From hyperplasia to T cell lymphoma

N Cerf-Bensussan, N Brousse, C Cellier

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).¹ A recent survey confirmed that non-Hodgkin lymphomas, although rare, are the main cause of mortality in CD.² 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.³ 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,⁴ and by Ashton-Key *et al* in non-lymphomatous ulcers of ulcerative jejunitis and in lymphomas, which later developed in these patients.⁵ Recent 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 GFD⁶ (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 (TCR γ) rearrangements and abnormal phenotype.⁷⁻⁸ The malignant nature of IELs in RS was demonstrated by the frequent association of RS with ulcerative jejunitis (30%), and the outset of EITCL sharing the same clonality and phenotype after several months or years in approximately 20% of cases.⁷⁻¹⁰ 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.⁶

The report of seven new patients by Farstad and colleagues¹¹ in this issue of

Gut, four with RS without overt high grade lymphomas and three with EITCL associated with TCR γ 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 CD3 ϵ but did not express surface CD3 ϵ , CD8, or TCR.⁸ Herein, in 2/4 cases of RS, IELs expressed a TCR β chain. These results are in keeping with previous reports,^{6,9} and our unpublished results, showing TCR β chain expression in 2/20 patients. Interestingly, flow cytometry in one patient studied herein as in our two cases, showed that TCR β 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.¹¹ 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, respectively⁷ (unpublished data). Daum *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 β F1 expression.⁶ 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+ β F1-CD8-CD4-, but with some cases being CD8+ or β F1+; rare cases are positive for both markers.^{4,6} 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 colleagues¹¹ 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.¹¹ 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.¹¹ These observations illustrate the difficulties in predicting outcome and in propounding an appropriate treatment in RS patients. Apart from CD30, other immunohistochemical 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.⁴ 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 patients⁷ and the poor prognosis of EITCL.¹ 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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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.¹ 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 the condition. However, careful questioning of these patients sometimes, but not always, suggests that they may have had a “forme fruste” of the disorder before their infection, raising the possibility that there may be two forms of the illness, one being an exacerbation of a pre-existing disorder and another where the condition appears to arise de novo. Ultimately, issues surrounding the natural history of PD-IBS will only be resolved by detailed prospective and retrospective studies, such as the one reported by Neal and colleagues² in this issue of *Gut* [see page 410].

Given that dysentery leads to irritable bowel symptoms, what mechanisms might be responsible? Do the dysenteric organisms cause the problem directly, and if so, are some more noxious than others? Alternatively, does the resulting diarrhoea lead to a form of non-specific sensitisation?

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.³ 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,⁴ 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.⁵ 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 Neal and colleagues,² 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.⁶ This is perhaps not surprising as it is now well recognised that stress can affect the immune, and hence the inflammatory response.⁷ 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.⁸

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,^{9,10} 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 unselected patients with IBS.¹¹

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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Colorectal cancer

Top down or bottom up? Competing management structures in the morphogenesis of colorectal neoplasms

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Modifier genes may influence the severity, or adenoma number, of familial adenomatous polyposis in humans through tumour initiation rather than progression

One of the earliest tumour suppressor genes to be identified was APC. Germline mutations in APC are found in familial adenomatous polyposis (FAP) and second hits lead to the development of often many hundreds of adenomas in the colon and rectum, some of which progress to cancer if

untreated. Many sporadic adenomas, and their ensuing carcinomas, show APC mutations, and FAP remains an important paradigm for the commoner sporadic form. Thus recent studies from the Tomlinson laboratory¹ show a very close linear relationship between the macroscopic—or naked eye—count of

adenomas in excised FAP colons and the count made microscopically from adenomas occupying one crypt (the *unicryptal* or *monocryptal* adenoma, fig 1) upwards. Such a close relationship strongly indicates that progression from microadenomas to macroscopic size is essentially random, that variation in disease severity (number of adenomas) results from differences in the number of microadenomas rather than disease progression, and importantly, that the selective advantages provided by different APC mutations act on tumour initiation rather than progression. A paper in this issue of *Gut*, also from the Tomlinson laboratory,² analyses the effects of putative modifier genes: the severity of the disease was related to the site of the mutation, as might be expected, but first degree relatives showed polyp counts which were more similar than more distant relatives [see page 420]. These observations indicate that modifier genes influence the severity of FAP, again through tumour initiation. Furthermore, the finding of a constant microadenoma density as the colon is traversed¹ suggests that initiation of FAP adenomas

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 copyright 2001 National Academy of Sciences, USA). (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 colleagues⁴). (G) How mutated clones expand in the colorectal epithelium by crypt fission.

are *spontaneous events* rather than environmentally produced, which of course has considerable potential implications for sporadic adenomas.

These observations underline the pivotal early events in colonic carcinogenesis: establishment of the mutant clone, its evolution to a microadenoma, and its development into a tumour recognisable by the naked eye. The molecular events associated with these stages are clear: in FAP a second hit in the APC gene is sufficient to give microadenoma development.³ But a further recent article from the Vogelstein laboratory⁴ has drawn on some earlier morphological studies to challenge contemporary concepts of how such mutant cells establish themselves and develop into an adenoma. Struck by the appearances in some early non-FAP adenomas (fig 1), dysplastic cells were seen only at the orifices and luminal surface of colonic crypts. Shih *et al* determined loss of heterozygosity (LOH) for APC, and nucleotide sequence analysis of the mutation cluster region of the APC gene was applied to microdissected well orientated histological sections of these adenomas. Not surprisingly perhaps, half the sample showed LOH in the upper portion of the crypts and most of these had a truncating APC mutation. Those cases without LOH showed a truncating mutation, again confined to the dysplastic epithelium at the crypt apex. Moreover, these cells showed intense proliferative activity, with nuclear localisation of β -catenin, supporting the presence of an

APC mutation in these apical dysplastic cells. Several earlier morphological studies have drawn attention to the same appearances,⁵⁻⁹ including those in FAP.⁹

This morphological and immunohistochemical profile was apparently virtually always present in nearly every crypt of each adenoma studied. Two models for adenoma morphogenesis were proposed (fig 1): in the first, mutant cells appear in the intracryptal zone between crypt orifices and, as the clone expands, cells migrate laterally and downwards to displace the normal epithelium of adjacent crypts. Alternatively, a mutant cell in the crypt base, classically the site of the stem cell compartment,¹⁰ migrates to the crypt apex where it expands as before (fig 1).

This "top down morphogenesis" has profound implications for concepts of stem cell biology in the gut. Most evidence indicates that crypt stem cells are found at the origin of the cell flux, near the crypt base.¹¹ Their repertoire includes all crypt cell lineages, metaplastic and reparative cell lineages, the genesis of new crypts and, as is widely believed, gastrointestinal tumours.¹² These proposals by Shih and colleagues⁴ either re-establish the stem cell compartment in the intracryptal zone or make the intracryptal zone a locus where stem cells, having acquired a second hit, clonally expand.

Where the concepts of Crabtree and colleagues^{1,2} and Shih and colleagues⁴ diverge is in the recognition of the earliest lesion, the unicryptal adenoma,

where the dysplastic epithelium occupies an entire single crypt.¹ These lesions are very common in FAP¹³ and while rare in non-FAP patients, have certainly been described.¹⁴ Here, a stem cell apparently acquires a second hit and expands—either stochastically or more probably because of a selective advantage to colonise the entire crypt. Such lesions are thus clonal.¹³ Similar crypt restricted expansion has been well documented in mice after ENU treatment,¹⁵ and also in humans heterozygous for the OAT (*O*-acetyl transferase) gene where, after LOH, initially half and then the whole crypt is colonised by the progeny of the mutant stem cell.¹⁶ Interestingly, OAT+/OAT- individuals with FAP show increased rates of stem cell mutation with clustering of mutated crypts.¹⁶

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,^{17,18} 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.¹⁷ Aberrant crypt foci, lesions which are putative precursors of adenomas, which can show *k-ras* and APC mutations,²⁰ grow by crypt fission^{21,22} as do hyperplastic polyps.²³ 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.⁴

Finally, there are other reasons for finding crypts containing a mixture of mutant and wild-type cells—or APC-/- and APC+/- cells. Bjerknes and colleagues²⁴ 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,¹⁷ interpreted as adjacent normal crypts transforming into adenomatous crypts. Since crypts are clonal units,¹³ this would explain the observation that some 75% of microadenomas in an FAP patient and in *Min* mice appear polyclonal.^{13,25}

The concept that the severity of the disease, or adenoma number, depends on initiation rather than progression,^{1,2} brings these early events into sharp focus. The debate also extends into how clonal patches of dysplasia spread in the

colon in ulcerative colitis²⁶—"top down" by lateral migration or "bottom up" by crypt fission, or both? Which management structure prevails will have considerable implications for gut biology.

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