient mice may be *more* susceptible than the congenic normal mice to certain forms of gastric injury, and that this increased susceptibility may be *independent* of the mutants' mast cell deficiency. Thus, WBB6F$_1$-*W/W^v* mice develop chronic ulcers of the gastric antrum, and papillomas of the forestomach, much more frequently than do the congenic normal (+/+) mice.[4,26] Moreover, bone marrow transplantation experiments in WBB6F$_1$ mice indicated that the increased susceptibility to these lesions represented a consequence of the *W/W^v* mutations independent of those affecting mast cells and other bone marrow-dependent elements.[26] WBB6F$_1$-*W/W^v* mice were also significantly more susceptible to the toxic effects of systemically administered indomethacin than were the congenic +/+ mice.[37] The difference in response was evident in normally fed mice, but not in mice starved prior to indomethacin treatment.[37] As in the case of spontaneous antral ulcers, the increased susceptibility of fed *W/W^v* mice to indomethacin-induced antral ulcers was not reversed when gastric mast cell populations were restored by transplantation of congenic +/+ bone marrow cells.[37]

WCB6F$_1$-*Sl/Sl^d* mice also develop chronic gastric antral ulcers and forestomach papillomas much more frequently than do the congenic +/+ mice.[26] If these problems, as in the *W/W^v* mice,[26] are independent of the mutant's mast cell deficiency, one might speculate that the *Sl/Sl^d* stomach exhibits a genetically determined increased sensitivity to the toxic effects of oral ethanol. According to this idea, the comparison between *Sl/Sl^d* mice and +/+ mice might be misleading, and the only way to detect the contribution of mast cells to the gastric damage observed after 100% ethanol in WCB6F$_1$ mice would be to test *Sl/Sl^d* mice in which gastric mast cells have been restored. The same point could be made concerning the experiments performed in *W/W^v* and congenic +/+ mice. Unfortunately, unlike *W/W^v* mice, *Sl/Sl^d* mice do not develop mast cells after transplantation of bone marrow cells derived from the congenic +/+ mice,[12] nor has any other strategy for replacing the mast cells of *Sl/Sl^d* mice been described.[15,35] As a result, the most critical experiment for clarifying the question of the role of mast cells in ethanol-induced gastric injury in WCB6F$_1$ mice cannot be performed. Thus, the experiments with the WCB6F$_1$ mice must be considered inconclusive with respect to the involvement of mast cells in the development of the ethanol-induced lesions.

By contrast, the data derived from the WBB6F$_1$-*W/W^v* mice, which can undergo repair of their gastric mast cell populations, strongly suggests that mast cell activation and/or disruption can augment the areas of ethanol-induced gastric mucosal injury provoked by 100% ethanol. These data are consistent with previous morphologic and pharmacologic studies implicating mast cell mediator release in the pathogenesis of many forms of acute gastric injury in several mammalian species.[6,8,9,39,41–43] But the observation that some ethanol-induced gastric injury can occur in the complete absence of mast cells clearly demonstrates that mast cells do not play an *essential* role in the pathogenesis of ethanol-induced gastric injury in the mouse. Our findings also suggest that the extent to which mast cells contribute to other forms of gastric injury may be difficult to predict on the basis of morphologic observations alone.

Several issues remain unresolved. One of these is to distinguish, in +/+ → *W/W^v* mice, between the effects of mast cell reconstitution and other consequences of bone marrow transplantation. We have shown that the short-term (17-day) consequences of bone marrow transplantation, including correction of the anemia of the *W/W^v* recipients, are not sufficient to normalize the response of +/+ → *W/W^v* mice to the injurious effects of oral ethanol. But we have not formally ruled out the theoretic possibility that a long-term consequence of bone marrow reconstitu-

tion other than restoration of gastric mast cell populations might contribute to the normalization of the response of +/+ → W/W^v mice to oral ethanol. One approach to this issue would be to use cultured, growth factor-dependent mast cells derived from $WBB6F_1$-+/+ mice to restore the gastric mast cell populations of $WBB6F_1$-W/W^v mice. We have reported that intravenous administration of cultured $WBB6F_1$-+/+ mast cells to $WBB6F_1$-W/W^v mice can partially restore the mutants' gastric mast cells.[13] If similar methods can be developed that permit complete restoration of the gastric mast cell populations of W/W^v mice, such mice might be useful in investigating the specific effect of mast cell repopulation, as opposed to other long-term consequences of bone marrow transplantation, in influencing the stomach's susceptibility to ethanol-induced injury.

Another task is to account for the mechanism(s) by which gastric mast cells might augment the area of mucosal injury produced by ethanol. One possibility is that mediators released by activated or damaged mucosal mast cells, perhaps through effects on the local vasculature,[1,6,18,39] promote mucosal hemorrhage. In preliminary studies, we have found that the development of ethanol-induced acute gastric erosion in both mast cell-deficient and congenic +/+ mice can be markedly (> 80%) reduced by the pretreatment of the mice with H_1 anti-histamines, whereas H_2 anti-histamines were without effect.[44] These findings suggest that H_1-dependent effects of histamine on the local vasculature might contribute to the development of some of the gross features of the ethanol-induced lesions. If the importance of histamine in the pathogenesis of these lesions can be confirmed, the finding also provides at least one explanation for the occurrence of acute gastric erosions in mast cell-deficient mice. The glandular stomach histamine content of $WBB6F_1$-W/W^v mice is ~ 50% that of the congenic +/+ mice, indicating that only about half the histamine in $WBB6F_1$ mouse glandular stomach is associated with mast cells.[4,5] Studies in the rat[45–47] and the dog[48,49] suggest that at least some of this "non-mast cell histamine" may be concentrated in enterochromaffin cells. W/W^v and congenic +/+ mice have similar numbers of gastric enterochromaffin cells.[4] Thus, release of histamine from non-mast cell stores[1,2] might contribute to the development of gastric lesions in mast cell-deficient mice challenged with ethanol.

Whatever the resolution of questions about the precise role of the mast cell in ethanol-induced gastric injury, our results clearly demonstrate that genetic factors can influence the susceptibility of mice to ethanol-induced gastric injury. Not only were there clear differences in the amounts of ethanol-induced gastric injury induced in mast cell-deficient $WBB6F_1$-W/W^v and $WCB6F_1$-Sl/Sl^d mice and the congenic normal (+/+) mice, but we also observed differences in the magnitude of the responses in genetically distinct mice containing mast cells ($WCB6F_1$-+/+ > $WBB6F_1$-*an/an* or -*an*/+ > $WBB6F_1$-+/+). The reasons for these variations in response are not clear, but might reflect the influence of multiple genetically determined factors.

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