Grains of truth: evolutionary changes in small intestinal mucosa in response to environmental antigen challenge

Michael N Marsh

'T... the scientist too scared to speculate boldly can hardly be said to be having a creative life at all'

... *Memoir of a Thinking Radish*

Peter B Medawar

I had been struck by Booth’s chapter in the first series volume of *Recent advances in gastroenterology*, entitled *Physiopathology of intestinal malabsorption*,1 in which he focussed on the striking differences between ‘flat’ and ‘convoluted’ coeliac mucosae as had been recently revealed through the ‘dissecting’ microscope. Importantly, the question arose as to whether each type of surface structure could be inter-related and hence part of the same pathological process. What was needed was some other technique endowed with high resolving power to show that these stages were distinctive, yet progressive, phases in the natural evolution of the coeliac lesion. Fortunately, the necessary technique for tackling this problem came with the introduction of the first commercial scanning electron microscope by Cambridge (Scientific) Instruments.2 The rest was easy. Nevertheless, at the time, I had no premonition that within a few years I should be asked by the editors to contribute,3 in the second volume of *Recent advances*, some of the findings that had arisen from my research with Chris (Fig 1). Despite such successes, particularly at a time when the scanning electron microscope had hardly realised its full potential as an experimental research tool, the work failed to provide clues about pathogenetic mechanisms concerned in mucosal flattening.

In the early 70s, however, interest in the immunological behaviour of the intestine was increasing. More specifically, the demonstration by Anne Ferguson that the local tissue density of intraepithelial lymphocytes (IEL) is increased in untreated coeliac disease mucosae (note that in numerical terms the absolute population of IEL was subsequently shown to be reduced) was one of the first major studies suggestive of an immunological component in pathogenesis.4 Interest in the importance of IEL was encouraged by their close proximity to the intestinal lumen, indicative of a role in ‘immune surveillance’ at this strategic host-environment frontier. Around that time the presence of large ‘immunoblastoid’ lymphocytes within the murine surface epithelium was discovered and, under colchicine blockade it was shown that many IEL are actively mitotic. Thus it seemed reasonable to suppose that IEL might be reactive to antigens traversing the intestinal epithelium. It followed that if coeliac disease was also the result of local immunisation and reactivity towards gluten peptides, then similar changes within the IEL pool should be demonstrable within the target of immunologically sustained damage – the upper intestinal mucosa of untreated coeliac patients.

In a detailed study of untreated coeliac mucosa similar changes within surface IEL were observed – that is these lymphocytes were enlarged and showed increased mitotic activity. Removal of gluten from the diet caused these alterations to return to their respective control ranges, indicating that they are gluten driven phenomena.

Shortly after that work was published,5 it was arranged for me to work with Professor V I Mathan who also, like myself, had recently been a research fellow in Bob Donaldson’s unit at Boston City Hospital, Massachusetts. I was interested in extending the same type of quantitative analysis to jejunal biopsies from patients with endemic tropical sprue, and helped by a grant from the British Council, I arrived at the Christian Medical College Hospital (Fig 2) in Vellore, South India in January 1982.

The mucosal lesion in tropical sprue closely resembles that of coeliac disease with effacement of villi, somewhat cuboidal surface enterocyte profiles, and crypt hyperplasia. Analysis of lymphoid populations gave identical results as in coeliac mucosae – large mitotic IEL that were reduced (in absolute terms) in surface epithelium, but increased within the crypts.6 On returning to England, I decided to investigate lymphocytes within coeliac crypts, because up to that time, they had never been quantified: all attention had been directed to surface epithelium and to the unproven assumption that gluten was directly damaging to it. With the aid of an MRC funded computerised image analysis system, work began. In addition to measuring mucosae from treated and untreated coeliacs, four control groups were included comprising (i) healthy volunteers (ii) healthy coeliac relatives (iii) disease controls with normal mucosae and (iv)
disease controls with flat (non-gluten induced) mucosae. The results clearly showed that the crypts of the small intestinal mucosa in coeliac disease were not only enlarged but also contained an increased population of large lymphocytes, their distribution corresponding to the expression of DR surface alloantigens. Others had shown that the phenotype of crypt IEL is identical to surface epithelial IEL. Thus, from this evidence, it seemed difficult to ascribe surface enterocyte damage to infiltrating IEL when an apparently identical infiltrate into crypt epithelium failed to influence cell division or differentiation or the novel expression of D-locus gene products by crypt enterocytes.

**Gluten challenge**

It seemed desirable, at this stage in our work, to lay the foundations for future studies concerned in identifying disease activating regions of gliadin by carrying out a series of graded oral challenges with Frazer’s fraction III (FF3), in order to reveal the detailed sequence of changes involved in the evolution of a flat mucosa. To this end, small groups of well treated coeliac patients with near-normal mucosae were challenged orally with doses of 0·1, 0·5, 1·0, 1·5, 3, 6, or 12g FF3, and biopsied before, and at 12, 36, 60 and 84h postchallenge.

With doses of 0·1 through 1·5g FF3, a time dependent, dose related increase of small, non-mitotic lymphocytes into villous epithelium at 12h occurred, but without alterations in mucosal architecture. These results were therefore similar to the findings in the less severe lesion of DH, as well as in 25% of our series of first degree coeliac relatives. At higher challenge doses (3g FF3) infiltration of villous and crypt epithelium predictably occurred, but now mucosal architecture was altered by the appearance of crypt hypertrophy at 12 hours postchallenge, but without change in villous height. Villous flattening first occurred later in the challenge series, at 60–84 hours with 6g FF3, and at 12h with 12g FF3. In all cases, crypt hypertrophy preceded villous flattening.

With this approach in which incremental challenges were used and the effects observed over a five day period per dose, the dynamic spectrum of immunopathological changes accompanying the evolution of a flat mucosa could be precisely identified. This evolution was clearly phasic, the first stage involving lymphocytic infiltration of normal mucosal structure, followed by crypt hypertrophy, and thence, by a third stage where mucosal destruction became evident.

It should be clear that such progressive changes are inconsistent with the so called 'haemolytic' view of flattening, in which crypt hypertrophy is seen as a response, and thus a *post hoc* event secondary to loss of villous height. Rather...
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**Figure 3:** Three distinctive patterns in mucosal immunopathology occur at either end of the gluten-sensitive spectrum. The 'infiltrative' (type 1) lesion, comprising normal crypts and villi, reveals infiltration of villous epithelium only by small, non-mitotic lymphocytes. It occurs in some first degree relatives of coeliac disease patients, and after small dose gluten challenge of coeliac patients in remission. The 'hyperplastic' (type 2) lesion, comprising normal villi and hyperplastic crypts that are both infiltrated by small and essentially non-mitotic lymphocytes, is seen in some DH patients without enteropathy, and is inducible in treated coeliac patients by moderate dose gluten challenge. The 'destructive' (type 3) lesion shows villous effacement and markedly hypertrophied crypts containing a population of large, mitotic lymphocytes. This lesion is typical of untreated coeliac disease and of 30–40% DH patients with enteropathy, and can be reproduced by high dose gluten challenge of well-treated coeliac patients. In the tropics, the majority of individuals exposed to a heavy intestinal microbial population develop an asymptomatic type 1 delayed-type (cell-mediated) intestinal lesion. Among these are presumably some people who, in due time and because of genetic factors, are predisposed to develop the flatter type 3 lesion of tropical sprue, associated with severe malabsorption. In intestinal giardiasis, some individuals may be apparently unaffected while others develop a characteristic type 1 lesion: only rarely is the 'flat' type 3 lesion evident.

The initial infiltrative stage is analogous to that observed experimentally in mild graft-versus-host lesions. At the later stage, villous flattening is accompanied by a reduction in the absolute number of IEL, which become larger and more actively mitotic. These features are similar to those seen in untreated coeliac patients, and to those elicited by severe graft-versus-host lesions. A similar series of changes can be defined with a sufficiently large series of mucosae from untreated patients with dermatitis herpetiformis, when arranged in descending order of surface epithelial volume. Analogous changes also occur spontaneously in the evolution of a flat mucosa in coeliac patients.

**Conclusions**

It is now evident that distinctive mucosal patterns typify experimental cell-mediated (T lymphocyte) reactions in small intestinal mucosa—the type 1 ('infiltrative') lesion, type 2 ('hyperplastic') lesion and the type 3 ('destructive') lesion (Fig 3). Similar lesions have been identified in coeliac and dermatitis herpetiformis patients, in some first degree coeliac relatives, and can be induced progressively during oral gluten challenges. These latter studies indicate (like the experimental models) that the development of a type 3 lesion requires transition through the milder type 1 and 2 lesions in which crypt hyperplasia (and hypertrophy) is an early and prominent feature. Recent work indicates that crypt hypertrophy is a rapid response to activation of lamina propria T cells restricted by MHC class II gene products. Despite this latter restriction element, it is apparent that the degree of tissue damage seen is dependent on antigen dose—that is, extent of MHC disparity in GVH, or dietary gluten intake in coeliac/DH patients.

It seems certain that a similar pattern of intestinal injury occurs in endemic tropical sprue—that is, type 3 lesion. At the other extreme, individuals with so-called tropical enteropathy and whose villi are infiltrated by small lymphocytes manifest a milder infiltrative type 1 cell-mediated...
the future, we should re-evaluate our perception of intestinal pathology in such terms, rather than by the continued use of subjective degrees of 'villous atrophy'. Such latter terminology no longer serves a useful purpose and moreover, obscures recognition of the fundamental changes occurring within small bowel mucosal in these conditions.

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