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 Bal-das et al (Gut 2000;47:628–31). Humoral screening of CD is coming closer to 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 vs 25% in coeliacs, with respect to total IEL) is not per se diagnostic of CD as it has been observed, although to a lower 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.

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.

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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 HLA typing, 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;48:839–50) 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 the widespread use of flexible sigmoidoscopy and the consequent removal of premalignant adenomas.

We recently carried out further analysis of data from the Northern Ireland Colorectal Cancer Registry for the years 1995–97. The results of this analysis are shown in table 1 together with our previously published data for the years 1976–78. All incidences were age standardised per 100,000 for each sex using the world standard population.

These values show that the age-standardised incidence of colonic carcinoma has increased in both sexes over the period studied (proximal more than distal) while the incidence of rectal cancer has remained relatively constant. These data suggest that the changing pattern of distribution of colorectal cancer which we have observed is unlikely to be due to a decreased incidence of distal and rectal cancers. These results may well represent a true increase in proximal colorectal cancers, although as Boland and Savides suggest, they could also be explained by a rising incidence in all subsites, with relative sparing of the distal colon and rectum due 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 Cunliiffe 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, α defensins (HNP 1–3) and lysozyme in epithelial cells of active IBD1 has fuelled interest in the molecular events behind defensive 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.1 Thirty five years ago, Azzopardi and Evans2 found mucin containing macrophages (denominated muciphages) in the colorectal 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 mucicarmine). 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 mucin. Until now, muciphages have been considered as a non-specific manifestation of mucosal damage.

We investigating the occurrence of those cells in rectal biopsies from patients with a variety of diseases, we found muciphages either scattered in the lamina propria mucosa or distributed in a more “organised” fashion underneath the superficial epithelium and along the base of the crypts, near the muscularis mucosae.

Immunostain (CD68) confirmed the macrophagic nature of those cells and histochemistry showed the presence of lysozyme rich granules in muciphages along the muscularis mucosae. In addition, the cytoplasm of muciphages was strongly positive for lysozyme (Lysozyme-Muramidase stain; Dako A/S, Denmark) (fig 1).

At this stage it should be stressed that the mucin contained in the goblet cells of the crypts, in the mucus of mucous producing adenocarcinomas of the rectum, and in the mucus from a ruptured colon diverticulitis were lysozyme negative. Thus the cytoplasm of muciphages contains not only mucins but also the antimicrobial peptide lysozyme.

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 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 hinder (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 colon</th>
<th>Distal colon</th>
<th>Rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1976–78</td>
<td>4.7</td>
<td>6.1</td>
<td>12.5</td>
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<tr>
<td>1995–97</td>
<td>9.5</td>
<td>8.4</td>
<td>12.2</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1976–78</td>
<td>4.7</td>
<td>5.8</td>
<td>6.0</td>
</tr>
<tr>
<td>1995–97</td>
<td>8.2</td>
<td>6.2</td>
<td>6.6</td>
</tr>
</tbody>
</table>
that muciphages may be an important source of antimicrobial peptides in mucosae 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-
dise a young investigator submitting an experimental and/or clinical research project in the fields of hepatobiliary and pancreatic disorder. The prize, awarded by an inter-
national committee, will be personally presented to the winner during the congress “Progressi in Chirurgia Epato Bilio Pancre-
atica” which will be held in Napoli on June 20–22, 2002. Travel expenses will be refunded to the winner. Applications, in English, should be sent to the Organising Secretariat (G.P. Pubbliche Relazioni s.r.l., Via San Pasquale a Chiaia 55, 80121 Napoli. Tel: +39 081 403457/ 411450; fax: +39 081 404036; email: g.p.congress@napoli.com) by 20 May 2002 and should include:

- Curriculum vitae of the applicant
- Research project (max three typewritten pages) including a financial plan to use the stipend
- Covering letter inclusive of formal applica-
tion
- Address where an acknowledgement of the receipt of the application and any further correspondence should be mailed, includ-
ing telephone, fax, and email address.
- Letter of nomination of a sponsor of a known reputation in the field of hepato pancreatic and biliary surgery.

Broad Medical Research
Program—Inflammatory Bowel Disease Grants
Funds for inflammatory bowel disease (IBD) research are available immediately from the Broad Medical Research Program of The Eli and Edyie I. Broad Foundation for innovative projects regarding etiology, therapy, or preven-
tion. Grants totalling approximately US$100,000 per year are available for basic or clinical projects. Larger requests may be considered. Initial letter of interest (no sub-
mision deadline), simple application, rapid (60 day) peer review, and funding. Criteria for funding includes new ideas or directions, sci-
entific excellence, and originality. Early exploratory projects, scientists not currently working in IBD, and/or interdisciplinary ef-
forts are encouraged. Further information: Mariana Poland, Research Administrator, Broad Medical Research Program, 10900 Wilshire Blvd., 12th Floor, Los Angeles, CA 90024-6532, USA. Tel: +1 310 954 5091; email: info@broadmedical.org; website: www.broadmedical.org

Falk Symposium No 128:
Exogenous Factors in Colonic Carcinogenesis
This will be held on 2–3 May 2002 in Würzburg, Germany. Further information: Falk Foundation e.V.-Congress Division, Lisenweg 6, 5, PO Box 6529, D-79041 Freiburg, Germany. Tel: +49 761 15 14 0; fax: +49 761 15 14 359; email: symposfa@falkfoundation.de

Artificial Oxygen Carriers—A
Clinical Future?
This conference will be held on 9 May 2002 in Edinburgh, UK. Further information: Rosemary Hector, Acting Consensus Conference Co-ordinator, Education and Standards De-

cartment, Royal College of Physicians of Edinburgh, 9 Queen Street, Edinburgh EH2 2LQ, 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
This will be held on 30–31 May 2002 in Mar-
seille, France. Further information: Nathalie
Fontant, Atelier Phenix, 41 rue Docteur Morucci, 13006 Marseille, France. Tel: +33 04 91 37 50 83; fax: +33 04 91 57 15 28; email: nfontant@aphenix.com

Endoscopic Oncology:
Gastrointestinal Endoscopy and
Cancer Management
This ASGE Annual Postgraduate Course will be held on 22–23 May 2002 in San Francisco, USA. Further information: American Society for Gastrointestinal Endoscopy. Tel: +1 978 526 8330; fax: +1 978 526 7521; email: asge@shore.net

11th International Symposium
on Hepatic Encephalopathy and
Nitrogen Metabolism
This meeting will be held on 30 May to 1 June 2002 in Amsterdam, The Netherlands. Further information: Secretariat, Nicolaes Tulp Insti-
tute, Academic Medical Center, PO Box 23123, 1100 DS Amsterdam, The Netherlands. Tel: +31 20 656 8385; fax: +31 20 696 3226; email: tulpinst@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-
ment, Erasme Hospital, Route de Lennik 808, B-1070 Brussels, Belgium. Tel: +32 (0)20 555 49 00; fax: +32 (0)20 555 4901; email: Beauprez@ulb.ac.be

EASL Monothematic Conference
on Vascular Function in Liver Disease
This conference will take place on 30 June to 2 July 2002 in London, UK. Further information: Professor Jordi Bruix, EASL Liaison Bureau, c/o Kees Neren, 17 rue du Cendrier, PO Box 1726, CH-1211 Geneva, Switzerland. Tel: +41 22 908 0486; fax: +41 22 732 2850; email: info@easl.ch; www.easl-
com. Deadline for abstract submission 15 May 2002. Further information: kmoece@ rfc.ucl.ac.uk; tel: +44 (0)207 433 2876.

5th International Workshop on
Pathogenesis and Host Response
in Helicobacter Infections
This will be held on 4–7 July 2002 in Helsingør, 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