LETTERS TO THE EDITOR

Absence of antibodies stimulating H_2-receptor mediated cyclic adenosine monophosphate (cAMP) production in peptic ulcer disease

SIR,—We read with great interest the paper by Burman *et al* (*Gut* 1991; **32**: 620–3). Using porcine gastric mucosal cells as an in vitro test system, the authors could not find any stimulatory effect on cyclic adenosine monophosphate (cAMP) production by sera or immunoglobulin (Ig) fractions of 57 patients with relapsing ulcer disease. As the authors reported, their results are in contrast with data obtained by De Lazzari *et al*.[1] This group found in duodenal ulcer patients antibodies stimulating cAMP production in enriched guinea pig parietal cells. Thus, De Lazzari *et al* have suggested that duodenal ulcer disease may be caused by gastric cell stimulating antibodies to H_2-receptors, which therefore are proposed as 'a new addition to the growing list of receptor antibodies in human diseases'.

By using another in vitro test system, we have also investigated the possible role of cAMP stimulatory antibodies in peptic ulcer disease. We tested the effects of Ig preparations on cAMP production in cultured human gastric tumour cells (HGT-1[2]; kindly provided by Dr C Gespach, INSERM U 55, Paris). These cells have H_2-receptors and are considered to be a useful tool for studies of cAMP mediated gastric acid secretion.[3,4] Igs were derived from sera of 36 peptic ulcer patients. The patients were classified as adequate (AR; n=16) and inadequate responders to ranitidine (IR; n=20) by intragastric pH monitoring.[5] Sera were not tested directly because of several undefined components which decreased cell viability from 91 to 52% after four hour incubation of the cells with 20% serum. IgG was isolated by column chromatography on protein G-sepharose and concentrated by microultrafiltration. Other proteins were removed and non-IgG was precipitated by ammonium sulfate (1·6 mol/l). The Igs were tested at concentrations of 4 (IgG) and 1 (non-IgG) mg protein/ml medium. HGT-1 cells were grown as monolayers and incubated for 10, 30, 60, 180, and 360 minutes with Igs in the presence of 1 mmol/l phosphodiesterase inhibitor (IBMX). Standard IgG (Behringwerke) was used as control. The total amount of cAMP was measured by radioimmunoassay (Amersham Buchler).

The basal value of cAMP production in HGT-1 cells was mean (SD) 10·7 (1·7) pmol/mg protein and was stimulated after 10 minutes exposure to histamine (10 µmol/l) to 80·4 (15·0) pmol/mg protein in all experiments (n=36). The stimulation could be blocked by the H_2-receptor antagonists cimetidine and ranitidine with IC50 values of 0·400 and 0·034 µmol/l, respectively, confirming the presence and specificity of H_2-receptors on this cell type.[6,7] No statistically significant stimulation of cAMP production could be obtained after incubation of HGT-1 cells with any Ig preparation tested (see Table).

Cyclic adenosine monophosphate (cAMP) production (pmol/mg protein) in human gastric tumour cells HGT-1 after incubation with Ig preparations derived from sera of ulcer patients with adequate and inadequate responses to ranitidine. (Values, mean (SD))

	Time (min)				
	0	10	30	180	360
Control	11·8 (0·5)	12·2 (1·0)	12·6 (1·3)	12·8 (1·7)	11·5 (1·0)
Adequate responders (n=16):					
IgG	10·4 (1·7)	10·7 (1·7)	10·4 (2·2)	12·0 (2·1)	11·4 (2·2)
Non-IgG	10·6 (1·2)	13·0 (1·6)	13·1 (1·3)	11·9 (2·0)	11·8 (2·5)
Inadequate responders (n=20):					
IgG	10·1 (1·7)	10·5 (1·1)	10·1 (1·8)	11·8 (2·5)	11·0 (1·9)
Non-IgG	10·7 (0·9)	12·7 (1·0)	12·3 (1·6)	11·7 (2·4)	11·7 (2·0)

Our results agree with those of Burman *et al*. In neither in vitro test systems was there evidence for cAMP stimulating antibodies in peptic ulcer patients. In addition, we conclude that antibodies to H_2-receptors do not cause an inadequate response to H_2-antagonists as has been assumed.[1] However, despite these results, it can not be totally ruled out that autoimmunological processes have a role in specific subpopulations of patients with peptic ulcer disease. Moreover, the differing results of Burman *et al* and our group on one hand and De Lazarri *et al* on the other may be caused by the different in vitro test systems used. In particular, De Lazzari *et al* failed to detect any cAMP stimulatory effects on antibodies, unless the parietal cell content in cell suspensions was at least 50%.

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Reply

SIR,—The letter of Drs A Sarem-Aslani, Ch Bergmann, S Walker, D Ratge, U Klotz, and H Wisser supports our paper recently published in *Gut*.[1] In contrast to a previous report by DeLazarri *et al*,[2] we could not find any evidence of gastric acid stimulatory antibodies in patients with severe ulcer disease. Our assay system of porcine gastric mucosal cells exhibited an approximately 20 fold increase in cAMP upon addition of 10^{-4} mol/l histamine. This system seems superior to that of DeLazzari *et al*, who used guinea pig gastric mucosal cells, which had to be enriched for parietal cells (at least 50%) in order to produce a measurable response, but then produced only a fivefold cAMP response to 10^{-3} mol/l histamine. As the results of the two studies were divergent, an argument of species specificity could be made. In this context, the data of Sarem-Aslani *et al*, using a human histamine responsive cell line is of great interest. The sensitivity of their system is comparable to that of ours and the conclusions are in total agreement. Thus, we can emphasise that although it is not possible to exclude histamine receptor stimulatory antibodies in exceptional cases, these are not a cause of severe ulcer disease in humans.

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Non-colonic symptomatology and the irritable bowel: is it really of diagnostic value?

SIR,—We read the paper by Maxton and colleagues on the diagnostic value of 'non-colonic' symptoms in the irritable bowel syndrome with interest.[1] We have some major concerns about their study, however, which we hope they can clarify.

Our first concern relates to the study design. No healthy controls were included for comparison; this makes it difficult to determine the clinical significance of 'non-colonic' symptoms, which are known to be common in otherwise healthy people.[2] The measurement of symptoms is also of concern because the reliability and validity of the symptom assessment was not documented. If the authors did not define and measure each of the symptoms carefully, these might have been interpreted in many different ways by the interviewer and the patients, and thus the discriminatory ability of

these symptoms inaccurately determined. The investigators also included patients with various organic diseases as controls. It is unclear, however, what symptoms led these patients to present, and it is likely that some of these patients also had irritable bowel syndrome (particularly the gall stone group who may have had incidental gall stones discovered during their evaluation). Such misclassification could have diluted the discriminant value of symptoms between patients with irritable bowel syndrome and the disease controls.

Our second concern relates to the statistical analyses. The authors seem to have relied largely on univariate analyses (χ^2 tests). As 78 tests of association were undertaken (13 symptoms times six comparison groups!), however, several spurious significant results could have been obtained just by chance alone.[3 4] One way to adjust for the number of tests undertaken would be to multiply each p value by the number of comparisons. When evaluated in this way, only p values less than 0·0006 would be significant at the 5% level. While the authors did use a multiple logistic regression analysis, it appears that gender and socioeconomic status (which are potential confounders) were not included in the model. Moreover, it would have been of interest to determine whether the same symptoms could distinguish irritable bowel syndrome from each of the control groups using logistic regression analysis; instead all the organic disease patients were lumped together (a criticism that the authors justifiably levy at past studies). In addition, their analysis is likely to have seriously overestimated the discriminatory ability of the symptoms identified; it is well recognised that estimates based on a single data set are typically biased towards optimism[5] and for this reason prospective samples need to be tested to confirm the discriminant value of any symptom model developed.

Finally, the authors' contention that multiplication of the relative risks can be used to estimate the 'overall risk' depends rather heavily on having the 'correct' model – for example, no other unobserved confounding variables and no interactions among the symptoms used in the model – but this was not documented in the article.

Dr Maxton and colleagues have provided some intriguing hypotheses, but based on the data presented the diagnostic value of 'non-colonic' features for irritable bowel syndrome remain, in our opinion, unclear.

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Reply

SIR,—Thank you for giving us the opportunity to reply to the letter from Dr Prather and colleagues. We have previously shown that the prevalence of the non-colonic symptoms referred to in the paper are very significantly more common in patients with irritable bowel syndrome than in normal controls and therefore felt it appropriate to repeat this work.[1] Each symptom was carefully defined before the project began and the exact wording used decided before patient recruitment. A single interviewer conducted the entire study to minimise possible differences of interpretation. Irritable bowel syndrome is a common disorder and, as Dr Prather suggests, could easily coexist with other organic diseases. In the absence of a diagnostic test for irritable bowel syndrome, there is no easy way of preventing or excluding this possibility. As Dr Prather rightly points out, however, this would reduce rather than increase the discriminant value of the symptoms in separating organic from functional bowel disease thus making our findings more reliable. The gall stone patients included in the study all had symptoms suggestive of this disorder at first clinic attendance which improved after cholecystectomy. None had gall bladder disease as an incidental finding.

The statistical problem of multiple comparisons is well known and the use of repeated statistical testing (without proper adjustment) to establish definitive relationships is inappropriate. In our study, however, univariate analysis was only used in the preliminary investigation of discriminant power. The main part of the study identified symptoms with a significant association with irritable bowel syndrome using multiple logistic regression, a technique which makes appropriate adjustment for the large number of symptoms under consideration. Although the numbers of subjects in each of the separate disease groups were large by the standards of previous publications, they were insufficient to allow discrimination from each control group using multiple regression analysis. As reported in the paper the effect of gender had little effect on the discriminant ability of the 'non-colonic' symptoms of irritable bowel syndrome. Socioeconomic group also did not differ significantly between irritable bowel syndrome and organic disease groups. The description of relative risk estimation was included only as an illustration of how the risks can be combined. No claim is made for the accuracy of these combined estimates (the associated confidence intervals would be wide) but they give some indication of the order of magnitude of the relative risks. We agree it would certainly be of value to test our symptom model on other groups of patients. We are continuing to do so and hope others will follow.

The answer to the question posed by Dr Prather and colleagues in the title of their letter is, yes, non-colonic symptomatology is indeed of discriminant value in separating irritable bowel syndrome from a large number of organic gastrointestinal diseases.

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1 Whorwell PJ, McCallum M, Creed FH, Roberts CT. Non-colonic features of irritable bowel syndrome. *Gut* 1986; **27**: 37–40.

BOOK REVIEW

Inflammatory bowel disease – diagnosis and treatment. By G Gitnick. (Pp 541; illustrated; £91.) New York: Igaku Shoin, 1991.

This is a multichapter (37), multiauthor (58), multinational (though predominantly US) textbook on ulcerative colitis and Crohn's disease. The editor's aim was to produce an up to date and concise clinical overview of these inflammatory bowel diseases, their diagnosis, and treatment. It is laid out in seven sections – aetiology and epidemiology, clinical features, diagnosis, prognosis, medical and surgical management, and management problems.

The book is extraordinarily uneven. Contrast the first section, 40 pages on genetics and probably the best chapter in the book, with the five pages on aetiology or seven pages on inflammatory mediators. The sections on clinical features and diagnosis encompass six chapters, which between them duplicate or triplicate the routine assessment of the two conditions. Some excellent endoscopic pictures are mixed with out of focus histopathology, but histopathological appearances are presumably regarded as so recherché that the section on dysplasia is not illustrated. A number of full, worthwhile, and extensively referenced sections – for example, those on the use of corticosteroids or on the natural history of these diseases, based not on anecdote but on the placebo arms of published studies – contrast strongly with, for example, the Tennessee experience on T cell apheresis. This is an uncontrolled study on 63 patients (from the reference list, I cannot see that it has appeared in any peer reviewed form) 'in which the chances of . . . undergoing spontaneous remission was statistically zero'. Few people who have experience of inflammatory bowel disease will recognise such a group.

There are better books on inflammatory bowel diseases, from both the scientific and the clinical view point.

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