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Autoimmunity and chronic pancreatitis

EDITOR,—It was with considerable interest that I read the article by Jalleh *et al* (*Gut* 1993; 34: 1452-7) on histocompatibility antigen expression in human chronic pancreatitis. The authors assessed the morphology of the pancreas and class I (HLA) and II (HLA-DR) histocompatibility antigen expression in surgical specimens obtained from a large series of patients with chronic pancreatitis (93 patients), comparing these with 10 patients undergoing surgical resection for the presence of neuroendocrine tumours of the head of the pancreas and with four patients with chronic obstructive pancreatitis. The authors showed that, in the specimens obtained from patients suffering from chronic pancreatitis: (a) the disease was focal in distribution; (b) in the early stage of the disease neither protein plugging of major ducts nor calcification of minute ducts were identified; (c) in the later stage of the disease sections comprised only a few residual epithelial elements together with nerves and vascular structures in dense fibrous connective tissue, together with focal calcifications; (d) in the early stage of the

disease HLA class I expression by pancreatic exocrine epithelial cells was seen in 82% of chronic pancreatitis specimens, HLA class II in 66%, and both in 57%, whereas no major histocompatibility complex expression was identified in control specimens; (e) in the positive specimens expression was confined to ductal and ductular (inter and intralobular) epithelium with no staining of acinar cells; (f) T lymphocyte infiltration was significantly more prominent in chronic pancreatitis compared with control specimens. These data confirm those obtained in a study conducted by our team in Verona,¹ which showed increased HLA-DR expression in pancreatic specimens from patients with chronic pancreatitis, together with the presence of T lymphocyte infiltration foci, mainly surrounding the pancreatic ducts.

These reports seem to lend support to the hypothesis recently expounded by Cavallini² suggesting that primary (that is, non-obstructive) chronic pancreatitis is pathogenetically attributable to an obliterating primary inflammatory fibrosis of the main or secondary pancreatic ducts, or both. The fibrosis may be induced by active mediators released by T lymphocytes activated by aberrant expression of HLA. This phenomenon, which presents a patchy distribution, may be responsible for partial or total obstruction of the outflow of pancreatic juice, which in turn causes stasis facilitating the intraductal formation of protein plugs and the subsequent precipitation of calcium salts. Several experimental studies have, in fact, shown that partial obstruction of the pancreatic ducts alone is capable of causing the formation of intraductal stones in the dog^{3,4} and in the rat.⁵

Although the data of the study by Jalleh *et al* need to be viewed with caution, as the authors themselves recommend, as aberrant ductal HLA expression may be secondary to an inflammatory phenomenon, we feel we should stress the fact that it was found in the early stage of the disease. Aberrant HLA expression was detected, in fact, in specimens with a histological picture compatible with early disease abnormalities. This mechanism therefore would seem to constitute an early pathogenetic factor in development of the disease.

As we see it, the abnormal HLA expression may result from a genetic defect. According to its expressivity, this genetic defect may be responsible, in the case of greater penetrance, for the juvenile forms of the disease (previously classified as hereditary) or, if there is less penetrance, for the classic form of disease, which manifest themselves above the age of 30 (adult chronic pancreatitis). Exogenous factors epidemiologically associated with chronic pancreatitis may contribute to HLA expression in genetically predisposed subjects (alcohol), or even accelerate the formation of intraductal stones (alcohol, smoking, diet) and thus have an impact on the progression of the disease. One last finding that should perhaps be emphasised is the lack of protein plugs or intraductal calcifications in specimens compatible with early disease abnormalities. This finding, together with a number of clinical features, such as the low incidence of calcifications in chronic alcoholic pancreatitis in the early stages of the disease, and biochemical considerations, such as the lack of disease in alcohol abusers with lower concentrations of lithostatin and the presence of protein microaggregates also in normal subjects, raises serious doubts as to the soundness of the

pathogenetic hypothesis put forward by the Marseille school.⁶ It is possible, as already postulated,² that the lithostatin abnormalities may be no more than an epiphenomenon related to chronic stasis secondary to ductal obstruction, parallel to the reduced acinar exocrine production of all the other proteic-enzymatic substances.

We believe that further, more thorough studies of an immunological and immunohistochemical type need to be conducted to confirm the possible autoimmune pathogenesis of primary chronic pancreatitis.

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Reply

EDITOR,—Thank you for the opportunity of replying to the letter by Cavallini *et al* in response to our paper describing the enhanced expression of major histocompatibility complex determinants in chronic pancreatitis. We agree with the correspondents' suggestion that predisposition of certain subjects to chronic pancreatitis may be congenital in origin. We do not yet have sufficient information, however, to suggest that the abnormal HLA expression we have identified in early chronic pancreatitis may be due, in itself, to a genetic defect. An alternative possibility is that enhanced HLA expression may be an epiphenomenon of the chronic inflammatory process and that the prime aetiology may lie in a genetic defect affecting another, although possibly related, molecule. For example, any of the endogenous molecules participating in the intracytoplasmic processing of cellular peptides or proteins (such as the heat-shock proteins) might be affected and hence permit inappropriate targets of immune recognition to develop within pancreatic epithelial cells.

Recently obtained data shortly to be published from our laboratory have clearly shown the enhanced and differential expression of transforming growth factor β_1 in human chronic pancreatitis.¹ These findings support the suggestion of Cavallini *et al* that potent mediators of an evolving inflammatory process probably promote the profound fibrosis characteristic of chronic pancreatitis while they are not aetiological factors. In this respect, we believe that enhanced expression

of cytokines such as transforming growth factor β_1 is likely to be an epiphenomenon of the inflammatory process.

Finally, we support the view of Cavallini *et al* that while calcification might contribute to the pathogenesis of chronic pancreatitis in its later stages, there is no well documented evidence fulfilling Koch's postulates that it is probably a significant aetiological factor in the disease. Hence, we also cannot support the hypothesis of the Marseilles school.²

We consider that the disease known as 'chronic pancreatitis' is not a single pathological entity but rather a group of different aetiologies and pathogenetic processes sharing a few common morphological end points.³ Within this overall group, we anticipate that a predisposition based upon an identifiable genetic abnormality is the likely primary aetiological factor responsible for at least a proportion of cases of chronic pancreatitis. Whether this defect occurs within the pathway of alcohol metabolism or is responsible for promoting an inappropriate cell mediated cytotoxic response to some pancreatic cellular antigen is presently unknown. Nevertheless, answers to such questions are vital if biologically appropriate treatment regimens for different aetiologically and pathogenetically distinct types of 'chronic pancreatitis' are to be developed and affected patients treated more rationally than at present.

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Diagnosis of invasive amoebiasis: renaissance of the morphology era

EDITOR,—In his leading article entitled Diagnosis of invasive amoebiasis time to end the morphology era (*Gut* 1994; 35: 1018-21), Professor Ravdin rightly emphasises that microscopy cannot differentiate between pathogenic and non-pathogenic strains of *Entamoeba histolytica*. This applies equally to the cysts, and cultured trophozoites. Professor Ravdin goes on to suggest that microscopy should be abandoned in the diagnosis of invasive amoebiasis. He has underestimated the diagnostic value of one important point, first described by Losch in 1875.¹ In clinical specimens from patients with amoebic dysentery trophozoites of *E. histolytica* may be seen with ingested red cells. These erythrophagocytic trophozoites are a specific feature of infection with invasive strains of *E. histolytica* and may be seen on microscopy of fresh faecal specimens or in fixed smears stained with Field's stain. The current recommendations for the diagnosis of amoebiasis are microscopical

examination of fresh stools and stained fixed faecal smears, and amoebic cultures of stool specimens.²⁻³ A study⁴ of patients with dysentery, diarrhoea, and asymptomatic carriage of pathogenic and non-pathogenic *E. histolytica* confirms that microscopy is a highly efficient diagnostic method for amoebic dysentery. The sensitivity and specificity of erythrophagocytic trophozoites, were 96% and 100% respectively, when compared with amoebic culture and subsequent zymodeme typing of the isolated strains to confirm pathogenicity. We agree with Professor Ravdin that there is a need for diagnostic methods that do not depend on the detection of intact parasites. Tests that detect faecal or circulating amoebic antigen, or both, would be suitable for this purpose. To validate such assays they must be compared with a gold standard. With the current state of knowledge the standard should include the finding of erythrophagocytic trophozoites. We do not agree that currently available antigen detection tests have supplanted microscopy in the diagnosis of amoebiasis. Many things have changed since 1875 but the findings of erythrophagocytic amoebic trophozoites in stool specimens remains the simplest, cheapest, and most reliable test in the diagnosis of amoebic dysentery. Welcome to the morphology era!

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Reply

EDITOR,—González-Ruiz *et al* have shown that in expert hands the detection of haematophagous trophozoites has a high predictive value in diagnosing infection by pathogenic *Entamoeba histolytica* strains.¹ This study is very helpful and reaffirms the value of microscopy of faecal samples when it is readily available and highly skilled. Unfortunately, in many areas of the world complete laboratory facilities are not readily accessible. In addition, clinical laboratories in community settings may vastly over-diagnose amoebiasis and report leucocytes in stool as *E. histolytica*.² Lastly, stool culture for *E. histolytica* has repeatedly shown that microscopy has a low sensitivity.^{3,4}

Clearly, there is a consensus that better, quantitative methodology is needed that ideally can be brought directly to the field. I suggested that in the future microscopy will

not be needed as serological studies for anti-amoebic antibodies and studies of antigen and DNA detection will be sufficiently developed and field tested.⁵ The gold standard for comparison of these new methods is stool culture and zymodeme determination, not stool microscopy for erythrophagocytic trophozoites as suggested by González-Ruiz and Bendall. Simple agglutination tests, based upon current enzyme linked immunosorbent assay and hybridisation technology, will evaluate for infection by multiple enteric parasites. This will be the most cost effective, sensitive, and specific methodology available for diagnosis of infections by '*E. dispar*' and *E. histolytica*. Currently, we should use practical criteria as suggested by González-Ruiz and Bendall, but in the 21st century, let us advance beyond the technology of the 19th century.

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Relation of acupuncture and vagal gastric acid secretion

EDITOR,—We read with interest the paper of Lux *et al* (*Gut* 1994; 35: 1026-9), which examines the effects of various forms of acupuncture on sham feeding stimulated acid output in healthy volunteers. These results are entirely in accord with our own findings, previously published both in abstract form (the first of which was in this journal six years ago!)^{1,2} and in a peer reviewed journal,³ that acupuncture produces a significant decrease in sham feeding stimulated acid output under randomised, placebo controlled conditions in humans.

In those studies we also showed that the effects of acupuncture decreased sham feeding stimulated acid output was through naloxone sensitive opioid mechanisms, involving vagal efferent pathways. Furthermore, acupuncture produced neither a decrease in gastrin release nor a diminished parietal cell sensitivity to gastrin. While we agree with Lux *et al* that the mechanism through which acupuncture exerts its effect is not fully elucidated, it seems to be at least in part through opioid pathways, which may be similar to the mechanisms participating in the analgesic properties of acupuncture.

In their report, Lux *et al* cite another of our publications as concluding that acupuncture accelerates peptic ulcer healing.¹ This is incorrect; the cited study was conducted in healthy volunteers to examine the effects of acupuncture on sham feeding stimulated acid output. In our comprehensive review of all the