Intestinal intraepithelial lymphocytes and anti-transglutaminase in a screening algorithm for coeliac disease

We have noticed the frequent publication of important advances in the serological screening of coeliac disease (CD), such as the interesting and useful technique described by Baldas et al (Gut 2000;47:628-31). Humoral screening of CD is coming closer than ever towards representing an affordable population wide strategy (Gut 2000;47:628-31), largely due to the identification of tissue transglutaminase (tTG) as the main—if not only—autoantigen for antiendomysial antibodies (EMA). This finding highlights the possibility of antigen specific testing and, today, determination of anti-tTG is a valid alternative to EMA.

However, we believe that the recent advances in the cellular component of the diagnosis of CD have been somewhat over looked. The study of intestinal intraepithelial lymphocytes (IEL) by flow cytometry has added specificity to mere histological study of the small bowel biopsy. It has been shown that CD is characterised by an important increase in the TcR-γδ IEL subset (or γδ IEL), a decrease in the natural killer (NK)-like subset and, depending on gluten intake, a considerable increase in the TcR-αβ IEL (αβ IEL) subset which constitute the majority of IEL. The increase in γδ IEL (average 4% in controls v 25% in coeliacs, with respect to total IEL) is not per se diagnostic of CD as it has been observed, although to a lesser extent, in food allergy and occasionally in other conditions. But CD is the only entity in which γδ IEL have been described as systematically, permanently, and markedly raised. The combined study of total, γδ, and NK-like IELs, that could be termed “IEL lymphogram”, allows for nearly 94% specificity and sensitivity in the diagnosis of CD after clinical suspicion. This technique, complementary to the diagnosis of symptomatic and silent CD, shows its real value in latent and potential presentations of the disease, and offers important data for the differential diagnosis from other enteropathies. It is noteworthy that the increase in IEL is the earliest detectable alteration in the mucosa, prior to the increase in lamina propria lymphocytes or architectural changes.

Many recent reviews have commented on these characteristic serological and cellular findings of CD but their incorporation into clinical practice is very different. While tTG testing is spreading, IEL phenotyping—particularly by flow cytometry—is still regarded as a research tool rather than a diagnostic test. We consider that the easy procedure of IEL procurement and phenotyping could be routinely performed in many medium sized hospitals, and we propose an initial screening algorithm that takes this “IEL lymphogram” into account (fig 1).

Screening would be based on tTG IgA determination, and seric IgA quantification if anti-tTG was negative. If there was an IgA deficiency, only IgG tests would then be performed. If serum and blood were obtained at the first visit and temporarily cryopreserved, many tests (serum IgA, AGA, EMA, HLA, IgE, other autoantibodies, etc) could be performed without the patient attending the clinic again.

The establishment of the putative diagnosis would be achieved by mandatory small bowel biopsy. But the IEL lymphogram would allow for serological and clinical evaluation of gluten withdrawal (and challenge) if it fitted into the coeliac pattern and histology showed a typical coeliac enteropathy. If the lymphogram shows normal values for γδ and NK-like IEL, it has a high negative predictive value of 95% against the existence of CD. If the interpretation of the immunohistological study is not straightforward, the classical ESPGAN criteria can be followed. We believe that this algorithm, which can be conveniently adapted to the needs of each centre, can correctly classify the vast majority of patients, saving time and money, and avoiding morbidity.

Acknowledgements

Our work was financed by the Spanish Fondo de Investigaciones Sanitarias (FIS), grants Nos 00/0196 (G Roy) and 01/9417 (F León).

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Figure 1 Proposal of an initial diagnostic algorithm for coeliac disease (CD). After screening with anti-transglutaminase (tTG), and taking into account the high negative predictive value of tTG, study of mandatory intestinal biopsy would include phenotyping of intraepithelial lymphocytes (IEL). The proportion of “total IEL” is calculated with respect to the cellularity of the epithelium while the proportions of “γδ IEL” and “natural killer (NK)-like IEL” are relative to the total IEL. The combined analysis of the pathology and the “IEL lymphogram” allows for a correct classification of >95% of patients after the first biopsy, reducing the need for subsequent invasive procedures. N, normal values.
The changing scope of colorectal cancer

We read with great interest the commentary by Boland and Savides (Gut 2001;48:489–501) on our paper “Flexible sigmoidoscopy and the changing distribution of colorectal cancer: implications for screening” (Gut 2001;48:322–5). The authors make several important points about the changing pattern of distribution of colorectal cancer and the possible reasons for the changes we observed. Our data showed an increased percentage of colorectal cancers diagnosed proximal to the splenic flexure between 1976–78 and 1990–97. As Boland and Savides point out, this change may be linked to a true increase in the incidence of proximal cancers or to a reduction in the incidence of distal and rectal tumours owing to either the protective effect of non-steroidal anti-inflammatory drugs or endoscopic polypectomy.

A Paneth cell surrogate?

We read with interest the article by Cunliffe et al (Gut 2001;48:176–85) on defensin 5 stored in normal Paneth cells and in metaplastic Paneth cells in inflammatory bowel disease (IBD). In recent years a great deal of interest has centred around Paneth cells as carriers of innate host defence, effective through their content of antimicrobial peptides and proteins.1 In humans, that mechanism seems to be conveyed by a complex system of proteins present in the granules of the Paneth cells: lysozyme, secretory phospholipase A, and probably α defensins (that is, cryptidins, so far recorded in mice).

The lysozyme rich granules in Paneth cells appears to be one of the main sources of antimicrobial peptide in the normal small bowel (where Paneth cells are normally present). Although such cells are not found in the normal colorectal mucosa, Paneth cell metaplasia may be present in the colorectal mucosa of some (but not all) patients with longstanding IBD. Demonstration of human neutrophil defensins (HNP 1–3) and lysozyme in epithelial cells of active IBD has fuelled interest in the molecular events behind defense mediated intestinal host defence.

Against that background, it may be of interest to point out that another source of cytoplasmic lysozyme has recently been unveiled.3 Thirty five years ago, Azzopardi and Evans4 found mucin containing macrophages (denominated muciphages) in the colonic mucosa. Those cells were described as normal phagocytes in an otherwise normal mucosa. The mucoprotein present in their cytoplasm stained with a variety of mucous colorants (alcian blue, aldehyde fuchsin, and mucicarmin). Muciphages which were subsequently found to be associated with mucosal abnormalities induced by an inflammatory disruption of the crypts would officiate as scavengers to keep the lamina propria free from the liberated mucus. Until now, muciphages have been considered as a non-specific manifestation of mucosal damage.

We investigated the occurrence of those cells in rectal biopsies from patients with a variety of diseases, we found muciphages either scattered in the lamina propria or associated with the base of the crypts, next to the muscularis mucosae.

In recent years, immunohistochemistry has shown the presence of lysozyme mRNA. Muciphages which were subsequently found to be associated with mucosal abnormalities induced by an inflammatory disruption of the crypts would officiate as scavengers to keep the lamina propria free from the liberated mucus. Until now, muciphages have been considered as a non-specific manifestation of mucosal damage.

The presence of lysozyme in muciphages suggests that those particular macrophages are not an accidental happening but expression of a more targeted active biological mechanism of lysozyme dependent mucosal host defence.

In some patients with IBD in remission, the topographical disposition of those lysozyme containing cells—between the mucosa and the underlying host (fig 1)—is noteworthy. That arrangement insinuates the possibility of an organised biological barrier (a “defensive barrier”) against a factor(s) entering the host through the rectal mucosa.

The fact that muciphages also contain lysozyme may open new vistas for those previously unattended cells. It is conceivable

Table 1 Incidence of proximal, distal, and rectal colorectal cancers in the years indicated. All incidences were age standardised per 100 000 for each sex using the world standard population.

<table>
<thead>
<tr>
<th>Year</th>
<th>Proximal colo</th>
<th>Distal colo</th>
<th>Rectum</th>
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<td>Year</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>1976–78</td>
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<td>9.5</td>
<td>12.5</td>
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<td>1976–78</td>
<td>6.1</td>
<td>8.4</td>
<td>5.8</td>
</tr>
<tr>
<td>1995–97</td>
<td>7.2</td>
<td>9.2</td>
<td>6.2</td>
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that muciphages may be an important source of antimicrobial peptides in mucosa in protracted remission from earlier inflammatory episodes.

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References

CORRECTION

NOTICES
Agostino Trapani International Prize
The Scuola Medica Ospedaliera Napoletana invites applications for the above international prize. A stipend of €7,000 (seven thousand Euros), generously offered by the Professor Trapani family, is available to subsi-
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• Covering letter inclusive of formal applica-
tion
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ing telephone, fax, and email address.
• Letter of nomination of a sponsor of known reputation in the field of hepato pancreatic and biliary surgery.

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Falk Symposium No 128: Exogenous Factors in Colonic Carcinogenesis
This will be held on 2–3 May 2002 in Wurzburg, Germany. Further information: Falk Foundation c.V-congress Division, Lennovenstr. 5, PO BOX 6529, D-79041 Freiburg, Germany. Tel: +49 761 15 14 0; fax: +49 761 15 14 359; email: symposia@falkfoundation.de

Artificial Oxygen Carriers—A Clinical Future?
This conference will be held on 9 May 2002 in Edinburgh, UK. Further information: Rose-
mary Hector, Acting Consensus Conference Co-ordinator, Education and Standards De-
partment, Royal College of Physicians of Edinburgh, 9 Queen Street, Edinburgh EH2 1LQ. Tel: +44 (0)131 225 7324; fax: +44 (0)131 220 3939; email: r.hector@rcpe.ac.uk

12th International Workshop of Digestive Endoscopy, Ultrasonography, and Radiology
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Endoscopic Oncology: Gastrointestinal Endoscopy and Cancer Management
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11th International Symposium on Hepatic Encephalopathy and Nitrogen Metabolism
This meeting will be held on 30 May to 1 June 2002 in Amsterdamb, The Netherlands. Further information: Secretariat, Nicolaes Tulip Ini-
tute, Academic Medical Center, PO Box 23123, 1100 DS Amsterdam, The Netherlands. Tel: +31 20 566 8385; fax: +31 20 696 3228; email: tulipinst@amc.uva.nl.

Gastroenterology and Endotherapy European Workshop: XXth Anniversary
This course will be held on 17–19 June 2002 in Brussels, Belgium. Further information: Nancy Beauprez, Gastroenterology Depart-
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EASL Monothematic Conference on Vascular Function in Liver Disease
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com. Deadline for abstract submission 15 May 2002. Further information: kmnoore@ rfc.ucl.ac.uk; tel: +44 (0)20 743 2876.

5th International Workshop on Pathogenesis and Host Response in Helicobacter Infections
This will be held on 4–7 July 2002 in Helsingor, Denmark. Further information: Dr Tina Ken Hansen, Department of Cardiology-Endocrinology E, Frederiksberg Hospital, Ndr. Fasanvej, DK-2000 Frederiksberg, Denmark. Fax: +45 3545 7708; email: helpatim@ biobase.dk
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_Gut_ 2002 50: 741-742
doi: 10.1136/gut.50.5.741-a

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