From hyperplasia to T cell lymphoma

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Disturbances in intraepithelial lymphocyte homeostasis in coeliac disease may lead to the emergence of lymphoid malignancies

Enteropathy-type intestinal T cell lymphomas (EITCL) are a recognised complication of coeliac disease (CD). A recent survey confirmed that non-Hodgkin lymphomas, although rare, are the main cause of mortality in CD. The mechanisms favouring the development of EITCL in CD patients but not in other chronic inflammatory bowel diseases remain elusive, but mounting evidence points to a profound disturbance in intraepithelial lymphocyte (IEL) homeostasis, leading to the emergence of lymphoid malignancies. A link between IELs and EITCL was first advocated in 1988 by Spencer et al who observed that most EITCL expressed the CD103 IEL marker. Two complementary observations suggested that EITCL derive from a reactive T cell population present in the intestine of CD patients: thus the same T cell clonal rearrangement was detected by Murray et al in EITCL and in the adjacent non-tumoral flat mucosa, and by Ashton-Key et al in non-lymphomatous ulcers of ulcerative jejunitis and in lymphomas, which later developed in these patients. The subsequent work in refractory sprue (RS) provided a missing link between IELs and lymphomas in CD.

RS is a coeliac-like enteropathy, primary or secondary resistant to a strict gluten free diet (GFD). Several conditions underlie villous atrophy resistant to GFD (Cellier et al, in preparation) but the majority of RS complicate CD and are associated with massive expansion of IELs with normal cytology but clonal T cell receptor γ (TCRγ) rearrangements and abnormal phenotype. The malignant nature of IELs in RS was demonstrated by the frequent association of RS with ulcerative jejunitis (30%), and the onset of EITCL sharing the same clonality and phenotype after several months or years in approximately 20% of cases. RS can thus be regarded as a “cryptic or low grade T cell lymphoma” derived from IELs, and draws a link between IEL hyperplasia, characteristic of CD, and EITCL. In some CD patients however, EITCL develop directly without this first intermediary step.

The report of seven new patients by Farstad and colleagues in this issue of Gut, four with RS without overt high grade lymphomas and three with EITCL associated with TCRγ clonal rearrangement (3/3) and phenotypically abnormal IELs (1/3) away from the tumour, concurs with previous work in RS and EITCL, and highlights two novel findings [see page 372]. One concerns the phenotype of IELs. Cellier et al initially reported that IELs in RS contained intracellular CD3ε, CD8, or TCRγ. Herein, in 2/4 cases of RS, IELs expressed a TCRβ chain. These results are in keeping with previous reports, and our unpublished results, showing TCRβ chain expression in 2/20 patients. Interestingly, flow cytometry in one patient studied herein as in our two cases, showed that TCRβ chains remain exclusively intracellular. Farstad et al also suggest that the lack of CD8 is not a constant feature of abnormal IELs in RS and EITCL. Using flow cytometry, we detected weak CD8 expression in a fraction of abnormal IELs in 30% of RS patients but only one and two of 20 cases were positive for CD8 by immunohistochemistry on fixed and frozen tissue sections, respectively (unpublished data).

Farstad et al in a recent report observed one case of CD8+ (CD56+)+ EITCL with CD8+ clonal IELs away from the tumour but concluded that clonality in EITCL and RS is generally associated with loss of CD8 and/or βF1 expression. Taken together, these data indicate that the phenotype of IELs in RS is very similar to that reported in EITCL, with the majority of cases being CD3+ βF1−CD8−CD4−, but with some cases being CD8+ or βF1+; rare cases are positive for both markers. These results emphasise the need to combine phenotype and molecular biology studies to investigate patients suspected of having RS or EITCL. They also support the idea that RS and EITCL share a common origin from IELs deprived of surface CD3-TCR complexes by a mechanism to be deciphered.

A second issue raised by Farstad and colleagues concerns the predictive value of CD30 expression. The outcome of RS is variable and not easily predictable. A small number of patients respond to therapy, mainly corticoids and parenteral nutrition, and can be maintained symptom free for years on a GFD in spite of persistent monoclonal IELs, as illustrated in patient Nos 1 and 2 described by Farstad et al. In contrast, some patients develop high grade lymphomas while in others abnormal IELs do not form tumours but disseminate to lamina propria, blood, and eventually to other organs. Furthermore, some patients who do not develop lymphomas, rapidly die from untractable malabsorption. In the two latter cases, classical chemotherapy is ineffective or even deleterious because the abnormal lymphocytes divide too slowly to be efficiently destroyed by drugs interfering with cell proliferation. Discovery of prognosis markers would thus be useful to adjust therapy.

Farstad et al suggest that CD30 allows the early detection of overt lymphoma. Firstly, they detected some CD30+ blast-like IELs and lamina propria cells away from the tumours in their three cases of EITCL. Secondly, in one patient with RS without overt lymphoma, the rapidly fatal outcome was associated with the presence of 25% CD30+ blast-like IELs. No CD30+ IELs were however observed in another RS patient with rapid severe outcome, whereas a third patient with some CD30+ IELs was improved by parenteral nutrition and a GFD. Finally, the patient with many CD30+ IELs did not benefit from one attempted cure of CHOP. These observations illustrate the difficulties in predicting outcome and in propounding an appropriate treatment in RS patients. Apart from CD30, other immunohistostaining markers may help to detect transformation from low to high grade proliferation, such as proliferation markers or p53 detected by Murray et al on small lymphocytes in the bowel adjacent to EITCL. Nevertheless, as in patient No 3 reported by Farstad et al, none of these markers may be useful in predicting untractable malabsorption in the absence of overt lymphoma. Functional analysis of abnormal IELs in RS may identify criteria predictive of their aggressiveness for the mucosa or new targets for therapy, a pressing need given the lack of current efficient treatment for severely sick RS patients and the poor prognosis of EITCL. Insight into the mechanism(s) disturbing IEL homeostasis in CD may help to decipher the links between inflammation and lymphoid malignancies and to design treatments able to prevent or cure these rare but most severe complications of CD.

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**Gut 2002;51:304–305**
Irritable bowel syndrome and the enteric nervous system

Infection and irritability

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A proportion of patients with irritable bowel syndrome report an apparent association between the onset of symptoms and a dysenteric illness

Chaudhary and Truelove were amongst the first to recognise that a proportion of patients with irritable bowel syndrome (IBS) report an apparent association between the onset of symptoms and a dysenteric illness. The concept of “post-dysenteric IBS” (PD-IBS) has now been widely accepted with claims suggesting that it accounts for anything up to 25% of the totality of symptoms following dysentery.

A retrospective analysis of the prevalence of gastrointestinal symptoms following microbiologically confirmed gastroenteritis suggested that there was no difference in the risk of IBS between bacterial species. However, it is noteworthy that up to half of the stool cultures obtained in some studies of PD-IBS may in fact be negative. This suggests that viruses or possibly other pathogens such as parasites may also be capable of provoking IBS in some individuals. There has been relatively little work addressing the possible role of viruses in IBS although it is tempting to speculate that such agents might be implicated in some cases, especially as symptoms of IBS are very common in chronic fatigue syndrome for which a viral aetiology has been proposed.

In addition to the role of different pathogens, it is possible that exposure to other factors which may be associated with the infection could be critical to the subsequent development of IBS. For instance, it has been shown that diarrhoea induced by polyethylene glycol can lead to rectal sensitisation, suggesting that diarrhoea per se is important. It is also well known that the use of antibiotics often results in diarrhoea, and there is evidence that patients suffering from dysentery treated with antibiotics are more likely to develop IBS compared with those who do not receive this form of therapy. Furthermore, this effect has been observed with antibiotics used for reasons other than dysentery. A further explanation for the deleterious effect of antibiotics in IBS might be alteration of the bacterial flora in the gut. If this is the case, one might predict that measures aimed at favourably modifying the gut flora might offer therapeutic promise, and reports of the beneficial effect of probiotics would seem to support this view.

Gastroenteritis can lead to disruption of the gastrointestinal mucosa resulting in excessive antigenic exposure and loss of the brush border. However, it is well known that the resultant disaccharidase deficiency is usually transient, and therefore unlikely to be contributory to the pathogenesis of PD-IBS. A similar mechanism may lead to bile salt malabsorption, and a group of patients with chronic gastrointestinal symptoms and documented bile salt malabsorption have been reported as relating the onset of their problems to an episode of gastroenteritis. Bile salt chelating therapy has been used in this particular situation with apparent success, although it is generally an unrewarding form of treatment for IBS in general. Food intolerance has also been reported as being important in some patients with IBS, but there is little evidence that an immune mediated process due to increased antigen exposure is involved. However, abnormal bacterial fermentation, a process capable of elaborating toxic short chain fatty acids, has been suggested as a possible cause of food intolerance, and may follow both gastroenteritis and antibiotic usage.

Psychosocial factors are known to be important in IBS. Several studies, including that of Neale and colleagues, which specifically relate to PD-IBS have shown that the risk of developing persistent symptoms following dysentery is related to the presence of psychopathology. Although gastroenteritis may lead to...
physiological changes that predispose to IBS, there is evidence that an adverse psychosocial milieu is necessary for the condition to fully develop. This is perhaps not surprising as it is now well recognised that stress can affect the immune, and hence the inflammatory response. Similarly, stress may increase intestinal permeability, an observation that may be particularly relevant as increased gut permeability has been demonstrated in some patients with PD-IBS.

It would seem reasonable to assume that whatever the triggering factor, an inherited predisposition for IBS might be necessary. This is suggested by the observation that IBS tends to cluster within families, although this could also be explained by environmental factors and indeed, similarities in health related behaviour have been observed between close relatives of those with IBS. Nevertheless, twin studies have shown an increased prevalence of IBS in mono compared with dizygotic twins, which might support a genetic background, but a study involving mono and dizygotic twins separated at birth would be required in order to reach a firm conclusion. Laboratory evidence also provides some support for the concept that inheritance is an important factor in the development of IBS. Studies on cytokines, which are known to be involved in the modulation of intestinal inflammation, have shown that mice lacking the interleukin 10 gene develop a spontaneous form of chronic enterocolitis, and that patients with ulcerative colitis are more likely to have genotypes associated with a lower production of interleukin 10. Similarly, a significantly reduced prevalence of the “high producer” gene for interleukin 10 has been reported in a group of unslected patients with IBS.

It is almost 40 years since Chaudhary and Truelove wrote their classic paper identifying the PD-IBS subgroup. We now know that female sex, younger age, prolonged duration of the initial illness, and psychological comorbidity appear to be important risk factors, and that sufferers usually have the diarrhoea predominant form of the condition. However, there is still much to learn, and emerging technologies will undoubtedly aid this process.

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crypts. Shih and colleagues, 13 have drawn attention to the same observations, 14–16 which can show k-ras and APC mutations, 17–20 grow by crypt fission 17,20 as do hyperplastic polyps. 21 But this concept does not exclude the possibility that the clone also expands by lateral migration and downward spread into adjacent crypts; this model of morphogenesis is conceptually quite different from that proposed by Shih and colleagues. 22 In this scenario, in sharp contrast, the mutated clone further expands, not by lateral migration but by crypt fission, where the crypt divides, usually symmetrically at the base, or by budding (fig 1). In several studies, fission of adenomatous crypts is regarded as the main mode of adenoma progression, certainly in FAP where such events are readily evaluated, 27–29 but also in sporadic adenomas. 19 In fact, the non-adenomatous mucosa in FAP, with only one APC mutation, shows a large increase in the incidence of crypts in fission. 24,25 Aberrant crypt foci, lesions which are putative precursors of adenomas, can show k-ras and APC mutations, 26 grow by crypt fission, 21,27 as do hyperplastic polyps. 21 But this concept does not exclude the possibility that the clone also expands by lateral migration and downward spread into adjacent crypts; this model of morphogenesis is conceptually quite different from that proposed by Shih and colleagues. 22 Finally, there are other reasons for finding crypts containing a mixture of mutant and wild-type cells—or APC−/− and APC+/− cells. Bjørkes and colleagues 26 found crypts harbouring cells staining both positively and negatively for APC protein in FAP although these were not spatially distinct, and were construed as crypts containing at least two stem cell lineages. Moreover, at the margins of FAP adenomas, serial section reconstruction has shown normal crypts in continuity with two or three adenomatous crypts, interpreted as adjacent normal crypts transforming into adenomatous crypts. Since crypts are clonal units, 21 this would explain the observation that some 75% of microadenomas in an FAP patient and in Min mice appear polyclonal. 26,27

The concept that the severity of the disease, or adenoma number, depends on initiation rather than progression, 28 brings these early events into sharp focus. The debate also extends into how clonal patches of dysplasia spread in the

Figure 1  [A] A monocryptal or unicryptal adenoma. [B] A three dimensional reconstruction of a unicryptal adenoma (inset) from serial sections, showing the adenoma in blue. Note that the adenomatous epithelium extends to the base of the crypt. [C] The mechanism of crypt fission in the normal colon whereby a crypt divides into two by this fission process. [D] A larger adenoma showing expansion by basal fission and budding. [E] Lateral migration at the margins of an adenoma, with adenomatous epithelium invading crypt territories (reproduced with permission from Shih and colleagues). 4 [F] “Top down models” of adenoma morphogenesis where either a single cell incurs APC inactivation, passes to the top of the crypt and proliferates, or transforms in situ at the top of the crypt. Both concepts lead to expansion of the clone in the intercrypt zone (from Shih and colleagues). 4 [G] How mutated clones expand in the colorectal epithelium by crypt fission.
colon in ulcerative colitis—"top down" by lateral migration or "bottom up" by crypt fission, or both? Which management structure prevails will have considerable implications for gut biology.

Gut 2002;51:306–308

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