

CD8⁺ T Cells Are Associated with Severe Gastritis in *Helicobacter pylori*-Infected Mice in the Absence of CD4⁺ T Cells[∇]

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***Helicobacter pylori* infection results in the development of chronic gastritis, and CD4⁺ T cells are a major component of the gastric cellular infiltrate. To examine whether CD4⁺ T cells are important in initiating and maintaining *H. pylori*-induced gastritis, mice deficient in CD4⁺ T cells (B6.BM1.GK 1.5 mice [GK 1.5 mice]) were infected with *H. pylori*. We found that as in normal mice, *H. pylori*-specific antibodies, mostly of the immunoglobulin M isotype, developed in GK 1.5 mice but were unable to cure *H. pylori* infection. Further, while the stomachs of *H. pylori*-infected GK 1.5 mice were more heavily infiltrated with CD8⁺ T cells and B cells, mice deficient in both CD4⁺ and CD8⁺ T cells developed mild inflammation comparable to the level observed for C57BL/6 mice. These observations suggest that CD4⁺ T cells may play an important role in regulating or suppressing gastric CD8⁺ T cells which, in the absence of CD4⁺ T cells, may mediate more-severe disease. These studies have revealed a potentially important role for CD8⁺ T cells in the gastric disease resulting from *H. pylori* infection.**

Helicobacter pylori colonizes approximately 50% of the world's population, resulting in persistent stomach inflammation in infected individuals (30). Chronic *H. pylori* gastritis can lead to the development of gastric and duodenal ulcers and, ultimately, gastric cancer (10, 14, 15). The growth of *H. pylori* is restricted to the stomach lumen and epithelium, a site poorly served by classical immune effectors, and the proximal duodenum, and successful attempts at bacterial eradication through therapeutic vaccination have not been achieved (29). Immunomodulation might provide an alternate means of reducing *H. pylori*-mediated disease through qualitative changes to the inflammation induced by chronic infection. Any rational attempt at immunomodulation will require a more complete understanding of the bacterial and host drivers of chronic inflammation and the cellular infiltrate which is characteristic of *Helicobacter*-associated gastritis.

CD4⁺ T cells are a major component of the gastric cellular infiltrate in both human and experimental animal models of *H. pylori* infection (5–7, 18, 27). Severe combined immunodeficiency (SCID) mice that receive adoptively transferred splenic CD4⁺ T cells develop gastritis after *Helicobacter* infection, but mice which received splenocytes depleted of CD4⁺ T cells do not (7, 20). Hence, CD4⁺ T cells are believed to be fundamental to the initiation and maintenance of gastric inflammation. We aimed to examine the role of CD4⁺ T cells with respect to both effector and regulatory function and to determine

whether gastritis was dependent on infiltration by CD4⁺ T cells. In this study, CD4⁺ T-cell-deficient mice were infected with *H. pylori* and examined for the development of gastritis.

MATERIALS AND METHODS

Mice. Female C57BL/6 mice were reared under specific-pathogen-free conditions at the animal facility of the Department of Microbiology and Immunology, The University of Melbourne. Female B6.BM1.GK 1.5 (GK 1.5) (33) and B6.BM1.GK/2.43 (GK/2.43) (31) mice were obtained from the Walter and Eliza Hall Institute (Melbourne, Australia) and housed at the animal facility of the Department of Microbiology and Immunology, The University of Melbourne. Mice were from 6 to 16 weeks old at the time of *H. pylori* infection, and age-matched naive mice were included in all experiments. All animal experiments were approved by The University of Melbourne Animal Ethics and Experimental Committee and complied with relevant legislation.

***H. pylori* infection, quantification from the stomach, and assessment of serum anti-*H. pylori* antibody titers.** The mouse-adapted *H. pylori* SS1 strain (16) was kindly provided by Hazel Mitchell (University of New South Wales, NSW, Australia). The methods used for laboratory bacterial culture, mouse infection, and isolation from mouse stomachs were as described previously (13). Total *H. pylori*-specific and *H. pylori* lipopolysaccharide (LPS)-specific antibody titers from mouse sera were determined by enzyme-linked immunosorbent assay (ELISA) as described before (13). Briefly, Maxisorp immunoplates (Nunc, A/S) were coated with 5 µg/ml *H. pylori* sonicate or purified LPS and the plates were blocked with skim milk and washed. Following incubation with serum samples, total *H. pylori*-specific antibodies were detected using sheep anti-mouse immunoglobulin (Ig) conjugated to horseradish peroxidase (Chemicon). Mouse sera were analyzed individually for *H. pylori*-specific ELISAs and pooled for LPS-specific ELISAs. IgG and IgM isotypes were determined in the same manner, except that rabbit anti-mouse IgG1 or IgG3 (ICN Biomedicals) was added after serum incubation followed by detection with goat anti-rabbit Ig conjugated to horseradish peroxidase (Sigma).

Purification of *H. pylori* SS1 for determination of anti-*H. pylori* LPS-specific antibody levels. *H. pylori* LPS was kindly provided by Rebecca J. Gorell (Department of Microbiology and Immunology, University of Melbourne) and was purified from *H. pylori* SS1 based on the methods described by Hitchcock and Brown (12). Briefly, *H. pylori* cells were lysed with LPS cell lysis buffer (1 M

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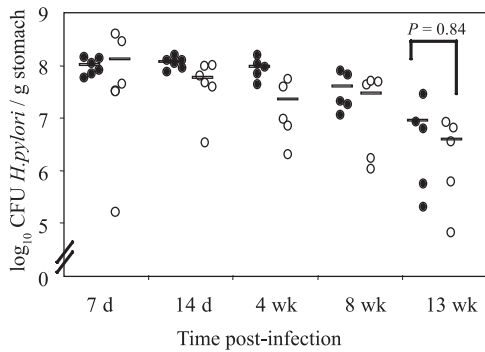


FIG. 1. Progression of *H. pylori* infection in GK 1.5 mice. *H. pylori* was isolated from the stomachs of C57BL/6 and GK 1.5 mice up to 13 weeks postinfection with *H. pylori* and enumerated by viable count. Represented are results from individual C57BL/6 (●) and GK 1.5 (○) mice, with the mean of each group represented by the bars. d, days; wk, weeks.

Tris-HCl [pH 6.8], 10% [vol/vol] glycerol, 4% [vol/vol] β -mercaptoethanol, 2% [wt/vol] sodium dodecyl sulfate, 0.0002% [wt/vol] bromophenol blue) before heat denaturation by boiling for 10 min. Proteins were digested with 0.5 mg/ml proteinase K (Sigma) and LPS was extracted with hot phenol before dialysis with water.

Assessment of stomach inflammation by histology. Mouse stomachs were dissected, fixed in formalin, embedded, sectioned, and stained with hematoxylin and eosin as described previously (13). Stomach sections were blindly graded by a pathologist using criteria defined elsewhere (28). Briefly, the severity of chronic inflammation was assessed and graded according to the density and distribution of lymphocytes. The inflammation scores used in the grading system were as follows: 0, none; 1, mild multifocal; 2, mild widespread or moderate multifocal; 3, mild widespread and moderate multifocal or severe multifocal; 4, moderate widespread; 5, moderate widespread and severe multifocal; and 6, severe widespread (28).

Analysis of gastric cellular infiltrate by flow cytometry. Lymphocytes were isolated from mouse stomachs by injecting 10 ml of phosphate-buffered saline (1.9 mM KH_2PO_4 , 8.1 mM Na_2HPO_4 , 150 mM NaCl, pH 7.4) into the mucosa as previously described (2). Cells were resuspended in fluorescence-activated cell sorter buffer (0.1% [wt/vol] bovine serum albumin, 0.1% [wt/vol] sodium azide in phosphate-buffered saline), counted by trypan blue dye exclusion, and stained with the appropriate antibodies. The antibodies used were anti-mouse CD3-fluorescein isothiocyanate (clone 145-2C11), anti-mouse CD4-Cy5 (clone H129.19), anti-mouse CD4-fluorescein isothiocyanate (clone RM4-4), anti-mouse CD8-allophycocyanin (clone 53-6.7), and anti-mouse CD19-phycoerythrin (clone 1D3) from BD Biosciences (San Diego, CA). Flow cytometry was performed using a BD FACSCalibur flow cytometer (BD Biosciences). Results were analyzed using the CELLQuest software (Becton Dickinson Immunocytometry Systems).

Statistics. Statistical significance was assessed by the nonparametric Mann-Whitney U test, and two-tailed *P* values of less than 0.05 were considered statistically significant.

RESULTS

Progression of *H. pylori*-induced disease in CD4-deficient mice. (i) *H. pylori* colonization and serum antibody levels. GK 1.5 transgenic mice constitutively produce the anti-CD4 GK 1.5 antibody, which results in the depletion of CD4^+ cells (33). Flow cytometric analysis of splenocytes and lymph node cells by us (data not shown) and others (32, 33) has shown that the percentages of CD4^+ cells in GK 1.5 mice are reduced to 0.4% and 0.1%, respectively. We infected GK 1.5 and C57BL/6 mice with *H. pylori* (five mice per group) and monitored the infection for up to 13 weeks. Throughout the experiment, *H. pylori* was isolated from the stomachs of all infected mice, and there was no statistical difference in colonization levels, measured by

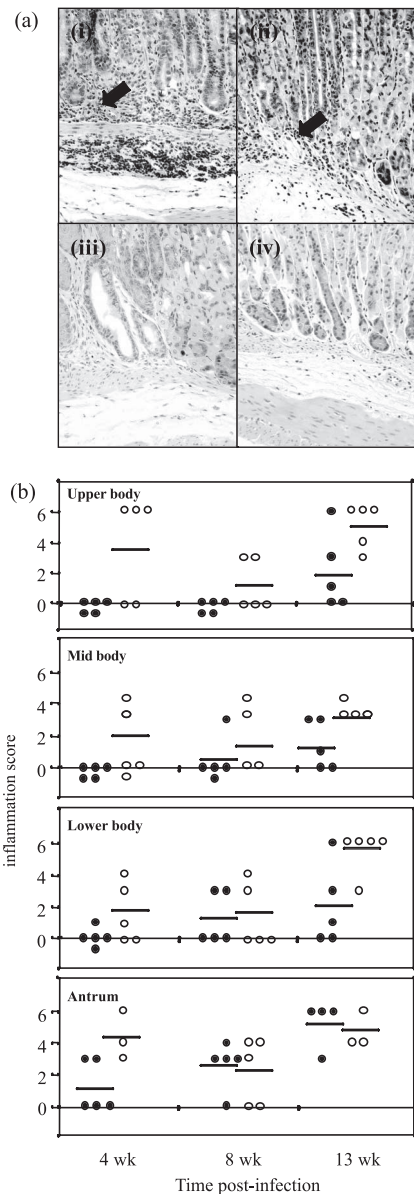


FIG. 2. Gastric pathology in *H. pylori*-infected GK 1.5 mice. The gastric pathology of *H. pylori*-induced gastric inflammation in GK 1.5 and C57BL/6 mice was assessed using hematoxylin and eosin-stained stomach sections, which were blindly graded by a pathologist and to which inflammation scores from 0 to 6 (28) were assigned. (a) Representative hematoxylin and eosin-stained stomach sections from 13-week-infected GK 1.5 (i) and C57BL/6 (iii) mice and from naive GK 1.5 (iii) and C57BL/6 (iv) mice. Arrows indicate lymphocyte infiltration. Magnification, $\times 20$. (b) Summary of inflammation scores for infected mice, with analysis of the various stomach regions. Represented here are individual C57BL/6 (●) and GK 1.5 (○) mice, with the mean of each group represented by bars. wk, weeks.

viable count, between the mouse strains at any time point during the experiment (Fig. 1). The circulating *H. pylori*-specific antibody responses indicated that the total anti-*H. pylori* antibody titers in both mouse strains increased progressively from 4 to 13 weeks after infection but, while the antibody levels detected in infected C57BL/6 mice were consistently higher than those in GK 1.5 mice, this difference was not statistically

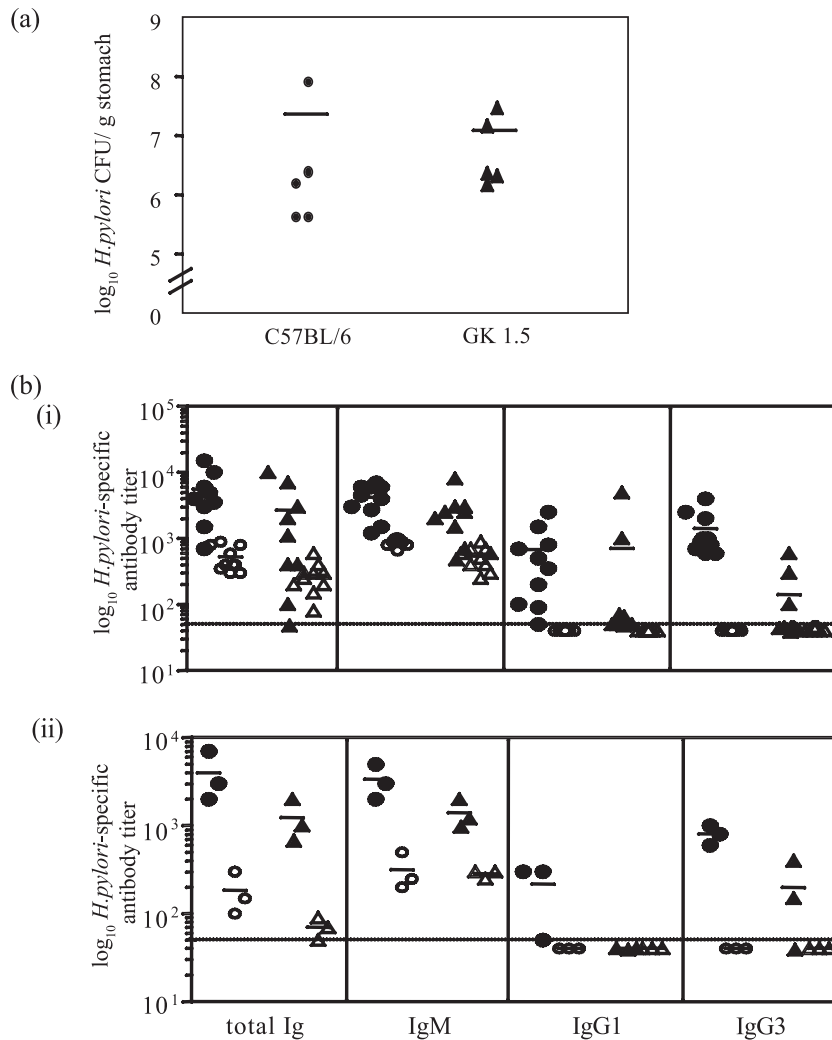


FIG. 3. *H. pylori* colonization and antibody responses in GK 1.5 and C57BL/6 mice 13 weeks after infection. (a) Viable counts of *H. pylori* isolated from mouse stomachs at 13 weeks postinfection were consistent with results from the previous experiment. *H. pylori* was not isolated from naive mice (data not shown). (b) Total *H. pylori* (i)- and *H. pylori* LPS (ii)-specific serum antibodies and their isotypes in C57BL/6 and GK 1.5 mice were measured by ELISA. Mouse sera were analyzed individually for *H. pylori*-specific ELISA and pooled for LPS-specific ELISA; each symbol represents a sample pooled from three mice. Represented are results from infected C57BL/6 (●) and GK 1.5 (▲), and naive C57BL/6 (○) and GK 1.5 (△) mice, with the mean of each group represented by the bars. The dashed line indicates the level of detection of the assay.

significant ($P = 0.15, 0.31,$ and 0.06 , comparing GK 1.5 mice and C57BL/6 mice at 4, 8, and 13 weeks, respectively [data not shown]).

(ii) **Assessment of gastric inflammation by histology.** Gastritis was not detected in uninfected mice of either strain (data not shown), but *H. pylori*-infected C57BL/6 and GK 1.5 mice developed characteristic *H. pylori*-induced inflammation, with infiltration of mononuclear cells into the stomach mucosa (Fig. 2a). Inflammation scores increased during the 13-week period of *H. pylori* infection in C57BL/6 mice. The gastritis observed for infected GK 1.5 mice appeared to be more severe than that in C57BL/6 mice. For example, the average inflammation score for C57BL/6 mice at 13 weeks postinfection for the lower body was 2.0, whereas the average inflammation score for the corresponding GK 1.5 mice was 5.4 (Fig. 2b).

Examination of *H. pylori*-specific antibody responses in CD4⁺ T-cell-deficient mice. The *H. pylori* infection experiment

was repeated to examine the immune response in C57BL/6 and GK 1.5 mice at 13 weeks after *H. pylori* infection. *H. pylori* colonization levels (Fig. 3a) and gastritis scores (data not shown) were consistent with the previous experiments. *H. pylori*- and LPS-specific antibody titers (total Ig, IgG1, IgG3, and IgM) were measured by ELISA and are shown in Fig. 3b. Anti-*H. pylori* antibody levels increased in both mouse strains following *H. pylori* infection (Fig. 3bi) ($P < 0.001$ and $P = 0.06$ for C57BL/6 and GK 1.5 mice, respectively, comparing infected to naive mice). However, C57BL/6 mice mounted a stronger antibody response when infected with *H. pylori* than did GK 1.5 mice ($P = 0.053$, comparing total Ig levels between infected C57BL/6 and GK 1.5 mice). The majority of the *H. pylori*-specific antibodies raised in GK 1.5 mice were of the IgM subtype, and minimal or no IgG responses were detected (Fig. 3bi). Since CD4⁺ T cells provide B-cell help for protein-specific antibody responses, *H. pylori* LPS-specific antibody

responses were determined in this study as a measure of T-cell-independent antibody responses. Figure 3bii shows that in both strains of mice, the majority of anti-LPS antibodies was of the IgM subtype with low IgG3 titers, while only *H. pylori*-infected C57BL/6 mice raised LPS-specific IgG1.

Examination of gastric infiltration in CD4⁺ T-cell-deficient mice by flow cytometry. Lymphocytes were isolated from five mice per group and analyzed by flow cytometry. Results from the analysis were expressed in terms of the percentages of specific cells detected (Fig. 4), which were subsequently extrapolated to absolute numbers (Table 1). It may be technically difficult to extract lymphocytes from nonlymphoid organs like the stomach for immunological assays, but gastric lymphocytes from infected animals could clearly be detected using the employed “ballooning” method, whereby the stomach mucosa is injected with medium (2) (Fig. 4a). Indeed, the lymphocyte population in the gastric cell preparation was identified using the forward- and side-scatter profiles of spleen cells obtained from uninfected C57BL/6 mice (Fig. 4a). Many cells were present in the lymphocyte gate in the gastric cell preparations from GK 1.5 mice compared to what was seen for C57BL/6 mice (Table 1). Analysis of cell surface marker expression, indicated in Fig. 4b and c, revealed that infected GK 1.5 mice had sixfold as many gastric B cells, defined by CD19 expression, and fourfold as many T cells, defined by CD3 expression, as C57BL/6 mice, although these differences were not statistically significant (Table 1). Analysis of the T-cell subsets showed that CD8⁺ T cells comprised a significant proportion of CD3⁺ cells within the GK 1.5 mouse stomach and were 30-fold more numerous than in C57BL/6 stomachs ($P = 0.032$). In contrast, the gastric mucosae of C57BL/6 mice were infiltrated with more CD4⁺ T cells than CD8⁺ T cells. In both mouse strains, the majority of the CD3⁺ cells in the stomach were double-negative (DN) cells (CD4⁻ CD8⁻). The CD4 deficiency in GK 1.5 mice was confirmed using the RM4-4 anti-CD4 antibody clone, which recognizes an epitope different from that recognized by the GK 1.5 and H129.19 antibodies. Although the DN T-cell population in infected GK 1.5 stomachs was also substantial, the dominant cellular infiltrate accounting for the increase in lymphocyte numbers in the GK 1.5 mouse stomachs appeared to be CD8⁺ T cells.

Examination of *H. pylori*-induced disease in mice deficient in both CD4⁺ and CD8⁺ T cells. (i) *H. pylori* colonization, serum antibody levels, and gastric inflammation by histology. We next infected GK/2.43 mice with *H. pylori* together with infecting C57BL/6 mice as controls. GK/2.43 mice are transgenic for the GK 1.5 and GK/2.43 antibodies that deplete CD4⁺ and CD8⁺ cells, respectively; hence, these mice are deficient in both CD4⁺ and CD8⁺ T cells (reference 31 and data not shown). Both GK/2.43 and C57BL/6 mice were colonized by *H. pylori* at 13 weeks postinfection ($n = 6$) (Fig. 5a), and there was no significant difference between the *H. pylori* burdens of the mouse strains ($P = 0.082$).

Assessment of gastric inflammation by histology revealed that naive mice did not develop gastritis (data not shown) and, although both C57BL/6 and GK/2.43 mice demonstrated mild inflammation at 13 weeks after infection, there was no apparent difference in the severities of inflammation seen for the mouse strains (Fig. 5b).

Helicobacter-specific antibody levels were significantly in-

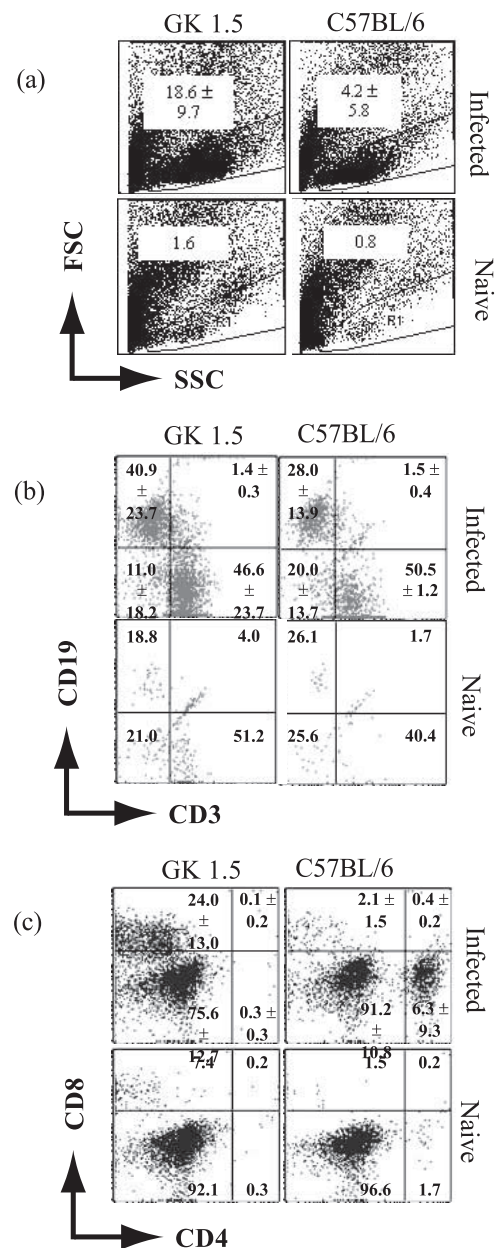


FIG. 4. Flow cytometric analysis of gastric lymphocytes from *H. pylori*-infected GK 1.5 mice. Flow cytometric analysis of gastric lymphocyte populations in GK 1.5 and C57BL/6 mice at 13 weeks after *H. pylori* infection ($n = 5$). Numbers within plots refer to mean percentages of cells \pm standard deviations (SD) for infected animals, and proportions of cells in pooled naive animals, within the gate or quadrant. (a) Forward- and side-scatter (FSC and SSC) profiles of stomach cell preparations with lymphocytes within the gate. (b) B (CD19⁺) and T (CD3⁺) lymphocytes in the stomachs of infected and naive GK 1.5 and C57BL/6 mice. (c) CD4⁺ and CD8⁺ cell populations within CD3⁺ lymphocytes from infected and naive animals.

creased after *H. pylori* infection ($P = 0.001$ and 0.013 for C57BL/6 and GK/2.43 mice, respectively, comparing infected to naive mice), and the total Ig and IgG antibody levels in infected C57BL/6 mice were significantly high compared to those for infected GK/2.43 mice ($P \leq 0.0003$) (Fig. 5ci). As with GK 1.5 mice, the *H. pylori*-specific antibodies elicited

TABLE 1. Analysis of cells by flow cytometry in stomachs of GK 1.5 and C57BL/6 mice during *H. pylori* infection^a

Mouse strain and infection status	No. of cells	No. of lymphocytes	No. of indicated lymphocyte:		No. of indicated CD3 ⁺ cell type:		
			CD19 ⁺	CD3 ⁺	CD8 ⁺	CD4 ⁺	CD4 ⁻ CD8 ⁻
GK 1.5							
Infected ^b	44.9 ± 19.6	9.4 ± 6.1	4.7 ± 3.2	4.2 ± 3.1	1.2 ± 0.9	0.006 ± 0.004	3.0 ± 2.2
Naive ^c	5.0	0.08	0.02	0.04	0.003	<0.001	0.04
C57BL/6							
Infected	34.2 ± 28.4	1.9 ± 3.4	0.8 ± 1.5	1.0 ± 1.7	0.04 ± 0.08	0.2 ± 0.4	0.7 ± 1.2
Naive	3.3	0.03	0.007	0.01	<0.001	<0.001	0.01

^a Results shown are per 10⁴ cells.

^b *H. pylori*-infected mice (13 weeks), mean ± SD (*n* = 5 per group).

^c Pooled results for age-matched naive mice (*n* = 5 per group).

following *H. pylori* infection in GK/2.43 mice were mostly of the IgM subtype, and no *H. pylori*-specific IgG1 or IgG3 was detectable. Further analysis of *H. pylori*-specific antibody responses revealed that in contrast to infected C57BL/6 mice, GK/2.43 mice elicited only LPS-specific IgM (Fig. 5cii).

(ii) **Examination of gastric infiltration in GK/2.43 mice by flow cytometry.** The gastric cellular infiltrate of infected GK/2.43 mice was also analyzed by flow cytometry and compared with that of infected C57BL/6 mice (*n* = 6 each). The results from the flow cytometric analyses are expressed in terms of percentages of specific cells detected (Fig. 6) and as absolute numbers in Table 2. The results indicated that there were approximately three times as many lymphocytes in the infected C57BL/6 mouse stomach as in GK/2.43 mice, whereas only few cells were isolated from naive mice from both strains. There were also more T cells than B cells in the infected stomachs in both strains of mice. There was a distinct population of CD4⁺ T cells in infected C57BL/6 mice but not in GK/2.43 mice. Most of the CD3⁺ cells isolated from the stomachs of both GK/2.43 and C57BL/6 mice were DN cells (95.1% and 92.1%, respectively). Unlike the GK 1.5 mouse, where transgenic expression of the GK 1.5 antibody resulted in the almost complete removal of CD4⁺ T cells, consistent with previous reports (32, 33), the mice expressing the anti-CD8 antibody (i.e., GK/2.43 mice) were not depleted of all CD8⁺ cells.

DISCUSSION

In this study, we examined the immune and inflammatory responses induced by *H. pylori* infection in mice lacking either CD4⁺ T cells or CD4⁺ and CD8⁺ T cells. As CD4⁺ T cells had been implicated in *H. pylori*-induced gastritis in several independent reports (7, 20), we hypothesized that CD4⁺ T-cell-deficient mice infected with *H. pylori* would not develop stomach inflammation. However, our results suggested that the absence of CD4⁺ T cells was linked to increased gastritis, dominated by an influx of CD8⁺ T cells, and further supported existing data that CD4⁺ T cells provide an important regulatory function in *Helicobacter*-induced gastritis.

GK 1.5 mice were used in our study as an in vivo model of CD4 deficiency. The immunological characteristics of GK 1.5 mice have been examined and summarized by Zhan et al. (32). Briefly, the absence of CD4⁺ cells in the periphery was confirmed by flow cytometry, using an anti-CD4 antibody that recognized an epitope different from that by the GK 1.5 antibody, and the percentage of CD4⁺ T cells present in the spleen

and lymph nodes was less than 0.1% (32). This is in accordance with our evaluation of GK 1.5 mice, where the percentage of splenic CD4⁺ T cells detected in uninfected GK 1.5 mice was 0.4% (*n* = 5) (data not shown). With regard to other immune cells, GK 1.5 mice were found to have comparable populations of CD8⁺ T cells, α/β and γ/δ T-cell-receptor-positive cells, B cells (B220⁺), and NK T cells but reduced numbers of dendritic cells (due to the depletion of CD4⁺ dendritic cells) compared to wild-type mice (32). Unlike CD4^{-/-} and major histocompatibility complex (MHC) class II^{-/-} mice, aberrant helper populations were absent in GK 1.5 mice, which raised only a low IgG response toward influenza A viral antigens and no detectable IgG responses toward simple antigens (32).

In this study, CD4⁺ T-cell deficiency had no apparent effect on *H. pylori* colonization, suggesting either that *H. pylori* colonization was independent of CD4⁺ T-cell activity or that another cell type (e.g., CD8⁺ T cells) could compensate for the lack of CD4⁺ T cells in controlling the growth of the bacterium. The former conclusion, if proven, would have important consequences for the development of vaccines which aim to reduce bacterial burden in gastric *Helicobacter* disease.

Circulating *H. pylori*-specific antibodies, although present in *H. pylori*-infected humans, are generally believed to play a minor role in protection against bacterial colonization. Clinical studies have revealed that, despite the development of a serological response, *H. pylori* infection is typically lifelong unless eradicated through antibiotic therapy. It has been shown that murine *H. pylori* disease and vaccine-mediated protection can occur in the absence of conventional B cells and their antibodies (9, 25). In our study, both infected GK 1.5 and C57BL/6 mice developed anti-*H. pylori* circulating antibodies, an unexpected result, as GK 1.5 mice lacked CD4⁺ T-cell help provided to B cells for the production of antigen-specific antibodies. A further analysis of the isotypes of *H. pylori*-specific antibodies revealed that most *H. pylori*-specific antibodies were of the IgM subtype. *H. pylori*-infected C57BL/6 mice also developed strong IgG (IgG1 and IgG3) responses, consistent with other studies (4, 26). In contrast, most GK 1.5 mice had barely detectable *H. pylori*-specific IgG antibodies. To further analyze the *H. pylori*-specific antibody response, we used *H. pylori* LPS as a CD4⁺ T-cell-independent antigen and demonstrated that many of the *H. pylori*-specific antibodies, mostly IgM, appeared to be LPS specific. In infected GK 1.5 mice, only LPS-specific IgM and IgG3 were detected. Taken together, these results suggest that the GK 1.5 mice have no residual CD4⁺ helper

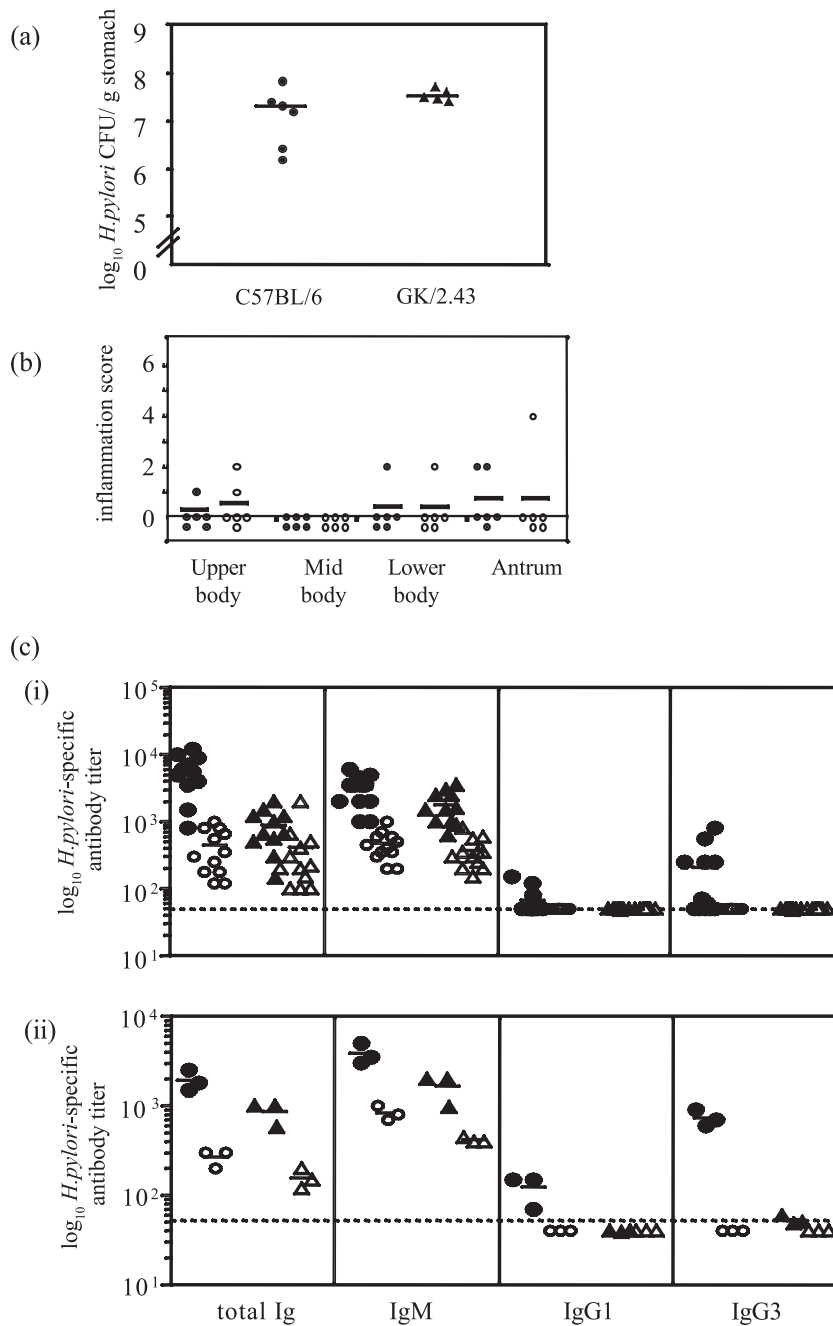


FIG. 5. *H. pylori* infection in GK/2.43 mice at 13 weeks postinfection. (a) *H. pylori* populations were enumerated in the stomachs of C57BL/6 and GK/2.43 mice by viable count at 13 weeks after infection. (b) Summary of gastric inflammation scores of *H. pylori*-infected GK/2.43 and C57BL/6 mice examined by histology, using a grading scale from 0 to 6 as described previously (28). No inflammation was noted for naive mice (data not shown). Represented are individual inflammation scores for C57BL/6 (●) and GK/2.43 (○) mice, with the mean of each group represented by the bars. (c) Total *H. pylori* (i)- and *H. pylori* LPS (ii)-specific serum antibodies and their isotypes in C57BL/6 and GK/2.43 mice were measured by ELISA. Mouse sera were analyzed individually for *H. pylori*-specific ELISA and pooled for LPS-specific ELISA; each symbol represents a sample pooled from three mice. Represented are results of infected C57BL/6 (●) and GK/2.43 (▲) mice and naive C57BL/6 (○) and GK/2.43 (△) mice, with the mean of each group represented by the bars. The dashed line indicates the level of detection of the assay.

responses. Further, our results imply that both T-cell-dependent and -independent *H. pylori*-specific antibodies may be ineffective in reducing bacterial colonization.

The gastritis that developed in $CD4^+$ T-cell-deficient mice after *H. pylori* infection appeared to be more severe than that seen for immunocompetent C57BL/6 mice, suggesting both

that there were other immune cells capable of infiltrating the stomach in large numbers and that the $CD4^+$ T cells, which normally infiltrate the stomachs of *H. pylori*-infected mice, might be negative regulators of gastric inflammation. Upon closer examination, we found that the predominant cells in the gastric infiltrate in infected GK 1.5 mice were $CD8^+$ T cells,

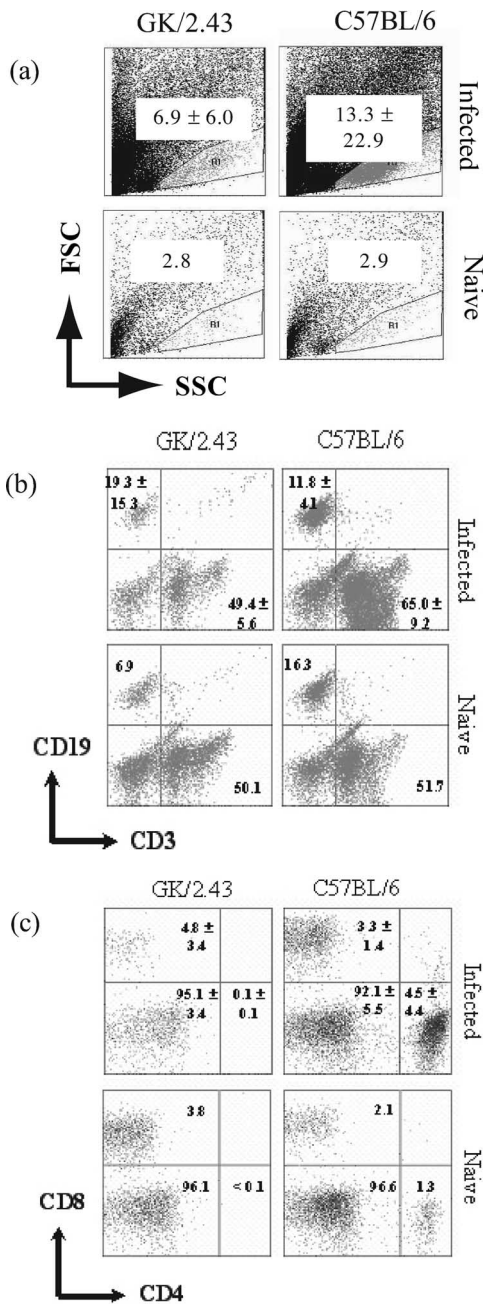


FIG. 6. Flow cytometric analysis of *H. pylori*-infected GK/2.43 mice. Flow cytometry was used to analyze the gastric lymphocyte populations in GK/2.43 and C57BL/6 mice 13 weeks after *H. pylori* infection. (a) Forward- and side-scatter (FSC and SSC) profiles of stomach cell preparations with lymphocytes within the gate ($n = 6$ for each group). Numbers within plots refer to mean percentages of cells \pm SD for infected animals, and percentages of cells in pooled naive animals, within the gate or quadrant. (b) B (CD19⁺) and T (CD3⁺) lymphocytes in infected and naive GK/2.43 and C57BL/6 mice. (c) CD4⁺ and CD8⁺ cell populations within CD3⁺ lymphocytes in infected and naive animals.

which were present in infected GK 1.5 mice at significantly high levels compared to C57BL/6 mice. Indeed, mice deficient in both CD4⁺ and CD8⁺ T cells developed only mild gastritis after *H. pylori* infection at 13 weeks, which was comparable to

the inflammation that developed in immunocompetent mice. Hence, our results appear to contradict those reported by Eaton et al. (7), where no gastric inflammation was observed for SCID mouse recipients of splenocytes depleted of CD4⁺ T cells. While the discrepancy between our results and those reported by Eaton et al. might be explained by the presence of a subset of CD4 helper cells in the GK 1.5 mice, this is highly unlikely, since extensive studies by Zhan et al. have demonstrated that GK 1.5 mice lack residual aberrant helper cells (32, 33). Further, our results show that GK 1.5 mice developed an IgM anti-*Helicobacter* antibody response. Therefore, the difference between this study and the work of Eaton et al. may also be explained by the use of different experimental systems. In contrast to SCID mice, the GK 1.5 mice have, except for the absence of CD4⁺ cells, a normal immune repertoire (32, 33). Nevertheless, our results agree with those of Pappo et al. (21), who noted that CD8⁺ T cells were the major infiltrating cellular group in the stomachs of immunized MHC class II-deficient mice which were subsequently challenged with *H. pylori*. Although a predominant role of gastric CD4⁺ T cells has been reported in many studies of *H. pylori*-induced inflammation (4, 7, 20), Hatz et al. compared the cellular constituents between lamina propria lymphocytes and intraepithelial lymphocytes in patients with *H. pylori*-associated gastritis and found that, while CD4⁺ T-cell numbers exceeded CD8⁺ T cells among lamina propria lymphocytes, the reverse was observed for intraepithelial lymphocytes, where CD8⁺ T-cell levels were higher than those for CD4⁺ T cells (11). Hence, the severe inflammation seen for humans and animals after *H. pylori* infection might be caused not only by MHC class II-restricted antigen-specific CD4⁺ T cells but also by CD8⁺ T cells which are activated by *H. pylori* antigens presented on MHC class I molecules that are expressed on virtually all cell types. Mice that were deficient in both CD4⁺ and CD8⁺ T cells developed a relatively mild gastritis compared with what was seen for GK 1.5 mice, suggesting that in the absence of CD4⁺ T cells, CD8⁺ T cells might be directly contributing to the inflammation typical of *Helicobacter*-associated gastritis.

The role of regulatory CD25⁺ CD4⁺ T cells (Tregs) in *H. pylori* infection has now been extensively analyzed, where Tregs reportedly suppress the immune response after *H. pylori* infection (8, 17, 24). Our results support these findings inasmuch as exacerbated inflammation was observed for mice that lacked classical Tregs (i.e., CD25⁺ CD4⁺ T cells). It has also been suggested that Tregs may modulate the gamma interferon (IFN- γ)-producing CD4⁺ T cells in the gastric mucosa (23). In their analysis of cytokine production by CD4⁺ and CD8⁺ T cells isolated from human gastric biopsy samples, Quiding-Jarbrink et al. reported that CD8⁺ T cells produced more IFN- γ on a per-cell basis than did CD4⁺ T cells and that IFN- γ was produced preferentially by CD8⁺ T cells (22). Hence, our findings together with those from Quiding-Jarbrink et al. suggest that the regulatory action of CD4⁺ T cells may be directed toward the IFN- γ -producing CD8⁺ T cells, which were found to infiltrate the infected GK 1.5 stomach in large numbers, rather than to other (proinflammatory) CD4⁺ T cells.

There have been relatively few studies that examined the role of CD8⁺ T cells in *H. pylori* infection. CD8⁺ T-cell numbers reportedly increase in the stomachs of individuals infected

TABLE 2. Analysis of cells by flow cytometry in stomachs of GK/2.43 and C57BL/6 mice during *H. pylori* infection^a

Mouse strain and infection status	No. of cells	No. of lymphocytes	No. of indicated lymphocyte:		No. of indicated CD3 ⁺ cell type:		
			CD19 ⁺	CD3 ⁺	CD8 ⁺	CD4 ⁺	CD4 ⁺ CD8 ⁻
GK/2.43							
Infected ^b	7.2 ± 6.5	0.8 ± 1.4	0.28 ± 0.6	0.34 ± 0.5	0.03 ± 0.05	<0.001	0.3 ± 0.5
Naive ^c	1.8	0.05	0.003	0.03	0.001	<0.001	0.02
C57BL/6							
Infected	12.3 ± 1.3	1.8 ± 3.2	0.16 ± 0.3	1.28 ± 2.4	0.06 ± 0.1	0.1 ± 0.2	1.1 ± 2.1
Naive	1.2	0.04	0.006	0.02	<0.001	<0.001	0.02

^a Results shown are per 10⁴ cells.

^b *H. pylori*-infected mice (13 weeks), mean ± SD (*n* = 6 per group).

^c Pooled results for age-matched naive mice (*n* = 6 per group).

with *H. pylori* (1), and the majority of human gastric CD8⁺ T cells had a memory phenotype (3). It is possible that CD8⁺ T cells contribute to *H. pylori*-induced pathology, as CD8 expression in *H. pylori*-infected children correlated positively with the severity of antral gastritis, and *H. pylori*-reactive CD8⁺ T cells from blood can be activated *in vitro* by *H. pylori* antigens (3, 19). Hence, it would be interesting (i) to examine the gastric CD8⁺ T cells found in GK 1.5 mice after *H. pylori* infection for antigen-specific activation and cytokine production, (ii) to investigate whether gastritis could be initiated after the adoptive transfer of CD8⁺ T cells into lymphopenic hosts such as RAG^{-/-} mice, and (iii) to examine the progression of *H. pylori* infection in mice deficient in CD8⁺ T cells.

In summary, we confirmed results from earlier studies showing that anti-*H. pylori* antibodies, whether generated via T-cell-dependent mechanisms or not, cannot eliminate *H. pylori* colonization. We also showed that, in the absence of CD4⁺ T cells, CD8⁺ T cells were capable of initiating and maintaining gastric inflammation following murine *H. pylori* infection and that the severity of gastric inflammation was significantly increased. These results imply that CD4⁺ T cells, usually present in large numbers in the stomachs of *H. pylori*-infected hosts, are likely to have a dominant role in regulating or suppressing the local inflammation caused by *H. pylori*, and the target cells of such regulation may be CD8⁺ T cells.

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