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Dominant Nonresponsiveness to *Helicobacter pylori* Infection Is Associated with Production of Interleukin 10 but Not Gamma Interferon

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***Helicobacter pylori*-induced gastritis is an essential precursor lesion for the development of peptic ulcers or gastric adenocarcinoma. We demonstrate that nonresponsiveness to *H. pylori* SS1 infection is dominantly inherited in mice. F_1 hybrid crosses between a nonresponder mouse and three responder strains all possessed the nonresponder phenotype. Secretion of interleukin-10 but not gamma interferon was associated with nonresponsiveness to infection.**

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 ulceration 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 corpal gastritis, but some strains such as BALB/c and CBA do not develop inflammation; we term these mice nonresponders. We further dissected the basis of this nonresponsiveness using F_1 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 F_1 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 F_1 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 F_1 hybrids. Thus, the inflammatory phenotype of all F_1 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)F_1 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)F_1 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 10^6/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,

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TABLE 1. Histopathological gradings of parental and F_1 hybrid mice with or without *H. pylori* infection.[a]

Mouse strain	Infection status	Antrum		Body		No. (mean ± SD) of:	
		Activity	CI	Activity	CI	Lymphoid aggregates	Gland abscesses
CBA	Control	0 (0)	0 (0)	0 (0)	0 (0)	0	0
	Infected	0 (0)	0 (0)	0 (0)	0 (0)	0	0
CBA × C3H/He	Control	0 (0)	0 (0)	0 (0)	0 (0)	0	0
	Infected	0 (0)	0 (0)	0 (0)	0 (0)	0	0
CBA × C57BL/6	Control	0 (0–1)	0 (0)	0 (0)	0 (0)	0	0
	Infected	0 (0)	0 (0–1)	0 (0)	0 (0–1)	0	0
SJL × CBA	Control	0 (0)	0 (0–1)	0 (0)	0 (0)	0	0
	Infected	0 (0–1)	0 (0)	0 (0–2)	0 (0–1)	0.1 ± 0.3	0
C3H/He	Control	0 (0–1)	0 (0)	0 (0)	0.5 (0–1)	0.2 ± 0.4	0
	Infected	1 (0–3)*	1 (0–2)*	1 (0–3)*	1 (0–2)*	0.3 ± 0.5	0.7 ± 1.0*
C57BL/6	Control	0 (0–1)	0 (0)	0 (0)	0 (0)	0	0
	Infected	1 (0–3)*	0.5 (0–2)*	1 (0–4)*	1 (0–3)*	0	0.9 ± 1.4
SJL	Control	0 (0)	0 (0)	0 (0–1)	0 (0)	0	0.2 ± 0.4
	Infected	2 (0–3)*	1 (0–4)*	2.5 (1–3)*	2 (1–3)*	0.6 ± 0.7*	1.1 ± 1.2*

[a] 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 ($P < 0.05$).

and 2.5 μg of amphotericin B [Bristol-Myers Squibb, Princeton, N.J.] per ml) with or without *H. pylori* or *H. felis* lysate at 5 μg/ml. After 2 days of incubation at 37°C with 5% CO_2, the supernatants were collected for the assessment of cytokines by standard enzyme-linked immunosorbent assay. Maxisorp immunoplates (Nunc, Roskilde, Denmark) were coated with anticytokine antibodies (Pharmingen, San Diego, Calif.) in bicarbonate buffer (pH 9.6). Wells were blocked with 1% (wt/vol) bovine serum albumin in phosphate-buffered saline. Culture supernatants or serial dilutions of recombinant IL-10 (Pharmingen) or IFN-γ (Sigma, St. Louis, Mo.) in complete medium were added to wells in duplicate. Biotinylated anticytokine antibodies (Pharmingen) in phosphate-buffered saline–bovine serum albumin were added, followed by streptavidin-alkaline phosphatase (Zymed, South San Francisco, Calif.). Color was developed by the addition of Sigma 104 phosphatase substrate tablets (Sigma) dissolved in diethanolamine buffer. Absorbance was read at 405 nm. A standard curve was plotted for each recombinant cytokine, from which cytokine levels in supernatants were determined.

Cytokine production was observed only following culture with *Helicobacter* antigens, and interestingly, the infectious status appeared to be irrelevant. There was virtually no difference in the cytokine patterns whether from uninfected or *H. felis*- or *H. pylori*-infected mice. This suggests that the host mounts an inherent response upon the first exposure to *Helicobacter* antigens, whether in the form of lysate or viable whole bacteria. This initial response apparently dictates the type of long-term immune response that develops.

Nonresponder CBA/Ca mice produced very low levels of IFN-γ but large amounts of IL-10 (Fig. 1). Responder C57BL/6 mice produced high levels of IFN-γ but little or no IL-10. Nonresponder F_1 hybrid CBA × C57BL/6 mice produced high levels of IFN-γ, significantly more than the CBA/Ca parent (one-way analysis of variance; $P < 0.001$) but not significantly more or less than the C57BL/6 mice. Inverse to this, F_1 mice produced high levels of IL-10 like the CBA/Ca parent but significantly more than the C57BL/6 mice ($P < 0.05$). Thus, regarding cytokine production, the nonresponder F_1 mice were true hybrids, secreting IL-10 like the CBA/Ca nonresponders, but surprisingly also produced IFN-γ levels indistinguishable from those of their responder C57BL/6 parents. IFN-γ would classically be expected to produce inflammation and has been shown to mask the detection of Th2 cytokines in *Helicobacter*-immunized mice (19). This raises the possibility that IL-10 can be a dominant factor in controlling *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 F_1 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

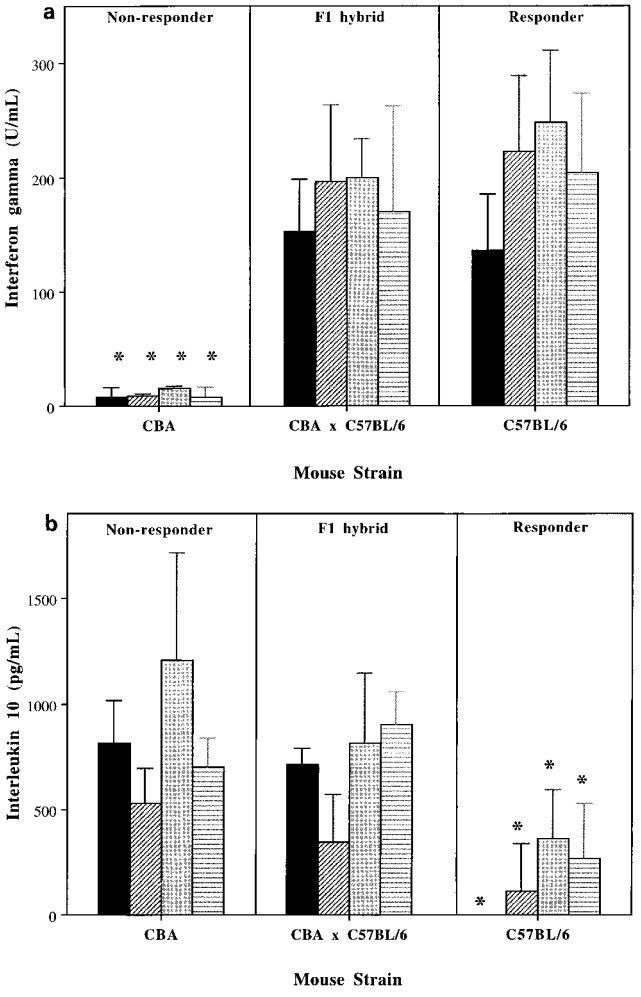

FIG. 1. Production of IFN-γ and IL-10 following in vitro stimulation of mice with *Helicobacter* antigens. Data shown represent the mean ± standard deviation for IFN-γ (a) and IL-10 (b) by enzyme-linked immunosorbent assay. All supernatants from cells cultured in medium alone were negative for both cytokines (not shown). Hf, *H. felis*; Hp, *H. pylori*. *, significantly less cytokine than the equivalent group of the other two strains ($P < 0.05$). ■, Uninfected and Hf, stimulated; ▨, Uninfected and Hp stimulated; ▨, Hf infected and Hf stimulated; ▤, Hp infected and Hp stimulated.

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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