STOMACH

Inhibitory effects of *Helicobacter pylori* infection on murine autoimmune gastritis

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Background and aim: Long term *Helicobacter pylori* infection leads to atrophic gastritis but the relation between *H pylori* infection and autoimmune related atrophic gastritis (AIG) remains unclear. We studied the effects of *H pylori* infection on the pathophysiology of AIG in mice.

Materials and methods: BALB/c nu/nu mice (n=40) with or without *H pylori* infection received splenocytes from neonatally thymectomised mice to induce AIG. Half of the mice were orally infected with *H pylori* prior to AIG induction. Histological findings, and local and systemic immune responses were serially evaluated.

Results: Two and six months after transfer, parietal cells in uninfected mice were depleted while those in infected mice were well preserved. The degree of gland atrophy ($p<0.01$), hyperplasia ($p<0.01$), gastric pH ($p<0.05$), and serum gastrin levels of infected mice were significantly lower than those of uninfected mice. Serum antiparietal cell antibody levels gradually decreased in infected mice, and were significantly lower than those of uninfected mice at six months ($p<0.05$). Real time polymerase chain reaction studies revealed significantly higher interleukin 4 ($p<0.05$) and transforming growth factor β ($p<0.05$) gene expression in the gastric mucosa in infected mice than in uninfected mice at both two and six months after AIG induction.

Conclusions: *H pylori* infection inhibited the development of AIG in mice. Th2-type immune responses and transforming growth factor β in the gastric microenvironment might be involved in the inhibitory effects of *H pylori* infection on the development of AIG, in which Th1-type responses have an important role.

Autoimmune gastritis (AIG) is a typical organ specific autoimmune disease,¹ producing autoantibodies against some molecules, including H⁺K⁺-ATPase and intrinsic factor.^{2,3} Histologically, AIG is characterised by a chronic inflammatory infiltration affecting only or predominantly the corpus mucosa and causing loss of parietal and chief cells from the gastric gland.¹ Patients with AIG often have complications such as gastric cancer, gastric carcinoid tumour,^{4,5} or pernicious anaemia accompanied by achlorhydria. The prevalence of AIG varies, being more common in Caucasians, particularly Scandinavians, in whom it accounts for up to 5% of patients with chronic gastritis or 1.9% of Western populations over the age of 60 years.^{6,7} In contrast, AIG is rarely encountered in South America or Asia^{8,9} where there is a high prevalence of *Helicobacter pylori* infection.^{10,11}

H pylori infection is thought to be involved in the development of chronic atrophic gastritis, peptic ulcer, gastric cancer, and lymphoma.^{12,13} In addition, several investigators report that *H pylori* infection may be involved in the development of AIG because of cross reactivity between *H pylori* and components of the gastric mucosa.¹⁴⁻¹⁶ In contrast, some clinical investigations demonstrated a low prevalence of *H pylori* infection among AIG patients, suggesting that *H pylori* infection is not likely to be an aetiological factor in AIG.¹⁷⁻¹⁹ Thus the relation between *H pylori* infection and AIG remains controversial.

Experimental AIG can be induced in BALB/c mice by thymectomy three days after birth. Murine AIG shares many pathological and clinical features with human AIG, such as selective loss of parietal cells from the gastric mucosa, lymphocytic infiltration, and production of autoantibodies to parietal cells.^{20,21} This animal model is induced by CD4⁺ T cells^{20,21} of the Th1 subtype.²² It is reproducible by adoptive

transfer of effector T cells into syngeneic nude mice,^{22,23} resulting in a more uniform disease model than in individually thymectomised mice. In the present study, therefore, to examine whether *H pylori* infection affects the development of AIG, we used nude mice with or without *H pylori* infection, transferred with effector T cells to induce AIG.

MATERIALS AND METHODS

Bacterial strain

H pylori (TN2GF4), isolated from a patient with a duodenal ulcer, was provided by Dr M Nakao (Pharmaceutical Research Division, Takeda Chemical Industries Ltd, Osaka, Japan). It was maintained in blood agar base No 2 with horse serum (5%, vol/vol) containing amphotericin B (2.5 mg/l), trimethoprim (5 mg/l), polymyxin (1250 IU/l), and vancomycin (10 mg/l). Bacteria for experimental inoculation were grown in Brucella broth supplemented with 2.5% heat inactivated fetal bovine serum at 37°C. Sterilised glycerol was added to the cultures at a final concentration of 15%, and cultures were maintained at -80°C until use.²⁴

Donor mice and AIG induction

Pregnant BALB/c mice were purchased from SLC (Shizuoka, Japan) and bred under specific pathogen free conditions. Neonatal thymectomy was performed three days after birth under

Abbreviations: AIG, autoimmune gastritis; ELISA, enzyme linked immunosorbent assay; PBS, phosphate buffered saline; IFN- γ , interferon γ ; IL-4, interleukin 4; TGF- β , transforming growth factor β ; PCR, polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

ether anaesthesia, as described previously.²³ Four months after thymectomy, serum antiparietal cell antibody levels were measured using an enzyme linked immunosorbent assay (ELISA). Eight mice with high serum antiparietal cell antibody titres were killed and diagnosed with typical AIG by histological examination. Total lymphocytes were prepared from freshly removed spleens for transfer, as described previously.²⁶

Recipient nude mice, *H pylori* infection, and disease transfer

BALB/c nu/nu mice were purchased from SLC. Twenty five mice were inoculated with 10^8 *H pylori* organisms into the stomach at eight weeks of age using a steel catheter, as previously reported.²⁵ Colonisation of bacteria was confirmed by May-Giemsa staining and a whole stomach bacterial culture system, as previously described.^{24, 25} Briefly, after the stomach was opened along the lesser curvature, the longitudinal half of the stomach was homogenised with physiological saline. An aliquot of dilutions was inoculated onto modified Skirrow's agar and incubated at 37°C for four days under microaerobic conditions. The density of infection was estimated by counting the number of colonies per plate and expressed as log colony forming units per gastric wall. Another 25 mice were inoculated with saline as a control AIG group.

Lymphocytes from the removed spleens were intravenously injected (1×10^7 per recipient mouse) into these mice three months after inoculation with *H pylori* or saline. Both groups of mice were isolated from one another and bred under specific pathogen free conditions. Mice were killed before (n=10), two months (n=20), or six months (n=20) after the lymphocyte injections.

Age matched normal nude mice served as normal controls (n=5). All animal experiments were approved by the Animal Ethics Committee of Kyoto University.

Measurement of gastric pH and histological examination

After 24 hours of starvation, mice were killed under ether anaesthesia and their stomachs were rapidly removed. The local pH in the corpus area of the stomach was measured with pH test paper (Advantac, Tokyo, Japan). The stomachs were fixed in neutral buffered formalin, embedded in paraffin wax, and cut into 4 µm thick sections. Sections were stained with haematoxylin-eosin.

The degree of gastritis was determined according to a modification of Sakagami's method using a semiquantitative scoring system.²⁷ "Chronic inflammation", characterised by infiltration of mononuclear cells, was graded from 0 to 3, where 0=no increase in inflammatory cells, 1=slight infiltration of the lamina propria by lymphocytes and plasma cells, 2=moderately dense infiltration, and 3=very dense infiltration. Quantitation of gland atrophy and hyperplasia was evaluated according to a modification of Judd's method.²⁸ Briefly, 10 complete longitudinal profiles of gastric units were selected at random from the body of the stomach in each mouse. The numbers of parietal cells (for atrophy) or total gland cells (for hypertrophy) per gastric unit were counted under high magnification. Data were expressed as the average number of cells per section of gastric unit for each animal.

Serological examination

Serum antiparietal cell antibody levels were measured by ELISA, as described previously.² Briefly, duplicate wells of microtitre plates (Corning Inc., Corning, New York, USA) were incubated with antigens (100 µg/ml), extracts prepared from the normal gastric mucosa, in phosphate buffered saline (PBS) for 24 hours at 4°C. The wells were blocked with PBS containing 2.5% non-fat dried milk and then incubated for 24 hours at 4°C with serial dilutions of sera. The wells were next

incubated with horseradish peroxidase labelled goat anti-mouse Ig (Cappel, Durham, North Carolina, USA) diluted at a predetermined concentration for 24 hours at 4°C. After rigorous washing, each well was reacted with a substrate (o-phenylenediamine) solution for 15 minutes. The reaction was terminated with 25 µl of 2 M H₂SO₄, and absorbency at 490 nm was determined with an ELISA reader. Serum levels of gastrin were measured by radioimmunoassay^{29, 30} with a commercially available kit (Gastrin-RIAKIT II; Dinabot, Tokyo, Japan).

Quantitation of mRNA expression (TaqMan real time PCR analysis)

Total RNA was extracted by the single step guanidium thiocyanate-phenol-chloroform method from the stomachs of five mice in each group. Extracted RNA preparations were reverse transcribed with MultiScribe Reverse Transcriptase (PE Applied Biosystems, Foster City, California, USA). The resultant cDNAs (50 ng/reaction) were analysed for expression of interferon γ (IFN-γ), interleukin 4 (IL-4), and transforming growth factor β (TGF-β) genes by TaqMan polymerase chain reaction (PCR) assay,³¹ using an ABI Prism 7700 sequence detection system (Perkin-Elmer, Foster City, California, USA). Reactions were incubated for two minutes at 50°C, denatured for 10 minutes at 95°C, and subjected to 40 two step amplification cycles with annealing/extension at 60°C for one minute, followed by denaturation at 95°C for 15 seconds. Amplifications used for IFN-γ, IL-4, and TGF-β covered base pairs 101–178, 198–269, and 445–530, respectively (numbering starts at the start codon), and were analysed with 6-carboxyfluorescein labelled probes. The oligonucleotides and TaqMan fluorogenic probes were: IFN-γ (sense, 5'-CTC TGA GAC AAT GAA CGC TAC ACA CT-3'; antisense, 5'-TGG CAG TAA CAG CCA GAA ACA G-3'; probe, 5'-CAT CTT GGC TTT GCA GCT TTG CAG CTC TTC CTC ATG-3'); IL-4 (sense, 5'-GGA GCT GCA GAG ACT CTT TCG-3'; antisense, 5'-GGC TTT CCA GGA AGT CTT TCA G-3'; probe, 5'-CTG CAC CAT GAA TGA GTC CAA GTC CAC A-3'); TGF-β (sense, 5'-CCT GCA AGA CCA TCG ACA TG-3'; antisense, 5'-CGA GCC TTA GTT TGG ACA GGA T-3'; probe, 5'-AAC GGA AGC GCA TCG AAG CCA TC-3'). All TaqMan PCR data were captured using Sequence Detector Software (PE Applied Biosystems), normalised by dividing copies per nanogram of the target gene by copies per nanogram of a housekeeping gene (glyceraldehyde-3-phosphate dehydrogenase (GAPDH), amplified using TaqMan Rodent GAPDH control reagents; PE Applied Biosystems), and expressed as copies per 1000 copies of GAPDH (mean (SEM)).

Immunohistochemistry

Immunohistochemical staining was performed on freshly frozen sections using the avidin- biotin immunoperoxidase method. Briefly, freshly frozen sections were fixed in acetone for 10 minutes, rinsed in PBS (pH 7.2), and incubated with the appropriate blocking agent for 20 minutes. Biotin conjugated goat antimouse B220, CD4, and CD8 (Pharmingen, San Diego, California, USA) monoclonal antibodies were applied to the sections for two hours. Sections were washed with cold PBS for 30 minutes and incubated with ABC (Vector Laboratories, Inc., Burlingame, California, USA) for 30 minutes according to the manufacturer's instructions. After washing with PBS, sections were reacted with a fresh mixture of 0.05% 3,3'-diaminobenzidine, and 0.005% H₂O₂ in Tris buffer (0.05 M, pH 7.6) for five minutes and washed with distilled water. Controls were incubated with normal goat serum (Cappel Laboratories, Cochranville, Pennsylvania, USA) instead of the monoclonal antibodies. These control samples produced negative results.

Statistical analysis

All results are expressed as means (SEM) for each sample. Differences in histopathological scores between control and

Figure 1 Colonisation of *Helicobacter pylori* in the gastric mucosa of BALB/c nu/nu mice. The number of *H. pylori* in the gastric mucosa was evaluated using a whole stomach culture system. Data are expressed as mean (SEM) log colony forming units (CFU) for the initial number of *H. pylori* inoculated into each mouse, for the number of *H. pylori* three months after inoculation (before induction of autoimmune gastritis (AIG)), and for the number of *H. pylori* two (2 mo) or six (6 mo) months after induction of AIG. **Significantly different compared with initial number of *H. pylori* ($p < 0.01$).

infected mice were analysed using the Mann-Whitney U test. Differences in cell number, gastric pH, serum antiparietal cell antibody levels, serum gastrin levels, and quantitative cytokine mRNA expression were analysed using Wilcoxon's *t* test. A two tailed *p* value of less than 0.05 was used to indicate statistical significance.

RESULTS

Bacterial colonisation

Three months after inoculation of nude mice with *H. pylori*, persistent colonisation was confirmed using a whole stomach bacterial culture system, although the number of bacteria was lower than that initially inoculated. Colonisation persisted even at two and six months after transfer of AIG although levels tended to decrease over time (fig 1). We also inoculated other nude mice in which splenocytes from AIG mice were transferred three months before infection, to evaluate the effects of *H. pylori* infection on established AIG. Colonisation was not detected however in any mouse using the culture system (data not shown).

Evaluation of gastritis

Three months after inoculation, there was no apparent gastritis in saline or *H. pylori* inoculated BALB/c nu/nu mice (fig 2A, 2B) although the whole stomach bacterial culture from *H. pylori* inoculated BALB/c nu/nu mice revealed persistent colonisation of *H. pylori* (fig 1).

Two months after transfer of splenic lymphocytes from neonatal thymectomised mice, histological findings of the stomachs of uninfected mice were typical of AIG, showing loss of parietal cells, proliferation of pit cells, and moderate to severe lymphocyte infiltration (fig 2C). In contrast with human AIG, the mucosa in murine AIG was hypertrophic with proliferation of pit cells following gland atrophy, and depletion of parietal and zymogenic cells. In contrast, histological findings of infected mice varied in the degree of atrophic and hyperplastic changes; parietal cells were relatively preserved, and the hyperplastic change was milder in *H. pylori* infected mice (fig 2D). Although there was no significant difference in chronic inflammation scores of the gastric mucosa between infected and uninfected mice (2.40 (0.16) *v* 2.70 (0.15)) (fig 3A), the average number of parietal cells in infected mice was significantly greater than that in uninfected mice (7.80 (1.67) *v* 2.30 (0.58); $p < 0.01$) (fig 3B), and the total number of cells per gastric unit in infected mice was significantly lower than that in uninfected mice (68.8 (7.04) *v* 101.7 (6.23); $p < 0.01$) (fig 3C).

Six months after lymphocyte transfer, all 10 uninfected mice developed typical AIG with loss of parietal cells, proliferation of pit cells, and moderate to severe lymphocyte infiltration (fig 2E). In contrast, parietal cells were relatively preserved in *H. pylori* infected mice at two months (figs 2F). Although there was no significant difference in chronic inflammation scores of the gastric mucosa between infected and uninfected mice (2.40 (0.22) *v* 2.70 (0.15)) (fig 3A), the average number of parietal cells in infected mice was significantly greater than that in uninfected mice (8.60 (1.38) *v* 2.44 (0.71); $p < 0.01$) (fig 3B), and the total number of cells per gastric unit in infected mice was significantly lower than that in uninfected mice (72.30 (7.21) *v* 102.30 (5.88); $p < 0.01$) (fig 3C).

Because there was considerable variation in the degree of gland atrophy and hyperplasia in *H. pylori* infected mice, we evaluated bacterial colonisation in the stomach of each infected and lymphocyte transferred mouse using the culture system. There was no difference in mucosal colonisation of *H. pylori* between mice with marked gastric hyperplasia ($n = 3$) and mice with minimal hyperplasia ($n = 3$) at either two or six months after disease transfer (data not shown).

Gastric pH and serum gastrin levels

At two and six months after disease transfer, gastric pH was significantly elevated compared with control mice, irrespective of *H. pylori* infection ($p < 0.05$). Gastric pH in infected mice however was significantly lower than that in uninfected mice two months after AIG induction (4.80 (0.63) *v* 6.45 (0.29); $p < 0.05$) (fig 4). Similarly, gastric pH in infected mice was significantly lower than that in uninfected mice six months after induction of AIG (4.80 (0.63) *v* 7.13 (0.25); $p < 0.01$) (fig 4).

Serum gastrin levels significantly increased to 198.0 (35.3) pg/ml in uninfected mice two months after transfer compared with control mice ($p < 0.01$). This increase is a characteristic feature of murine AIG but serum gastrin levels in infected mice tended to be lower than those in uninfected mice (108.8 (30.3) pg/ml; $p = 0.07$) (fig 5). Six months after transfer, serum gastrin levels increased markedly to 322.5 (52.0) pg/ml in uninfected mice ($p < 0.01$). The increased serum gastrin levels in transferred mice, however, were significantly decreased by *H. pylori* infection (144.8 (36.3) pg/ml *v* uninfected mice; $p < 0.05$) (fig 5).

Serial changes in antiparietal cell antibody titres

Serum antiparietal cell antibody levels in mice were serially evaluated using ELISA. All mice had increased serum antiparietal cell antibody levels at two months after transfer, irrespective of *H. pylori* infection. Uninfected mice maintained high serum antibody titres throughout the experiment without significant changes. Specific absorbance antiparietal cell antibody levels were 0.709 (0.044) at two months after transfer, 0.710 (0.039) at four months, and 0.731 (0.033) at six months. In contrast, serum antiparietal cell antibody levels in infected mice decreased significantly at four and six months after transfer. Specific absorbance levels were 0.804 (0.029) at two months, 0.633 (0.044) at four months ($p < 0.05$ *v* two months), and 0.593 (0.062) at six months ($p < 0.05$ *v* two months). Serum antibody levels in infected mice were significantly lower than in uninfected mice at six months after transfer ($p < 0.05$) (fig 6).

Cytokine messages in the gastric mucosa

Cytokine mRNA profiles in the gastric mucosa were determined by TaqMan real time PCR. IFN- γ was upregulated by AIG transfer at both two and six months compared with normal mice. Two months after transfer, relative IFN- γ gene expression levels in the *H. pylori* infected group tended to be higher than those in the uninfected group (26.7 (14.1) *v* 15.0 (3.1) copies/1000 copies of GAPDH) although this was not

Figure 2 Histopathological findings of the stomach of BALB/c nu/nu mice with (C–F) or without (A, B) autoimmune gastritis (AIG) transfer (haematoxylin-eosin stain, original magnification $\times 100$). Gastric tissue from BALB/c nu/nu mouse was inoculated with saline (A) or *Helicobacter pylori* (B) three months previously. There was no apparent gastritis in saline or *H. pylori* inoculated BALB/c nu/nu mice. (C) Two months after transfer of splenic lymphocytes from neonatal thymectomised mice, histological findings of the stomachs of uninfected mice were typical of AIG, showing loss of parietal cells, proliferation of pit cells, and moderate to severe lymphocyte infiltration. (D) In contrast, parietal cells were relatively preserved, and the hyperplastic changes were milder in *H. pylori* infected mice. (E) Six months after transfer, all 10 uninfected mice developed typical AIG with loss of parietal and chief cells, proliferation of pit cells, and moderate to severe lymphocyte infiltration. (F) In contrast, parietal cells were relatively preserved, and the hyperplastic changes were milder in *H. pylori* infected mice at six months.

significant. Six months after transfer there was no difference in relative IFN- γ gene expression between infected and uninfected mice (16.7 (2.3) ν 16.8 (4.6) copies/1000 copies of GAPDH) (fig 7A). IL-4 was also upregulated by AIG transfer at both two and six months compared with control mice. IL-4 gene expression levels in infected mice were significantly higher than those in uninfected mice at both two months (25.7 (8.2) ν 3.6 (0.7) copies/1000 copies of GAPDH; $p < 0.01$)

and six months (5.2 (1.3) ν 2.2 (0.6) copies/1000 copies of GAPDH; $p < 0.05$) (fig 7B). TGF- β was constitutively expressed in the gastric mucosa, and significantly upregulated in infected mice compared with uninfected mice at both two months (245.5 (87.3) ν 71.6 (18.9) copies/1000 copies of GAPDH; $p < 0.05$) and six months (212.0 (69.0) ν 86.5 (15.1) copies/1000 copies of GAPDH; $p < 0.05$) after disease transfer (fig 7C).

Figure 3 Effects of *Helicobacter pylori* infection on inflammation, gland atrophy, and hyperplasia in BALB/c nu/nu mice with autoimmune gastritis (AIG) transfer. (A) There was no significant difference in chronic inflammation scores of the gastric mucosa between *H. pylori* infected and uninfected mice either two (2 mo) or six (6 mo) months after induction of AIG. (B) The number of parietal cells per gastric unit was significantly greater in *H. pylori* infected mice compared with uninfected mice at both two and six months after induction of AIG. (C) The total number of cells per gastric unit was significantly smaller in *H. pylori* infected mice compared with uninfected mice at both two and six months after disease transfer. * $p < 0.05$, ** $p < 0.01$.

Immunohistochemistry

Histological findings indicated non-destructive gastritis in infected mice and destructive gastritis in uninfected mice although there was no difference in chronic inflammatory scores. The major phenotype of the infiltrating cells in the body mucosa was CD4 positive-CD8 negative in both

Figure 4 Effects of *Helicobacter pylori* infection on gastric pH in autoimmune gastritis (AIG) transferred BALB/c nu/nu mice. At two (2 mo) and six (6 mo) months after AIG induction, gastric pH was significantly elevated compared with control mice, irrespective of *H. pylori* infection. Gastric pH in infected mice however was significantly lower than in uninfected mice. * $p < 0.05$, ** $p < 0.01$.

Figure 5 Effects of *Helicobacter pylori* infection on serum gastrin levels in autoimmune gastritis (AIG) transferred BALB/c nu/nu mice. Serum gastrin levels increased significantly in uninfected mice two months (2 mo) after transfer. Serum gastrin levels in infected mice tended to be lower than those in uninfected mice ($p = 0.07$). Six months (6 mo) after transfer, serum gastrin levels markedly increased in uninfected mice. The increased serum gastrin levels in lymphocyte transferred mice however were significantly decreased by *H. pylori* infection. * $p < 0.05$, ** $p < 0.01$.

uninfected and infected transferred mice. In contrast, clusters of B220 positive cells were partially observed in infected mice but not in uninfected mice (fig 8).

DISCUSSION

Although mucosal atrophy is a characteristic feature of both *H. pylori* induced gastritis and AIG, the relation between *H. pylori* infection and AIG is controversial. Several investigators have suggested that *H. pylori* infection initiates an autoimmune process in the gastric mucosa, especially in parietal cells³² and, moreover, that *H. pylori* eradication induces cure of AIG.³³ Indeed, patients with chronic *H. pylori* gastritis with body mucosal atrophy have serum autoantibodies that react with gastric $H^+K^+-ATPase$.¹⁴ *H. pylori* lipopolysaccharide contains Lewis y and/or Lewis x antigens similar to those expressed by human gastric epithelial cells, especially in the glycosylated $H^+K^+-ATPase$ beta subunit.^{14 34 35} The hypothesis that *H. pylori* induces autoantibodies through common expression of Lewis antigens seems unlikely however because binding of serum

Figure 6 Effects of *Helicobacter pylori* infection on antiparietal cell antibody titres in autoimmune gastritis (AIG) transferred BALB/c nu/nu mice. All mice had increased serum antiparietal antibody levels two months (2 mo) after transfer, irrespective of *H pylori* infection. Uninfected mice maintained high serum antiparietal cell antibody levels throughout the experiments without significant changes. In contrast, serum antiparietal cell antibody levels in infected mice significantly decreased at four (4 mo) and six (6 mo) months after disease transfer. Serum antibody levels in infected mice were significantly lower than those in uninfected mice at six months. Parietal cell antibody titre was determined by measuring absorbance at 490 nm using an ELISA reader. Data are expressed as means (SEM). * $p < 0.05$, ** $p < 0.01$.

anticanalicular autoantibodies is not decreased by preabsorption with *H pylori*.^{14 36 37} In contrast, several epidemiological and clinical studies suggest that *H pylori* infection is not likely to be involved in AIG because the prevalence of *H pylori* infection in patients with pernicious anaemia is extremely low.^{17–19 38} It is also worth noting that AIG is rare in Asia and South America where the prevalence of *H pylori* infection is high.^{8–11}

Genetic background may be a factor affecting the development of AIG,⁸ and might cause geographic diversity of the prevalence of AIG. In mice, the incidence of experimental AIG depends on the mouse strain, suggesting the importance of genetic factors.^{39 40} In this study, we examined whether *H pylori* infection affects the development of AIG using a genetically identical host.

A possible reason for the low prevalence of *H pylori* infection in patients with pernicious anaemia is that *H pylori* cannot colonise in severely atrophic gastric mucosa with AIG.^{41 42} Supporting this idea, *H pylori* did not colonise the murine gastric mucosa with established AIG. In humans however progression of AIG is very slow, spanning 20–30 years, and median age at diagnosis is 60 years.⁷ Because most cases of *H pylori* infection are established in childhood, it is natural that a longstanding *H pylori* infection precedes the onset of AIG. In this study therefore we inoculated nude mice with *H pylori* three months before transfer of lymphocytes from AIG mice.

Human AIG is characterised by chronic infiltration of inflammatory cells within the fundic mucosa, loss of parietal cells from the fundic gland, and the presence of circulating autoantibodies to parietal cells.¹ Murine experimental AIG can be induced by thymectomising BALB/c mice on the third post-natal day. It shares common pathological and clinical features with human AIG, including lymphocytic infiltration and selective loss of parietal cells from the fundic mucosa, production of autoantibodies to parietal cells, elevated gastric pH, and hypergastrinaemia.^{20 21} Murine AIG is induced by CD4⁺ Th1 cells, recognising the alpha or beta subunit of H⁺K⁺-ATPase of parietal cells, and can be induced in syngeneic nude mice by adoptive cell transfer.^{22 43} BALB/c mice are relatively resistant to the development of chronic gastritis following infection with *H pylori*.^{27 44} In the present study however using this nude

Figure 7 Effects of *Helicobacter pylori* infection on mucosal cytokine messages in autoimmune gastritis (AIG) transferred BALB/c nu/nu mice. The cytokine mRNA profiles in the gastric mucosa were determined using real time polymerase chain reaction. Data are expressed as means (SEM) for copies of mRNA/1000 copies of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Interferon γ (IFN- γ) was upregulated by AIG transfer at both two and six months compared with normal mice. There was no significant difference in relative IFN- γ gene expression between infected and uninfected mice at either two or six months after disease transfer (A). Interleukin 4 (IL-4) was also upregulated by AIG transfer at both two and six months compared with control mice. IL-4 gene expression levels in infected mice were significantly higher than those in uninfected mice both at two and six months after disease transfer (B). Transforming growth factor β (TGF- β) was constitutively expressed in the gastric mucosa, and significantly higher in infected mice than in uninfected mice both at two and six months after disease transfer (C). * $p < 0.05$, ** $p < 0.01$.

mouse model, we provided compelling evidence that *H pylori* infection inhibits the development of characteristic features of murine AIG, including destruction of parietal cells, elevation of gastric pH, production of antiparietal cell antibodies, and increase in serum gastrin.

This animal model clarifies the effects of *H pylori* infection on progression of AIG rather than the role of *H pylori* infection in the initiation of AIG because the lymphocytes transferred to nu/nu mice have already been activated in mice with established AIG. Our findings are however compatible with a previous report that concomitant *H pylori* infection might be protective before the autoimmune lesion develops into total gastric atrophy.²⁵

Figure 8 Immunohistochemical staining of gastric mucosa of uninfected (A–C) or *Helicobacter pylori* infected (D–F) autoimmune gastritis (AIG) transferred mice (original magnification $\times 400$). Each section was incubated with antimouse CD4 (A, D), CD8 (B, E), or B220 (C, F) monoclonal antibody. The major phenotype of the lymphocytes infiltrating the gastric mucosa was CD4 positive-CD8 negative in both uninfected and *H. pylori* infected mice (A, B, D, E). In contrast, B220 positive cells partially infiltrated the gastric mucosa of infected mice while no cells stained for B220 in the gastric mucosa of uninfected mice (C, F).

In this experiment, BALB/c nu/nu mice did not develop gastritis due to *H. pylori* infection because they had no T lymphocytes which cause chronic inflammation in gastric mucosa infected with *H. pylori*. In contrast, mice that received splenocytes from AIG mice can respond to *H. pylori* because the lymphocytes transferred from the donor mice can be stimulated by *H. pylori* in recipient mice. Indeed, serum anti-*H. pylori* antibody levels were elevated in infected AIG transferred mice whereas there was no elevation of serum anti-*H. pylori* antibodies in infected non-transferred nu/nu mice (data not shown).

In the present study, in addition to IFN- γ expression (a Th1 response marker), there was upregulation of gastric IL-4 expression (a Th2 subtype marker) and TGF- β by *H. pylori* infection in AIG mice. Although Th1 cells are predominant not only in AIG^{22–45} but also in *H. pylori* induced gastritis,^{47–52} several lines of evidence suggest that Th2 cytokines also have an important role in the pathophysiology of *H. pylori* induced gastritis.^{27–53–54} Th1 and Th2 responses are mutually exclusive.⁵⁵ Thus it is reasonable to speculate that preservation of parietal cells in *H. pylori* infected AIG mice is due to suppression of Th1 function by activation of Th2 responses by *H. pylori* infection, causing inhibition of parietal cell specific Th1 effectors. In previous reports, lymphoid follicles, including B cells, formed in the gastric mucosa of neonatally thymectomised mice infected with *H. pylori* and were accompanied by local upregulation of a Th2-type cytokine, IL-4.^{25–56} The present study also demonstrates that a population of B cells emerges in the inflamed gastric mucosa of mice infected with *H. pylori* (fig 8). Our result is similar to a recent finding that progression of *H. pylori* induced gastritis and gastric atrophy mediated by Th1 cells is modulated by concurrent parasite infection, which induces Th2 responses.⁵⁷

In the present study, *H. pylori* infection upregulated TGF- β expression in the gastric mucosa of AIG mice. TGF- β has a wide variety of biological actions,⁵⁸ including anti-inflammatory effects.^{59–60} TGF- β is involved in active suppression as a

Th3 cytokine,⁶¹ and inhibits Th1 responses such as IL-12 induced T and NK cell proliferation, or IFN- γ production.^{62–66} Indeed, systemic administration of TGF- β prevents Th1 cell mediated autoimmune diseases.^{67–69}

Supporting our data, Lindholm *et al* reported that the numbers of TGF- β specific intraepithelial and lamina propria cells in gastric biopsy samples were higher in *H. pylori* infected than in uninfected subjects.⁷⁰ Lymphocytes that express cell surface bound TGF- β , rather than secreted TGF- β , mediate suppression of CD4⁺CD25⁺ T cells, which are considered to be effector cells in AIG.^{21–71} Although the source of TGF- β was not specified in this study, upregulation of TGF- β in the gastric mucosa by *H. pylori* infection might have an important role in the prevention of the development of AIG.

In conclusion, our data support the hypothesis that *H. pylori* infection suppresses the development of AIG. This may explain why the number of AIG patients is low in countries such as Japan where the prevalence of *H. pylori* infection is very high, although one must also consider differences between human AIG and the murine model.

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