STOMACH

Human peripheral and gastric lymphocyte responses to *Helicobacter pylori* NapA and AphC differ in infected and uninfected individuals

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Gut 2005;54:25–32. doi: 10.1136/gut.2003.025494

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Revised version received
31 March 2004
Accepted for publication
10 May 2004

Background: In this study, we identify the nature of the immunological response of human peripheral blood mononuclear cells (PBMC) and lamina propria gastric lymphocytes (LPL) to two *Helicobacter pylori* antigens, the neutrophil activating protein (NapA) and alkyl hydroperoxide reductase (AphC). These antigens were identified and selected for study based on the observation that serological recognition of these proteins was associated with *H pylori* negative status in humans.

Aims: The aim was to study the serological, proliferative, and cytokine responses of PBMC and LPL, obtained from *H pylori* infected and uninfected individuals, to these antigens.

Methods: Patient serum, PBMC, and LPL were used to determine antibody isotype, and proliferative and cytokine responses to recombinant forms of NapA and AphC using western blotting and ELISA.

Results: Western blotting revealed antibody reactivity to recombinant NapA and AphC among the *H pylori* negative population studied. Both the proliferative and interferon γ responses of PBMC and LPL to NapA and AphC were significantly higher in *H pylori* negative compared with *H pylori* positive subjects. Analysis of the IgG subclass profiles to both antigens revealed a T helper 1 associated IgG3 antibody response in uninfected individuals. However, interleukin 10 production was greater in *H pylori* positive individuals in response to these antigens.

Conclusions: Taken together these data are consistent with an immune response to these antigens skewed towards a T helper 1 response in the uninfected cohort.

Helicobacter pylori specifically colonises human gastric epithelium, is a major cause of chronic gastritis, and is strongly associated with peptic ulcer disease and the development of gastric cancer.^{1–3} Colonisation of the gastric epithelium by the bacterium results in an inflammatory reaction consisting of elements of both the humoral and cellular immune response. However, the immune response mounted by the host is ineffective in eliminating *H pylori* from the stomach lumen.⁴ Eradication of the organism is believed to be a rare event once colonisation is established. In addition to strain dependent gene expression by *H pylori*, host factors are also thought to influence disease outcome. The vast majority of individuals colonised by *H pylori* elicit a measurable systemic antibody response that may reflect the specificity of those antibodies produced at the gastric mucosa.⁵ The Ig classes and subclasses of these circulating anti-*H pylori* antibodies are consistent with a prolonged chronic mucosal infection, with IgG and IgA predominating and IgM antibodies rarely observed.^{6–9} Despite the production of such antibodies, the infection persists and gastritis progresses chronically. However, following eradication of *H pylori*, specific antibody levels decline slowly¹⁰ but can be detected by immunoblot for at least two years post eradication.¹¹ Reinfection is accompanied by a rapid rise in antibody titre.¹² These observations support the view that anti-*H pylori* antibodies are not protective and only reflect the chronicity of infection. Of note, reports in the literature indicate that spontaneous eradication of *H pylori* can occur, particularly in the paediatric population^{8 13–19} Of the two documented ingestion studies^{20 21} one reported elimination of an acute infection whereas the other proceeded to develop chronic colonisation. Little attention has been paid however to the systemic and humoral immune responses of *H pylori* uninfected seropositive individuals to *H pylori* antigens.

In this paper, we demonstrate that *H pylori* negative individuals have detectable antibody responses to several *H pylori* antigens, including the neutrophil activating protein (NapA; HP0243, The Institute for Genomic Research annotation, www.tigr.org) and alkyl hydroperoxide reductase (AphC, HP1563). We present the proliferative and cytokine (interleukin 10 (IL-10), interferon γ (IFN- γ)) responses of human peripheral blood mononuclear cells (PBMC) and lamina propria lymphocytes (LPL) to NapA and AphC in *H pylori* positive and negative individuals. The different immune responses to these antigens by both cohorts may have implications for disease progression.

MATERIALS AND METHODS

Materials

All antibodies were obtained from Sigma Chemical Co. (Poole, Dorset, UK), Dako Ltd (High Wycombe, UK), or the Binding Site Ltd (Birmingham, UK). All other chemicals and solvents, except where indicated, were obtained from Sigma. Reagents for DNA manipulation were obtained from either Promega Corporation (Madison, Wisconsin, USA) or New England Biolabs (Beverly, Massachusetts, USA). Recombinant urease B subunit (UreB) was obtained from Austral Biologicals (California, USA).

Abbreviations: IFN- γ , interferon γ ; IL-10, interleukin 10; LPL, lamina propria lymphocytes; NapA, neutrophil activating protein; PBMC, peripheral blood mononuclear cells; rUreB, recombinant urease B subunit; AphC, alkyl hydroperoxide reductase; PBS, phosphate buffered saline; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; HPS, *H pylori* sonicate

Sera samples

Serum samples were obtained from individuals undergoing gastrointestinal endoscopy at St James's Hospital, Dublin. Infection in these patients was determined and confirmed by histological examination of endoscopic biopsy specimens, CLO testing, and culture of the bacterium *in vitro*. The studies described herein were approved by the ethics committee of the Federated Dublin Voluntary Hospitals. Serum samples were also collected from the cohort of patients described below for PBMC and LPL and additional immunoblotting studies.

Subjects used for PBMC/LPL studies

Sixty patients with dyspepsia (30 females, 30 males; age range 18–67 years (median 40)) were studied. All of these patients were attending for upper gastrointestinal endoscopy. All patients had antral biopsies performed to obtain gastric LPL. None of the patients had received non-steroidal anti-inflammatory drugs, bismuth compounds, or antibiotics in the preceding 12 months. Patients with evidence of malignant disease or immunosuppression were excluded. *H. pylori* was identifiable in tissue sections by haematoxylin-eosin staining. Seropositivity for *H. pylori* was determined by ELISA.

Absorption of sera

Sera (diluted 1/50 with phosphate buffered saline (PBS)) were absorbed with a pooled mixture of two clinical isolates of *H. pylori* in addition to the reference strain NCTC 11638, *Escherichia coli* (K12), or *Campylobacter jejuni* (clinical isolate) by incubating a suspension of the bacteria (10^9 bacteria/ml; McFarland standards) in PBS (pH 7.5) with patient sera for two hours at room temperature with gentle mixing. The bacteria were removed from suspension by centrifugation (12 000 *g*, three minutes). Additionally, for some experiments (figs 2, 4), sera were adsorbed with a whole cell sonicate of *H. pylori* (pooled strains N6 and NCTC 26695) or sonicates of *C. jejuni*, *Enterobacter aerogenes*, *Salmonella*

typhimurium, or *Yersinia pseudotuberculosis*. In this case, bacteria were harvested in PBS and subjected to sonication (3×30 second bursts, amplitude setting 10 μ m on a MSE Soniprep 150). The sonicates were diluted to an OD of 0.8 (600 nm) and used for adsorption studies. Sera were diluted 1/50 in the sonicate and incubated overnight (4°C) with rotary mixing. Prior to use for immunoblotting, the various adsorbed sera were diluted with blocking buffer (PBS, 10% (w/v) non-fat milk powder and Tween-20 (0.01%, v/v)) to give a final 1/100 dilution of each serum sample.

Bacterial strains and growth conditions

The clinical isolates of *H. pylori* used in this study were isolated from antral biopsies obtained from patients attending the Gastroenterology Clinic at St James's Hospital, Dublin. *H. pylori* was grown as described previously.²² A clinical isolate of *C. jejuni* from a patient with *C. jejuni* enteritis and a reference strain (HS:19) were grown for two days on campylobacter selective supplement (Oxoid, Basingstoke, UK) at 42°C. *E. coli* K12 was purchased from Gibco (Grand Island, USA) and was grown under standard conditions on LB agar plates. *E. aerogenes* (NCTC 9528), *S. typhimurium* (ATCC 19585), and *Y. pseudotuberculosis* (IP 2627) were grown on LB agar.

Western blotting and SDS-PAGE

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting were performed essentially as described previously.^{22, 23} Immunoblots were processed and developed by enhanced chemiluminescence. For N terminal sequencing, proteins were electroblotted to ProBlott.

Purification of NapA and AphC

Both the NapA and AphC antigens were purified from whole *H. pylori* (strain 11638; 25 mg) on the basis of molecular weight using preparative continuous elution SDS-PAGE on a Model 491 Prep-Cell (BioRad, Hemel Hempstead, UK), as recommended by the manufacturer.

Cloning of the *H. pylori* napA gene

Genomic DNA was extracted from *H. pylori* (NCTC 11638) as described previously.²⁴ Oligonucleotide primers specific for the 5' and 3' termini of the *napA* gene were generated. The forward primer (F) was designed to incorporate an *NdeI* restriction endonuclease site while the reverse primer (R) incorporates a *BamHI* restriction site. The primer sequences were: F, 5'-GAA GGA CTT CAT ATG AAG ACA TTT G -3' and R, 5'-CGT GAA TGG ATC CTC ATG CTG ACT TCT -3'. The *napA* gene sequence was amplified in a "hotstart" polymerase chain reaction (PCR) using 50–100 ng of *H. pylori* DNA. A "touchdown" PCR procedure²⁵ was utilised. The reaction products were purified on a 4% low melting point agarose gel and recovered following β -agarase 1 digestion. Approximately 3 μ g of purified DNA fragment corresponding to the *napA* gene was then digested with the restriction enzymes *NdeI* and *BamHI*, each of which occurs only once on the amplified fragment.

Cloning of aphC

The following primers were used to amplify the entire sequence of *aphC* for cloning in an expression vector (pET16b: Novagen, Madison, USA). Forward primer: 5' GAC TGA TAG CAT ATG TTA GTT ACA AAA CTT GC-3'; reverse primer: 5'-AGC TTA ATG GAT CCT TCT TAA AGA TAT TCT GCA ACG-3'. The forward primer was modified to include an *NdeI* site and the reverse had a built in *BamHI* site. The insert was amplified, digested with the appropriate enzymes, and ligated into the expression vector pET16b.

Figure 1 Serum samples screened for the presence of anti-*Helicobacter pylori* IgG antibodies. Western blots of whole *H. pylori* (50 μ g/lane) probed with serum obtained from *H. pylori* infected (A) and *H. pylori* uninfected (B) cohorts are shown. All sera were diluted 1:100 in phosphate buffered saline containing fat free dried skimmed milk (5% w/v). Each track represents an individual strip of PVDF membrane which had been incubated with a different serum sample. Blots were developed by enhanced chemiluminescence.

Figure 2 Adsorption of sera from infected and uninfected seropositive subjects. Serum (40 μ l) from either uninfected (sera 1, 2, 3) or infected (serum 4) individuals was either untreated (C) or adsorbed with sonicates of *H pylori* (Hp), *C jejuni* (Cj), *E aerogenes* (Ea), *S typhimurium* (St), or *Y pseudotuberculosis* (Yp) prior to probing blots of whole *Helicobacter pylori* (NCTC26695) with each serum sample. Primary IgG was detected with horseradish peroxidase conjugated rabbit antihuman IgG (1/3000) and developed by enhanced chemiluminescence.

Expression and purification of the recombinant products

The expression vector used was pET16b (Novagen); 1.6 μ g of the vector were digested using *Nde*I and *Bam*HI. Approximately 200 ng of pET16b were ligated to approximately 100 ng of the appropriate insert DNA with 3 units of T4 DNA ligase at 20°C for 16 hours. The products of this reaction were used to transform competent *E coli* XL1-blue cells. Plasmids with appropriate inserts were used to transform *E coli* expression hosts (BL21 DE3 and NOVAbblue DE3). Overexpression was induced by the addition of IPTG (1 mM). The antigens were purified as recommended by the manufacturer on Ni-NTA agarose.

IgG ELISA

Polyclonal IgG ELISA

All steps were performed at room temperature. ELISA plates (Nunc Maxisorp, Roskilde, Denmark) were coated with recombinant NapA or AphC (1 μ g/ml; 50 μ l/well) in PBS (pH 7.4) for three hours. After washing with PBS and blocking with bovine serum albumin (3% w/v; 150 μ l/well) in PBS for one hour the plates were washed with PBS and serum (50 μ l; diluted 1/50 in PBS) was added to duplicate wells and incubated for one hour. Controls consisted of wells with PBS alone and *H pylori* sonicate (1 μ g/ml) as negative and positive controls, respectively. Peroxidase conjugated antihuman IgG (1/5000) was added and incubated for one hour after which time the plates were washed with PBS and the colour reaction was initiated by addition of TMB (50 μ l). After 10 minutes the reaction was terminated by addition of 1 M- H_2SO_4 (50 μ l) and the colour intensity was measured at 450 nm.

IgG subclass ELISA

Detection of specific IgG subclasses was achieved by adding 50 μ l of alkaline phosphatase conjugated anti-IgG subclasses (IgG 1–4) at a dilution of 1/5000 in PBS. The colour reaction was initiated by addition of 50 μ l p-nitrophenyl-phosphate (1 mg/ml in 10% diethanolamine buffer, pH 9.8) and incubated in the dark for 10 minutes. The reaction was terminated by addition of 50 μ l NaOH (3 M). The plates were read at 405 nm.

PBMC and LPL proliferation studies

Venesections were performed for isolation of PBMC which were subsequently separated from other blood products by Ficoll hypaque density gradient centrifugation as described previously.²⁶ Viability of PBMC was consistently >95%. To assess antigen specific lymphocyte proliferation, 1×10^6 /ml

PBMC were cultured at 37°C in 5% CO_2 in 96 well U bottom microplates in a total volume of 200 μ l for three days either alone or in the presence of OKT3 (1:50 dilution), PHA (10 μ g/ml), *H pylori* sonicate (3 μ g/ml for PBMC and 300 μ g/ml for LPL), NapA (1 μ g/ml), AphC (1 μ g/ml), recombinant urease B (rUreB) (1 μ g/ml), or β -galactosidase (1 μ g/ml), essentially as described previously.²⁶ Gastric LPL were isolated and used in proliferation studies as previously described.²⁶ The optimal stimulatory concentration for each recombinant antigen was predetermined for both recombinant NapA and AphC (range 0.05–3 μ g/ml) using samples of PBMC and LPL obtained from *H pylori* infected (n = 4) and uninfected individuals (n = 4) and in both cases was found to be 1 μ g/ml (data not shown).

Measurement of IFN- γ and IL-10 secretion by PBMC and LPL

PBMC (1×10^6 /ml) and LPL (4×10^5 /ml) were cultured either alone or in the presence of the antigens described above for three days at 37°C in 5% CO_2 . The culture supernatants were collected and stored at -80°C prior to quantifying the amounts of IFN- γ and IL-10 present using commercially available ELISA kits (Cambridge Bioscience, UK).

Statistical analysis

The significance of the difference between the results obtained with *H pylori* positive and *H pylori* negative individuals was evaluated using the Mann-Whitney U test/Wilcoxon and independent Student's *t* test.

RESULTS

Immunoblotting of serum obtained from *H pylori* infected and uninfected subjects

Cohorts of sera obtained from *H pylori* infected and uninfected individuals were screened for anti-*H pylori* IgG antibodies by western blotting. All of the *H pylori* infected individuals examined recognised a heterogeneous population of *H pylori* antigens (fig 1A). Similarly, sera obtained from subjects known to be uninfected were found to be immunoreactive against *H pylori* antigens but to a lesser extent (fig 1B compared with fig 1A). The anti-*H pylori* immunoreactivity of sera from both infected and uninfected cohorts was immunodepleted almost completely only by pre-adsorption of the sera with whole *H pylori* extracts (fig 2, lane Hp in all panels). Adsorption of the same sera with *C jejuni* resulted in some but considerably less immunodepletion compared with adsorption with *H pylori*, and very little was seen when the samples were adsorbed with *E aerogenes*, *S typhimurium*, or *Y pseudotuberculosis* (fig 2). Also, the ability of rabbit polyclonal

Figure 3 *C jejuni* and *E coli* antigens recognised by anti-*Helicobacter pylori* antiserum and elimination of cross reactivity by adsorption with *H pylori*. (A) Western blot of *H pylori* (lane 1), *C jejuni* (lane 2), and *E coli* (lane 3) probed with rabbit anti-*H pylori* antiserum. A sonicate of each bacterium (50 µg) was subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis and immunoblotting, as described in the methods section. (B) Effect of adsorbing serum obtained from a *H pylori* infected subject (lanes 1–3) and a subject negative for *H pylori* (lanes 4–6) with *E coli* (lanes 1 and 4), *H pylori* (lanes 2 and 5), or *C jejuni* (lanes 3 and 6).

anti-whole *H pylori* antiserum to cross react with *C jejuni* and *E coli* antigens was examined by western blotting (fig 3A). Anti-*H pylori* antiserum recognised a reduced number of antigens on both *E coli* and *C jejuni* compared with *H pylori* itself. Specifically, the antiserum recognises proteins of molecular mass 72, 50, 40, 36, and 25 kDa on *C jejuni* and proteins of molecular mass 200, 116, 45, and 38 kDa on *E coli* (fig 3A). Of these, only three proteins (70 and 25 kDa from *C jejuni* and 200 kDa from *E coli*) showed pronounced cross reactivity. Additional adsorption experiments demonstrated that *E coli* also failed to significantly deplete anti-*H pylori* seroreactivity (fig 3B).

Identification of two antigens recognised by serum from *H pylori* negative subjects

Preparative continuous elution SDS-PAGE was used to fractionate whole *H pylori* on the basis of molecular size.

Figure 4 Immunoreactivity of neutrophil activating protein (NapA) and alkyl hydroperoxide reductase (AphC) with sera from uninfected seropositive subjects. Untreated sera (1/50) were used to probe blots of recombinant NapA (A, B) or AphC (E). Selected sera from these individuals were also adsorbed with the bacterial sonicates described in the legend to fig 2, prior to probing blots of NapA (C, three sera and D, three sera) or AphC (F, four sera). The blots were processed as described in fig 2.

Two immunoreactive antigens were identified by probing immunoblots of protein fractions with serum from *H pylori* uninfected individuals. N terminal amino acid sequencing of two of the seroreactive antigens revealed one to be NapA and the other, AphC. Recombinant forms of both antigens were subsequently generated and used for further studies. Almost all sera from uninfected subjects had IgG that reacted with both recombinant NapA (fig 4A, B) and AphC (fig 4E). Furthermore, this immunoreactivity was completely depleted only when the sera were pre-adsorbed with *H pylori*. Some immunoreactivity against NapA (fig 4C, D) and AphC (fig 4F) appears to be adsorbed partially, but incompletely, by other bacteria.

IgG subclass responses to Nap A and AphC

Both total IgG and IgG subclass responses to *H pylori* recombinant NapA and AphC were analysed in sera from *H pylori* infected and uninfected individuals by ELISA. IgG immunoglobulins to NapA and AphC were present in the serum of both *H pylori* positive and *H pylori* negative subjects (fig 5A, B). Interestingly, *H pylori* negative individuals had a

Figure 5 Total IgG and IgG subclass responses to neutrophil activating protein (NapA) and alkyl hydroperoxide reductase (AphC). Total IgG response and the various IgG subclass responses to NapA (A) and AphC (B) in serum obtained from *Helicobacter pylori* positive and *H. pylori* negative individuals are shown. IgG levels were determined by ELISA, as described in the methods section and the absorbance axes represent readings obtained at 450 nm for total IgG and 405 nm for the IgG subclasses.

significantly greater total IgG response to both NapA ($p<0.01$) and AphC ($p<0.01$) compared with the infected cohort. Analysis of the subclass specificity of the IgG response to NapA demonstrated that uninfected subjects had a significantly higher IgG2 ($p<0.05$) and IgG3 response ($p<0.01$) whereas the infected cohort had a higher IgG4 response ($p<0.01$) (fig 5A). A similar pattern was seen in the subclass responses to AphC (fig 5B). There were no significant differences in the IgG1 responses to these antigens in either study group.

Proliferative responses of PBMC and LPL to NapA and AphC

The proliferative responses of PBMC to NapA, AphC, and *H. pylori* sonicate (HPS) were significantly higher ($p<0.05$ in all three cases) in *H. pylori* negative compared with *H. pylori* positive subjects (fig 6A). In contrast, the proliferative responses to rUreB were not significantly different between *H. pylori* positive ($n=10$) and *H. pylori* negative ($n=10$) patients. Similarly, the proliferative responses of LPL to NapA, AphC, and HPS were significantly higher ($p<0.05$ in all three cases) in *H. pylori* negative compared with *H. pylori* positive patients (fig 6B) whereas there were no significant differences between the two cohorts in the proliferative responses observed to rUreB. No significant differences were found between the two groups after stimulation with PHA, OKT3, or β -galactosidase (table 1). β -Galactosidase was included as a control histidine tagged fusion protein.

Induction of IFN- γ and IL-10 production by *H. pylori* HPS, NapA, AphC, and rUreB in PBMC and LPL

Human PBMC and LPL were incubated with NapA, AphC, rUreB, and HPS to determine the effect of these antigens on

Figure 6 Proliferative responses of peripheral blood mononuclear cells (PBMC) and lamina propria lymphocytes (LPL) to neutrophil activating protein (NapA) and alkyl hydroperoxide reductase (AphC). The proliferative responses of PBMC (A) and LPL (B) to *Helicobacter pylori* sonicate (HPS) (3 μ g/ml for PBMC and 300 μ g/ml for LPL), NapA (1 μ g/ml), AphC (1 μ g/ml), and recombinant urease B subunit (rUreB) (1 μ g/ml) are shown for both *H. pylori* positive and *H. pylori* negative subjects. Results are expressed as [3 H]-thymidine incorporation (cpm) into PBMC (2×10^6 /ml) and LPL (4×10^5 /ml) cultured for three days in the presence of the indicated antigens. All samples were measured in triplicate.

cytokine production. IFN- γ production by PBMC from *H. pylori* negative patients stimulated with either NapA ($p<0.05$), AphC ($p<0.01$), or HPS ($p<0.05$) was significantly higher compared with *H. pylori* positive patients (fig 7A). In contrast, PBMC from *H. pylori* positive subjects produced significantly more IL-10 when activated with either NapA ($p<0.05$), AphC ($p<0.05$), or HPS ($p<0.05$) compared with *H. pylori* negative patients (fig 8A).

IFN- γ production by LPL was significantly higher in the *H. pylori* negative cohort after stimulation with NapA ($p<0.0001$), AphC ($p<0.01$), or HPS ($p<0.05$) compared with *H. pylori* positive individuals (fig 7B). IL-10 production by LPL was significantly higher in the *H. pylori* positive group after stimulation with NapA ($p<0.05$), AphC ($p<0.05$), or HPS ($p<0.05$) compared with the *H. pylori* negative group (fig 8B).

Of note, NapA and AphC induced significantly higher IFN- γ production by both PBMC and LPL from *H. pylori* negative subjects when compared with rUreB.

DISCUSSION

Eradication of *H. pylori* is thought to be a rare event once colonisation is established, yet there are indications in the literature that this does occur.^{13–19} Given the high incidence of *H. pylori* infection in the broad population it is likely that some individuals eliminate the infection without intervention. This is a contentious issue however but one that may have implications relating to the actual incidence of exposure as many such individuals would remain seropositive but undetected. Moreover, in many instances, *H. pylori* negative individuals are classified as such based solely on serological EIA, a technique prone to error.^{27–28} In addition, cross reactivity with other bacterial species decreases specificity (for example, see Feldman and colleagues²⁹). More sensitive

Table 1 Proliferative responses of PBMC and LPL to PHA, OKT3, and β -galactosidase: [3 H]-thymidine incorporation (cpm)

	PBMC			LPL		
	HP+ve	HP-ve	p Value	HP+ve	HP-ve	p Value
PHA	20574 (3974)	25645 (4735)	0.14	8378 (2086)	7360 (1744)	0.34
OKT3	10112 (2318)	12556 (2166)	0.31	5475 (666)	4745 (1043)	0.56
β -gal	651 (110)	670 (124)	0.39	601 (165)	605 (173)	0.96

HP, *Helicobacter pylori*; PBMC, peripheral blood mononuclear cells; LPL, lamina propria lymphocytes; β -gal, β -galactosidase.
Results are expressed as [3 H]-thymidine incorporation (cpm) into PBMC and LPL cultured for three days. LPL (4×10^5 /ml) were cultured with autologous irradiated (2500 rads) PBMC (2×10^6 /ml) in the presence of IL-2 (2 IU/ml). All samples were measured in triplicate and are shown as mean (SEM) (n=30). PHA was used at a concentration of 10 μ g/ml and 5 μ g/ml for PBMC and LPL, respectively. OKT3 was used at a dilution of 1:50 and β -galactosidase was 1 μ g/ml.

detection techniques such as immunoblotting combined with enhanced chemiluminescence³⁰ facilitate detection of low levels of specific antibodies not detected by ELISA.^{31–33} Additionally, immunoblotting has enabled investigators to differentiate between age related changes in antigen recognition.^{34–36}

An antibody response to bacterial antigens is one indicator of prior exposure to an organism. Infection with *H. pylori* at a subclinical level and consequent elimination of the infection has been proposed to account for serological recognition of *H. pylori* antigens in some uninfected subjects.³⁷ In this study, we showed that *H. pylori* uninfected subjects had circulating antibodies (IgG) to several *H. pylori* antigens, including NapA and AphC. Immunodepletion and cross reactivity studies indicated that the IgG response was *H. pylori* directed as seroreactivity could only be substantially eliminated by

adsorption with *H. pylori* but not with *C. jejuni*, *E. coli*, *E. aerogenes*, *S. typhimurium*, or *Y. pseudotuberculosis*. Specific polyclonal antisera (rabbit) to *H. pylori* only reacted weakly with *C. jejuni* and *E. coli*. Similarly, others have demonstrated little cross reactivity with anti-*H. pylori* serum and other prokaryotes (*Streptococcus sanguis*, *Salmonella typhimurium*, *Campylobacter fetus*, *Nisseria meningitidis*, *Haemophilus influenzae*, *Staphylococcus aureus*, and *Yersinia enterocolitica*).^{17–38} It is not possible however to exclude cross reactivity with other gastrointestinal commensals given the many hundreds of such organisms inhabiting the gastrointestinal tract. However, the *H. pylori* directed specificity of the anti-NapA and anti-AphC IgG response is supported by the inability of other bacteria, including *C. jejuni*, to immunodeplete NapA and AphC antibodies from uninfected patient sera, even

Figure 7 Interferon γ (IFN- γ) production by peripheral blood mononuclear cells (PBMC) and lamina propria lymphocytes (LPL) in response to neutrophil activating protein (NapA) and alkyl hydroperoxide reductase (AphC). IFN- γ production by PBMC (A) and LPL (B) obtained from *Helicobacter pylori* positive or *H. pylori* negative individuals in response to the indicated antigens. All antigens were used at 1 μ g/ml. Supernatants were collected from cultured PBMC and LPL after 72 hours and stored at -80°C . IFN- γ was measured in the supernatant by ELISA. All samples were measured in duplicate. Results are expressed as mean (SEM). rUreB, recombinant urease B subunit; HPS, *H. pylori* sonicate.

Figure 8 Interleukin 10 (IL-10) production by peripheral blood mononuclear cells (PBMC) and lamina propria lymphocytes (LPL) in response to neutrophil activating protein (NapA) and alkyl hydroperoxide reductase (AphC). Levels of IL-10 produced by PBMC (A) and LPL (B) from *H. pylori* positive and *H. pylori* negative subjects in response to the antigens indicated are shown. All antigens were used at 1 μ g/ml. Supernatants were collected from cultured PBMC and LPL after 72 hours and stored at -80°C . IL-10 was measured in the supernatant by ELISA. All samples were determined in duplicate. Results were expressed as mean (SEM). rUreB, recombinant urease B subunit; HPS, *H. pylori* sonicate.

though homologues of AphC and NapA from *C jejuni* exhibit the highest degree of identity to *H pylori* AphC and NapA, at 67% and 38%, respectively.

As prospective data were not available for this study there is an inherent uncertainty in unequivocally ascribing the observed antibody responses to *H pylori* to a high incidence of prior exposure to the bacterium. Although spontaneous eradication/transient colonisation of *H pylori* infection has been documented in paediatric populations^{14 16 19 39-42} it is thought to be a less frequent event in adults, yet a significant rate (7.7%) of IgG seroreversion was found in a young and middle aged Danish population.⁸ Others too have detected anti-*H pylori* antibodies in uninfected individuals by immunoblotting (for example, see Nilsson and colleagues⁴³). One possible explanation for seroreversion in the absence of therapeutic intervention is the widespread use of antimicrobials for other infections with secondary clearance of *H pylori*. However, there is evidence to suggest that this is unlikely to account for all cases of apparent spontaneous eradication.^{44 45} Strain variation, host genetic factors, and gastric atrophy have also been proposed to account for some cases of seroreversion.⁴⁶⁻⁴⁹

In addition to antibody responses, both the proliferative and cytokine responses of NapA and AphC stimulated PBMC and LPL were influenced by the infection status of the individuals. Both the gastric and peripheral lymphoproliferative responses of the uninfected seropositive group were significantly greater than those observed for infected individuals in response to NapA, AphC, HPS, but not rUreB. Furthermore, NapA, AphC, and HPS activated but not rUreB treated PBMC and LPL from uninfected subjects secreted significantly more IFN- γ than infected subjects, observations that are similar to previous studies with various preparations and extracts of *H pylori*.^{26 50 51} Others too have shown that gastric biopsy samples from uninfected dyspeptic patients have more IFN- γ secreting T cells than infected samples, suggesting that IFN- γ type responses might be protective.⁵² A number of groups have also reported suppressed lymphocyte responses from infected subjects compared with negative controls,^{26 50 51} possibly due to an altered T cell response secondary to infection or, alternatively, the production of immunosuppressive factor(s) by the pathogen. This is in agreement with our present data showing preferential IL-10 secretion by infected individuals.

Finally, as the cytokine profile during infection is documented to play a regulatory role with respect to immunoglobulin production, including subclass and isotype switching, it was of interest to determine the NapA and AphC specific IgG subclass pattern in *H pylori* infected and uninfected subjects. Both NapA and AphC preferentially, but not exclusively, elicited a stronger IgG3 response in uninfected subjects and a significantly stronger IgG4 response in *H pylori* positive individuals. Secretion of IgG4 by human PBMC is known to be suppressed by IFN- γ in vitro⁵³ but enhanced by IL-10,⁵⁴ a major regulatory cytokine. Studies on various infections including Lyme disease,⁵⁵ rubella^{56 57} and mumps⁵⁸ indicate a close association between IFN- γ and IgG3 production. Furthermore, the potent opsonising and complement fixing properties of IgG3 have prompted speculation that IgG3 positivity may play a role in disease resolution, particularly in the case of Lyme borreliosis. In this regard it is interesting to note in this study that *H pylori* negative subjects displayed a predominantly T helper 1-like cytokine and antibody response to NapA and AphC. However, it appears likely that the IgG subclass distribution will be influenced by the biochemical/antigenic properties of the molecules based on the antigen specific cytokine profiles observed in this present and other studies.

In summary, these findings may have implications with regard to protective immunity. In animal studies NapA has been identified as a protective vaccine⁵⁹ and it will be of interest to determine whether AphC demonstrates similar properties. However, identification of different immune responses to *H pylori* antigens in *H pylori* negative and positive populations suggests that the nature of the immune response to *H pylori* exposure may have an influence on patient/disease outcome.

ACKNOWLEDGEMENTS

The authors acknowledge Professor D Weir for the biopsy material, Dr A Moran and John Ferris for *C jejuni* and Dr Ken Whelan for various samples of gut flora. This work was supported by a Health Research Board grant (YSA).

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PostScript

LETTERS

ITPA genotyping test does not improve detection of Crohn's disease patients at risk of azathioprine/6-mercaptopurine induced myelosuppression

The thiopurine drugs azathioprine (AZA) and 6-mercaptopurine (6-MP) are effective for the treatment of inflammatory bowel disease (IBD) and their prescription is increasing. Haematotoxicity, which can lead to potentially life threatening bone marrow suppression, represents the most serious side effect of thiopurine therapy. It has been attributed to the accumulation of active cytotoxic metabolites of AZA/6-MP, collectively called 6-thioguanine nucleotides, resulting from a deficiency in thiopurine catabolism specifically catalysed by the thiopurine S-methyltransferase (TPMT) enzyme. Genotyping tests are now available to identify deficient and intermediate methylators who are, respectively, homozygous and heterozygous for non-functional alleles of the TPMT gene. As pointed out by Lennard in the leading article (*Gut* 2002;51:143-6), it is clear that myelosuppression may be caused by other factors in addition to variable TPMT.

Since the identification of the molecular basis of inosine triphosphate pyrophosphatase (ITPase) deficiency,¹ a clinically benign condition characterised by abnormal accumulation of inosine triphosphate in erythrocytes, the possibility of a correlation between thiopurine toxicity and ITPase deficiency has been raised. Complete ITPase deficiency was found to be associated with a homozygous missense 94C>A mutation that encodes a Pro³²Thr exchange, whereas an intronic IVS2+21A>C polymorphism was shown to have a less severe effect, homozygotes retaining 60% ITPase activity. It was then postulated that in ITPase deficient patients treated with thiopurine drugs, a 6-thio-ITP metabolite could accumulate resulting in toxicity.¹ A recent study in 62 patients with inflammatory bowel disease reported a significant association between the ITPA 94C>A polymorphism and AZA related adverse effects, specifically flu-like symptoms, rash, and pancreatitis.² No correlation was observed with occurrence of neutropenia but only 11 patients were studied. We previously reported TPMT genotype analysis in 41 Crohn's disease

(CD) patients who had experienced leucopenia during AZA/6-MP therapy.³ Even though this study confirmed the efficiency of TPMT genotyping in identifying patients at risk of developing myelosuppression, it also highlighted its limitations, as only 27% of patients carried mutant alleles of the TPMT gene that were associated with enzyme deficiency. This prompted us to investigate the occurrence of ITPA mutations in this series of patients in order to evaluate whether genotyping of the ITPase gene could improve the detection rate of patients at risk of thiopurine myelotoxicity.

Our population comprising 41 patients with CD has been described in detail previously.³ Briefly, all patients had either leucopenia (white blood cell count <3000/mm³; n = 24) or thrombocytopenia (platelets <100 000/mm³; n = 30), or both (n = 14), leading either to discontinuation of treatment or reduction of dose by 50% or more during AZA (n = 33) or 6-MP (n = 8) treatment. Patients were genotyped for the ITPA 94C>A and IVS2+21A>C mutations according to a previously described procedure based on endonuclease digestion of polymerase chain reaction products.¹ Distribution of the 41 patients according to their ITPA genotype is presented in table 1 and compared with that of a previously published control population of 100 healthy Caucasians.¹ Allele frequencies in the CD population were 0.085 for the 94C>A mutation and 0.12 for the IVS2+21A>C mutation, similar to frequencies observed in the control population (0.06 and 0.13, respectively). There was no significant difference in the genotypes distribution between the two populations, which confirmed the lack of association between ITPase deficiency and myelosuppression during thiopurine therapy. Due to the retrospective nature of the study, no correlation with other side effects could be investigated.

In conclusion, application of ITPA genotyping tests does not seem to improve the identification of patients at risk of myelosuppression with AZA/6-MP therapy. Although we believe that conventional TPMT genotyping tests should still be applied before the initiation of thiopurine treatment, further work is needed on the role of other candidate genes that may be involved in thiopurine haematotoxicity.

Acknowledgements

We thank N Ferrari and A Vincent for their assistance in performing the study and the members of the GETAID for recruiting patients in the study.

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doi: 10.1136/gut.2004.055947

Competing Interests: None declared.

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Small bowel malignancy at diagnosis of coeliac disease

We were very interested in the paper by Rampertab *et al* (*Gut* 2003;52:121-14) and the correspondence by Hawdle *et al* (*Gut* 2004;53:470). Their data are quite similar to ours, from the Italian Registry of Complications of Coeliac Disease.

We collected information on 1968 patients over 18 years of age (mean age at diagnosis: 36.7 years; female/male ratio 3:1), diagnosed with coeliac diseases between January 1982 and December 2002 at 20 Italian clinical centres specialised in gastrointestinal disease. The diagnosis was made according to revised ESPGHAN criteria.¹ We found five (0.25%) patients with a small bowel malignancy at the time of diagnosis of coeliac disease. Age range was 49-69 years (mean 59 years) with a predominance of females (4:1). Survival rate was very poor as three patients died within 36 months of diagnosis.

These results indicate that there is an increased risk of developing small bowel malignancy in patients with coeliac disease. This correlation was confirmed by the female/male ratio. In fact, while small bowel neoplasms are usually more frequent in males, in our population four of five cases were female. Moreover, mean age at diagnosis of these cases was higher than that of patients overall, emphasising that the risk of a neoplasm increases with longstanding coeliac disease.

Table 1 Distribution of ITPA genotypes in 41 Crohn's disease (CD) patients and 100 healthy Caucasians

ITPA genotype	CD patients (n = 41)	Control population† (n = 100)
Wt/Wt	26 (0.63)*	64 (0.64)
Wt/94C>A	6 (0.15)	10 (0.10)
Wt/IVS2+21A>C	7 (0.17)	24 (0.24)
94C>A/94C>A	0 (0.00)	0 (0.00)
IVS2+21A>C/IVS2+21A>C	1 (0.02)	0 (0.00)
94C>A/IVS2+21A>C	1 (0.02)	2 (0.02)

*Values in parentheses represent genotype frequencies.

†The control population comprised 100 healthy Caucasians who were genotyped in a previous study.¹

In conclusion, early diagnosis of coeliac disease should be made to prevent small bowel neoplasms from developing, and screening for this cancer should be carried out at diagnosis of coeliac disease, especially in patients diagnosed during adulthood.

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Hypergastrinaemia in patients infected with *Helicobacter pylori* treated with proton pump inhibitors

We read with interest the commentary by McColl on *Helicobacter pylori* infection and long term proton pump inhibitor (PPI) therapy (*Gut* 2004;**53**:5–7).

It is remarkable that he did not mention gastrin although hypergastrinaemia is a result of reduced gastric acidity¹ as well as *Helicobacter pylori* infection,² and that patients with *H pylori* infection treated with PPI have additive hypergastrinaemia.³ Hypergastrinaemia predisposes to gastric carcinoids in animals^{4–5} and humans^{6–7} as well as to malignant ECL cell derived tumours (gastric carcinomas) in animals⁸ and humans.^{9–10}

Interestingly, the carcinogenic effect of *H pylori* infection may be completely explained by its hypergastrinaemic effect,¹¹ a work where McColl was one of the authors. Furthermore, the increased gastric cancer frequency in moderate hypergastrinaemic INS-GAS mice concomitantly infected by *H pylori* infection¹² may also be caused by increased hypergastrinaemia in infected mice.¹³

To conclude, it is odd that gastrin was not taken into consideration when discussing the risk of gastric cancer following treatment with PPI in patients infected with *H pylori*. Animal as well as human studies linking gastrin to gastric cancer give support for a strategy where *H pylori* is eradicated in patients on long term PPI treatment.

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Competing Interests: None declared.

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Terminal ileal biopsies should not be used to document extent of colonoscopic examination

We commend the British Society of Gastroenterology and the authors for the excellent publication of guidelines for the management of inflammatory bowel disease in adults (*Gut* 2004;**53**(suppl V):vi–16). However, we feel that their recommendation for routine terminal ileal biopsy is inappropriate. Although it is important to biopsy the terminal ileum if there is macroscopic evidence of an abnormality, their statement that "a terminal ileal biopsy performed at colonoscopy documents the extent of examination" is not recommended practice, due to the potential risk of variant Creutzfeld-Jacob disease transmission from prion proteins which are prevalent in the lymphoid tissue of Peyer's patches in the ileum. Although the use of disposable forceps may reduce the risk of transmission, there could still be contamination of the intubation channel of the colonoscope and prion protein is resistant to the standard endoscopic cleaning process.¹ If the extent of examination needs to be documented, then a photograph of the ileocaecal valve or ileal mucosa is preferable.

It is worth emphasising that prion protein may be present in any part of the gastrointestinal tract² and random biopsy of gastrointestinal mucosa for reasons other than confirming an endoscopic abnormality or excluding microscopic colitis is not accepta-

ble. Similarly, for surveillance colonoscopy where multiple biopsy is recommended, the risk benefit ratio of this policy must be supported by the clinical indications.

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IgG food antibodies should be studied in similarly treated groups

The recent paper by Atkinson and colleagues (*Gut* 2004;**53**:1459–1464) regarding IgG food antibodies and irritable bowel syndrome (IBS) fails to compare like with like. Regardless of the IgG results, the treatment group excluded significantly different foods to the control group, particularly those foods which appear to exacerbate symptoms of IBS. Of particular concern is the "yeast exclusion" diet. A low yeast diet is not a recognised diet in standard textbooks of dietetics and nutrition. However, alternative practitioners offering such a "yeast exclusion diet" sometimes recommend exclusion of a wide range of foods, such as: bakery products, alcoholic beverages, many other beverages including commercial fruit juices, cereals, condiments, dairy produce, fungi, meat products (hamburgers, sausages, and cooked meats made with bread or breadcrumbs), yeast extracts (Bisto, Marmite, Oxo, Bovril, Vegemite, gravy browning, and all similar extracts), all B vitamin preparations, and sometimes, most worryingly, "sugar foods" (sugar, sucrose, fructose, maltose, lactose, glycogen, glucose milk, sweets, chocolate, sweet biscuits, cakes, candies, cookies, puddings, desserts, canned food, packaged food, hamburgers, honey, mannitol, sorbitol, galactose, monosaccharides, polysaccharides, date sugar, turbinado sugar, molasses, maple syrup, most bottled juices, all soft drinks, tonic water, milkshakes, raisins, dried apricots, dates, prunes, dried figs, and other dried fruit).

Therefore, regardless of IgG antibody status, the dietary restrictions in one group are not controlled for by the other group, and hence the conclusion may not be valid.

It would also be helpful to know if any of the patients with IgG antibodies to a particular antigen also had IgE antibodies to the same antigen.

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Competing Interests: None declared.

IgG antibodies to foods in IBS

We read with interest the article by Atkinson *et al* (*Gut* 2004;53:1459–64). The authors describe an important advance in our understanding of the putative role of inflammation in irritable bowel syndrome (IBS). However, we wonder whether their conclusion that assay of IgG antibodies may have a role in identifying candidate foods for elimination to treat patients with IBS may be a step too far. The four foods to which the patients most commonly formed antibodies and hence the four foods most commonly eliminated from the “true diet” were yeast (86.7%), milk (84.3%), whole egg (58.3%), and wheat (49.3%). The “sham diet” involved eliminating foods to which the patients had not formed antibodies and, therefore, in the sham group the exclusion rates for yeast, milk, whole egg, and wheat were very low (0%, 1.3%, 26.7%, and 8% respectively). It is therefore difficult to assess whether a diet excluding these foods would have led to symptomatic improvement in all patients, regardless of their antibody status.

Furthermore, the foods to which the study group commonly formed antibodies were similar to those already identified as leading to symptomatic benefit in patients with IBS when excluded from their diet. In a review cited by Atkinson and colleagues,¹ it was noted that in eight trials of exclusion diets in IBS, seven identified dairy products and five identified wheat as worsening symptoms. It is not clear whether the difference in improvement in symptoms seen in the current study between true and sham groups can be explained simply by the omission of these foods. This could in practice eliminate the need for antibody testing.

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Competing Interests: None declared.

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Influence of dietary factors on the clinical course of inflammatory bowel disease

Jowett *et al* reported in their elegant study on the role of diet in maintaining remission in patients with ulcerative colitis (*Gut* 2004;53:1479–84). Surely the effect of diet has an essential, but often forgotten, role in altering the course of disease in all types of inflammatory bowel diseases. This role does not necessarily act by maintaining patients in remission clinically, but perhaps more importantly by minimising the activities of the disease and rendering it quiescent.

We have recently reported a case of active stricturing Crohn's disease in an adult female patient with high stoma output.¹ She was treated solely with casein base formula (Modulen IBD-Nestle, Vevey, Switzerland) for three weeks. Her stoma output was reduced from 2800 ml to 400 ml per day by

day 10. Serum albumin and serum protein significantly increased also. She subjectively felt better and pain free and stopped her opiate and non-opiate formula. The casein based formula is a nutritionally complete formulation containing a natural anti-inflammatory growth factor, transforming growth factor $\alpha 2$. The mechanism for inducing remission in our patient was possibly inhibition of expression of MHC class II protein in downregulating the inflammatory response.²

Previous studies have shown that there is a decrease in plasma antioxidant defences in all types of inflammatory bowel disease.³ This is mirrored by an increase in free radical peripheral leucocyte DNA damage. It is therefore possible that the casein based formula acts as an antioxidant to minimise the oxidative stress that occurs in patients with active Crohn's disease. Another possible mechanism is that this formula may have a role as a prebiotic by stimulating the activity of bacteria which are already present in the gut.

Remission induced in our case study highlights the part played by a casein based formula in the management of adult Crohn's disease. The encouraging result demonstrates the need to treat similar cases with dietary measures first. This opportunity should not be missed as it may well obviate the need for surgical intervention or administration of potent pharmacotherapeutic agents which carries the risk of several comorbidities.

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Competing Interests: None declared.

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Identification of ferroportin disease in the Indian subcontinent

Haemochromatosis is a common inherited disorder of iron metabolism, characterised by excessive iron absorption and deposition in tissues. The majority of cases are associated with mutations in the *HFE* gene and inherited in an autosomal recessive manner.¹ Autosomal dominant forms of haemochromatosis have been reported, mainly associated with mutations in the *ferroportin 1* gene.² This syndrome, termed type 4 haemochromatosis or more recently ferroportin disease,³ is usually characterised by an early increase in serum ferritin with normal transferrin saturation. Iron accumulation is most prominent in Kupffer cells and other macrophages, in addition to hepatocytes. Some patients do not tolerate venesection

therapy well and can develop anaemia. Hereditary iron overload disorders appear to be uncommon in Asia. Secondary iron overload due to beta thalassaemia is relatively common in the Indian subcontinent. However, primary iron overload disorders and *HFE* mutations appear to be rare and cases have not been well characterised in this region.^{4,5} We identified a patient from the Indian subcontinent with features typical of ferroportin disease.

A 36 year old female of Sri Lankan origin presented for a routine medical examination in December 2003. She was found to have an elevated serum ferritin of 3145 $\mu\text{g/l}$. Her serum iron (17.1 $\mu\text{mol/l}$) and transferrin saturation (29%) were normal. Liver functions tests, blood glucose, and thyroid studies were all normal. Physical examination was normal and she had no significant past medical history or risk factors for iron overload.

C282Y, H63D, and S65C *HFE* gene mutations were all negative and she had no family history of iron overload. Her mother and three siblings all had normal serum ferritin levels. Her father died of ischaemic heart disease aged 48 years.

A magnetic resonance imaging scan showed hepatic iron overload. Liver biopsy showed grade 3–4 iron deposition within hepatocytes and Kupffer cells; no fibrosis or cirrhosis was evident (fig 1). The hepatic iron concentration was 17 700 $\mu\text{g/g}$ dry weight and hepatic iron index was 9.1.

Venesection therapy was initially poorly tolerated with the development of anaemia following the first two 500 ml venesections. Her haemoglobin is now stable on a programme of 300–500 ml venesections every three weeks.

The features of ferroportin disease in this patient led us to sequence the *ferroportin 1* gene, as previously described.⁶ Analysis of the DNA sequence revealed a heterozygous three base pair deletion (TTG) in exon 5. This is the same deletion, V162del, described by us and others in haemochromatosis patients from Australia, the UK, Italy, and Greece.^{6–9}

This is the first report to identify V162del or indeed any *ferroportin 1* mutation in an individual from the Indian subcontinent. Identification of V162del in an Asian patient confirms that this mutation is likely to be the most common mutation of *ferroportin 1* and the most common cause of non-*HFE* associated haemochromatosis. The wide geographical distribution of this mutation suggests that it is a recurrent mutation that has repeatedly arisen in distinct populations, probably by slippage mispairing.

Iron overload in this patient was typical of ferroportin disease. At the time of diagnosis she was asymptomatic and had no fibrosis on liver biopsy. Whether fibrosis or clinical complications will develop with age if iron stores are not depleted is unclear.

In conclusion, we have identified the V162del mutation of *ferroportin 1* in a fifth geographical location, emphasising that this mutation is the most common and widely distributed mutation which causes non-*HFE* haemochromatosis. We have identified V162del in a region where iron overload disorders have not been well characterised. Analysis of this and other *ferroportin 1* mutations may be useful in the study of iron overload disorders in this region and may be the basis of hitherto unexplained cases of iron overload.

Figure 1 Liver biopsy sections from our patient stained with (A) haematoxylin and eosin and (B) Perls' Prussian blue (magnification 100×). Grade 3–4 iron is prominent in hepatocytes and Kupffer cells.

Acknowledgements

This work was supported in part by grants from the National Health and Medical Research Council of Australia (953219), the National Institutes of Health, USA (5R01DK057648-02), and the Haemochromatosis Society of Australia to VNS.

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doi: 10.1136/gut.2004.060988

Competing Interests: None declared.

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BOOK REVIEW

Morson and Dawson's Gastrointestinal Pathology, 4th edn

Edited by D W Day, J R Jass, A B Price, *et al.* Massachusetts: Blackwell Publishing, 2003, £175.00, pp 692. ISBN 0-632-04204-4

Why do people buy a book such as this, which involves a not inconsiderable financial outlay (even if you box clever and make it tax deductible)? I think for two main reasons—firstly, for use as a bench book, and secondly, for information on the pathological basis of gastrointestinal disease for interest, teaching, or indeed research purposes.

On the first criterion, this book succeeds, usually quite brilliantly. As a *vade mecum* on gastrointestinal pathology it should be on the shelf of every pathologist who engages in the reporting of such material. In my view, the book is more user friendly than the

competition—Fenoglio-Preiser and Goldman to name but two—and is certainly more readable. I would therefore extol its virtues unreservedly in this respect.

On the second criterion, as a source book, I suppose the correct word is patchy. Some sections, for example that on colorectal tumours, is admirable in this respect, whereas other sections are more limited in scope and even cursory in their treatment of the pathobiology. There is also the problem of the unavoidable intrinsic delay in producing such a book, resulting in reference lists which are some years away from the publication date. I am aware however that my personal outlook is not that of most individuals who will purchase this volume so I am probably being over critical. It is, after all, quintessentially a bench book, and excellent at that.

However, I do have one real beef. In any multi-author work there is bound to be variation, but here we are not told which one of the stellar caste were responsible for which section or chapter. Of course we can make informed guesses about the Barrett's or colorectal carcinoma sections, but who did the GIST bit? Because of some (minor) errors in the criteria for the diagnosis of malignancy, I have tried to berate a number of authors who have all denied responsibility, and blamed someone else—usually the author(s) absent at the time. Not good enough.

I have to concede however that the authors have succeeded in producing perhaps *the text* in gastrointestinal pathology, which is a credit to both themselves and the discipline in the UK. I congratulate them.

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CORRECTIONS

doi: 10.1136/gut.2003.025494corr1

In the January 2005 issue of *Gut*, one of the author's names of the paper entitled Human peripheral and gastric lymphocyte responses to *Helicobacter pylori* NapA and AphC differ in infected and uninfected individuals (H J Windle, Y S Ang, V A Morales, R McManus, and D Kelleher. *Gut* 2005;**54**:25–32) was cited incorrectly. V A Morales should read V Athie-Morales. The journal apologises for this mistake.

doi: 10.1136/gut.2003.026807corr1

In the December issue of *Gut* fig 1 in the paper by AJG Bell *et al.* (Human lymphocyte stimulation with pouchitis flora is greater than with flora from a healthy pouch but is suppressed by metronidazole. *Gut* 2004;**53**:1801–1805) is incorrect. The labels for fig 1C are inverted; the squares should have been labelled HetNon and the triangles HetPM. The legend is also incorrect because the label for flora grown on agar without metronidazole is HetNon, not HetP as stated.