Half of the world’s population is infected with the stomach-dwelling bacterium Helicobacter pylori (18). Why some individuals develop symptomatic disease, such as peptic ulcer or gastric adenocarcinoma (4, 9), while most H. pylori-infected hosts present with asymptomatic gastritis is not fully understood. The various disease manifestations are clearly multifactorial, with bacterial and environmental factors being important (7, 8, 15), but host genetic factors also exert significant influence (2, 20).

We and others have shown previously that Helicobacter felis infection of inbred mice with different genetic backgrounds induces a dichotomy of inflammatory responses (12, 20, 21, 23). Most mice respond to H. felis infection with severe inflammation; we term these mice responders. We further dissected the basis of this nonresponsiveness using F1 hybrid mice, crossing three responder strains with nonresponder CBA/Ca mice. Infection of these mice with H. felis demonstrated that the nonresponder phenotype was dominantly inherited, and we hypothesized that “suppressive” mechanisms exist which can inhibit the normal inflammatory response induced by H. felis infection (23).

Here we report that dominant inheritance of nonresponsiveness to H. pylori infection also occurs in mice. Additionally, we examined the cellular response which associates with the nonresponder phenotype, using both H. pylori (the human pathogen) and H. felis, which induces greater inflammation in responder mice.

Mice of the parental strains CBA/Ca, C3H/He, C57BL/6, and SJL and the F₁ hybrid strains CBA × C57BL/6, CBA × C3H/He, and SJL × CBA (maternal × paternal) were bred in the School of Microbiology and Immunology Animal Facility, University of New South Wales. Protocols involving animal experimentation were approved by the Animal Care and Ethics Committee at the University of New South Wales. Eight females of each strain were infected with H. pylori SS1 as previously described (17), with four noninfected controls. Six months postinfection, mice were sacrificed and gastritis was assessed histologically on blinded sections stained with hematoxylin and eosin (13). Each stomach was graded for activity (neutrophils) and mononuclear inflammatory cells in the antrum and body as follows: 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; 6, severe widespread. The total number of lymphoid follicles and gland abscesses in each section was counted.

Examination revealed neutrophilic and mononuclear cell infiltration into the gastric tissue of infected C3H/He, C57BL/6, and SJL mice (Table 1). Infected mice of these strains also presented with gland abscesses, and SJL mice developed significant lymphoid aggregates. All these parameters were significantly increased from those of CBA/Ca and all F₁ hybrid mice (Kruskal-Wallis: P < 0.05), which had either extremely mild or no gastritis (Table 1). There was no significant difference between CBA/Ca mice and the F₁ hybrids. Thus, the inflammatory phenotype of all F₁ strains was the same as that of the nonresponder CBA/Ca parent and different from those of their respective responder parents.

A Th1 immune response is responsible for cell-mediated immunity, is proinflammatory, and is marked by the production of cytokines including gamma interferon (IFN-γ) and interleukin-12 (IL-12). IL-12 is a key cytokine in the induction of a Th1 response leading to the production of IFN-γ. IFN-γ has been shown to be produced in the stomachs of both humans and mice infected with H. pylori (3, 11, 22) and is almost certainly a key factor in driving Helicobacter-induced gastritis. IL-10 can inhibit the production of IL-12 and thus downregulate the proinflammatory Th1-type response (10).

Thus, the IFN-γ and IL-10 cytokine response to Helicobacter infection was assessed in CBA/Ca, C57BL/6, and their (CBA × C57BL/6)F₁ hybrid mice (Walter Eliza Hall Institute, Melbourne, Australia). Ten females of each strain were infected with H. felis or H. pylori or left uninfected for 3 months. Histopathological examination, as described above, confirmed that in contrast to the responder C57BL/6 mice, the nonresponder CBA/Ca and (CBA × C57BL/6)F₁ hybrid mice developed no gastritis whether infected with H. felis or H. pylori (data not shown).

Spleen cell suspensions were depleted of red cells by hypotonic shock in water, and remaining splenocytes were cultured at 10⁶/ml in complete medium (RPMI 1640 medium [Gibco BRL, Gaithersburg, Md.] with 10% fetal calf serum, 2 mM glutamine, 50 IU of penicillin/ml, 50 μg of streptomycin [Trace Biosciences, Castle Hill, New South Wales, Australia] per ml, 100 μg of L-glutamine [Gibco BRL], and 50 μg of gentamicin [Sigma, St. Louis, Mo.,] and incubated at 37°C for 48 h. Cells were harvested, washed, and stained with fluorescein isothiocyanate-conjugated anti-CD3 (clone 145-2C11), phycoerythrin-conjugated anti-CD4 (clone RPA-T4), phycoerythrin-conjugated anti-CD8 (clone 53-6.7), and 7-aminoactinomycin D (clone 7-AAD; Becton Dickinson, San Jose, Calif.) antibodies. CD3, CD4, and CD8 expression were assessed by flow cytometry.
Evidence from knockout mice deficient in IL-10 also suggests a role for this cytokine in controlling the inflammatory response to infection with *Helicobacter hepaticus* (16) and *H. felis* (5). There may be unknown consequences of the complete lack of IL-10 for immune development in these mice; thus, an advantage of the F1 hybrid model is that the mice used are immunocompetent. The demonstration of an association of cytokine profile with inflammatory response supports the *Helicobacter*-induced gastritis. The production of IFN-γ was apparently irrelevant with its proinflammatory effects clearly overridden, possibly by the inhibitory activity of IL-10.

The same situation may exist in humans, with several studies showing the production of both IL-10 and IFN-γ in response to *H. pylori* (1, 14). Bodger et al. reported higher production of IL-10 in *H. pylori*-infected individuals than in noninfected persons and those with *Helicobacter*-negative gastritis. IL-10 was associated with the severity of inflammation; the authors proposed that the increased secretion of this cytokine may be part of the host’s attempt to control the inflammation (6).

Evidence from knockout mice deficient in IL-10 also suggests a role for this cytokine in controlling the inflammatory response to infection with *Helicobacter hepaticus* (16) and *H. felis* (5). There may be unknown consequences of the complete lack of IL-10 for immune development in these mice; thus, an advantage of the F1 hybrid model is that the mice used are immunocompetent. The demonstration of an association of cytokine profile with inflammatory response supports the knockout mouse data and allows greater confidence in concluding that IL-10 is an important factor controlling *Helicobacter*-induced gastritis.

In summary, we have confirmed that host genetic mechanisms that influence inflammation in *H. felis*-infected mice also control responses to the human pathogen *H. pylori*. Using these models, we have demonstrated that production of IL-10 but not of IFN-γ is associated with the nonresponsiveness of certain strains of mice to *Helicobacter* infection. Determining

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**TABLE 1. Histopathological gradings of parental and F1 hybrid mice with or without *H. pylori* infection.**

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Infection status</th>
<th>Antrum</th>
<th>Body</th>
<th>No. (mean ± SD) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Activity</td>
<td>CI</td>
<td>Activity</td>
</tr>
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<td>Control</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
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<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>CBA × C3H/He</td>
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<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
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<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>CBA × C57BL/6</td>
<td>Control</td>
<td>0 (0–1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>0 (0)</td>
<td>0 (0–1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SJL × CBA</td>
<td>Control</td>
<td>0 (0)</td>
<td>0 (0–1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>0 (0–1)</td>
<td>0 (0)</td>
<td>0 (0–2)</td>
</tr>
<tr>
<td>C3H/He</td>
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<td>0 (0–1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>1 (0–3)*</td>
<td>1 (0–2)*</td>
<td>0 (0)</td>
</tr>
<tr>
<td>C57BL/6</td>
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<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>1 (0–3)*</td>
<td>0.5 (0–2)*</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SJL</td>
<td>Control</td>
<td>0 (0)</td>
<td>0 (0–1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>2 (0–3)*</td>
<td>1 (0–4)*</td>
<td>2.5 (1–3)*</td>
</tr>
</tbody>
</table>

*Values for neutrophil infiltration (activity) and mononuclear cell infiltration (CI) are from a 6-point scale: 1, mild multifocal; 2, mild widespread or moderate multifocal; 3, mild widespread and moderate or severe multifocal; 4, moderate widespread; 5, moderate widespread and severe multifocal; 6, severe widespread. Data are nonparametric and are expressed as median values (ranges). *, significantly greater than value for noninfected controls (P < 0.05).
the basis of unresponsiveness will allow us to better understand *H. pylori* pathogenesis and may provide vital information regarding individuals at risk of developing the more severe complications of an *H. pylori* infection.

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REFERENCES