The role of major histocompatibility complex (MHC) class I- and class II-restricted functions in *Helicobacter pylori* infection and immunity upon oral immunization was examined in vivo. Experimental challenge with *H. pylori* SS1 resulted in significantly greater (*P* < 0.025) colonization of MHC class I and class II mutant mice than C57BL/6 wild-type mice. Oral immunization with *H. pylori* whole-cell lysates and cholera toxin adjuvant significantly reduced the magnitude of *H. pylori* infection in C57BL/6 wild-type (*P* = 0.0083) and MHC class I knockout mice (*P* = 0.0048), but it had no effect on the *H. pylori* infection level in MHC class II-deficient mice. Analysis of the anti-*H. pylori* antibody levels in serum showed a dominant serum immunoglobulin G1 (IgG1) response in immunized C57BL/6 wild-type and MHC class I mutant mice but no detectable serum IgG response in MHC class II knockout mice. Populations of T-cell-receptor (TCR) αβ⁺ CD4⁺ CD8⁻ cells localized to gastric tissue of immunized C57BL/6 wild-type and MHC class I knockout mice, but TCRαβ⁻ CD4⁺ CD8⁺ cells predominated in the gastric tissue of immunized MHC class II-deficient mice. These observations show that CD4⁺ T cells engaged after mucosal immunization may be important for the generation of a protective anti-*H. pylori* immune response and that CD4⁺ CD8⁻ and CD4⁻ CD8⁺ T cells regulate the extent of *H. pylori* infection in vivo.

**MATERIALS AND METHODS**

**Helicobacter pylori** infection and immunization schedules. Groups (*n* = 10) of 6- to 8-week-old *C57Bl/6* mice were challenged by oral gavage with *H. pylori* SS1 (19; kindly provided by A. Lee, the University of New South Wales). Previous studies demonstrated that this strain of *H. pylori* challenge doses resulted in 100% infection rates and represented >10⁵ 50% infective doses in C57Bl/6 mice. The experiment was terminated 2 weeks after *H. pylori* challenge.

**Preparation of *H. pylori* antigens.** *H. pylori* SS1 was grown on tryptic soy agar (TSA) plates (Becton Dickinson, Cockeysville, Md.) containing 5% sheep blood (Remel, Lenexa, Kans.) and 100 μg of vancomycin, 3.3 μg of polymyxin B, 200 μg of bacitracin, 10.7 μg of nalidixic acid, and 50 μg of amphotericin B (Sigma Chemical Co., St. Louis, Mo.) per ml. The plates were incubated for 72 to 80 h at 37°C in 10% CO₂ and 5% O₂ in a Trigas incubator (Queue Systems, Asheville, N.C.). The bacteria were then harvested, inoculated into brucella broth (BBL; Becton Dickinson) supplemented with 5% fetal bovine serum (Intergen, Purchase, N.Y.), and shaken at 120 rpm at 37°C in the Trigas incubator. Cultures were grown to an optical density of 800 nm (OD₈₀₀) of 0.3 (ca. 5 × 10⁶ CFU/ml) and diluted in brucella broth for inoculation. Prior to use, *H. pylori* cells were analyzed in wet mounts to assess motility and morphology and subjected to urease, catalase, and oxidase tests.

**Assessment of *H. pylori* infection.** Longitudinal segments of gastric tissue were homogenized in 0.5 ml of brucella broth, and replicate serial 10-fold dilutions were plated on *Helicobacter*-selective blood agar plates. The plates were incubated (37°C in a Trigas incubator), and quantitation of the CFU was performed 5 to 7 days later. For determination of infection with a urease assay, segments of antrum, including the corpus-antrum junction, were incubated in urea broth as described elsewhere (15). After 4 h, the extent of color change was recorded in an automated enzyme-linked immunosorbent assay (ELISA) reader at OD₅₄₀.
TABLE 1. *H. pylori* infection in C57BL/6 wild-type and MHC mutant mice

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Infection level</th>
<th>No. of mice positive/total no.</th>
<th>Quantitative <em>H. pylori</em> culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6 wild type</td>
<td>1.51 ± 0.41</td>
<td>10/10</td>
<td>1.12 ± 0.37</td>
</tr>
<tr>
<td>β2m−/− MHC class I deficient</td>
<td>1.87 ± 0.52</td>
<td>10/10</td>
<td>2.41 ± 0.38</td>
</tr>
<tr>
<td>Aβ MHC class II deficient</td>
<td>2.14 ± 0.54</td>
<td>9/10</td>
<td>2.59 ± 0.55</td>
</tr>
</tbody>
</table>

a Mice were challenged per os with 10^6 CFU of *H. pylori* SS1. After 2 weeks, gastric tissues were examined for infection level by a quantitative urease assay.

b Mean OD_{550} ± standard error of the mean for each group.

c Quantitative *H. pylori* culture was defined as an OD_{550} > 2 standard deviations above the group mean OD_{550} from unchallenged control mice.

d Culture was performed on segments of gastric tissue at 2 weeks after *H. pylori* challenge as described in Materials and Methods.

RESULTS

*H. pylori* infection in MHC class I and class II mutant mice. The contribution of MHC class I or class II functions to experimental infection with *H. pylori* was examined. Challenge of MHC knockout mice and of C57BL/6 wild-type mice with *H. pylori* resulted in 90 to 100% infection rates (Table 1). Evaluation of the magnitude of colonization by quantitative bacterial culture showed a significantly increased *H. pylori* burden in MHC class I (*P* = 0.017) and MHC class II (*P* = 0.025) mutant mice compared to that in C57BL/6 wild-type mice (Table 1).

Protective immunity in MHC mutant mice with *H. pylori* antigens. The ability of oral immunization to limit the severity of *H. pylori* infection was investigated. Whereas oral immunization with *H. pylori* lysate antigens resulted in a significant reduction of the *H. pylori* infection level in C57BL/6 wild-type (*P* = 0.0083) and in MHC class I-deficient (*P* = 0.0048) mice relative to those immunized with CT alone, immunization of MHC class II mutant mice with *H. pylori* lysate antigens had no effect on the magnitude of *H. pylori* infection (Fig. 1). Oral immunization of wild-type and MHC-deficient mice with CT did not affect the *H. pylori* density relative to treatment of the corresponding strain with buffer alone (not shown).

Immune response in MHC knockout and C57BL/6 control mice. Oral immunization with *H. pylori* cell lysate antigens resulted in the development of a serum IgG anti-*H. pylori*...
antibody response of greater magnitude in C57BL/6 wild-type mice than in MHC class I mutant mice (Fig. 2). A higher level of serum IgG1 than IgG2a anti-H. pylori was consistently elicited as a function of oral immunization of wild-type and MHC class I mutant mice, but MHC class I knockout mice exhibited a depressed serum IgG1 and IgG2a response compared to C57BL/6 wild-type mice. In contrast, gastric H. pylori-specific IgA antibody levels were greatest in MHC class I mutant mice (Fig. 2). Serum IgG and gastric IgA anti-H. pylori antibody responses were not measurable in immunized MHC class II knockout mice. Challenge of buffer- or CT-treated MHC class I knockout and wild-type responder mice with 10^6 H. pylori organisms stimulated low anti-H. pylori antibody responses in serum (IgG1 levels, 0.30; IgG2a levels, 0.05 [OD405]) and gastric (0.025) compartments.

Phenotypic composition of gastric resident leukocytes in immunized MHC mutant mice. The distribution of mononuclear leukocytes infiltrating gastric tissue in C57BL/6, MHC class I-deficient, and MHC class II knockout mice was investigated by immunohistochemistry. Populations of T-cell-receptor (TCR) αβ^+ CD4^+ cells were the dominant T cells resident in the antral mucosa of C57BL/6 wild-type and MHC class I knockout mice (Table 2). In contrast, a high frequency of TCRαβ^+ CD4^+ CD8α^+ cells were observed scattered in the antral mucosa and epithelia of MHC class II mutant mice (Table 2). While approximately 10 to 20% of the gastric leukocytes expressed the α4 integrin subunit, the majority of infiltrating leukocytes in wild-type and MHC mutant mice exhibited the CD54 activation marker (Table 2). Analyses of the IgA^+ B-cell populations showed that immunized wild-type and MHC knockout mice accumulated equivalent frequencies of IgACC in the gastric antrum. Examination of gastric tissue from MHC knockout mice with anti-H-2Kb and -H-2Db MAb or with anti-I-Ab MAb confirmed the absence of MHC class I or class II expression in the epithelium and gastric resident leukocytes in the appropriate mutant mice.

DISCUSSION

Infection with Helicobacter spp. results in the recruitment of CD4^+ and CD8^+ T cells in gastric tissue (2, 14). The accumulation of gastric Th1-type H. pylori-specific CD4^+ cells has been proposed to account for their failure to generate protective immunity and to contribute to disease pathogenesis (2, 9). Although peripheral T cells derived from infected mice are unable to mediate clearance when adoptively transferred into H. felis-infected recipients (21), the present findings, showing that MHC deficiency exacerbates H. pylori infection, suggest an

![FIG. 2. Antibody responses in serum (A) and in gastric secretions (B) of wild-type and MHC knockout mice. Groups of immunized mice were challenged with live H. pylori. Two weeks later, the serum IgG1 (open bars), serum IgG2a (solid bars), and gastric IgA (striped bars) were quantitated by ELISA. The bars show the mean OD_405 for each group, and the brackets indicate 1 standard error of the mean.](http://iai.asm.org/)

| TABLE 2. Phenotypes of the infiltrating gastric leukocytes in immunized C57BL/6 wild-type and MHC mutant mice^a^ |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Mouse strain                    | Mean no.° of positively labeled cells/mm² of gastric antrum ± SEM with:  |
|                                 | TCRαβ            | CD4             | CD8α            | CD49d           | CD54            | IgACC           |
| C57BL/6 wild type               | 31 ± 3           | 25 ± 6          | 2 ± 0           | 7 ± 2           | 24 ± 3          | 37 ± 4          |
| β2m^-/- MHC class I deficient    | 25 ± 1           | 20 ± 3          | 1 ± 1           | 3 ± 1           | 16 ± 2          | 26 ± 8          |
| A^b MHC class II deficient      | 82 ± 15          | 1 ± 1           | 68 ± 11         | 17 ± 4          | 44 ± 4          | 57 ± 13         |

^a^ Mice were immunized with H. pylori lysate antigens and CT adjuvant and subsequently challenged with 10⁹ CFU of H. pylori SS1. Gastric biopsies were excised 2 weeks later and were frozen for immunohistochemical staining with the MAb indicated.

^b^ Mean numbers of positively labeled cells/mm² of gastric antrum from groups of five mice.
important regulatory role for CD4+ and CD8+ T cells and/or their secreted cytokines during the early course of infection. Oral immunization of MHC class II mutant mice with H. pylori antigens resulted in a significant reduction of the gastric H. pylori burden and in the generation of anti-H. pylori serum IgG and gastric IgA antibody. In contrast, MHC class II-deficient mice were unable to respond to oral antigenic stimulation and remained persistently infected with H. pylori. Because MHC class I-deficient mice are virtually devoid of peripheral CD4+ CD8+ and intestinal CD8+ intraepithelial lymphocytes (6, 31), while MHC class II mutant mice essentially lack mature CD4+ T cells in peripheral lymphoid organs (16), the present study strongly implicates CD4+ T cells in the protection against H. pylori infection upon mucosal immunization. The findings in immunized MHC class II knockout mice of a high frequency of gastric TCRαβ+ CD4+ CD8+ CD54+ T cells further support the notion that antigen-specific CD4+ T cells activated after mucosal immunization may be involved in protective immunity against H. pylori in vivo. Although the events in T-cell activation, recirculation, and cytokine secretion are not understood, a low incidence of αβ+ T cells was observed in gastric tissue of immunized wild-type and MHC knockout mice. The αβ integrin can be coexpressed either with the β1 subunit to form VLA-4, which mediates leukocyte transendothelial migration (28), or with the β7 integrin subunit to direct lymphocyte homing via the mucosal vascular addressin MadCAM (3). The small numbers of αβ+ TCRαβ+ cells suggested that T-cell populations recruited into gastric tissue after immunization may be largely derived from peripheral lymphoid organs, rather than recirculating from Peyer’s patches or the intestinal lamina propria.

Previous studies have suggested that mucosal IgA (8, 24) or IgG (13) antibody may mediate immunization-induced protection from Helicobacter infections, and this might explain the absence of effective immunity in MHC class II-deficient mice reported here. However, the relationship between antibody level and the extent of host protection against Helicobacter infections is discordant (20), and oral immunization may confer protection from H. felis infection in B-cell-deficient μMT mice (22). Furthermore, while MHC class I mutant mice show a less-efficient serum antibody response than do wild-type mice (28) and, as shown in this study, also exhibit depressed responses to anti-H. pylori IgG responses after oral immunization, the extent of protective efficacy against H. pylori was comparable to that observed in wild-type mice. The finding that orally immunized MHC class II mutant mice generated a frequency of gastric IgACC equivalent to that of wild-type and MHC class I mutant mice is consistent with observations that CD4-deficient mice fail to respond to oral antigenic stimulation despite having a normal complement of mucosal IgA B cells (18). Thus, while orally immunized wild-type and MHC knockout mice harbored equivalent frequencies of IgACC, the magnitude of serum or gastric antibody responses was not predictive of the extent of protective immunity.

A common histopathologic feature in H. felis-immunized mice is the progressive infiltration into gastric tissue of CD4+ and, to a greater extent, CD8+ T-cell populations of unknown function (12). The observations reported here that gastric tissue of MHC class II knockout mice contained a high incidence of CD4+ CD8+ CD54+ activated T cells within 2 weeks of H. pylori challenge may help to define the functional contribution of CD8+ cells to gastritis and epithelial-cell damage in the absence of CD4+ T cells. While gastric leukocyte infiltration appears to be a hallmark of protective anti-Helicobacter immunity in murine models, the H. pylori infection density in humans has been found to be associated with the degree of gastritis (1), and patients infected with the human immunodeficiency virus (HIV) show a lower incidence of gastritis and a lower prevalence of H. pylori infection relative to HIV-negative subjects (5, 11). Because T-cell recruitment into murine gastric tissue may be mediated by residual antigenic stimulation by live Helicobacter organisms in previously immunized hosts (12) and because CD4+ cells can regulate mucosal inflammatory reactions (27), the findings reported here raise the possibility that CD4+ T-cell populations generated during immunization in vivo may play an important role in limiting the severity of an H. pylori infection. Currently, it is not clear whether the lack of immune protection identified in MHC class II knockout mice is related to a deficit of CD4+ T cells in peripheral and gastric tissues or whether it is associated with elimination of class II MHC-restricted antigen presentation by gastric epithelia. However, both the current findings and the observation that T cells can reduce the H. felis burden when adoptively transferred into unimmunized recipients (21) strongly implicate CD4+ T cells in the protection against H. pylori infection.

ACKNOWLEDGMENTS

We thank Maria Uria-Nickelsen for helpful discussions and Heather Kamp for technical assistance with bacterial quantitation.

REFERENCES


H. PYLORI INFECTION IN MHC KNOCKOUT MICE 341

Editor: R. N. Moore