Occurrence of Gastric Ulcers in Gnotobiotic Piglets Colonized by *Helicobacter pylori*

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Received 8 December 1994/Returned for modification 7 February 1995/Accepted 2 March 1995

Archival gastric tissues from 10 of 39 gnotobiotic piglets colonized with *Helicobacter pylori* 26695 contained stromal leukocytic inflammation along with ulcers and erosions. In contrast, gastric tissues from 54 archival control piglets were devoid of both inflammation and ulcers. These data confirm the hypothesis that *H. pylori* infection alone is ulcerogenic.

Peptic ulceration is a common, chronic, recurrent, and occasionally life-threatening disease of poorly defined pathogenesis in humans (22, 36). Marshall and Warren (31) first suggested that ulcers may be a consequence of *Helicobacter pylori* infection rather than the result of stress, alcohol and tobacco consumption, or a diet high in salt and low in fiber (16, 30). While it is generally believed that *H. pylori* infection and associated inflammation account for virtually all NSAID-negative ulcers (1, 4, 7, 27, 28, 33, 37, 40), scientific proof for this hypothesis is lacking. Instead, investigators have relied upon epidemiologic data, concurrence observations (3, 18, 37, 43), and studies which emphasize bacterial products and metabolites (5, 29, 35, 38, 39, 45) or host tissue responses to them (6, 17, 26, 36) as causes of ulcerogenesis. The strongest evidence that infection, inflammation, and ulceration are linked is derived from antimicrobial trials with patients with endoscopically confirmed ulcer disease (19, 20, 24, 34, 36). Successful eradication is accompanied by a reduced rate of recurrence of ulcers 1 year later; failure to eradicate the bacterium results in high ulcer relapse rates (20, 21, 32). In spite of this strong association, ulcers occur in only a small fraction of infected individuals, a finding which suggests that *H. pylori* infection alone is not exclusively responsible for human ulcer disease (24, 26), and none of these associative studies completely excludes outside or unidentified influences as pivotal to ulcerogenesis.

H. pylori colonizes gnotobiotic piglets and produces gastritis histologically indistinguishable from that in humans (7, 17, 23, 41, 42), except for a reduced intensity of neutrophilic infiltrates in the former (2, 23). The piglet model is useful for delineation of bacterial colonization (virulence) factors (9–14) and is invaluable to the development of antimicrobial regimens. During the course of these antimicrobial trials, gastric ulcers and erosions were identified in several infected piglets. The microbe-free gnotobiotic environment combined with the absence of known ulcerogenic risk factors, including the confounding effects of other microbial products, indicates that *H. pylori* possesses intrinsic ulcerogenic ability and constitutes the basis of this retrospective study.

Bacterial inocula for the per os challenge consisted of 2.0 ml of brucella broth containing 10⁹ CFU of porcine-passaged *H. pylori* 26695, a motile, urease-positive (11, 12, 14) strain which

produces vacuolating cytotoxin (14) and possesses the cytotoxin-associated protein CagA ulcer marker (3a). In all, archival data for a total of 39 piglets from 17 litters colonized with *H. pylori* 26695 and constituting positive inoculum control groups from experimental therapy trials were studied (Table 1). Piglets were divided by gender and randomly assigned to treatment groups thereafter. Prior to inoculation, all piglets were rendered temporarily achlorhydric with cimetidine (60 mg/kg of body weight). Concurrent data for piglets contaminated with unrelated microbes were excluded, as were data for colonized piglets which received oral nonphysiologic excipients and data for proprietary-drug-treated piglets, even if the last expressed ulcers and remained culture positive for *H. pylori*. Archival gastric tissues from 27 piglets orally inoculated with noncolonizing strains of *H. pylori* and 27 microbially sterile uninfected piglets served as controls.

Stomachs were inspected for gross lesions prior to fixation in 10% neutral buffered formalin. Grossly detectable mucosal erosive or ulcerative lesions (Fig. 1) were infrequent (0 of 6 PID 3 piglets, 0 of 4 PID 14 piglets, and 3 of 29 PID 28 piglets) in gnotobiotic piglets colonized with *H. pylori* 26695 and were absent in the control groups. However, microulcers were fre-

TABLE 1. Summary of gross, microscopic, and cultural findings in gnotobiotic piglets colonized with *H. pylori* 26695

Piglets ^a	No. of ulcers and/or erosions in gastric tissue ^b		No. of <i>H. pylori</i> organisms (10 ⁶ CFU/g of gastric tissue)	
	Gross	Microscopic	Ulcers	No ulcers
PID 3 (<i>n</i> = 6)	0	0		25.8
PID 14 (<i>n</i> = 4)	0	2	34.0	11.4
PID 28 (<i>n</i> = 29)	3	8	8.5 ^c	6.7

^a A control group of uninfected piglets or piglets inoculated with noncolonizing *H. pylori* strains showed no signs of ulcers and/or erosions when examined at 8 days of age (*n* = 5), 13 to 15 days of age (*n* = 7), 13 to 24 days of age (*n* = 27), 23 to 27 days of age (*n* = 12), and 48 days of age (*n* = 3). One neonatal (3-day-old) germfree piglet contained two epithelial lesions compatible with microulcers or erosions (20a). No microbes were recovered from this piglet. The group 13 to 15 days of age contained an isolator (*n* = 4) contaminated with an unidentified yeast species (20a). The group 13 to 24 days of age contained piglets inoculated with the following noncolonizing strains: *H. pylori* Tx30a (*n* = 6) (14), urease-negative mutant *H. pylori* 26UI (*n* = 10) (12), and a nonmotile variant (*n* = 11) (11) of *H. pylori* 26695.

^b Ulcer is defined as penetration of the gastric mucosa into the lamina propria with a strong secondary tissue response; erosion defined as a loss of epithelium with a minimal underlying inflammatory response. Values are numbers of occurrences.

^c Mean CFU excludes one ulcer-bearing piglet with 5.6 × 10¹⁰ CFU/g.

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FIG. 1. Gross appearance of a large gastric ulcer in an *H. pylori*-infected gnotobiotic piglet. The deep surface of the ulcer crater is devoid of epithelium and consists of necrotic debris, exudate, and immature granulation tissue. A small epithelial erosion is also present (arrow). Both lesions were located along the greater curvature of the stomach at the approximate junction of the fundus and antrum. Total scale bar length = 1 cm.

quently detected in stained histologic sections (2 of 4 PID 14 piglets and 5 of 29 PID 28 piglets). Gastric epithelia of infected but not of control piglets exhibited focal mucus depletion (23), apical vacuolation (14), necrosis with or without associated

FIG. 2. Focal erosive epithelial lesion in the glandular gastric mucosa of an *H. pylori*-infected gnotobiotic piglet. The lesion is adjacent to an underlying developing inflammatory response in the lamina propria. (Hematoxylin and eosin stain; magnification, $\times 510$).

FIG. 3. Focal epithelial cytoplasmic vacuolation (arrowheads) in the gastric mucosa of an *H. pylori*-infected piglet; the vacuolation overlies inflammation and a developing lymphoid follicle. (Hematoxylin and eosin stain; magnification, $\times 525$).

microerosions (Fig. 2), and squamoid epithelial transformation over expanding submucosal lymphoid nodules (Fig. 3). In sections containing microulcers, tissue damage extended below the epithelial basement membrane, involving the lamina propria (Fig. 4). Both the lateral and deep margins were infiltrated with neutrophils. Desquamated debris, entrapped mucus, epithelia, and bacterial colonies were contained within ulcer craters. Replicate sections of microulcers stained with Masson's trichrome stain were negative for collagen deposition. Gastric tissue sections from all actively infected piglets showed evidence of a stromal leukocytic inflammatory response (2, 23) involving focal to diffuse lymphoplasmacytic cellular infiltrates, lymphoid follicles with germinal centers, and rare neutrophils and eosinophils. In several instances, microulcers developed within or over inflammatory foci; in others, there was no obvious relationship between ulceration and gastric inflammation. As reported elsewhere (2, 9–14, 23), gastric tissues from sterile gnotobiotic piglets and from piglets inoculated with noncolonizing strains of *H. pylori* were devoid of inflammatory cell infiltrates, their mucosal surfaces were intact, and their epithelia were normal.

Gastric ulcers have been reported to occur in ferrets infected with *Helicobacter mustelae* (25), in cheetahs cocolonized with *Helicobacter acinonyx* and a *Gastrospirillum* ("*Helicobacter heilmannii*") sp. (15), and in humans infected with *H. pylori* (1, 4, 18, 24, 27, 31, 33, 36, 37, 41). In all of these, the contributions

FIG. 4. Microulcer in the gastric mucosa of a gnotobiotic piglet infected with *H. pylori* 28 days previously. The ulcerative lesion extends downward, involving the muscularis mucosa (arrows). The ulcer crater is filled with mucus, desquamated epithelial debris, and colonies of bacteria. The margins of this lesion are infiltrated with neutrophils. (Hematoxylin and eosin stain; magnification, $\times 133$).

of the bacterial infection, host immune and inflammatory responses, diet, concurrent infection with other gastric microbes, and confounding factors such as alcohol and tobacco use cannot be determined. In contrast, *H. pylori*-colonized gnotobiotic piglets are confined to an environment which excludes all known outside ulcerogenic influences, and occurrence of ulcers in them constitutes direct proof that *H. pylori* alone is ulcerogenic.

The incidence of ulcers or erosions in archival tissues from colonized piglets was low (10 of 39 piglets), although higher than that reported for infected humans. In pigs, the small size and acute nature of the ulcers made them difficult to detect, even in formalin-fixed tissues. It is likely that the frequency would be higher if a detailed search of all gastric tissue was made by multiple sampling along the lesser curvature and antrum and/or by serial section examination of existent tissue blocks. The failure to detect gastric ulcers in our first study (23) is unexplained; however, the ability to detect infrequent events such as formation of ulcers improves as the number of piglets examined increases.

In humans, host and microbial factors contribute to ulcerogenesis. The importance of the host is exemplified by the tendency of ulcers to exhibit familial linkage (22, 25, 36). Our piglets are derived from genetically outbred parental stock, and no obvious difference between ulcer-bearing and ulcer-

free piglets was apparent. Human epidemiologic and pathologic studies correlate duration of infection, intensity of the inflammatory response, and estimated bacterial burden in the stomach to ulcer development (3, 7, 22, 24–26, 36, 37, 43); it is widely believed that *H. pylori*-associated gastritis precedes or promotes development of peptic ulceration (6–8, 17, 29, 35, 36, 38, 40). The piglet data do not prove that inflammation is a prerequisite for ulcerogenesis but do show that ulcers were not detected in inflammation-negative control piglets or in piglets infected with 26695 for only 3 days. Microbial factors also contribute to peptic ulceration. Bacterial sonicates are epitheliotoxic (44), and epidemiologic data suggest that in vivo expression of cytotoxin, the CagA gene or its product, or high bacterial loads in the stomach (22) promote ulcerogenesis. Proof that CagA is essential for ulcerogenesis must be obtained by in vivo testing of isogenic mutants. The development of clinically significant peptic ulceration is a multistep process. An absence of collagen deposition within porcine ulcer craters suggests that ulcers may well develop and then resolve without obvious long-term effects. Perhaps the event critical to ulcerogenesis is not mucosal damage but rather how the mucosa responds to injury. The piglet model permits examination of various facets of experimental ulcer disease with the ultimate aim to determine (and thus thwart) the ulcerogenic process in humans.

Portions of this research were supported by grant RO1 3950 from the NIDDKD, PHS.

We acknowledge the excellent technical assistance of Susan S. Ringler, Judith Younger, and Bryan Kessler.

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