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CLEARANCE OF *HELICOBACTER PYLORI* INFECTION THROUGH IMMUNISATION: THE SITE OF T CELL ACTIVATION CONTRIBUTES TO VACCINE EFFICACY*

THOMAS G. BLANCHARD[1,2], JULIA C. EISENBERG[2], and YUKO MATSUMOTO[1]

Departments of [1]Pediatrics and [2]Pathology,
Case Western Reserve University School of Medicine, Cleveland, Ohio, USA

SUMMARY

H. pylori vaccine development has progressed rapidly in animal models. Both *H. pylori*-associated pathogenesis and protective immunity are $CD4^+$ T cell dependent, with no discernable phenotypic difference to distinguish pathogenic T cells from protective T cells. Functionally however, protective T cells promote enhanced inflammation upon *H. pylori* challenge. Additionally, only mouse models such as phagocyte oxidase- or IL-10-deficient mice that respond to *H. pylori* infection with intense gastritis are capable of demonstrating spontaneous eradication of the bacteria. These data, combined with recent descriptions of down-regulatory T cells in infected humans and mice, support an emerging model of *H. pylori* pathogenesis in which *H. pylori* induces inflammation that is limited by regulatory T cells in the stomach. Immunisation therefore may succeed by activating T cells in peripheral lymph nodes that are capable of promoting qualitatively or quantitatively different inflammation when recruited to the stomach. Evidence in support of this model will be discussed.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is one of the world's most successful pathogens, infecting greater than 50% of the earth's population (*Marshall*, 1995). Prevalence of infection ranges from 20% in some developed nations to greater than 90% in some developing nations. *H. pylori* is a Gram-negative bacterium whose primary niche is the human gastric mucosa, where it resides in the mucus and on the surface of gastric epithelial cells. A direct role for *H. pylori* in gastritis and peptic ulcer disease has now been established through the successful culture of *H. pylori* from gastric biopsies (*Marshall* and *Warren*, 1984), the fulfilment of Koch's postulates in human volunteers (*Marshall* et al., 1985; *Morris* and *Nicholson*, 1987), and numerous studies documenting the complete and permanent remission of ulcers following antimicrobial therapy (*NIH Consensus Conference*, 1994). *H. pylori* is also recognised as a risk factor for the development of gastric adenocarcinoma and has been categorised by the

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World Health Organisation as a Class I human carcinogen (*World Health Organization*, 1994).

A number of antimicrobial therapies have been developed for treatment of *H. pylori* infection, with eradication rates ranging from 60% to over 90%. These therapies typically include at least two antibiotics and a proton pump inhibitor, and must be taken several times per day for up to 14 days. The complexity of therapy however, often results in poor patient compliance, and the cost of these drugs is prohibitive in nations where *H. pylori* is endemic. Additionally, significant resistance to antibiotics such as clarithromycin and metronidazole are already being reported. Finally, from an immunologic perspective, even successful eradication therapy does not protect the host from potential re-infection, nor protect asymptomatic hosts at risk for developing gastric cancer. Therefore, interest in a *H. pylori* vaccine is quite high.

HOST RESPONSE

H. pylori infection induces histologic gastritis in all infected individuals (*Dooley* et al., 1989), with subgroups progressing to symptomatic gastritis and peptic ulcer disease. The inflammation has both an acute and chronic character, with a monocytic and polymorphonuclear component remaining prevalent after lymphocytes are recruited to the mucosa. *H. pylori* infection is typically associated with focal neutrophil infiltration of the gastric epithelium, most often in the gland necks (*Warren*, 2000). The lamina propria becomes infiltrated with lymphocytes, normally absent from the stomach, which may then form a moderately diffuse pattern extending the full thickness of the mucosa. Lymphocytes will also occasionally form focal patterns, and the development of lymphoid follicles with germinal centres has been noted. Long-term manifestations of infection involve changes in the architecture of the epithelial cell monolayer, including disorganisation of the epithelial cells, atrophy, and metaplasia.

In addition to the persistent inflammation that accompanies *H. pylori* infection, a strong adaptive immune response also develops. The presence of *H. pylori*-specific serum IgG antibodies remains one of the quickest and simplest methods for detecting *H. pylori* infection. Studies performed on gastric biopsies and washings have also demonstrated the presence of *H. pylori*-specific IgA at the gastric mucosa (*Rathbone* et al., 1986; *Wyatt* et al., 1986; *Blanchard* et al., 1999a,b). Numerous studies have also documented strong *H. pylori*-specific T cell responses using lymphocytes isolated from infected individuals (*Karttunen* et al., 1990,1995; *Karttunen*, 1991; *Sharma* et al., 1994; *Fan* et al., 1994; *Di Tommaso* et al., 1995; *D'Elios* et al., 1997; *Lindholm* et al., 1998; *Sommer* et al., 1998; *Bamford* et al., 1998) (Table 1). Both peripheral blood mononuclear cells (PBMC) and lamina propria mononuclear cells (LPMC) from gastric explants respond to *H. pylori* stimulation *in vitro* by secretion of cytokines or by proliferation. These studies routinely result in a predominance of interferon-γ-producing T cells, consistent with *H. pylori* inducing a Th1 mediated, pro-inflammatory response.

Table 1: T cell cytokine and proliferation response following *in vitro* stimulation with *H. pylori* antigen is characterised by IFN-γ production

Cells	Assay	*H. pylori* positive patient		*H. pylori* negative patient	Reference
PBMC	ELISA ^{3}H-thymidine	↑IFN-γ[a] Proliferation	< <	↑IFN-γ Proliferation	*Karttunen* et al, 1990
PBMC	ELISA ^{3}H-thymidine	↑TNFα ↑IL-2 Proliferation	< = <	↑TNFα ↑IL-2 Proliferation	*Karttunen*, 1991
PBMC	^{3}H-thymidine	Proliferation		Proliferation	*Sharma* et al., 1994
PBMC, LPMC	ELISA ^{3}H-thymidine	↑IFN-γ Proliferation	< <	↑IFN-γ Proliferation	*Fan* et al., 1994
LPMC	ELISPOT	↑IFN-γ	<	↑IFN-γ	*Karttunen* et al., 1995
PBMC and LPMC (T cell clones)	^{3}H-thymidine	↑Proliferation		n.d.[b]	*Di Tommaso* et al., 1995
LPMC (T cell clones)	RT-PCR + ELISA	↑IFN-γ ↑TNFα ↑IL-4		-IFN-γ[c] -TNFα -IL-4	*D'Elios* et al., 1997
LPMC (T cell clones)	Immunohisto- chemistry	↑IFN-γ ↑TNFα ↑IL-4	 =	-IFN-γ -TNFα ↑IL-4	*Lindholm* et al., 1998
LPMC	Flow cytometry	↑IFN-γ ↑IL-4		n.d.	*Sommer* et al., 1998
LPMC	Flow cytometry	↑IFN-γ ↑IL-2		n.d.	*Bamford* et al., 1998

[a] ↑ indicates in increase following *in vitro* stimulation.
[b] n.d. indicates not determined.
[c] - indicates little or no cytokine was detected.

VACCINE PROTOTYPES IN ANIMAL MODELS

In the early stages of *H. pylori* vaccine research, immunologists and microbiologists had at least two reasons to doubt the potential success of such a vaccine. First, because *H. pylori* is a non-invasive mucosal pathogen, successful vaccination would most likely require oral delivery. Previous vaccine research had established that to stimulate efficacious immunity in gastrointestinal tissue, direct immunisation of mucosal tissue was required, optimally through oral immunisation. This complicated vaccine design, as ingested proteins are poor immunogens, and the acid environment of the stomach must be traversed to gain access to the lymph tissue-rich intestines. This problem had hindered the development of oral vaccines in humans for years, and had yet to be successfully overcome. Second, the *H. pylori*-induced adaptive immune response is ineffective following natural infection. Since *H. pylori* is able to persist in the face of an active immune response, it seemed unlikely that stimulation of a similar immune response through immunisation would be effective.

Oral vaccine research in animals

The development of a *Helicobacter* mouse model with the cat pathogen, *H. felis* (*Lee* et al., 1990), allowed researchers to test the efficacy of vaccination in mice (*Czinn* et al., 1993; *Chen* et al., 1992). The vaccination protocol was based upon an experimental Sendai virus model in which the mucosal adjuvant, cholera toxin, was combined with viral antigen to stimulate immunity in the upper respiratory tract of mice (*Nedrud* et al., 1987). A similar protocol effectively stimulated an anti-Helicobacter humoral response when cholera toxin was combined with Helicobacter proteins and delivered orally to mice (*Czinn* and *Nedrud*, 1991). When applied to the *H. felis* challenge model, nearly 80% of the mice were found to be protected from chronic infection (*Czinn* et al., 1993; *Chen* et al., 1992). Although these experiments were performed with crude bacterial lysate, several other laboratories soon expanded these studies to include successful immunisations with purified Helicobacter proteins such as the *Helicobacter* urease enzyme (*Michetti* et al., 1994; *Ferrero* et al., 1994) and heat shock protein (*Ferrero* et al., 1995).

Several laboratories also demonstrated that infected mice could be therapeutically immunised to accomplish eradication of the bacteria (*Corthesy-Theulaz* et al., 1995; *Doidge* et al., 1994). This concept was strengthened when a similar study was performed on ferrets infected with endogenous *H. mustelae* (*Cuenca* et al., 1996). The therapeutic immunisation experiments were of profound importance because they demonstrated that vaccination succeeds not because it induces an immune response prior to infection, but because immunisation must induce a quantitatively or qualitatively different immune response than normally induced by chronic infection.

Despite the excitement generated by these and most other *H. pylori* vaccine experiments, enthusiasm has always been tempered by two observations. First, when immunised mice are challenged with *Helicobacter* bacteria they respond with gastric inflammation that is histologically indistinguishable from the inflammation that accompanies natural infection. This response is termed "post-immunisation gastritis" and it can persist for months after the challenge organisms have been eradicated, although it eventually does dissipate (*Garhart* et al., 2002). Second, protection is often incomplete. In many experiments protective immunity has been defined as a significant reduction in bacterial load. In fact, in one experiment, where antibiotic therapy was applied to protected mice, there was a rapid remission of post-immunisation gastritis, suggesting the presence of *Helicobacter* organisms that went undetected by enzyme indicators and culture techniques (*Ermak* et al., 1997). Both of these observations illustrate the need to develop a better understanding of *H. pylori* pathogenesis and immunity.

By the mid-1990s, clinical isolates of *H. pylori* had been successfully adapted to several animal models including mice and some nonhuman primates. All early observations previously made in the *H. felis* model were confirmed and expanded with *H. pylori* (*Marchetti* et al., 1995; *Ghiara* et al., 1997). As a general rule, all of these immunisations have relied upon some variation of the original protocol, a purified or crude protein antigen combined with either cholera toxin or *E. coli* heat labile toxin (LT), given in multiple doses to the recipient animal prior to or subsequent to challenge.

Alternatives routes of mucosal immunisation

Cholera and *E. coli* LT enterotoxins are potent adjuvants for protein antigens delivered orally in animal models. Both increase the immunogenicity of protein antigens without having to form covalent linkages or emulsions, and less than 10 μg is required to retain adjuvanticity. Small doses of enterotoxin however are sufficient for toxicity when given to humans, as demonstrated in a recent clinical trial testing a therapeutic *H. pylori* vaccine (see clinical trials) (*Michetti* et al., 1999). Side effects such as diarrhoea and cramping may occur. Therefore, efforts at developing a safe and efficacious vaccine for *H. pylori* in humans have moved towards avoiding the inherent risk involved in taking oral enterotoxin. One strategy has been to develop *E. coli* LT with point mutations that reduce or eliminate toxicity without reducing adjuvanticity (*Marchetti* et al., 1998). This strategy has met with some success and is currently under further development.

A second strategy has been the search for alternative routes of immunisation. Both rectal and intranasal immunisations have been tested to induce mucosal immunity that disseminates to the stomach upon challenge with *H. pylori* in mice (*Kleanthous* et al., 1998). There is evidence in the mouse model that intranasal immunisation is more efficacious than the oral immunisation (*Garhart* et al., 2003a). The rectal and intranasal immunisation protocols are similar to oral immunisation in that multiple doses are required and a bacterial toxin adjuvant is necessary. However, the success of these alternative routes of mucosal immunisation is actually a major advance in vaccine development, since they require less antigen in mice (100 μg for intranasal versus 2 to 4 mg for oral) and the risks associated with the toxin adjuvant are significantly reduced.

Systemic immunisation against H. pylori infection

Intranasal immunisation, although successful in mice, remain experimental and controversial in humans. A mucosal adjuvant is still required and intranasal application does not preclude ingestion of some part of the vaccine, consequently still exposing the patient to risk for toxicity. Additionally, recent reports indicate that CT and LT enterotoxins can target the central nervous system via the olfactory epithelium and nerves, and can induce histologic inflammation within the olfactory bulb (*Fujihashi* et al., 2002). Therefore, we and others have pursued the possibility of employing

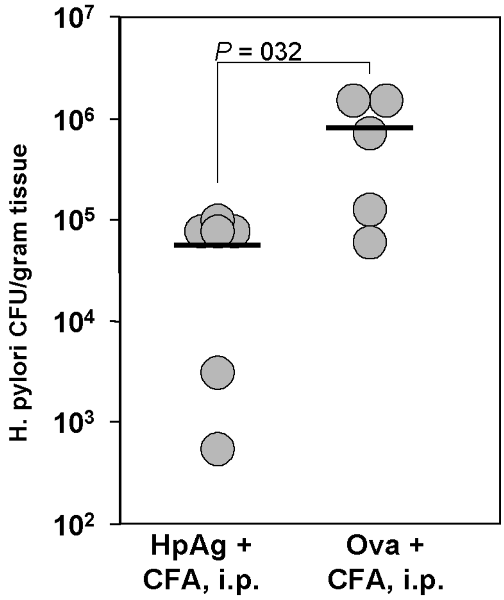

Figure 1. Systemic immunisation of mice against *H. pylori* reduces the bacterial load. Mice were immunised i.p. with a single dose of 100 µg of either *H. pylori* lysate or ovalbumin emulsified in complete Freund's adjuvant. Mice were challenged with 10^7 *H. pylori* 28 days post-immunisation and the number of colony forming units in gastric biopsies was determined 28 days post-challenge. Statistical analysis was performed by ANOVA.

traditional systemic vaccination to induce protective immunity against *H. pylori*. We have found that both intraperitoneal and subcutaneous prophylactic immunisations can result in significant reduction in bacterial load by four weeks after challenge of mice with infectious *H. pylori* (*Gottwein* et al., 2001; *Eisenberg* et al., 2003). Similar levels of protection can be induced by either Th1 (Freund's complete adjuvant) or Th2 (aluminium hydroxide or Freund's incomplete adjuvant) polarising vaccine regimens. An example of this immunity is shown in Figure 1 where mice were immunised with either *H. pylori* lysate or ovalbumin protein emulsified in complete Freund's adjuvant and given a single injection of 100 µg protein by intra-peritoneal injection. Mice were challenged with $1x10^7$ CFU *H. pylori* 28 days after immunisation and then assessed 28 days after challenge. Although immunisation did not provide sterilising immunity, there was a significant reduction in bacterial load (p= 0.032). We have achieved similar reductions when immunising neonatal mice within 24 hours of birth (*Eisenberg* et al., 2003), thus demonstrating the potential application for young children prior to contracting *H. pylori*. Several additional laboratories have demonstrated success with other adjuvants (*Guy* et al., 1998; *Weltzin* et al., 2000).

The results of these systemic immunisation experiments provide valuable insight into *H. pylori* immunity. Whereas systemic immunisation typically fails when applied against other mucosal pathogens, they can be efficacious against *H. pylori*. Thus it appears that immunisation by almost any route, including oral (which targets the Peyer's Patches of the small intestine), intranasal, rectal, and systemic can generate some degree of protective immunity when applied to mice. The relevant feature of a successful *H. pylori* vaccine therefore might not be stimulation of the mucosal immune response, but rather stimulation of an immune response in a tissue or lymph node designed to optimise immune responsiveness. This concept will be discussed further below.

CLINICAL TRIALS

The early success of oral vaccination against *H. felis* and *H. pylori* in mice led to the rapid development of a prototype oral vaccine for use in humans. Doses of either 180, 60, or 20 mg of recombinant *H. pylori* urease was administered with 5 μg *E. coli* LT and given to infected volunteers as an oral therapeutic vaccine (*Michetti* et al., 1999). Vaccination was delivered in four doses similar to the protocol used for mice. The vaccine significantly enhanced the number of circulating *H. pylori*-specific IgA-secreting cells over those in placebo immunised control volunteers demonstrating immunogenicity. Most encouraging was the significant reduction in bacterial load of urease LT-immunised subjects compared to control volunteers ($p=0.032$). Enthusiasm was somewhat dampened by the prevalence of diarrhoeal episodes induced by the *E. coli* LT adjuvant. Sixty six percent of the volunteers who completed the study experienced some level of diarrhoea, but the study confirmed the possibility of positive influence on gastric immunity in humans through oral vaccination. Several additional clinical trials have now been performed by other laboratories in which vaccine formulations were shown to be immunogenic as well. However, none have achieved the efficacy of the original study. Buoyed by the promise of this initial study, a new generation of trial vaccines is now being developed and tested. A more thorough understanding of *H. pylori* immunity will aid in the development of a better human vaccine.

IMMUNE EFFECTOR MECHANISMS IN *H. PYLORI* IMMUNITY

One means of optimising a vaccine for *H. pylori* would be to specifically design a vaccine to enhance that aspect of the immune system that mediates the protective immune response. Many studies have now been performed to elucidate how the immune system actually eradicates *H. pylori* once stimulated by immunisation. The focus has been to identify effector mechanisms or cells that are essential for protection, and to differentiate those factors from their counterparts that are also present during the chronic inflammation that accompanies natural infection.

The role of antibodies in the protective immune response

Since *H. pylori* predominantly resides at the apical surface of the gastric epithelium, the types of known immune effector mechanisms that might actually come into contact with *H. pylori* seem limited. The existence of tight junctions between epithelial cells severely limits the ability of leukocytes to cross the epithelium. Polymeric IgA however, is transported across the epithelium via the polymeric immunoglobulin receptor and released into the lumen. Although no correlation had been established between IgA levels and protective anti-*H. pylori* immunity, IgA seemed the most likely immune effector molecule for interacting with *H. pylori* to mediate protection. However, in our studies with IgA-deficient mice, protective immunity was achieved at a level similar to that in wild type mice (*Blanchard* et al., 1999c). Because secretory IgM levels were found to compensate for the lack of IgA, we subsequently repeated these experiments with total antibody knock-out mice. Our results were consistent with those of others using the same model in that lack of antibody production in mice did not compromise the ability of an oral vaccine to induce protective immunity (*Ermak* et al., 1998; *Sutton* et al., 2000). Therefore, although secreted antibody may contribute to *H. pylori* immunity, it is not required.

The role of T cells in the protective immune response

The cellular requirements for protective immunity have been difficult to identify. Two studies using MHC I knockout mice and MHC II knockout mice have suggested the requirement for $CD4^+$ cells but not for $CD8^+$ cells in generating protective immunity (*Pappo* et al., 1999; *Ermak* et al., 1998). We found that injection of Helicobacter-primed $CD4^+$ T cells was sufficient to transfer protective immunity to otherwise immunodeficient $rag1^{-/-}$ mice (*Gottwein* et al., 2001). These studies demonstrate that T cell help is required to generate an adaptive immune response but do not advance our insight into the mechanism of protection. To further refine our understanding, many groups have used mice deficient in specific cytokines or cytokine receptors to elucidate which T cells may be most important in providing protective immunity. The most widely studied of the T cell cytokines have been IL-4 and IFN-γ, but it is now apparent that neither of these cytokines is essential to induce the protective immune response (*Lucas* et al., 2001; *Akhiani* et al., 2002; *Sawai* et al., 1999; Garhart, 2003a,b).

The role of innate host factors in the protective immune response

The importance of innate factors in *H. pylori* immunity has only recently been addressed. However, as discussed below, it may be that immunity is accomplished through the enhancement of inflammation by appropriately activated T cells. It is important therefore, to determine how innate factors may be contributing to *H. pylori* immunity. Two recent studies have demonstrated that although inducible nitric oxide synthase (iNOS) is upregulated in inflamed gastric tissue following challenge, iNOS deficient mice can be effectively immunised against *H. pylori* (*Garhart* et al., 2003a; *Blanchard* et al., 2003). This was true even when mice were deficient in both iNOS and phagocyte oxidase, the two primary host innate anti-bacterial defence mechanisms (*Blanchard* et al., 2003). In a separate study, mast cells have been shown to be unnecessary to achieve protection in mice from *H. pylori* through vaccination (John Nedrud and Steve Czinn, personal communication).

One non-T cell, pro-inflammatory factor that does seem to be necessary for protection is IL-12. Two separate laboratories have now demonstrated that mucosal immunisation of IL-12-deficient mice fails to induce significant protection as compared to non-immunised control mice (*Garhart* et al., 2003a; *Akhiani* et al., 2002). Both groups employed the IL-12 p40 subunit knockout to eliminate the formation of biologically active heterodimeric p70. Elimination of p40 also prevents formation of IL-23. Whether IL-12, IL-23, or both are required for the induction of protection remains to be determined. Regardless, whereas both IFN-γ and

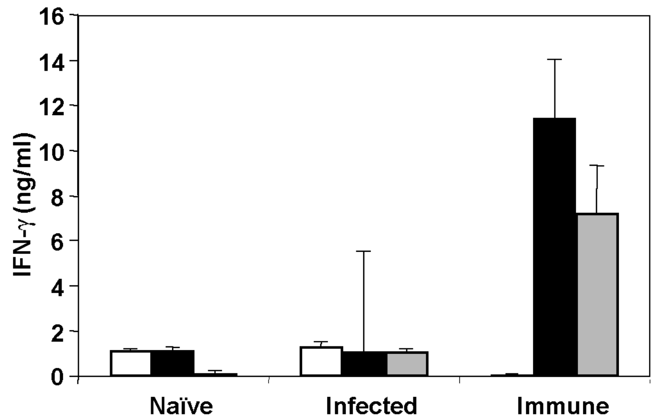

Figure 2. Memory T cells from immune mice produce IFN-γ in response to antigen presentation by mucosal epithelial cells. MODE-K epithelial cells (1x10^4) were combined with 1 x 10^6 $CD4^+$ spleen cells from naïve, infected, or immune mice and pulsed with either PBS (white bars) or *H. pylori* lysate (black bars). To demonstrated class II-restricted antigen presentation anti-MHC-II blocking antibody was also tested (gray bars). Supernatants were assessed after 48 hours for IFN-γ by ELISA.

IL-12 p40 knockout mice are capable of generating inflammation in response to *H. pylori* challenge, only IL-12 p40 is required to induce a protective state. These findings indicate that the character of the inflammatory response in IFN-γ knockout mice is qualitatively different than that in IL-12 p40 knockout mice.

H. PYLORI-ASSOCIATED INFLAMMATION AND IMMUNOREGULATION

Most efforts at defining *H. pylori* immunity have focused on identifying a specific effector mechanism. Another interesting possibility is the ability of *H. pylori* to down-regulate the inflammatory or immune response. This concept may seem counter-intuitive since studies in both mice and humans routinely report that infection with *H. pylori* results in *H. pylori*-specific IFN-γ producing T cells, and infection induces both inflammation and adaptive immune mechanisms. However, close inspection of the data suggests that *H. pylori* may in fact suppress the immune response, or at least the aspect of the immune response required for eradication of the bacteria. This was evident in several early studies in which it was demonstrated that T cells from infected patients responded no better than T cells from seronegative patients with regard to *H. pylori*-induced cytokine production and proliferation (Table 1). In several studies, cells from control donors actually responded as well as, or significantly stronger than cells from infected donors with more IFN-γ production or proliferation in recall assays against *H. pylori* antigen (*Karttunen* et al., 1990; *Karttunen*, 1991; *Karttunen* et al., 1995; *Fan* et al., 1994; *Sharma* et al., 1994). This observation perhaps did not garner the attention it deserved and latter studies have focused exclusively on T cells or T cell clones from infected individuals.

In mice, the data has tended to establish *Helicobacter* infection results in strong T cell reactivity *in vitro* compared to T cells from naïve mice. Several of those studies were performed with the *H. felis* mouse model (*Mohammadi* et al., 1996; *Fox* et al., 2000) but one laboratory reported *H. pylori*-infected mice had a significant increase in IFN-γ production in recall assays compared to naïve control mice (*Smythies* et al., 2000). Our own studies in the *H. pylori* mouse model demonstrate only weak induction of IFN-γ production by T cells from infected animals. Whereas we have been able to detect cytokines such as IFN-γ and IL-2 in response to *H. pylori* infection, these responses are significantly weaker than those induced by our immunisation strategies (*Eisenberg* et al., 2003). Others have also noted increased IFN-γ production in immunised mice compared to infected control mice (*Garhart* et al., 2003a; *Goto* et al., 1999). We have noted these differences regardless of the type of antigen presenting cell used to activate T cells. Figure 2 illustrates that antigen presentation by a mouse gastrointestinal epithelial cell line, to mimic what may be occurring in the gastric mucosa, induced low levels of IFN-γ by $CD4^+$ T cells from *H. pylori*-infected mice while immunised mice responded with significantly greater levels of cytokine. This IFN-γ production was partially diminished by anti-MHC class II antibody. As discussed above, IFN-γ is not required for induction of protective immunity. Nevertheless, it remains a good marker for a pro-inflammatory response when present.

$CD25^+$ Immunoregulatory T cells

In support of an immunoregulatory capacity for *H. pylori,* there is new evidence in both mice and humans that *H. pylori*-specific T regulatory cells are present in the infected host and actually work to limit the T cell or inflammatory response to *H. pylori*. Thus, when peripheral blood mononuclear cells (PBMC) from infected patients were examined *in vitro* and compared to non-infected donor PBMC, proliferation and IFN-γ production were equivocal between the two groups (*Lundgren* et al., 2003). However, when PBMC were depleted of $CD25^+$ cells (a cell phenotype implicated as a suppressive regulatory T cell), the remaining cells responded in a significantly stronger manner than non-infected controls in recall assays for proliferation and IFN-γ production. These studies were taken a step further in mice where lymph node cell populations were transferred to nude mice recipients prior to challenge with *H. pylori* (*Raghavan* et al., 2003). If $CD25^+$ cells were removed from the lymph node cells prior to transfer, the mice developed significantly more inflammation and ultimately had significantly fewer bacteria in the gastric mucosa following challenge. Therefore, in the absence of immunisation there are cells present that are capable of reducing the bacterial load in the gastric mucosa.

IL-10 producing regulatory T cells

A second type of suspected immunoregulatory cell is the IL-10 producing T cell. Intestinal colonisation of $IL\text{-}10^{-/-}$ mice with normal bacterial flora results in pronounced colitis suggesting that under normal circumstances a population of IL-10 producing T cells must prevent this inflammation. T cells that produce high amounts of IL-10 have been termed Tr1 cells and have been isolated from both mice and humans (*Groux* et al., 1997; *Muminova* et al., 1999). We have recently shown that IL-10 producing regulatory T cells may also be present in the stomach in response to *H. pylori* infection. Infection of the mouse stomach with *H. pylori* results in persistent infection, but only mild inflammation. Figure 3 illustrates that infection of $IL\text{-}10^{-/-}$ mice, however,

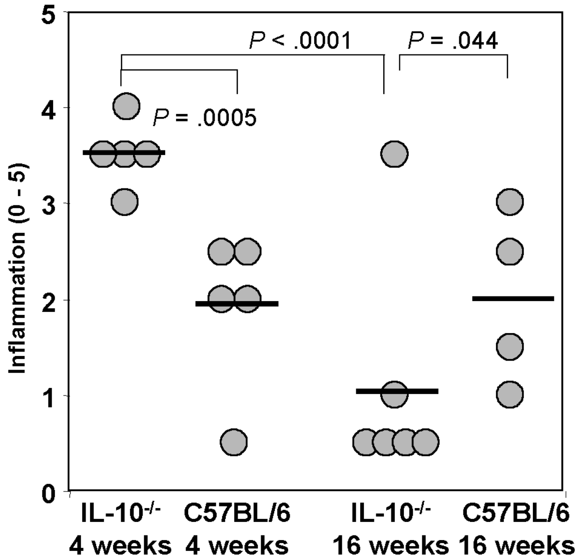

Figure 3. Il-$10^{-/-}$ mice develop severe inflammation relative to C57BL/6 mice in response to *H. pylori* infection. Mice were inoculated on two consecutive days with $1x10^7$ CFU *H. pylori* SS1. Subsets of each group were sacrificed and examined at either 4 weeks or 16 weeks post-inoculation and assessed for inflammation by examination of H&E stained sections. Statistical analysis was performed by ANOVA.

results in significantly greater inflammation by 4 weeks post inoculation (p=0.0005). Additionally, the *H. pylori* are spontaneously eradicated from these mice, but not from wild-type mice (data not shown). Spleen cells from the IL-$10^{-/-}$ mice also produce significantly greater levels of IFN-γ than wild type counterparts. By 16 weeks post-inoculation, in the absence of *H. pylori*, the inflammation in the IL-$10^{-/-}$ mice is significantly reduced (p<0.0001). Wild type mice, which remain infected, maintain a constant level of gastritis, significantly greater than the IL-$10^{-/-}$ mice at 16 weeks. Similar results with regard to bacterial load and inflammation in the IL-$10^{-/-}$ model have been reported by others (*Chen* et al., 2001).

Inflammation and immunoregulation

As stated above, challenge of immunised mice results in post-immune gastritis, which can be significantly greater than the gastritis induced by natural infection, at least within the first several weeks of challenge (*Garhart* et al., 2002). While some consider this a detriment to vaccination, the gastritis does dissipate over time. It may be that since the gastric mucosa lacks any organised or diffuse lymphoid structures, inflammation is essential to recruit the appropriate T cells to the stomach. Also, as previously mentioned, transfer of CD25-deficient lymph node cells to nude mice increases the inflammatory response following *H. pylori* challenge, as well as reducing the bacterial load, providing further evidence that inflammation may hold the key to *H. pylori* eradication (*Raghavan* et al., 2003). This concept is strengthened by our IL-$10^{-/-}$ studies in which eradication of the *H. pylori* was again accompanied by significant increases in gastritis.

We have recently described another model in which mice are able to spontaneously eradicate *H. pylori* from the

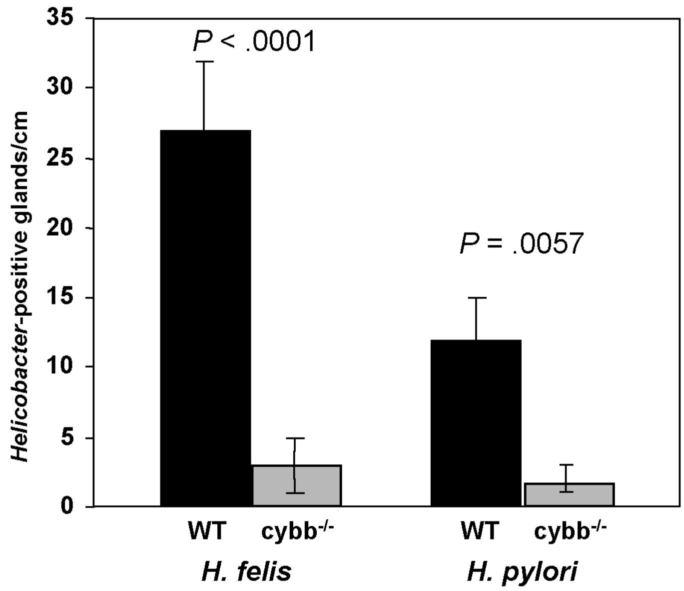

Figure 4. Phagocyte oxidase-deficient mice (cybb$^{-/-}$) respond to Helicobacter infection with severe inflammation and a reduced bacterial load relative to C57BL/6 mice. Mice were inoculated with 1x10^7 CFU *H. pylori* SS1 or *H. felis* CS1 and sacrificed at 21 days post-inoculation for inflammation and bacterial load. Bacterial load was determined by direct enumeration of infected glands by examination of silver-stained histologic sections. Statistical analysis was performed by ANOVA.

gastric mucosa (*Blanchard* et al., 2003). Neutrophils and macrophages from NADPH phagocyte oxidase deficient mice (cybb$^{-/-}$) lack the ability to generate superoxide anions, a primary innate cellular antimicrobial defence mechanism (*Pollack* et al., 1995). This mouse line serves as an experimental model for human chronic granulomatous disease. Typically these mice have increased susceptibility to bacterial infection and delayed bacterial clearance when experimentally infected with bacteria (*Pollack* et al., 1995). When these mice are infected with either *H. pylori* or *H. felis* however, the inflammatory response is significantly greater than in wild-type controls. The bacterial load in these mice drops significantly, and in some cases the *Helicobacter* organisms are eradicated from the gastric mucosa within three weeks of infection (Figure 4). Although iNOS expression in the gastric tissue of mice with gastritis is elevated, mice deficient in iNOS, resembled wild type mice and similarly failed to eradicate *H. pylori*. Thus, the cybb$^{-/-}$ mouse is only the second mouse model described to date (in addition to the IL-10$^{-/-}$ mouse) capable of spontaneously eradicating *H. pylori*. Both models develop pronounced gastritis in response to infection.

A NEW MODEL OF *H. PYLORI* PATHOGENESIS AND IMMUNITY

Recent reports indicate that a reduction in *H. pylori* numbers in the gastric mucosa requires pro-inflammatory events. These have included the pres-

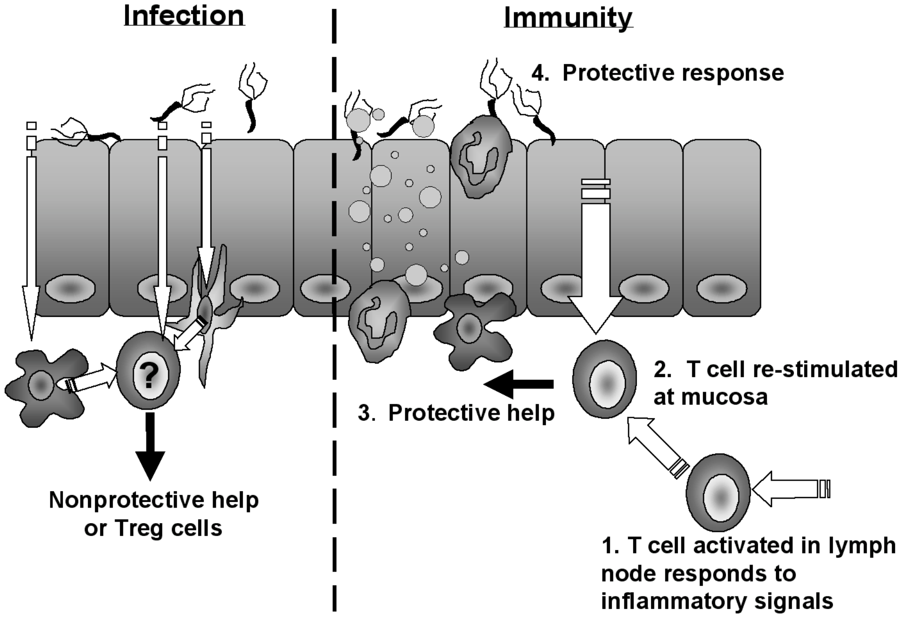

Figure 5. Model for *H. pylori* pathogenesis and immunity. *H. pylori* infection of the gastric mucosa results in activation of T cells recruited to the lamina propria (left side of figure). Antigen presentation may occur via MHC II-expressing epithelial cells, dendritic cells that bridge the tight junctions, or by macropohages that scavenge for bacteria and bacterial products that breech the epithelial monolayer. The activated T cells fail to elicit an effective immune response. Immunisation activates T cells in lymph nodes or other peripheral tissues resulting in fully active helper cells (right side of figure). Challenge of the gastric mucosa recruits these T cells to the site of inflammation where effective help results in a protective inflammatory response.

ence of post-immunisation gastritis when immunised mice are challenged (*Michetti* et al., 1994; *Pappo* et al., 1995; *Garhart* et al., 2002; *Goto* et al., 1999), a requirement for IL-12 in developing protective immunity (*Garhart* et al., 2003a; *Akhiani* et al., 2002), increased IFN-γ production upon challenge of immunised mice (*Goto* et al., 1999; *Garhart* et al., 2003a; *Eisenberg* et al., 2003; *Gottwein* et al., 2001), and spontaneous eradication only in mice that develop robust gastritis in response to infection (*Blanchard* et al., 2003; *Chen* et al., 2001). Therefore, previous theories that the induction of protective immunity requires a shift in immune character from a Th1 to a Th2 response, or even a mixed Th1/Th2 response, no longer accommodate the accumulating data. Additionally, when one considers that *H. pylori* infection does in fact stimulate *H. pylori*-specific T cells but fails to eradicate the infection, while immunisation by a number of different routes results in significant reduction in the *H. pylori* burden, a new model for *H. pylori* pathogenesis and immunity begins to emerge.

Whereas previous theories have promoted a Th1/Th2 dichotomy for pathogenesis and immunity, it is possible that *H. pylori*, while inducing a Th1 dominated response, survives in the stomach because it actually limits the inflammatory and immune response through the induction of *H. pylori*-specific immunoregulatory T cells. The studies mentioned above using IL-$10^{-/-}$ mice and describing $CD25^{+}$ regulatory T cells in both mice and humans support this hypothesis. We propose that activation of T cells in the gastric mucosa results in a population of down-regulatory cells that limits both the inflammatory and immune response (Figure 5). When immunisations are applied however, activation of the T cells occurs in peripheral lymph nodes where activation of these T regulatory cells is not favoured. When the T cells initially activated in lymph nodes are recruited to the gastric mucosa as a result of *H. pylori* challenge, they are capable of promoting either a more severe inflammatory response or a qualitatively different immune response than is induced by natural infection.

This theory is consistent with what we know about immunoregulation of the intestinal mucosa. To prevent detrimental immunity and inflammation from occurring in response to normal indigenous bacterial flora, specific T cells down-regulate the response to those antigens resulting in maintenance of immunologic quiescence (*Khoo* et al., 1997; *Groux* et al., 1997; *Chen* et al., 1994; *Powrie*, 1995; *Powrie* et al., 1993). It is believed that conditions in the lamina propria such as antigen presentation by epithelial cells, the presence of IL-10 and TGF-ß, and immunoregulatory dendritic cells favour the induction of the regulatory T cells. Similar events may occur in the gastric mucosa. In fact, the increased incidence of gastro-oesophageal reflux disease following *H. pylori* eradication has led to speculation that *H. pylori* may have formed a symbiotic relationship with humans, and could be seen by the host as normal flora (*Blaser*, 1999). In this respect, the fraction of individuals that develop symptomatic gastritis and peptic ulcer disease may represent those individuals that have an aberrant response to *H. pylori,* in the same way that patients who suffer from inflammatory bowel disease (IBD) are believed to react inappropriately to their own intestinal flora. Further studies regarding the immunoregulation of the gastric mucosa should continue to improve our understanding of how protective immunity is accomplished against *H. pylori,* and will most likely be essential for the development of an efficacious vaccine for use in humans.

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