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The Role of T Cell Subsets and Cytokines in the Pathogenesis of *Helicobacter pylori* Gastritis in Mice\(^1\)

Kathryn A. Eaton,\(^2\) Megan Mefford, and Tracy Thevenot

Gastritis due to *Helicobacter pylori* in mice and humans is considered a Th1-mediated disease, but the specific cell subsets and cytokines involved are still not well understood. The goal of this study was to investigate the immunopathogenesis of *H. pylori*-induced gastritis and delayed-type hypersensitivity (DTH) in mice. C57BL/6-Prkdc\(^{scid}\) mice were infected with *H. pylori* and reconstituted with CD4\(^+\), CD4-depleted, CD4\(^+\)CD45RB\(^{bigh}\), or CD4\(^+\)CD45RB\(^{low}\) splenocytes from wild-type C57BL/6 mice or with splenocytes from C57BL/6\(\text{Ifn}-\gamma/\text{Ifn}-\gamma\) or C57BL/6\(\text{Il}-10/\text{Il}-10\) mice. Four or eight weeks after transfer, DTH to *H. pylori* Ag was determined by footpad injection; gastritis and bacterial colonization were quantified; and IFN-\(\gamma\) secretion by splenocytes in response to *H. pylori* Ag was determined. Gastritis and DTH were present in recipients of unfractionated splenocytes, CD4\(^+\) splenocytes, and CD4\(^+\)CD45RB\(^{bigh}\) splenocytes, but absent in the other groups. IFN-\(\gamma\) secretion in response to *H. pylori* Ag was correlated with gastritis, although splenocytes from all groups of mice secreted some IFN-\(\gamma\). Gastritis was most severe in recipients of splenocytes from IL-10-deficient mice, and least severe in those given IFN-\(\gamma\)-deficient splenocytes. Bacterial colonization in all groups was inversely correlated with gastritis. These data indicate that 1) CD4\(^+\) T cells are both necessary and sufficient for gastritis and DTH due to *H. pylori* in mice; 2) high expression of CD45RB is a marker for gastritis-inducing CD4\(^+\) cells; and 3) IFN-\(\gamma\) contributes to gastritis and IL-10 suppresses it, but IFN-\(\gamma\) secretion alone is not sufficient to induce gastritis. The results support the assertion that *H. pylori* is mediated by a Th1-biased cellular immune response. The Journal of Immunology, 2001, 166: 7456–7461.

*Helicobacter pylori* is a human gastric pathogen that can cause gastritis, peptic ulcer disease, or gastric cancer. The organism was first associated with disease in 1983 (1), but despite significant advances in the understanding of the biology of *H. pylori*, the factors that determine the outcome of infection in an individual host are still poorly understood. In recent years, evidence has accumulated to suggest that in both human patients and animal models, host cellular immune response is an important determinant of the outcome of infection. Infected individuals express proinflammatory cytokines in their gastric mucosa (IFN-\(\gamma\), IL-8), and gastric mucosa is infiltrated with proinflammatory Th1-biased lymphocytes as well as neutrophils and other inflammatory cell types (2–5).

Because of the difficulty of performing invasive studies in humans, much of our understanding of the immune basis of *H. pylori*-related disease comes from studies in mice. Like humans infected with *H. pylori*, mice infected with the closely related *Helicobacter felis* respond to infection with infiltration of Th1-biased lymphocytes into the gastric mucosa and spleen. IFN-\(\gamma\) becomes elevated in tissue, and splenic Th cells respond to *H. pylori* Ag by secreting high levels of IFN-\(\gamma\) and undetectable levels of IL-4 (6). Furthermore, when infected with *H. felis*, IFN-\(\gamma\)-deficient genetic knockout mice fail to develop any gastritis (7, 8), while IL-10 knockout mice develop gastritis that is much more severe than in wild-type mice (9). Finally, splenic lymphocytes from nonresponder mouse strains that do not develop gastritis in response to gastric helicobacters express IL-10, but not IFN-\(\gamma\), in response to *H. pylori* Ag (10). These data suggest that Th1-biased cellular immune responses largely determine the outcome of gastric *Helicobacter* infection in mice.

Ig response does not appear to determine outcome of infection. Mice that are genetically unable to produce Ab respond to both infection and vaccination identically to normal mice (11, 12), whereas IFN-\(\gamma\)-deficient mice are not able to suppress bacterial infection to the same extent as wild-type mice (7). Finally, adoptive transfer of CD4\(^+\) lymphocytes into *H. felis*-infected mice exacerbates gastritis in the recipients (6). Thus, cellular rather than humoral immune responses control both gastritis and suppression of colonization.

Data from our laboratory demonstrate that *H. pylori*-infected mice respond similarly to humans and to *H. felis*-infected mice. Adoptive transfer of splenocytes from C57BL/6 mice to congenic SCID mice results in preferential engraftment of T cells, severe gastritis with concurrent development of a delayed-type hypersensitivity (DTH)\(^3\) response to *H. pylori* Ags, and development of a blastogenic response of CD4\(^+\) splenocytes to *H. pylori* Ag (13). In contrast, humoral immune response develops late and does not correlate with gastritis. Gastritis in infected recipient mice is widespread and rapidly developing. It resembles gastritis in *H. pylori*-infected humans both morphologically, with infiltration of lymphocytes, macrophages, and neutrophils, atrophy of gastric parietal cells, and epithelial erosions, and immunologically, with elevation of IFN-\(\gamma\) production by stimulated lymphocytes.

The purpose of the current study was to investigate the immunopathogenesis of severe gastritis in *H. pylori*-infected recipient mice.
SCID mice. H. pylori-infected recipient SCID mice received unfractonated splenocytes, CD4⁺ splenocytes, or CD4-depleted splenocytes to determine whether Th cells, non-T cells, or both are capable of inducing gastritis. In addition, the role of CD4⁺ cells expressing the surface marker CD45RB was evaluated. Finally, the role of T cell-derived IL-10 and IFN-γ in suppression or promotion of gastritis mice was determined.

Materials and Methods

Mice

C57BL/6, C57BL/Pkdc-scid (SCID), C57BL/6J[129Pt]+/− (IL-10-deficient), and C57BL/6IFN-γ−/− (IFN-γ-deficient) mice, 4–6 wk old, were purchased from The Jackson Laboratory (Bar Harbor, ME). Immune incompetence of SCID mice was verified by the absence of murine IgG, as detected by ELISA (13). Mice were kept in sterile microisolator cages in a barrier facility, and fed sterile water and Teklad lab chow ad libitum (Teklad, Madison, WI). A total of 240 mice were used in this study. All procedures involving animals were approved by the Ohio State University institutional laboratory animal care and use committee.

Bacteria

H. pylori strain SS1, a mouse-adapted human isolate, was grown on 5% sheep blood agar plates or in Brucella broth with 10% FCS. For preparation of sonicates and for mouse inoculation, bacteria were grown in broth overnight at 37°C in a microaerobic environment with gentle agitation. Bacterial sonicates for footpad injection and T cell stimulation were prepared as previously described (13).

Bacterial infection and adoptive transfer

Details of the adoptive transfer mouse model used in this study have been previously published (13). Briefly, splenocytes from normal, uninfected C57BL/6 mice were transferred to H. pylori-infected SCID mice by ip injection. Adoptive transfer was performed 4 wk after oral inoculation of SCID mice with 10⁶ CFU of live, broth-cultured H. pylori. To ensure approximately equivalent numbers of engrafted T cells, each mouse received 1 × 10⁶ unfractonated splenocytes (20% CD4⁺ T cells, as determined by flow cytometry; Ref. 13), 2 × 10⁶ CD4⁺ splenocytes, or 8 × 10⁶ CD4-depleted splenocytes. This protocol results in engraftment of T or T and B lymphocytes and development of cellular or cellular and serologic immune responses to H. pylori in the recipient mice (13). In contrast to infected normal C57BL/6 mice, which develop minimal to mild cellular immune responses and mild gastritis, infected SCID recipients of unfractonated splenocytes develop strong cellular immune responses that result in DTH responses to H. pylori Ags and severe gastritis within 4 wk of adoptive transfer (13).

Cell fractionation

Spleens were removed immediately after death and placed in sterile HBSS, pH 7, at 4°C. Splenocytes were isolated by disaggregation and hypotonic lysis, washed in PBS, and stained with FITC- or PE-labeled Ab against CD4, and in some cases CD45RB, as previously described (13). Cells were separated with a Coulter EPICS 753 dye laser cell sorter (Coulter Pharmaceutical, Palo Alto, CA). The CD4⁺ fraction contained 95–98% CD4⁺ cells, and the CD4-depleted fraction contained <1% CD4⁺ cells, as determined by remaining and recounting. For fractionation according to CD45RB expression, double-labeled cells were sorted first to collect CD4⁺ cells, and then the CD4⁺ fraction was separated into two fractions according to intensity of CD45RB fluorescence, as described by Morrissey (14). Two populations of cells were recognizable based on fluorescence intensity. The brightest staining 40% was designated CD45RBhigh and the dullest staining 15% was designated CD45RBlow.

Experimental design

Infected or uninfected SCID mice received either unfractonated (total) splenocytes, CD4⁺ splenocytes, CD4 splenocytes fractionated according to CD45RB expression, or CD4-depleted splenocytes. All splenocytes were from uninfected C57BL/6, C57BL/6[129Pt]+/−, or C57BL/6IFN-γ−/− mice. Uninfected mice (both recipient and donor groups) developed no lesions, no serum immune responses, and no footpad swelling in response to H. pylori Ag. Results from these groups are not shown in all cases. Recipients of unfractonated splenocytes, CD4⁺ splenocytes, CD4-depleted splenocytes, or splenocytes from C57BL/6IFN-γ−/− mice were killed 4 or 8 wk after transfer. Recipients of CD45RBhigh or CD45RBlow splenocytes or splenocytes from IL-10-deficient mice were killed 4 wk after transfer. In these groups, lesions were maximal by 4 wk after transfer, and 8–wk groups were not included. IL-10-deficient mice and C57BL/6 controls were killed 8 wk after inoculation. Each experimental and control group had between 5 and 20 mice. The number of mice in individual experiments is indicated in Figs. 1–5.

To measure DTH, mice were given 10 μg of H. pylori sonicate by injection into the hind footpad 1 day before sacrifice. The opposite footpad received sterile saline. Twenty-four hours later, footpad thickness was measured with a dial thickness gauge, and the difference in thickness between the control and sonicate-treated footpad was recorded. At sacrifice, serum was collected and stored at −20°C until use. Spleens were removed and placed in cold sterile HBSS for splenocyte isolation, as described above. Stomachs were aseptically removed and bisected along the greater and lesser curvatures. One half of the stomach was homogenized, and bacterial colonization was determined by plating serial dilutions. The other half of the stomach was divided longitudinally into 1- to 2-mm-wide strips, and immersed in Formalin for histologic examination.

ELISA

H. pylori-specific serum IgG and IgM were determined as previously described (13). Briefly, plates were coated with 100 μg/ml H. pylori SS1 sonicate for 48 h at 4°C, washed, blocked with blocking buffer (1 g/L gelatin in 0.1 M PBS + 0.02% sodium azide), washed again, and incubated for 90 min at 37°C with terminal mouse sera diluted 1/50. Plates were washed again, incubated with alkaline phosphatase-conjugated goat anti-mouse IgM or IgG (Bio-Rad, Richmond, CA), and washed, and alkaline phosphatase was detected with an alkaline phosphatase substrate kit (Bio-Rad), according to the manufacturer’s instructions.

Lymphocyte culture and IFN-γ ELISA

Splenocytes were isolated by disaggregation and hypotonic lysis (13). To ensure sufficient numbers of cells for analysis, spleens from five recipient SCID mice per group were combined. Thus, each assay represented five individual mice. Nonrecipient SCID spleens had no identifiable splenic lymphocytes, and thus could not be evaluated. Between one and five assays (representing 5–25 different mice) were evaluated for each experimental group. Sterile 96-well culture dishes were seeded with 1 × 10⁶ washed splenocytes/well in RPMI 1640, and Ag (1 μg/ml or μg/mi) and mitogen (Con A, 25 μg/ml) was added. Ag was either sonicated H. pylori prepared as described above or whole washed H. pylori cells resuspended to 1 × 10⁶ cells/ml in PBS with 1% Formalin added. Control wells were treated with medium alone. Splenocytes were incubated for 5 days at 37°C in 5% CO₂, and the medium were removed and stored at −70°C until used. For IFN-γ determinations, a commercially available kit was used according to the manufacturer’s instructions (PharMingen, San Diego, CA).

Histologic evaluation

For quantification of gastritis, hematoxylin and eosin-stained sections were scored for lymphocytic and neutrophilic inflammation and gastric epithelial metaplasia, as previously described (13). Briefly, sections were examined under ×200 magnification, and the percentage of fields containing inflammatory infiltrate sufficient to displace glands (gastritis), neutrophilic infiltration (polymorphonuclear), and/or loss of normal fundic morphology with replacement by undifferentiated mucus-type glands (metaplasia) was determined. In this way, the extent of inflammatory lesions, which correlates with severity, was quantified. This method is less subjective than semiquantitative scoring methods because percentage of affected mucosa can be quantified based on presence or absence of lesions rather than an estimate of severity. Results reflect differences in mouse groups, as previously demonstrated (13, 15).

Statistics

Group means were compared by nonparametric methods (Mann-Whitney U test) or by ANOVA with Fisher’s protected least significant difference to compare individual groups. Values in the text are expressed as mean ± SD. Statistical significance was set at p < 0.05.

Results

C57BL/6 splenocyte subsets in infected recipient SCID mice

Four weeks after transfer, infected SCID recipients of either unfractonated splenocytes or CD4⁺ splenocytes from C57BL/6 donors developed marked gastric inflammation compared with nonrecipient mice or uninfected controls (Fig. 1). All three indicators
of gastric damage, gastritis, neutrophil infiltration, and metaplasia, were increased. In contrast, recipients of CD4-depleted splenocytes did not develop gastric lesions ($p = 0.961$, compared with nonrecipient controls). Fractionation of CD4$^+$ cells according to the CD45RB marker also revealed differences between the subsets (Fig. 1). Recipients of CD4$^+$CD45RB$^{hi}$ splenocytes developed gastritis that did not differ significantly from recipients of CD4$^+$ or unfractionated splenocytes, whereas recipients of CD4$^+$CD45RB$^{lo}$ splenocytes did not develop gastric lesions. Gastric lesions in mice killed 8 wk after transfer were similar to lesions in mice killed after 4 wk (data not shown).

Indicators of immune response also differed between the groups. Humoral immune response, as indicated by $H. pylori$-specific IgG, developed only in recipients of unfractionated splenocytes, confirming that only this group received both T and B lymphocytes (Fig. 2). The only other mice to develop $H. pylori$-specific Abs were recipients of CD4-depleted splenocytes killed 8 wk after transfer. These mice developed low levels of IgM, but maturation of the immune response did not occur, most likely because of the absence of Th cells. In contrast to humoral immune response, cellular immune response as measured by DTH response correlated with severity of gastric lesions. Four weeks after transfer, mean footpad swelling in recipients of unfractionated splenocytes (3.2 ± 3.4 mm) and CD4$^+$ splenocytes (5.3 ± 4.1 mm) was significantly greater than in recipients of CD4-depleted splenocytes (0.7 ± 0.5 mm) and nonrecipients (0.2 ± 0.2 mm) ($p < 0.0001$).

Bacterial colonization was inversely correlated to extent of gastric lesions. Gastritis in recipients of unfractionated splenocytes was associated with suppression of colonization both 4 and 8 wk after transfer ($p < 0.0001$ compared with nonrecipients, Fig. 3; 8-wk interval not shown). Similarly, 4 wk after transfer, colonization was suppressed in recipients of CD4$^+$ or CD4$^+$CD45RB$^{hi}$ splenocytes, but not in recipients of CD4$^+$CD45RB$^{lo}$ splenocytes (Fig. 3).

Response of sensitized splenocytes to $H. pylori$ Ags in vitro

IFN-γ secretion in vitro in response to $H. pylori$ Ags (either Formalin-fixed bacteria or sonicate) partly correlated with extent of gastritis and DTH response. The greatest $H. pylori$-induced IFN-γ secretion was by splenocytes recovered from infected SCID recipients of CD4$^+$ splenocytes, the group that also developed the strongest DTH response, and the most extensive gastric lesions (Fig. 4). Splenocytes from both infected and uninfected C57BL/6 and SCID recipient mice secreted IFN-γ, although secretion was significantly higher in splenocytes from infected mice (Fig. 4). This suggests that $H. pylori$ Ags induce both specific and nonspecific responses from host splenocytes. Both specific and nonspecific IFN-γ responses to Formalin-fixed (surface) Ag were consistently higher than response to sonicated (soluble) Ag (Fig. 4), but these differences were not significant. Surprisingly, splenocytes recovered from infected recipients of CD4-depleted splenocytes or
CD4⁺CD45RBlow splenocytes and from uninfected recipients of CD4⁺ splenocytes also secreted IFN-γ in response to *H. pylori* Ag, despite the absence of DTH response and gastritis. Failure of splenocytes from recipients of CD4⁺CD45RBlow cells to respond to either Con A or anti-CD3 despite their CD4⁺ phenotype has been previously reported (16). Nonrecipient mice could not be evaluated because they had insufficient numbers of splenocytes.

**Recipients of splenocytes from IL-10- or IFN-γ-deficient mice**

In both donor IL-10-deficient mice and SCID recipients of IL-10-deficient splenocytes, gastritis, neutrophilic infiltration, and epithelial metaplasia were more extensive than in wild-type controls and recipients (Fig. 5). Concurrently, bacterial colonization was more effectively suppressed in IL-10-deficient mice than in C57BL/6 mice (5 × 10⁴ CFU/g compared with 2.37 × 10⁷ CFU/g, *p* = 0.016) and in recipients of IL-10-deficient splenocytes compared with recipients of normal splenocytes (4 ± 5 × 10⁴ compared with 1.1 ± 1.2 × 10⁶ CFU/g, *p* > 0.0001). In contrast, IFN-γ-deficient splenocytes induced less extensive gastric lesions (Fig. 5) and suppressed colonization less extensively than wild-type splenocytes. Four weeks after transfer, only small differences were present, but by 8 wk after transfer, gastritis was significantly less in recipients of IFN-γ-deficient splenocytes (*p* = 0.0002, Fig. 5) compared with recipients of normal splenocytes. Colonization was greater in recipients of IFN-γ-deficient splenocytes (4.8 ± 5.8 × 10⁶ CFU/g) than in recipients of normal splenocytes (1 ±

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**FIGURE 3.** Bacterial colonization 4 wk after transfer in SCID recipients of total and fractionated splenocytes. Bacterial colonization was inversely correlated with gastritis (see Fig. 1). Colonization was lowest in recipients of unfractionated (total), CD4⁺, or CD4⁺CD45RBhigh splenocytes, and highest in nonrecipients and in recipients of CD4-depleted or CD4⁺CD45RBlow splenocytes. The number in parentheses is the number of mice per group.

**FIGURE 4.** IFN-γ secretion in response to T cell mitogens and *H. pylori* Ags by splenocytes from C57BL/6 and recipient SCID mice. A, IFN-γ secretion by splenocytes from infected C57BL/6 mice was significantly higher than secretion by splenocytes from uninfected mice, but less than secretion by SCID recipient mice (B). B, Splenocytes from both infected and uninfected SCID recipients of CD4⁺ splenocytes secreted IFN-γ in response to *H. pylori*, but secretion by cells from infected mice was significantly higher in recipient mice. C, Splenocytes from all groups of infected recipients secreted IFN-γ in response to *H. pylori*. Secretion by recipients of CD4⁺ cells was higher than secretion by the other recipient groups. Cultured cells were stimulated by Formalin-killed whole *H. pylori* (HP Formalin) or by *H. pylori* sonicate (HP Sonicate). Con A and CD3 were positive controls. The number in parentheses is the number of mice per group.
2 × 10^5 CFU/g), but the difference did not reach statistical significance.

Discussion
A central finding of this study is that CD4^+ Th cells are both necessary and sufficient for induction of severe gastritis in infected recipient SCID mice. We previously showed that adoptive transfer of unfraccionated splenocytes from C57BL/6 mice to H. pylori-infected congenic SCID mice results in rapidly progressive, severe gastritis in the recipients (13). This inflammation is dependent on both splenocytes and H. pylori infection, and is accompanied by development of a DTH response to H. pylori Ags and suppression with eventual eradication of bacterial infection (15). No gastritis develops in either nonrecipient mice or uninfected recipient SCID mice. In the current study, we have extended these findings by demonstrating that the cells responsible for these changes are in the CD4^+ cell population, and that they are identifiable by strong expression of the CD45RB marker. CD4-depleted cells and CD4^+CD45RB^low cells do not induce gastritis, DTH, or bacterial suppression.

The clear role of CD4^+ /CD45RB^high T cells in induction of gastritis, demonstrated in this study, is evidence that gastritis due to H. pylori is most likely representative of a common mechanism characteristic of several other inflammatory diseases, including inflammatory bowel disease (IBD) and possibly autoimmune diseases in mice (14, 16–20). These diseases differ in the inciting Ag (luminal bacteria in the case of H. pylori gastritis and IBD, and self Ags in the case of autoimmune diseases), but appear similar in that they are caused by an imbalance in progressor and regulatory CD4 subsets. Adoptively transferred progressor cells expressing CD4 and high levels of CD45RB surface markers induce inflammation in immunodeficient recipients, while regulatory cells expressing CD4, in some studies CD25 (IL-2R, an activation marker) (16), and low levels of CD45RB do not induce inflammation, and in fact protect recipients from inflammation induced by CD45RB^high cells (17). It remains to be determined whether activated CD45RB^low cells are protective against H. pylori gastritis as they are in other diseases, but the similarity of these diseases is strongly suggestive of a common mechanism. In contrast to the studies cited above in which transfer of CD4^+ or CD4^+CD45RB^high splenocytes alone induced IBD, we did not observe lower bowel disease in our mice (data not shown). The absence of enteric helicobacter species in our mice may explain this difference. It is likely that enteric helicobacter were at least in part responsible for IBD in recipient mice in the published studies (21).

The data reported in this study support assertions that cellular rather than humoral immunity determines the outcome of H. pylori infection. Several studies have shown that failure of Ig production (in μMT-deficient and MHC II-deficient mice) has no effect on either gastritis or bacterial colonization (11, 12, 22), but the absence of immune cells completely eliminates gastritis (13, 22), suggesting that cellular rather than humoral immune response determines outcome of infection. More specifically, several studies done in mice infected with the related bacterium, H. felis, have indicated that cellular immune responses are central. Mohammadi et al. (5, 6) showed that adoptive transfer of CD4^+ cells to immunocompetent mice exacerbated gastritis, and Roth et al. (22) showed that transfer of CD4^+ cells induced gastritis in H. felis-infected immunodeficient C57BL/6 RAG1^−/− mice. We have extended these findings to H. pylori-infected mice and confirmed that humoral immunity is not necessary for full expression of gastritis.

Like other studies (2, 3, 5, 7, 8), our data indicate a role for IFN-γ in induction of gastritis. The expression of IFN-γ by CD4^+ splenocytes in response to H. pylori Ags is consistent with previous studies indicating that H. pylori induces a Th1-biased immune response and is associated with increased levels of IFN-γ in infected humans and mice (2, 3, 7, 8, 10). In addition, our finding that 8 wk after transfer, splenocytes from IFN-γ-deficient mice induced less extensive gastritis than did splenocytes from wild-type mice indicates at least some role for IFN-γ. This was true even though normal SCID mice have NK cells that may secrete IFN-γ. It indicates that IFN-γ secretion by sensitized T cells themselves is necessary, and is congruent with previous studies demonstrating the absence of gastritis in IFN-γ-deficient mice (7, 8).

Despite these indications that IFN-γ has a role in H. pylori gastritis, we also showed that IFN-γ secretion alone is not sufficient to induce gastritis. H. pylori Ags stimulated IFN-γ secretion by splenocytes from all mice examined, including infected and uninfected C57BL/6 and infected and uninfected SCID recipients of either CD4^+ or CD4-depleted lymphocytes, despite the absence of either DTH or gastritis in some of these mice. This finding suggests that while IFN-γ may be necessary for induction of gastritis, it is not sufficient. Unlike previous studies (23), we did demonstrate significantly higher IFN-γ secretion in infected mice and in mice given CD4^+ and CD4^+CD45RB^high cells compared with the other cell populations, suggesting that sensitization of lymphocytes...
may enhance secretory response. However, clearly there is a strong nonspecific component as well.

These results are not unprecedented. A recently published study investigating the inheritance of \textit{H. pylori} nonresponsiveness in mice demonstrated that while IFN-\(\gamma\) secretion correlated with gastritis in inbred parental mouse strains, splenocytes from hybrid F1 of gastritis. The authors of that study suggested that high levels of IL-10 also demonstrated in F1 mice may have masked the proinflammatory effects of IFN-\(\gamma\), presumably by suppressing TNF-\(\alpha\) or other macrophage products. The role of other costimulatory or regulatory cytokines in \textit{H. pylori} gastritis must be further examined.

The Th1 bias of \textit{H. pylori} gastritis is further supported by our finding that IL-10-deficient mice in this study developed more severe gastritis than did normal control mice, and that recipients of IL-10-deficient splenocytes developed more severe gastritis than did recipients of normal splenocytes. These findings are compatible with previous studies in which \textit{H. felis} induced more severe gastritis in IL-10-deficient mice than in normal mice (9). A new finding in the current study was that splenocytes from IL-10-deficient mice were more effective at induction of disease and suppression of colonization than were splenocytes from normal mice. However, this difference was small, and it suggests that transferred splenocytes from normal mice contain few IL-10-secreting cells. However, this difference was small, and it suggests that transferred splenocytes from normal mice contain few IL-10-secreting cells. Thus, even in splenocytes from normal mice, the regulatory effect of IL-10 in SCID recipients is small, and loss of this regulatory control in IL-10-deficient mice only results in a small exacerbation of gastritis.

The primary new findings presented in this study are that CD4\(^+\) lymphocytes are necessary and sufficient for induction of gastritis in \textit{H. pylori}-infected SCID mice, that high expression of CD45RB is a marker for gastritis-inducing CD4\(^+\) cells, that IFN-\(\gamma\) secretion alone in response to \textit{H. pylori} is not sufficient to induce gastritis by splenocytes, and that splenocytes from IL-10-deficient donors induce gastritis that is somewhat more severe than splenocytes from normal donors. These results are compatible with previous findings in both mice and humans suggesting that \textit{H. pylori} gastritis is a Th1-mediated disease induced by contact with bacterial Ags, and suppressed by IL-10. Finally, these results add to the increasing body of data that suggest that differences in severity of disease between different patients may be attributable to differences in local Th immune responses. Evaluation of this hypotheses will require examination of human tissues from patients with varying responses to \textit{H. pylori} colonization.

References


