Can the response to eradication therapy in *Helicobacter pylori* infection be predicted?

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The failure to eradicate *Helicobacter pylori* infection with antibiotic therapy has become a major clinical problem, not entirely accounted for by either poor compliance or antibiotic resistance. Recognition that failed eradication is one outcome of the host-parasite relationship focuses attention on impaired host protection as a determinant of nonresponse to antibiotics. A secreted interleukin (IL)-4 whole blood assay was developed to determine whether persistent infection was contributed to by impaired cytokine responses. The blood assay was shown to correlate well with mucosal organ cultures. Significantly lower levels of IL-4 were detected in the whole blood assays in 11 subjects with failed eradication compared with subjects with successful eradication ($P<0.05$). This latter group underwent a Th1 to Th0 'switch', which appears to be important to successful eradication. Detection of subjects at risk for failing to eradicate infection with standard combination therapy, by virtue of low secreted IL-4 in whole blood cultures, may have clinical value.

Key Words: *Failed eradication;* Helicobacter pylori; *Immunoglobulin G 2 antibody*

Peut-on prévoir l'issue du traitement suppressif d'*Helicobacter pylori*?

L'échec du traitement suppressif des infections à *Helicobacter pylori* par l'antibiothérapie est devenu un problème clinique important, qu'on ne peut attribuer entièrement à un manque d'observance thérapeutique ou à la résistance aux antibiotiques. Le fait de reconnaître que l'échec de la suppression résulte de la relation hôte-parasite fait ressortir l'absence de protection adéquate de l'hôte comme facteur déterminant de non-réaction aux antibiotiques. Un test de dosage de l'interleukine 4 (IL-4) sur sang entier a été mis au point pour savoir si une réaction inadéquate des cytokines pouvait expliquer la persistance de l'infection. Le dosage sanguin est en étroite corrélation avec les cultures d'organes muqueux. Des taux significativement bas d'IL-4 ont été décelés chez 11 sujets chez qui la suppression avait échoué comparativement aux sujets chez qui la suppression avait porté fruit ($P<0,05$). Ces derniers sont passés de Th1 à Th0, ce qui semble un facteur important pour la réussite du traitement. Le repérage des patients susceptibles de ne pas réagir favorablement à la suppression de *H pylori* par la polythérapie usuelle à cause d'un faible taux d'IL-4 dans les cultures sur sang entier pourrait s'avérer utile sur le plan clinique.

The role of *Helicobacter pylori* infection in the production of mucosal damage has largely been considered within a simple infection paradigm because eradication has appeared to be a predictable outcome of antibiotic therapy. Various antibiotic regimens claim eradication rates in excess of 90% and in vitro *H pylori* is sensitive to many antibiotics. Eradication failure is discussed in terms of antibiotic resistance and poor compliance. However, it is clear that the contemporary notion that *H pylori* infection directly causes mucosal disease, and that a course of antibiotics to which the organism is sensitive will cure the disease, is too simplistic. Thus, in general clinical experience, a more realistic eradication rate is 60% to 70% and the failure to attain mucosal sterility cannot simply be explained by patterns of antibiotic resistance (1). An increasingly large pool of patients with persistent infections due to failed therapy has become a significant medical problem, causing confusion in the minds of clinicians, which increases as the cases become less common.

Much of the confusion is due to a failure to recognize that the spectrum of mucosal disease reflects outcomes of a complex host-parasite relationship. A recent editorial, subtitled 'The swings and roundabouts of peptic ulcer disease' (2), did not identify host factors or altered bacterial physiology as contributors to changes seen in the epidemiology of peptic ulcer disease. Patients with chronic gastritis and *H pylori* infection are prone to eradication failure – indeed antibiotic therapy in this group remains controversial (3). We observed one-quarter of a group of unselected patients with nonulcer dyspepsia and chronic gastritis who were '*H pylori*-negative' by gold standard diagnostic criteria, yet were shown by polymerase chain reaction analysis to be colonized by nonurease secreting *H pylori*, with intermediate levels of mucosal inflammation and antibody levels, but with coccoid-shaped bacteria in their tissue sections (4). The characteristics of 'coccoid *H pylori*' reflect an appropriate adapted form in response to protective immunity, with features that include reduced susceptibility to antibiotics (5). A dynamic relationship between *H pylori* and the mucosal immune response is suggested by possible reactivation of latent infection in human and animal models involving stress (unpublished data, 6).

Knowledge of mucosal immune mechanisms that limit infection, condition adaptive forms of *H pylori*, determine the pattern of clinical disease and modulate eradication efficiency, is limited. The identification of relevant immunological markers, how-

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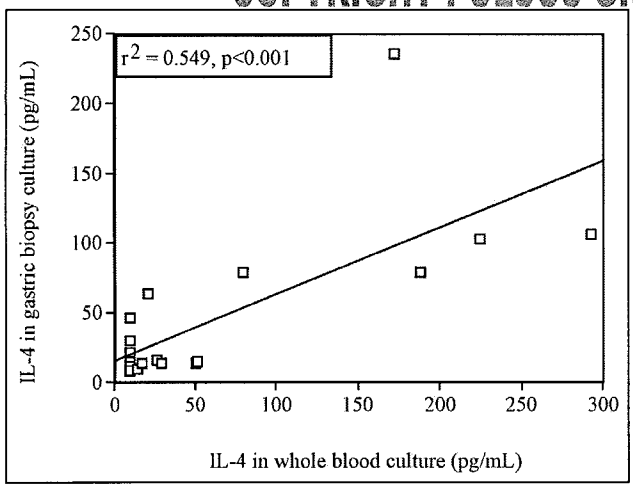

Figure 1) *Interleukin (IL)-4 secreted in blood cultures correlates with secretion from gastric mucosal organ culture*

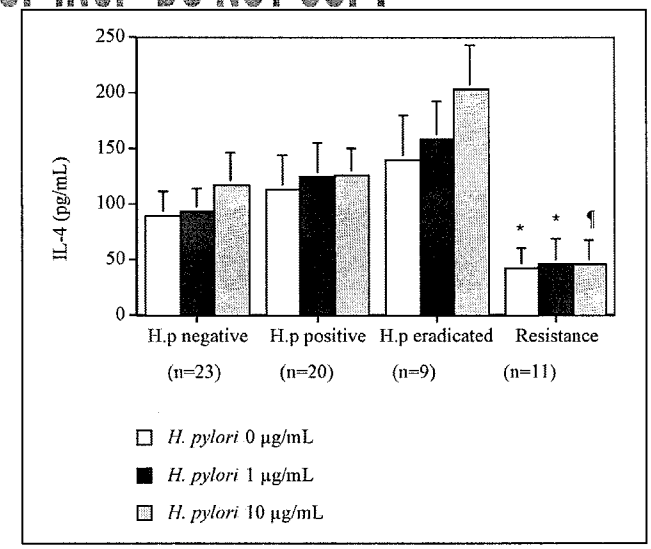

Figure 2) *Interleukin (IL)-4 secretion from blood cultures related to* Helicobacter pylori *(Hp) status. *P<0.05; ¶P<0.01*

ever, would have clinical value in the assessment of disease with respect to outcome and response to therapy. Mucosal antibody is an accurate monitor of contemporary colonization, but does not appear to have a significant role in limiting established infection. We therefore assessed T lymphocyte function in terms of patterns of cytokine secretion using interferon-gamma (INF-γ) and interleukin (IL)-4 as markers of T helper cell (Th) 1 and Th2 activity, respectively. Most mucosal T cells in patients infected with *H pylori*, secrete cytokines characteristic of a Th1 response (7). Following successful eradication, the cytokine pattern switches to one more characteristic of a balance between Th1 and Th2 cells, the so-called Th0 response (8). Mucosal cultures from patients with gastric cancer or precancer showed an extreme Th2 polarization, with high levels of IL-4 and an absence of IFN-γ (9). These observations suggested that variation of the mucosal T lymphocyte response to *H pylori* infection may play a critical role in determining the pattern of mucosal disease, the physiology of the bacteria and the response to therapy. In studies of host-parasite relationships at mucosal surfaces in relation to *Candida albicans* and *Chlamydia pneumoniae*, IL-4 secretion appeared to be critical to both the protection (10) and the promotion of inflammation (unpublished data).

Just as it has long been recognized that infection in patients with immune deficiency is characterized by poor clinical response to otherwise appropriate antibiotics, we asked the question "is there a mucosal immune defect in *H pylori*-infected patients who fail to eradicate infection with standard antibiotic regimens?" To address this question, and to identify clinical and laboratory markers to predict the risk of eradication failure, two studies are described.

CLINICAL STUDY

A retrospective assessment of the files of 392 *H pylori*-positive patients was made to determine the likely value of clinical characteristics in identifying those at risk for 'first round' eradication failure (189 with successful eradication, 203 with eradication failure). The following features were significantly different (P<0.05) between the groups: eradication failure with a longer duration of symptoms (6.06 years versus 3.04 years); consumed regular alcohol less often (39% versus 62%); had a higher use of antibiotics in the recent past (35% versus 16%); had a higher recent use of proton pump inhibitors (17% versus 8%); were of Middle Eastern origin (17% versus 8%) and had a higher incidence of *H pylori*-negative diagnosis at the time of endoscopy (ie, a negative Clotest) (21% versus 9%).

IMMUNOLOGICAL STUDY

Patients

Fifty-two *H pylori*-positive patients with dyspepsia and 11 patients with persistent *H pylori* infection following one or more courses of antibiotics were studied.

Comparison of blood and mucosal IL-4 response

A secreted IL-4 whole blood assay based on the ligation of CD40 ligand on Th2-committed cells by CD40 on platelets (Secril-4 Alert, VRI BioMedical, Australia) was used to detect IL-4 secreted from blood T cells, and modified to measure IFN-γ in the supernatant and to include addition of *H pylori* antigen at 1 and 10 µg/mL to stimulate antigen reactive cells). To validate the use of blood cytokine responses, IL-4 secretion from whole blood cultures was compared with levels in gastric mucosa cultures (Figure 1). A positive correlation was found (r^2=0.55; P<0.001).

IL-4 and IFN-γ production in whole blood culture

Significantly lower levels of IL-4 were detected in whole blood cultures (Figure 2) from those with eradication failure compared with patients in whom *H pylori* infection was successfully eradicated (P<0.05, 0 µg/mL and 1.0 µg/mL *H pylori* Acid Glycine Extract (AGE) antigen; P<0.01, 10 µg/mL antigen. Patients with successful eradication showed a trend toward an increase in IL-4 secretion, especially with antigen stimulation. The low level of IL-4 secretion in those with ongoing infection was unrelated to the number of courses of therapy.

There was no difference in the levels of IFN-γ between the different groups, although a trend toward lower levels of 'spontaneous' secretion was noted in those with successful eradication (Figure 3).

Anti-*H pylori* antibody

Both serum and saliva immunoglobulin G (IgG) antibody levels were significantly lower six to eight weeks following eradication therapy (P<0.05). For both saliva and serum

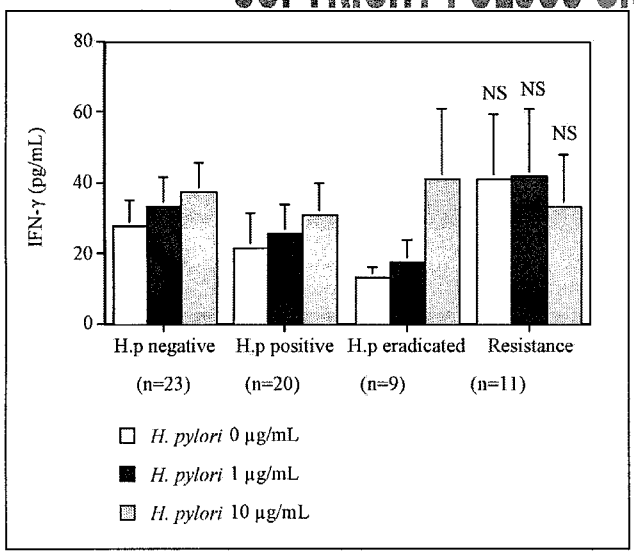

Figure 3) *Interferon (IFN)-gamma secretion from blood cultures related to* Helicobacter pylori *(Hp) status. NS Not significant*

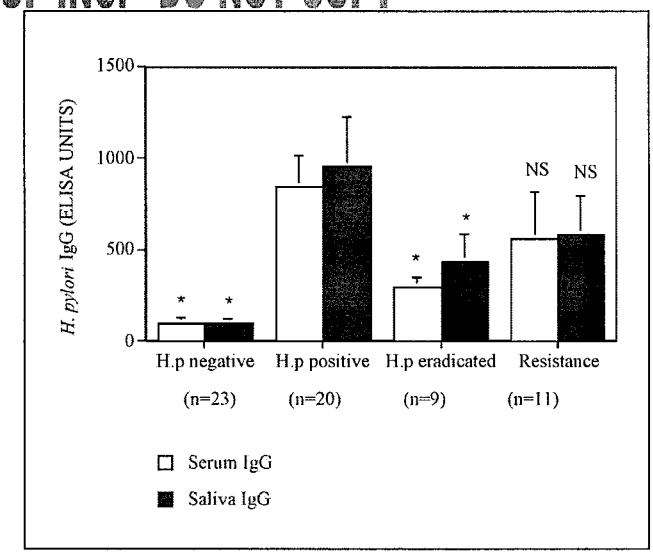

Figure 4) *Antibody to* Helicobacter pylori *(Hp) (Immunoglobulin G [IgG]) in serum and saliva related to* H pylori *status. NS Not significant. *P<0.05 related to* H pylori*-positive subjects*

antibodies, a trend toward lower levels of antibody in those failing to eradicate infection was observed (Figure 4). In a prospective study of saliva IgG subclass antibody six weeks following antibiotic therapy compared with pretherapy levels equated at 100%, IgG2 subclass antibody selectively and significantly ($P<0.05$) was not reduced in patients with eradiaction failure compared with those undergoing successful eradication (Table 1).

TABLE 1
Saliva *Helicobacter pylori* antibody levels after antibotic treatment

	H pylori IgG (%)	*H pylori* IgG1 (%)	*H pylori* IgG2 (%)
Eradication group (n=18)	72.38	73.44	64.06
Eradication failure (n=6)	60.81	77.18	93.27

IgG Immunoglobulin G

COMMENT

These studies add to existing clinical data that identify patients at risk for eradication failure, and for the first time identify a mucosal defect that predisposes patients to eradication failure. The clinical characteristics are consistent with previous observations, and relate to both bacterial and host factors. A possible protective effect of regular alcohol ingestion (50-150 gm alcohol/week) could be related to either a direct effect on bacteria, or a direct or indirect effect on mucosal protection. A prospective study is currently aimed at the development of a clinical index to define risk of eradication failure, so as to tailor optimal primary eradication therapy.

Recognition that a mucosal immune defect predisposes patients to eradication failure has clinical importance. The selective secreted IL-4 deficiency with sustained secretion of INF-γ supports the concept that the switch from a Th1 to a Th0 cytokine pattern accompanying effective eradication is linked to enhanced protection. A similar mucosal response in immune mice following oral challenge with C *albicans*, has shown protection to include a positive paracrine loop involving IL-4 and nitric oxide (11). The data in these studies show not only a low baseline IL-4 secretion, but also an absence of the 'switch' and of the antigen-induced enhancement of cytokine secretion, which characterize an effective response to eradication (8). It is not clear whether the defective 'switch' characteristics reflect the primary defect of low IL-4 secretion or persisting bacterial colonization. About 25% of normal patients have low (or nondetectable) levels of IL-4 secretion in this assay (unpublished data), which suggests that the 'defect' observed is constitutive in a subgroup of the population. Identification of this group as a method of planning effective eradication therapy is being assessed (eg, those with high IL-4 levels may require less intense and shorter term antibiotic regimens, while those with low levels and impaired host protection may require more extensive sensitivity assessment and more vigorous antibiotic strategies). The relationship between the high level of drug resistance following eradication failure and low IL-4 secretion is being studied. Anti-*H pylori* antibody was lower (though not significantly) in those with failed eradication, consistent with a mucosal immune defect. A selective retention of IgG2 subclass antibody in saliva six weeks after failed eradication therapy differed significantly from the reduction noted after successful eradication. The clinical value of this observation needs to be confirmed.

CONCLUSIONS

Failure to eradicate *H pylori* infection by standard antibiotic strategies is a significant clinical problem, with an increasing pool of patients with persistent infection. The failure to respond to antibiotics can no longer be explained in terms of antibiotic sensitivity profile and drug compliance, but rather as a particular outcome of the host-parasite relationship operating at the gastric mucosal surface. Patients failing to eradicate the infection have a defect in the normal Th1 to Th0 switch that follows effective eradication, and this defect is predicted by a low level of secretion of IL-4 from circulating Th2 cells. These cells in turn are in equilibrium with the mucosal T cell pool. Reduced anti-*H pylori* antibody in patients failing to eradicate infection probably also reflects a mucosal immune

deficiency. Low levels of secreted IL-4 in this patient group appear to reflect the lower quartile of levels in the population, which is similar in proportion to antibiotic nonresponders in *H pylori*-positive patients. The identification of these patients before therapy is being assessed as a logical step in designing individualized treatment strategies.

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