Helicobacter pylori with a Truncated Lipopolysaccharide O Chain Fails To Induce Gastritis in SCID Mice Injected with Splenocytes from Wild-Type C57BL/6J Mice

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The goal of this study was to determine whether *Helicobacter pylori* lipopolysaccharide (LPS) O-chain polysaccharide contributes to gastritis in a mouse model. C57BL/6J or C57BL/6-*Prkdc*^{scid} (severe combined immunodeficient [SCID]) mice were inoculated with *H. pylori* strain SS1 or SS1::0826kan, in which a β -1,4-galactosyltransferase (HP0826), an LPS biosynthetic enzyme, had been disrupted. *H. pylori* strain SS1::0826kan expresses truncated LPS lacking O chain. Recipient SCID mice were given C57BL/6J splenocytes by intraperitoneal injection. Bacterial colonization, gastric lesions (gastritis, neutrophilic infiltration, and gastric epithelial metaplasia), cellular (delayed-type hypersensitivity) and humoral immune responses to *H. pylori* sonicate, and gastric gamma interferon (IFN- γ) mRNA expression were quantified. Recipient SCID mice colonized by *H. pylori* strain SS1 developed extensive gastritis with loss of normal fundic gland morphology. In contrast, gastric mucosa of recipient SCID mice colonized by *H. pylori* strain SS1::0826kan was not statistically distinguishable from that of uninfected recipient mice. Delayed-type hypersensitivity and humoral immune responses were detected in infected mice inoculated with wild-type SS1, but not with SS1::0826kan. IFN- γ transcription was lower in mice infected with SS1::0826kan than in mice infected with SS1. In this model of rapidly progressive gastritis due to *H. pylori*, the O chain contributed to the extent of gastritis and to the host immune response. These data support a role for *H. pylori* LPS O chain in direct induction of the host immune response leading to gastritis and gastric damage and are in contrast to protein antigens, such as urease and *cag* products which do not contribute to gastritis in mice.

Lipopolysaccharide (LPS), the major component of bacterial endotoxin, consists of a surface-expressed O-chain polysaccharide that is composed of oligosaccharide repeating units, a core oligosaccharide, and a lipid A backbone. In many gram-negative bacterial species, lipid A is considered the biologically active moiety of endotoxin and the cause of the endotoxic effects, including fever, nonspecific immunostimulation, the Schwartzman reaction, and death. The O-polysaccharide portions of the molecule are associated with B-cell stimulation and humoral immune response. However, *Helicobacter pylori* lipid A differs structurally from the lipid A of enterobacteria (41), and the endotoxic activity of its LPS is 100- to 1,000-fold lower. Thus, lipid A of *H. pylori* is much less likely to have a major pathogenic effect (6, 20, 33, 45). In contrast, it is the high-molecular-weight O-polysaccharide side chains of *H. pylori* LPS that have been implicated in colonization and/or pathogenesis of *H. pylori*-related disease.

Several O-chain polysaccharide variants may be important in determining host immune response and host range. The best studied are a group of fucosylated poly-N-acetyllactosamine O chains known as Lewis antigens. These structures are identical to blood group antigenic determinants found on human gastric

epithelial cells, and there has been speculation that they contribute to the pathogenesis of disease (40). For example, phase variation in Lewis antigen expression by *H. pylori* may allow colonizing organisms to adapt to their host, facilitating long-term colonization (2–4, 55). Alternatively, cross-reacting antigens may lead to host autoimmunity and thus exacerbate disease (42). In addition to Lewis antigens, *H. pylori* expresses other O-chain antigens that may also be predictive of disease. For example, Yokota et al. have identified several O antigens that appear to correlate with disease as do the Lewis epitopes (57, 58). In a study by Logan et al. (29), an *H. pylori* mutant expressing truncated LPS failed to induce a serological response in mice, indicating low immunogenicity and potentially a role in pathogenesis, at least in mice. Thus, there is experimental evidence that some O-antigen epitopes contribute to disease due to *H. pylori*, although the specific epitopes and the mechanism whereby this occurs are still being debated.

Clinical evidence notwithstanding, the suggestion that O chains mediate *H. pylori*-related disease presents a quandary. As noted above, O antigen is a polysaccharide antigen, and as such, is considered B cell specific, mediating humoral, but not cellular responses. *H. pylori* gastritis, in contrast, is now well established as a T-cell-dependent disease, resulting from induction of Th1-type CD4⁺ T cells (11, 14, 24, 34, 46). Recent evidence has indicated that LPS can, in fact, stimulate gamma interferon (IFN- γ) production from T cells in an antigen-dependent manner, suggesting that LPS could induce adaptive cellular and humoral immunity (32, 43, 54). IFN- γ production in these studies was attributed for the most part to lipid A, but

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in one study, IFN- γ induction by high concentrations of LPS was shown to be CD14 independent (43), suggesting that a lipid A-independent mechanism for T-cell stimulation may also exist. Thus, it is possible that the polysaccharide O antigen plays a role in *H. pylori*-associated disease. The purpose of the present study was to use a mouse model of severe gastritis to determine whether *H. pylori* O chain contributes to disease in mice; ultimately, the goal is to identify possible mechanisms whereby a B-cell antigen mediates a T-cell-mediated disease.

In a previous study (29), Logan et al. demonstrated that *H. pylori* O chain contributes to colonization fitness but is not essential for colonization by *H. pylori*. A β -1,4-galactosyltransferase enzyme was identified and functionally characterized and was shown to direct the addition of Gal to GlcNAc of the Lewis antigen precursor on the poly-*N*-acetylglucosamine chain of the O antigen (29). Inactivation of this enzyme resulted in a semirough mutant lacking O chains. In this study, we used the β -1,4-galactosyltransferase mutant to determine the roles of O chain in gastritis and delayed-type hypersensitivity (DTH) reactions.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study were the wild-type *H. pylori* strain SS1 and strain SS1::0826kan, an isogenic nonpolar mutant of SS1 inactivated by insertional mutagenesis of a kanamycin cassette into the β -1,4-galactosyltransferase gene (HP0826). Strain SS1 is a mouse-virulent isolate originally isolated from a human patient (27). It colonizes mice reproducibly to a density of 10^7 to 10^8 CFU/g of gastric mucosa, depending on the mouse strain (16, 27). The mutant strain SS1::0826kan expresses truncated O chain and does not colonize immunocompetent mice as well as the parental strain does (29). Construction and characteristics of the mutant strain SS1::0826kan are described elsewhere (29). Briefly, insertional mutagenesis resulted in a nonpolar mutant that did not express the β -1,4-galactosyltransferase gene. The resulting mutant strain synthesized a truncated LPS with a normal core polysaccharide, indicating that the β -1,4-galactosyltransferase gene is not involved in core biosynthesis. This structure was capped with only GlcNAc and fucose residues and did not produce the extended fucosylated polyglucosamine O-chain structure found in the parent strain. No other genetic or phenotypic differences were detected between the wild-type and mutant bacteria. Bacteria were cultured on 5% sheep blood agar or in brucella broth with 10% fetal bovine serum at 37°C in a microaerobic environment.

Mice. Four- to 6-week-old female C57BL/6J and C57BL/6J-*Prkdc*^{scid} (severe combined immunodeficient [SCID]) mice were purchased from Jackson Laboratory, which maintains a helicobacter-free colony. They were housed in sterile microisolator cages in barrier hoods and offered sterile lab chow (Teklad) and water ad libitum. For bacterial inoculation, mice were given 0.2 ml of brucella broth containing approximately 10^8 CFU of live broth-cultured *H. pylori* SS1 or SS1::0826kan by oral inoculation. For adoptive transfer, the spleens were removed from donor C57BL/6J mice and disaggregated in cold (4°C) Hanks buffered salt solution. Cells were washed, counted, and adjusted to 10^7 CFU/ml, and recipient SCID mice were given 0.1 ml by intraperitoneal injection. To determine DTH reactions to *H. pylori* antigens, mice were given 10 μ g of *H. pylori* sonicate in 30 μ l of phosphate-buffered saline (PBS) by injection into a rear hind footpad. The opposite hind pad received PBS alone. Before and 24 h after injection, footpad thickness was measured with a dial thickness gauge, and the results were expressed as the change in thickness (in millimeters) (16). DTH is an indication of the cell-mediated immune response.

Experimental design. This study used a total of 70 mice in three groups: wild-type C57BL/6J mice, SCID mice, and SCID mice that had received splenocytes from wild-type C57BL/6J mice by intraperitoneal injection (recipient SCID mice) 4 weeks after bacterial inoculation. These mouse strains were used on the basis of extensive evidence that they represent a rapid progressive model of severe gastritis due to *H. pylori* (14, 16). Each mouse group was divided into three subgroups: uninfected control, *H. pylori* strain SS1-infected, and SS1::0826kan-infected mice. In recipient SCID mice, DTH responses were evaluated by footpad injection of antigen 24 h prior to sacrifice. C57BL/6J and SCID mice do not develop DTH responses (16), and therefore, they were not evaluated by footpad injection. Thirteen weeks after bacterial inoculation, serum was collected and

stored at -20°C, the mice were killed, and their stomachs were aseptically removed and bisected. One-half of each stomach was weighed, and bacterial colonization was quantified by plate count (16). Strips of mucosa from the other half were immersed in formalin for histologic examination or snap-frozen for reverse transcriptase PCR (RT-PCR).

ELISA. Commercially available antibodies to Lewis X and Y antigens were used to detect *H. pylori* expression of O-antigen chains in Western blots and by enzyme-linked immunosorbent assays (ELISA). In addition, the serologic response of mice to *H. pylori* antigens was determined by ELISA as previously described (16). For determination of mouse immunoglobulin G (IgG), 96-well ELISA plates were coated with SS1 sonicate for 48 h at 4°C. The plates were washed, incubated with mouse serum diluted 1:50, washed, and incubated with peroxidase-labeled goat anti-mouse immunoglobulin. Binding was detected with the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt detection system according to the manufacturer's recommendations (Pierce, Inc., Rockford, Ill.). Results are expressed as optical density at 450 nm (OD₄₅₀). For detection of Lewis antigen expression, ELISA plates were coated as described above with either strain SS1 or SS1::0826kan and incubated with commercially available antibody directed against either Lewis X (BG-7) or Lewis Y (BG-8) antigen (both antibodies from Signet Pathology Systems, Inc., Dedham, Mass.). After the wells were washed with PBS three times, alkaline phosphatase-conjugated anti-mouse IgM was used to probe for the primary antibodies. The primary antibodies were detected with the 5-bromo-4-chloro-3-indolylphosphate (BCIP)/Nitro Blue Tetrazolium (NBT) phosphate substrate kit (Kirkegaard & Perry Laboratories, Gaithersburg, Md.), and the OD₄₅₀ was recorded.

Histopathology. For quantification of gastric lesions, strips of mucosa from the greater and lesser curvature (serosa side down) were allowed to adhere to paper towels and were fixed by immersion in 10% neutral buffered formalin and embedded in paraffin. Sections (5 μ m thick) were stained with hematoxylin and eosin, and the percentage of gastric mucosa affected by neutrophilic infiltration (polymorphonuclear leukocytes [PMN]), gastritis, and metaplasia (loss of fundic morphology and replacement by undifferentiated mucus-type epithelium) were quantified as previously described (16). Slides were examined in a blind manner by two pathologists (K. A. Eaton and R. A. Peterson), and the average score was reported. Greater than 10% affected mucosa was considered biologically significant (16).

RT-PCR. The levels of IFN- γ and glyceraldehyde-6-phosphate dehydrogenase (GAPDH) transcripts were determined for recipient SCID mice infected with the wild-type *H. pylori* strain SS1 and the mutant strain SS1::0826kan. Total RNA was isolated from snap-frozen gastric glandular tissue using a commercial RNA isolation kit (Qiagen, Inc., Valencia, Calif.). Five micrograms of total RNA was reverse transcribed using mouse Moloney leukemia virus RT enzyme (Gibco-BRL, Carlsbad, Calif.). One microgram of total cDNA was amplified by the PCR using specific forward and reverse oligonucleotide primers for murine IFN- γ and GAPDH. A commercial SYBR-green I amplification kit (Roche Molecular Biochemicals, Indianapolis, Ind.) was utilized on the LightCycler PCR system (Roche Molecular Biochemicals). Unknown test samples were amplified in parallel with serial dilutions of control cDNA for IFN- γ and GAPDH. Unknown sample concentrations were determined from the standard curves of the control cDNA. IFN- γ transcript concentration was reported as a copy number per 100 ng of cDNA.

Statistics. Means were compared by *t* test or by analysis of variance with Fisher's protected least significant difference to correct for multiple comparisons.

RESULTS

O-antigen expression by *H. pylori*. ELISA confirmed the loss of Lewis antigen expression consequent to O-chain loss in *H. pylori* strain SS1::0826kan. Lewis antigen expression (amount of IgG measured by OD₄₅₀) in mice infected with *H. pylori* strain SS1 incubated with anti-Lewis Y was 0.962 ± 0.290 , but no Lewis antigen expression in mice infected with SS1::0826kan was detected.

Bacterial colonization. In C57BL/6J and SCID mice, colonization by strain SS1::0826kan was significantly lower than colonization by strain SS1 ($P = 0.0088$ and $P = 0.0027$, respectively) (Fig. 1). In contrast, in recipient SCID mice, colonization by SS1::0826kan did not differ significantly from colonization by SS1 ($P = 0.091$). The difference in colonization

FIG. 1. Colonization density of *H. pylori* SS1 and SS1::0826kan (SS1::0826) in C57BL/6J, SCID, and recipient SCID mice. C57BL/6J and SCID mice were similar in that SS1 colonized these mice better than SS1::0826kan did. Colonization density of the two bacterial strains was reversed in recipient SCID mice, possibly because of suppression of SS1 colonization by severe gastritis (see text). Each point represents one mouse.

between recipient SCID mice and the other two strains is most likely due to suppression of SS1 colonization by severe gastritis in recipient mice (see below).

Gastritis. Gastritis affecting more than 10% of the gastric mucosa was not detected in uninfected mice or nonrecipient SCID mice (not shown). In C57BL/6J mice, mild gastritis and PMN (neutrophil) infiltrate and minimal gastric metaplasia were present in mice infected with strain SS1 and in mice infected with strain SS1::0826kan (Fig. 2). Consistent with previous findings, the lesions in C57BL/6J mice were mild and multifocal, and there were no differences in the lesions of mice in infected groups. In contrast to SCID and C57BL/6J mice, severe and extensive lesions were present in recipient SCID mice infected with strain SS1 (Fig. 3). As in previous studies, PMN infiltration and gastritis in many of these mice approached 100% of the gastric mucosa and were accompanied

FIG. 2. Gastric disease in recipient C57BL/6 mice colonized by wild-type *H. pylori* SS1 and by LPS mutant strain SS1::0826kan (SS1::0826). Indicators of gastric disease (PMN, gastritis, and metaplasia) in C57BL/6 mice were mild. The different values for mice infected with the SS1 and SS1:0826kan strains were not statistically significant. The values shown are the means $\pm$ standard errors of the mean (error bars).

FIG. 3. Gastric disease in recipient SCID mice colonized by wild-type *H. pylori* SS1 or LPS mutant SS1::0826kan (SS1::0826). In recipient SCID mice, severe PMN infiltration, gastritis, and metaplasia developed in mice colonized by SS1 but not in mice colonized by SS1::0826kan. There were significantly fewer lesions in mice colonized by SS1::0826kan than in mice colonized by SS1 (P values indicated), but the number of lesions in SS1::0826kan-infected mice was not significantly different from that in uninfected mice. The values shown are the means $\pm$ standard errors of the mean (error bars).

by extensive gastric mucosal metaplasia (Fig. 4C). Some uninfected recipient SCID mice had PMN infiltrates, but neither gastritis affecting more than 10% of the gastric mucosa nor metaplasia developed in these mice. In contrast to recipient SCID mice infected with SS1, lesions in recipient mice infected with SS1::0826kan were mild. PMN infiltration, gastritis, and metaplasia were present in these mice but were markedly and significantly less extensive than in SS1-infected mice ($P = 0.0002$, 0.0004 , and 0.0050 , respectively [Fig. 3]). The extent of lesions in mice infected with SS1::0826kan was not significantly greater than in uninfected mice.

Immune response. Because nonrecipient SCID mice do not develop immune responses, anti-*H. pylori* IgG was measured only in C57BL/6J and recipient SCID mice. The anti-*H. pylori* IgG responses of uninfected recipient SCID mice and mice infected with *H. pylori* strain SS1 or SS1::0826kan are shown in Table 1. Recipient SCID mice infected with SS1 developed the greatest response, significantly different from both uninfected mice ($P < 0.0001$) and recipient SCID mice infected with SS1::0826kan ($P = 0.0031$). Recipient SCID mice infected with SS1::0826kan developed IgG responses that were significantly

TABLE 1. Immune response of recipient SCID mice colonized by *H. pylori* SS1 or SS1::0826kan

Bacterial strain	IgG (OD ₄₅₀)	DTH response (mm ^a)	Gastric mucosal IFN- γ mRNA (copy number/ng of total cDNA)
None	0.191 $\pm$ 0.110	0.120 $\pm$ 0.035	Not detected
SS1	1.196 $\pm$ 0.163	0.435 $\pm$ 0.084	(2.17 $\pm$ 2.48) $\times 10^5$
SS1::0826kan	0.262 $\pm$ 0.066 ^b	0.183 $\pm$ 0.068 ^b	(1.83 $\pm$ 0.02) $\times 10^{1c}$

^a Difference between paw thickness before and 24 h after injection of *H. pylori* antigen (see Materials and Methods).

^b Significantly different from the values for both uninfected and SS1-infected mice ($P < 0.05$) (see text).

^c Significantly different from the value for SS1-infected mice ($P < 0.05$) (see text).

FIG. 4. Histologic photomicrographs of the gastric fundus of an uninfected recipient SCID mouse (A), and recipient SCID mice infected with *H. pylori* SS1::0826kan (B) or SS1 (C). (A) The gastric fundus from the uninfected mouse is normal, demonstrating dense populations of parietal cells in the mid-glandular region (bracket). (C) Gastritis in the SS1-infected mouse is characterized by loss of parietal cells, gastric mucosal hypertrophy (approximately twice the thickness of uninfected mucosa) and mucosal metaplasia, and wide-

greater than those of uninfected mice ($P = 0.032$) and less than those of SS1-infected mice ($P = 0.0031$). SS1-infected C57BL/6J mice also developed anti-*H. pylori* IgG antibody (0.639 ± 0.395).

Because recipient SCID mice are the only group to develop DTH responses, footpad tests were performed only in this group (Table 1). Footpad swelling in response to *H. pylori* antigens was greatest in recipient SCID mice infected with strain SS1 and was lowest in uninfected mice. Recipient SCID mice infected with strain SS1::0826kan developed swelling that was significantly greater than that of uninfected mice ($P = 0.032$) but significantly less than that of mice infected with SS1 ($P = 0.0031$).

IFN- γ in gastric mucosa. IFN- γ transcription in the gastric mucosa in recipient SCID mice infected with strain SS1 was detected (Table 1) and was markedly and significantly greater than IFN- γ transcription in the stomachs of recipient SCID mice infected with strain SS1::0826kan ($P = 0.009$). IFN- γ transcription was not detected in the stomachs of uninfected recipient SCID mice.

DISCUSSION

A long-standing challenge in *H. pylori* research is to explain the marked differences in the outcome of infection in different individuals. In some people, *H. pylori* is associated with severe manifestations, such as peptic ulcer disease and cancer, while others develop only mild, asymptomatic gastritis. Increasing evidence implicates both bacterial and host factors in the outcome of disease, but the details of these influences and their relative importance remain unclear. Differences in disease outcome in individual human patients is mirrored in animal studies showing that in both outbred monogastric and inbred mouse strains, the host species and/or strain is a determinant of the severity of disease. *H. pylori* strains that cause severe disease in one host or species may cause minimal or no disease in another (15), and differences in responsiveness in inbred strains of mice are now well established (35, 50, 52). That said, it is also clear that some bacterial factors contribute to disease. Strong epidemiologic associations suggest that a number of putative virulence factors, including LPS, VacA, and the products of the *cag* pathogenicity island contribute to severe manifestations of disease (8, 47, 48). In vitro studies demonstrate a variety of effects of bacterial products on cultured cells that could be associated with disease in vivo (9, 10, 25, 28, 44). Therefore, a major focus of interest has been to identify potential differences in bacterial strains and to determine how these differences could explain differences in the outcome of infection.

The potential role of *H. pylori* O antigens in human disease has engendered considerable study. Of particular interest is the finding that many strains of *H. pylori* express O antigens that cross-react with Lewis antigens, complex glycoconjugates that

spread mixed inflammatory infiltrate, indicated by the arrows. (B) Gastritis (arrows) and mucosal metaplasia (bracket) are also present in the mouse infected with SS1::0826kan, but lesions were rare, and their extent was not statistically different from uninfected mice (see text). Original magnification, $\times 200$.

are expressed on the surfaces of many human cells, including those of the gastric surface epithelium. This cross-reactivity has generated much research interest, and of the *H. pylori* O antigens, the cross-reacting Lewis antigens have received the most attention.

There are several major hypotheses regarding the potential role of Lewis antigens in *H. pylori*-related disease. O antigens are polysaccharides and as such, are expected to induce a humoral immune response in infected hosts. *H. pylori* strains that express Lewis antigens induce antibodies that cross-react with host Lewis antigens (5), and these are the antibodies that have been implicated in disease. Some investigators suggest that such cross-reacting antibodies could induce autoimmune-like gastritis (5). Support for this suggestion comes from identification of autoantibodies against gastric epithelium in helicobacter-infected humans and mice (5). Alternatively, cross-reactive Lewis antigens could protect the bacteria by preventing a host immune response (38). Finally, it has been suggested that Lewis antigens mediate adherence of *H. pylori* to the gastric epithelium, thus aiding delivery of pathogenic molecules to the host cell (39).

Clinical evidence to support these suggestions is conflicting. In one study, people infected with *H. pylori* that expressed Lewis antigen had more severe disease than people infected with *H. pylori* that did not express Lewis antigen (38). This suggests that Lewis antigens may be associated with disease but does not indicate the mechanism. Several studies have shown an association between expression of antibodies to Lewis antigens (21, 22, 53, 60) or other O antigens (57) with severe disease due to *H. pylori*. Not all clinical studies have demonstrated an association, however (23, 51), and as in the study cited above, these correlative studies do not suggest a mechanism. Overexpression of anti-Lewis antibodies in mice has been associated with development of gastritis (5), suggesting a direct role for antibodies in disease. Finally, *H. pylori* O antigens that do not mimic Lewis antigens have been reported (1, 26, 37, 58), and serological response to non-Lewis O antigens has been associated with clinical disease (57).

Thus, studies of the role of host immunologic response to Lewis antigens in gastritis have shown some disease associations but have focused on serologic immune response. However, there is mounting evidence suggesting that the host antibody response has a minimal role in gastritis induction. Experiments with genetically engineered mice that are incapable of making specific antibody have demonstrated that both protective and pathogenic immunity are dependent on cellular immune responses (46). Adoptive transfer studies have shown that disease is dependent on CD4⁺ T cells and that B cells are irrelevant for disease in mice (14). Correlative studies in humans indicate that cellular immunity to *H. pylori* antigens is associated with severe disease and that the proinflammatory cytokines and characteristics of the immune response in the human stomach are compatible with a Th1, or cell-mediated, immune response (11). These findings are difficult to reconcile with the suggestion that a polysaccharide antigen induces or exacerbates gastritis.

In *H. pylori*-infected recipient SCID mice, the animal model used in this study, gastric disease is consistent, severe, and quantifiable (16). Further, the immunologic pathogenesis of gastritis in recipient SCID mice is similar to that of gastritis

due to *H. pylori* in humans. In both mice and humans, gastritis and gastric epithelial lesions are CD4 and Th1 dependent and are associated with elevated IFN- γ secretion and cellular immune responses to *H. pylori* antigens (14). Eaton et al. (12) used the adoptive transfer model to evaluate the role of urease in the immunopathogenesis of *H. pylori* gastritis, and surprisingly, showed that neither the urease activity of the colonizing strain nor density of colonization affected the severity of gastritis. The dependence of gastritis on O chain LPS demonstrated in this study is the first evidence we have found to implicate a bacterial virulence factor in this mouse model of severe disease due to *H. pylori*.

The results reported here indicate that *H. pylori* O antigen is a major determinant of both immune response and disease outcome in recipient SCID mice. In contrast to wild-type *H. pylori* strain SS1, strain SS1::0826kan expressing truncated LPS failed to induce gastric lesions that were significantly different from background lesions in uninfected recipient SCID mice. Further, the absence of O chains resulted in much weaker serologic and cellular immune responses. These results are in contrast to previous studies that demonstrated no role for urease, bacterial colonization density, or *cag*-related proteins in disease due to *H. pylori* (12, 13). In a recent study, for example, Eaton et al. demonstrated that *H. pylori* mutants with weak urease activity were similar to the LPS mutant tested here in that they had diminished colonization density, but unlike the LPS mutant, they induced gastritis that was indistinguishable from that induced by the parental *H. pylori* strain (12). Thus, despite virtually identical densities of colonization, mutants with diminished urease activity cause gastritis in mice, while mutants with truncated LPS do not. This indicates that, unlike urease, O antigen is a determinant of severity of gastritis in this model.

In a previous study (12), evaluating the colonization ability of strain SS1::0826kan, Eaton et al. showed that the mutant did not colonize as well as the wild type did and, interestingly, that mice colonized by mutant *H. pylori* failed to mount a serologic immune response. In the present study, we confirmed the observation that the loss of O chain results in greatly diminished serologic immune response by the host; in addition, we observed that the cell-mediated immune response, as evaluated by DTH response and gastric IFN- γ expression, is also lower than the response of mice with LPS O chain. Finally, we used a model of severe gastritis to demonstrate that the decreased immune response is associated with greatly diminished gastritis in mice infected with mutant *H. pylori* compared to mice infected with wild-type *H. pylori*.

Because polysaccharide antigens are considered B-cell antigens and the preponderance of the evidence indicates that *H. pylori* gastritis is a T-cell-mediated disease, the outcome of the present study was unexpected. In both humans (11, 24, 30) and mice (14, 34), Th1-polarized local and systemic responses correlate highly with disease (in contrast to serologic responses which do not correlate with disease). In mice, T cells (14), but not B cells or antibody (7, 46), are necessary and sufficient for disease induction as well as for clinical response to vaccination. Therefore, although there is epidemiologic evidence in the human host for a correlation between O antigen and disease due to *H. pylori*, our results remained unexpected.

The mechanism for the apparent involvement of a polysac-

charide antigen in a disease mediated by cellular immunity is still speculative. As indicated above, simple differences in colonization density have been ruled out by a previous study showing no correlation between colonization density and gastritis in an *H. pylori* urease mutant (12). It is possible that O-chain-mediated disease may be due to nonspecific immune stimulation by O chain leading to upregulation of immune response to all bacterial antigens, including protein antigens. In such a model, the O chain would be necessary to allow full expression of the cellular immune response to *H. pylori* protein antigens such as urease and *cag*-associated proteins. This model would explain why the lack of production of O chain and consequent generalized suppression of antibacterial immune responses decrease the inflammatory response to all bacterial antigens, resulting in mild or absent disease, while deletion of one or a few of these antigens (e.g., urease or Cag antigens), as was done in previous studies (12, 13) would not have a clinically evident effect.

The suggestion that LPS O chain has nonspecific immunostimulatory effects is not unprecedented. Classically, LPS is considered a T-cell-independent antigen with direct effects on macrophages and B cells, and lipid A is considered the active principle. Because *H. pylori* lipid A has low biologic activity, it is not considered a major virulence factor in this way. However, there is some evidence that LPS in general, and polysaccharides specifically, may contribute to cellular and humoral immune responses. In an early study, *Bacteroides fragilis* LPS was shown to induce IFN- γ in murine splenocyte cultures, indicating T-cell stimulation not described in studies of enterobacterial LPS (56). In another study, LPS polysaccharide from *Serratia marcescens* induced proliferation of splenocytes and IFN- γ secretion. The effects were enhanced by coinubation with interleukin-2, consistent with a T-cell effect (19). More recent studies have demonstrated LPS receptors on T cells (31, 36), upregulation of major histocompatibility complex class II molecules on B cells by *Brucella abortus* LPS O chain (17), and possibly other effects of O antigen on cellular immunity (18, 59). Furthermore, direct antigen-presenting cell-mediated and antigen-specific stimulation of T cells by LPS and lipid A has recently been described (32, 43, 54). Such stimulation may in part be CD14 independent (43), suggesting that moieties other than lipid A could have an effect. Finally, evidence that LPS from bacteria that differ markedly from *E. coli* (as does *H. pylori*) may have markedly different effects continues to emerge. In a recent study, for example, LPS from *Porphyromonas gingivalis*, an organism that differs taxonomically from *E. coli* was shown to have receptor affinity and biologic activity that contrasted markedly from those of enterobacterial LPS (49). Thus, the well-studied effects of *E. coli* LPS and lipid A may not reflect the range of possible activities of this macromolecule. The *H. pylori* results reported herein along with previous reports of O-chain effects of other gram-negative bacteria may implicate a previously unrecognized role for bacterial polysaccharide in host immune response and disease.

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