Commentaries

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Anticipating the onset of inflammatory bowel disease

The great interest in identifying susceptibility genes in inflammatory bowel diseases (IBD) has recently triggered the aggregation of family trees throughout Europe and the United States. This has led to a better knowledge of empirical risk and clinical characteristics of familial IBD. One of the most consistent findings in families with Crohn's disease was early onset in most affected children of an affected parent, with an average difference of 15 years. This may be a more general feature of familial IBD, as shown in this issue by Lee *et al* (see page 808) who observed a similar time span (16–18 years) between generations in 50 pure Crohn's disease, 51 pure ulcerative colitis and 36 mixed disease families.

Genetic anticipation has been suggested as a possible explanation for this finding.[1] This term denotes a decrease in the age of onset and an increase in severity as a disease is passed through generations.[2] Genetic anticipation has been described in monogenic neurological illnesses such as Huntington's disease, myotonic dystrophy and more recently Friedreich's ataxia. In these diseases, there is firm evidence that anticipation reflects the effects of genetic factors and has a true molecular basis. Amplification of DNA triplet repeats within or adjacent to the disease gene occurs in successive generations and this DNA instability is associated with increasing disease severity and earlier age of onset.[3] The list of conditions exhibiting anticipation has grown in the past few years, and recognition of anticipation would be very important for a proper understanding of inherited susceptibility in IBD.

There are difficulties in defining biological anticipation in polygenic disorders such as IBD as the epidemiological data supporting genetic anticipation may be subject to many biases, particularly in retrospective studies.[4 5] Increased awareness and diagnostic acuity may influence age at diagnosis in the later generation. In Lee *et al*'s study and in a previous series[6] there was a shorter time interval between symptom onset and age at diagnosis in children than in parents although the difference only accounted for two years. This ascertainment bias was controlled by Heresbach and colleagues,[7] who compared the differences in age at diagnosis in second degree relatives and in parent and child. They found a similar inter-generation difference in the second degree relatives (16.5 years) and in first degree relatives (14.2 years) which was consistent with anticipation. Another complicating factor which is inherent in retrospective studies is the differential age at interview, which results in a greater chance of finding a later age at diagnosis in the older generation (recall bias).[5] A third potential bias is the preferential ascertainment of parents with late age at diagnosis. The median age at IBD diagnosis in the affected parents was higher in all published familial series (41 years in Lee *et al*'s study) than that observed in sporadic cases. It might well reflect a selection bias (also called truncation bias) with less recruitment of parents with early onset and reduced fertility.[4] This bias was displayed in two studies in which parent–child pairs were stratified according to age at diagnosis in the parents.[6 7] No anticipation persisted in the "early diagnosed" parent–child pairs but did in a Jewish subsample. Finally, the main confounder in these familial studies, which was tackled properly by Lee *et al*, is the inadequate follow up of the younger generation. Using regression analysis Lee *et al* showed an inverse correlation between age at diagnosis and calendar year of birth both for patients with familial and non-familial IBD. They thus showed that IBD occurring later in life in the younger generation was not taken into account: the children have not yet lived through their years of susceptibility. This ascertainment bias artificially lowered the mean age of onset of the younger generation. Additional evidence against genetic anticipation was provided by the observation that there was no difference between disease extent (a marker of disease severity) in parent–child pairs. This is consistent with the similarity in clinical characteristics of familial and sporadic IBD. In a recent study no difference was observed between 152 patients with a positive familial history of Crohn's disease and 1164 sporadic cases with regard to the importance of medical treatment and the incidence and extent of excisional surgery.[8]

In summary, assertion of genetic anticipation by mere observation of age at diagnosis and disease severity in complex disorders such as IBD is difficult.[9] Lee *et al*'s study was itself not without methodological pitfalls such as the use of more than one child per parent. This does not mean that genetic anticipation is definitely excluded. As seen in Huntington's disease, unstable DNA may be present even when the anticipation pattern is similar to regression to the mean, and cases of negative anticipation do occur.[10] Expansion of the (CAG)n repeat has been reported in Crohn's disease[11] and in keeping with genetic heterogeneity, subsets of patients (e.g. Jewish) could yet exhibit genetic anticipation.[7] In clinical practice, the only message available so far for affected parents is that the relative risk of IBD for their children is increased by a factor of 10 to 15 compared with the general population. Presently, we suggest genetic counselling should not be provided about age of onset and disease severity in IBD.

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Intolerance of the dirty intestine

The mucosal immune system faces the delicate task of co-existence with a luxuriant commensal intestinal bacterial flora (10^{12} bacteria/g faeces in the colon, and roughly 10^3 different species, with anaerobes predominating). Yet a protective immune response to invasive enteric pathogens is also mandatory. Of course, any commensal organism can become a pathogen in appropriate circumstances, and the magnitude of this balancing act is illustrated by the similarity between proteins of the harmless commensal *Escherichia coli* and its pathogenic derivatives (or the *Shigella* genus). The essential differences between innocent and harmful bacteria reside in toxin production and qualities of adherence to, or penetration of, the intestinal epithelial cell layer.

The barrier function of the intestine is clearly disrupted in active inflammatory bowel disease (IBD), with evidence for greater systemic penetration by commensal organisms. Whether abnormal intestinal permeability precedes intestinal inflammation in individuals predisposed to IBD, is still unclear, although it is a major feature of Crohn's disease relapse. The "passive" intestinal barrier (of the epithelial cell layer linked by junctional complexes and overlaid with mucus) is certainly not (in real life) as fixed as one might suppose.[1] We constantly face insults to barrier function, either through our own actions (ingestion of non-steroidal anti-inflammatory drugs, alcohol) or from intestinal pathogens. Epithelial cells are also actively involved in defence through chemokine and cytokine release. It is easy to see how a vicious cycle following penetration by luminal bacteria or their products might arise, ending in chronic IBD, yet most of us avoid it.

Over the past six years the study of genetically manipulated rodents has contributed enormously to the understanding of the circumstances which predispose to intestinal inflammation. Ablating the function of a large number of different immunological genes (including interleukin (IL) 2, IL-10 or αT cell receptor) or inserting HLA-B27 each independently renders the animal liable to develop spontaneous intestinal inflammation that may usually be attenuated or avoided by breeding and keeping the animals in very clean (SPF) or germ-free facilities. Although genetic loci linked to human IBD have been described, the hunt for the genes themselves continues, so many of the animal genetic abnormalities may be somewhat artificial. Nevertheless, they do provide support, in well defined conditions, for the concept that upsetting the delicate balance between the mucosal immune system, the epithelial cell layer and the commensal bacterial flora results in chronic intestinal inflammation.

Duchmann and coworkers, in this issue (see page 812), have examined the reactivity of T cell clones, derived from IBD intestinal mucosa, against commensal bacteria. It is clear that the mucosa of active Crohn's disease contains an increased proportion of activated T cells,[3] and T cell cloning has generally proved a powerful immunological technique as it provides a culture of T cells with a single receptor with specificity for short (9–15 amino acid) peptide epitopes. From such clones the major antigenic determinants for helper and cytotoxic T cells in viral and bacterial infections have been elucidated.

In general, working out the specific antigens to which CD4+ T cells in the intestinal mucosa respond is a formidable task, complicated by their very poor antigen specific proliferative responses in culture and the diversity of the luminal antigen mixture. Nevertheless, Duchmann and colleagues have previously presented data that T cells isolated from the intestinal mucosa of control subjects will proliferate in vitro in response to relatively crude fractions of bacteria isolated from the intestinal (heterologous) flora of a different individual, but not from their own flora. Patients with Crohn's disease, however, have intestinal T cells capable of responding to their own (autologous) flora. Thus Crohn's disease could be interpreted as a failure of mucosal tolerance to the indigenous flora, an idea in keeping with the data from the animal models and with the clinical effectiveness of faecal stream diversion. Despite the diversity of the human intestinal microflora, there is considerable homology between proteins of related species and common carriage of many species by different individuals, so the differences that bring about responses to the heterologous flora in normal subjects are still unclear.

An important technical issue surrounds the way in which the T cell clones were derived in this paper. It is difficult to get T cells from the intestinal mucosa to proliferate well in response to antigen, so to produce clones of identical cells the stimulation process had to be non-specific—phytohaemagglutinin (PHA) followed by expansion on irradiated allogenic feeder cells. Although the idea is to obtain representative clones, the responses to bacterial sonicates may not reflect the antigen specificities of the initial T cells. With this caveat, there are three main results. Firstly, there was considerable cross reactivity in the response of CD4+ clones to anaerobic (*Bifidobacterium* and *Bacteroides*) and aerobic enterobacteria. Secondly, the authors show that their previous observation whereby T cells from patients with IBD respond to crude preparations of the autologous flora, can be applied (in some cases) at the level of T cell clones. Thirdly, they analysed which bacterial species within a heterologous mixed isolate could stimulate a T cell clone from a patient with ulcerative colitis and showed that aerobic enterobacteria were mainly responsible and curiously some colonies of a bacterial species (e.g. *E coli*) might stimulate this clone whereas others would not.

So, on the one hand, there seems to be cross reactivity in the proliferative responses of T cell clones from patients with IBD between different bacterial species, and, on the other, the responses to the heterologous flora involve many common aerobic species. Where is the window in which "tolerance" to the autologous flora develops or collapses at the T cell level? A substantial amount of work will be needed to unravel this question. The beauty of T cell clones is that specificities to individual protein molecules (or other structural bacterial components) can be determined (if bacterial proteins are first purified), and this could sort out the molecular basis of cross reactivity and detect the window(s) in the autologous/heterologous flora in individual cases. Unfortunately each person is likely to be different because of the diversity of MHC class II in the