Immunocytes, Enterocytes and the Lamina Propria: an Immunopathological Framework of Coeliac Disease

M. N. MARSH, DM, FRCP

Senior Lecturer in Medicine, University of Manchester, and Consultant Physician, Salford District Health Authority

Almost a century has passed since Samuel Gee described the clinical features of a coeliac child[1]. Were he alive today he would doubtless be amazed by the advances of recent years, but perhaps no less perplexed than many a contemporary by the exquisite biochemical and immunological twists and turns that make study of coeliac disease such a fascinating challenge.

The Dutch paediatrician Dicke[2] first realised the aetiological importance of wheat flour and its constituent protein, gluten, in causing gross damage to the upper intestinal mucosa. Descriptions of this lesion were made possible by the introduction of the peroral biopsy technique, and centred on the abnormally small, irregular and disorganised epithelial cells and their damaged organelles, i.e. distorted microvilli, swollen mitochondria and endoplasmic reticulum, and prominent lysosomes. At the time, such appearances seemed entirely consistent with one of Frazer's views[3] that failure of complete digestion of gluten yields an oligopeptide that damages the enterocyte.

Nowadays, there is hardly any support for this view[4] and the quest for the elusive 'peptidase' remains unfulfilled. It is difficult to reconcile these biochemical premises with the fact that the entire depth of the mucous membrane is abnormal. Another objection, notwithstanding the more recent observations that gluten does not damage the unprimed enterocyte in organ culture[5,6], is that enterocytes of treated mucosa reveal no persisting abnormality that identifies them as the faulty party[7,8]. Conversely, the 'tolerance' of some treated patients to further prolonged gluten exposure conflicts with the idea of an inborn metabolic error within the enterocyte.

Other coeliac patients develop splenic atrophy, carcinomas and multi-focal histiocytic tumours of the intestinal tract, unusual complications that are more compatible with a chronic immunological disorder[9].

Historically, 1970 was the year when immunological views of pathogenesis began to assume prominence. This followed the realisation expressed in Booth's now classic description of the coeliac enterocyte[10], that a digestive view of gluten-sensitivity, as hinted at by Frazer, was no longer useful as a working hypothesis.

If coeliac disease results from local immunological reactions to gluten, the cytological appearances of the target tissue, the jejunal mucosa, should contain the most important clues about such mechanisms.

Gluten and the Humoral Response

The humoral response to gluten is T lymphocyte dependent, and genetically regulated[11,12]. Although many normal individuals are 'immunised' to gluten (and other dietary proteins) both locally and systemically, these antibodies clearly do not cause coeliac disease. The higher circulating antibody titres in coeliac patients compared with those in normal individuals are probably a reflection of genetic background; similar parallels exist, for example, in chronic liver disease, diabetes mellitus, myasthenia gravis and external allergic alveolitis[4].

In the mucosa there is a marked rise in the number of IgA and IgM plasma cells (Fig. 1), which is probably a non-specific, polyclonal expansion due to long-standing mucosal inflammation. Anti-gluten IgM and IgA antibody are synthesised within the mucosa[13] but the relevance of local IgG-anti-gluten antibodies[14] is uncertain. Since the number of IgA and IgM cells falls after gluten restriction, although the mucosa may still remain flat[15], local antibody formation cannot be a key factor in the pathogenesis of the coeliac lesion. Moreover, the recent description of coeliac disease in a patient with profound immunodeficiency[16] also makes this unlikely. It is more probable that the basis of coeliac disease is dependent on cellular immunity.

Cell-Mediated Immune Reactions in the Small Intestine

Few experimental studies of cell-mediated immunity (CMI) within the gastrointestinal tract have been made; not surprisingly, our knowledge of local CMI to gluten is uncertain. The observation that 'flattening' of the mucosa in certain immunologically-driven models, such as worm infestation[17] and allograft rejections [18,19], is T lymphocyte-dependent provides some insight into the mechanism of mucosal flattening in coeliac disease.

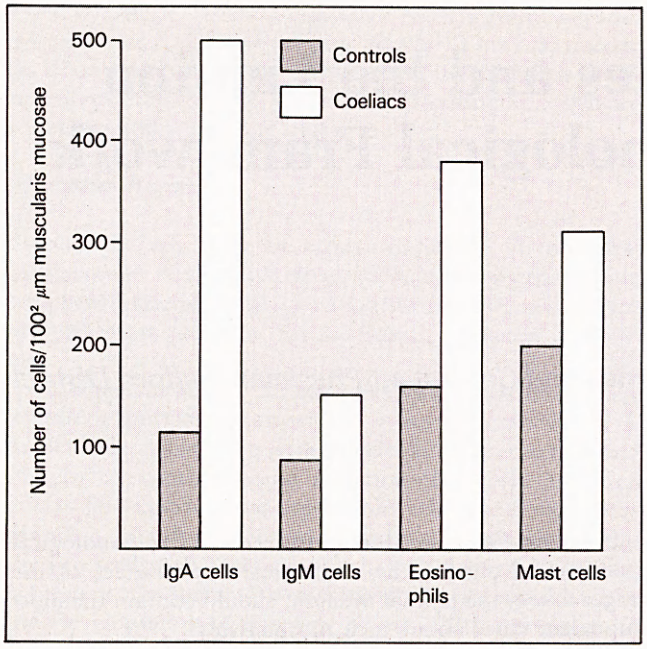


Fig. 1. *Comparative quantitative data for numbers of lamina propria cells within control and untreated coeliac mucosae.*

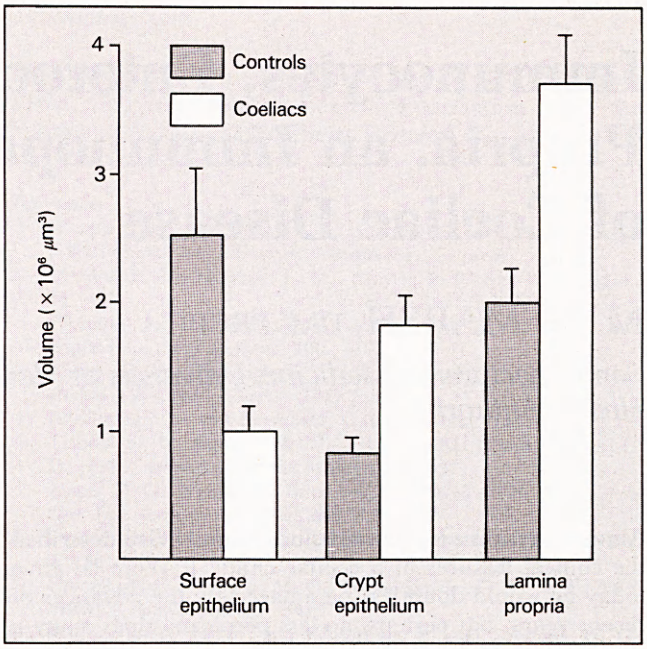


Fig. 2. *This diagram shows group mean volumes ($\times 10^6$ cubic microns) for surface epithelium, crypt epithelium, and lamina propria, all measurements being related to $100^2\mu m$ test area of muscularis mucosae. Note that volume of lamina propria is almost doubled in coeliac disease.*

Some years ago it was reported that supernates from cultured fragments of coeliac mucosa contain factor(s) inhibitory to macrophage migration (MIF)[20]. However, it is now known that such inhibitory factors are elaborated not only by sensitised T lymphocytes, but by B lymphocytes and other non-immunological cell types. Neither this, nor subsequent[21] work allows us to infer the presence of activated mucosal T lymphocytes.

For years it has been generally held that lymphocytic infiltration of the epithelium is a major hallmark of the established coeliac lesion. I believe this view may not be entirely valid, so it cannot be assumed that the infiltration is a specific reflection of local lymphocyte-mediated reactivity to gluten. The difficulty stems from methods[22,23] which have been widely, if somewhat uncritically, used to count epithelial lymphocytes.

The intestinal mucosa is a dynamic, three-dimensional structure, a fact often forgotten when its cytological aspects are viewed in the two-dimensional framework of a tissue section. The problem is to quantitate abnormal coeliac mucosae both before and during gluten restriction, and make valid comparisons with normal mucosae.

Our quantitative method, based on 1μm epon sections viewed with a $\times 100$ oil-immersion objective, refers all measurements to a constant test area ($100^2\mu m$) of muscularis mucosae. This permits determination of volumes (V) of villous (or surface) epithelium, crypt epithelium and lamina propria overlying the test area (Fig. 2) and the number of cell types (N) within their respective tissue compartments (Fig. 1). In this way, 'absolute' data are obtained which allow direct comparisons to be made between all types of mucosae, irrespective of their surface morphology. Quantitative observations of this kind make redundant the continued use of irrelevant and inappropriate images of mucosal abnormality, such as 'villous atrophy'. Moreover, we are able to imagine these volumes as cubes, which helps to distinguish between the number (N) of cells within any tissue compartment (volume V), and their resultant density (D). Density is dependent on N and V through the relationship D = N/V; thus, increases in D may not tell us anything about N.

Using this type of analysis, we showed conclusively[24] that the number of epithelial lymphocytes within coeliac mucosae is not raised (i.e., N remains constant) thus confirming results of an earlier independent study by Whitehead's group[25]. It can be seen, therefore, that the density (D) of these lymphocytes rises simply because there are fewer enterocytes comprising the surface epithelium (i.e., V is reduced). Clearly, counting epithelial lymphocytes per 100 enterocytes, or unit length of basement membrane, does not tell us precisely what we really want to know, which is whether the absolute pool of lymphocytes is increased or not. If we insist on counting epithelial lymphocytes in this fashion, we must ensure that there are no changes in villous height (epithelial volume), otherwise the results and conclusions drawn from them may be invalid.

However, in our recent challenge work in which very small doses of a peptic-tryptic digest of gluten (100-1,500 mg) were administered orally, we have demonstrated a dose-dependent rise in the total number of epithelial lymphocytes (N) within coeliac mucosa between 12 and 48 hours[26]. This is the first occasion in which a graded response of epithelial lymphocytes to gluten has been described, and we are continuing to analyse this gluten-responsive phenomenon in more detail.

Other evidence of epithelial lymphocyte 'activation' in

coeliac disease, and its gluten-dependency, rests on the demonstration of a 5-6 per cent increase in immunoblasts within epithelium (Fig. 3)[27] and the fact that the rate of

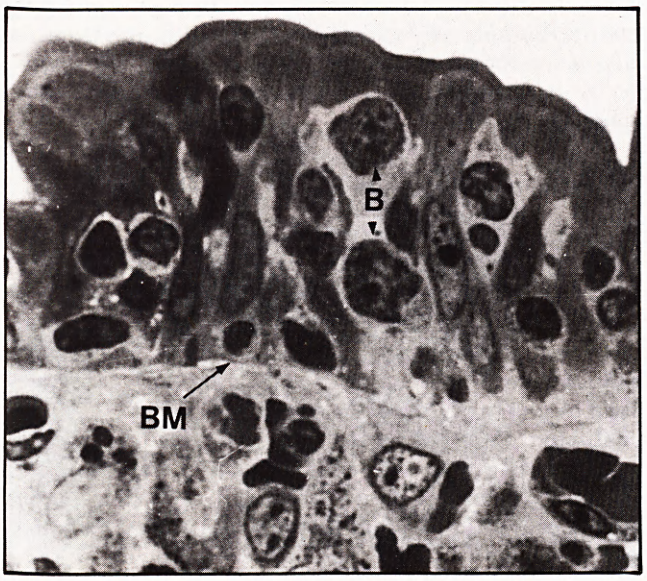


Fig. 3. *A 1μm epon section of untreated coeliac mucosa, showing marked heterogeneity of epithelial lymphocytes. B = immunoblasts. BM = basement membrane.*

mitotic proliferation of these lymphocytes is elevated in comparison with disease-control mucosae [27,28]. These aspects of lymphocyte behaviour in untreated coeliac mucosa, first shown by high-resolution light microscopy on 1μm epon sections, had been completely overlooked in all previous histological and electron microscopic studies of the coeliac lesion.

In addition to the blastogenic and mitotic responses of epithelial lymphocytes, we also showed that the turnover of lymphocytes across the epithelial basement membrane was significantly raised in coeliac disease compared with controls[27]; this effect was reversed by gluten restriction. The increased number of perforations observed in basement membrane by scanning electron microscopy[29] is the morphologic correlate of the increased traffic of lymphocytes into and away from the epithelium.

These observations indicate that the behaviour of epithelial lymphocytes in coeliac disease is markedly different from control mucosae in terms of their (a) dose-dependent accumulation within the epithelium, (b) degree of blast-transformation, (c) rate of mitotic activity, and (d) turnover across the basement membrane. These precise morphometric data clearly say much more about what these lymphocytes are doing than mere counts of their relative numbers within the epithelium.

There is substantial evidence of marked changes in lymphocyte physiology within the target tissue of the upper intestine, and of these changes being gluten-driven. Taken together with the observation that mucosal flattening is also T-lymphocyte dependent, there is persuasive support for the view that the coeliac lesion must result from a local cell-mediated reaction to gluten.

Lymphocyte Mitotic Index as a Prospective Marker of Coeliac Disease

The gluten-dependent mitotic activity of epithelial lymphocytes (Fig. 4) is an intriguing aspect of their disturbed

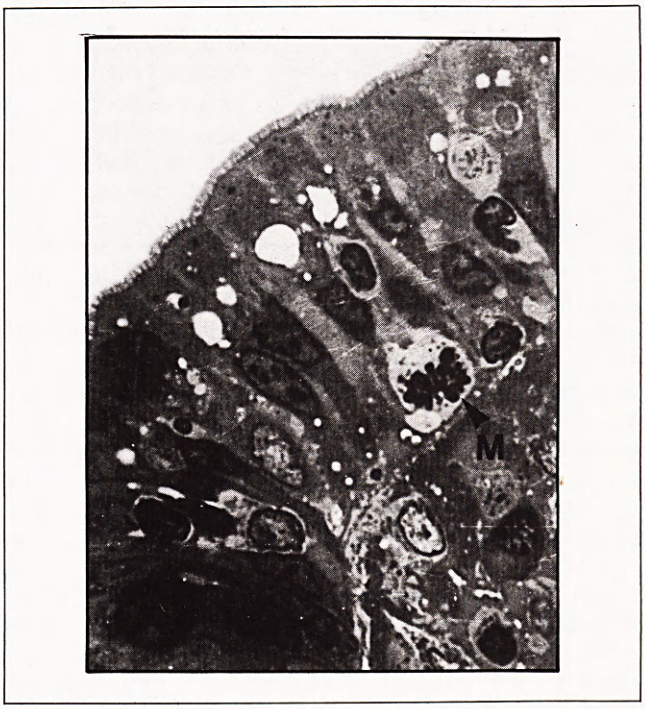


Fig. 4. *This 1μm section shows a mitotic epithelial lymphocyte (M).*

pattern of behaviour, and one studied in a wide range of small intestinal biopsies performed on approximately 200 adult and child patients.

A basal mitotic index (MI) (per cent mitotic figures/3,000 epithelial lymphocytes per specimen) exceeding 0.2 per cent prospectively identifies flat coeliac mucosa, while any flat mucosa with an MI of less than 0.2 per cent can be confidently deemed, from the outset, not to be due to gluten-sensitivity. Using this simple, direct approach, many patients with conditions that mimic coeliac disease (Crohn's jejunitis; lymphoma; immunodeficiencies; cystic fibrosis; cow's milk protein intolerance) are spared an unnecessary trial of gluten-free diet, and hence additional biopsies of jejunal mucosa[28,30,31].

Another observation was that several late or poor responders had a high mitotic index on their initial biopsy: in these circumstances, and especially in older patients in whose mucosa villous regeneration may take several years to occur, a high mitotic index strengthens the need to persist with a gluten-free diet. Conversely, in those patients whose response is prompt, or in whom a rapid growth spurt occurs, additional random follow-up biopsies may be entirely obviated. Finally, the criteria for diagnosis of childhood coeliac disease, as proposed by the European Association of Paediatric Gastroenterologists[32], are made redundant by this prospective index.

Over the years various screening tests for coeliac

disease have been proposed[4,29] but the mitotic index appears to correlate exclusively with gluten-sensitivity and thus identifies those patients for whom gluten restriction is rational treatment. Here we see a means of evaluating coeliac disease in terms of a consistent 'immunologic' abnormality rather than in non-specific terms such as mucosal flattening, which has been described in nearly 30 other clinical and experimental settings[4].

The proliferation of epithelial lymphocytes in coeliac mucosa is seen to be linked directly to the elaboration of mitogenic lymphokines by antigen(gluten)-stimulated lymphocytes. Further evidence for this view is provided by study of jejunal biopsies obtained from an immunodeficient, gluten-sensitive patient[28,30]. Mitotic indices in the initial flat biopsy (0.43 per cent) fell after a period of gluten restriction (0.1 per cent) and rose again (0.67 per cent) after a further period on a normal diet. Clearly this man's classic coeliac disease is most unlikely to be due to humoral or complement-dependent mechanisms[30]; his lymphocytes behave like those of other coeliac patients, and not like those of patients with immunodeficiency and severe mucosal lesions[24] in which gluten-sensitivity has no aetiological role.

Some Comments on Gluten Challenge

It has become customary to use gluten challenge for diagnostic purposes and as a means of elucidating pathological mechanisms of mucosal damage.

There are drawbacks to the diagnostic use of gluten challenge apart from the discomfort imposed by repeated biopsies, particularly in very young children. The incisiveness of the test is blunted in many patients[33,34] whose mucosa deteriorates only after long periods of gluten feeding, so the optimal timing of the post-challenge biopsy is never predictable. Wider use of the mitotic index may lead to the abandonment of this investigation.

No substantive advances in our understanding of the immunopathological basis of coeliac disease appear to have come from the use of 'megaton' challenges of gluten (20-40g). In addition to descriptions of non-specific changes (cellular disruption; reduced brush border enzyme activity) or frank mucosal destruction, there have been several unconvincing reports of increased 'counts' of epithelial lymphocytes that reflect reductions in villous height.

Others[35,36] have claimed that so-called Type III (antigen-antibody-complement) reactions underlie the tissue destruction, since mucosal abnormalities become maximal 4-12 hours after challenge. To base such conclusions on time alone is not entirely acceptable since, with good microscopic technique, early changes beginning at 2-4 hours are seen to initiate classic cell-mediated 'delayed-type' responses to intracutaneous tuberculin[37]. Furthermore, if it were supposed that Arthus-type reactivity is responsible for the coeliac lesion, the model would have to be a tissue, and not a vascular, example of the phenomenon; the tempo of the former is slower than that of its vascular counterpart[38]. Hence, studies based on an assumed reaction time starting about 4 hours after challenge are unimpressive[36]. Moreover, it is strange that in these reports[35,36] anti-gluten antibody was not shown to be present within the mucosa when challenge occurred. The failure to confirm the presence of a factor so necessary to the formation of complexes substantially invalidates the basis of these claims. Finally, as in other genuine examples of Arthus reactions[38-40], the electron microscope should reveal deposition of complexes within the lamina propria; such evidence has never been obtained[41].

The Inflammatory Component of the Coeliac Lesion

Our approach to gluten challenge has been radically different from other workers in that we have used very small doses of Frazer's fraction III[26], thus producing only minimal tissue injury. This approach has permitted the demonstration of new and relevant structural abnormalities hitherto obscured in studies in which immense doses of gluten were employed[35,36]. On the basis of these latter observations, it is proposed that the coeliac lesion is largely due to an intense inflammatory reaction that is presumed to be triggered by T lymphocyte-gluten interactions within the lamina propria. Indeed, the presence of numerous DR + macrophages (or dendritic presenting cells) and T4 + helper cells within the lamina propria provides the cellular basis for such events, and hence for the subsequent lymphokine-induced inflammatory and cellular changes that characterise the coeliac lesion.

Intra-epithelial Blebs

Our 'mini-challenges' have led to the formation of multiple intra-epithelial blebs, in addition to marked oedema of the lamina propria (Fig. 5). The size of these blebs varied considerably: in some the electron microscope showed destruction of the underlying basement membrane; up to 10 per cent of the basement membrane (per $100^2\mu$m muscularis mucosae) could be denuded of epithe-

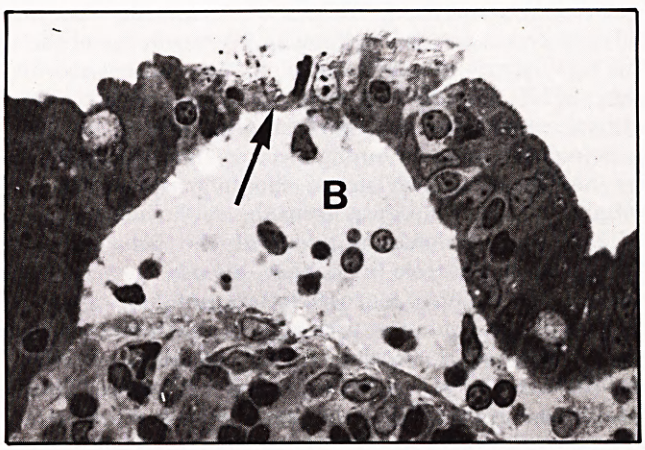


Fig. 5. *Post-challenge coeliac biopsy. The large intra-epithelial bleb (B) contains numerous cells; there is no detectable basement membrane. The epithelial 'dome' shows focal necrosis (arrow).*

lium. Control specimens showed no blebs, thus excluding effects of fixative tonicity, general autolytic or traumatic damage, or suction artefact imposed by the biopsy procedure. Large expanses of epithelium containing numerous lymphocytes assumed mushroom-like excrescences within the epithelium (Fig. 6); in others, central necrosis of the raised epithelial dome was apparent (Fig. 5).

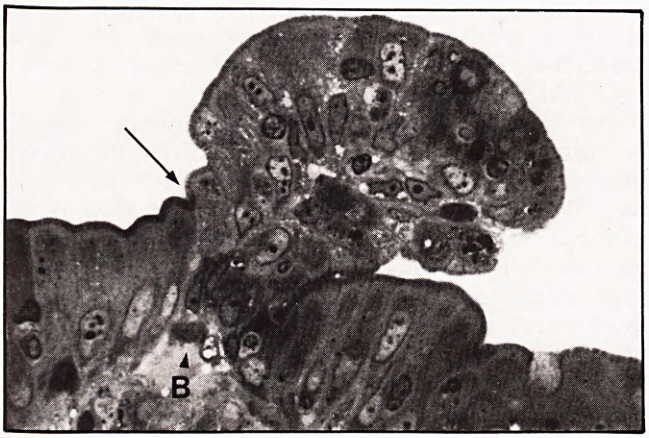


Fig. 6. *This micrograph shows focal extrusion of a large clump of effete enterocytes, overlying residual bleb (B). Note different staining characteristics of extruded cells compared with those still attached to basement membrane (arrow).*

It thus seems that the mechanism of increased cell loss and destruction involves production of intra- and sub-epithelial oedema, loosening of the attachment of enterocytes to their substratum and, in more intense reactions, destruction of the basement membrane and extensive ballooning of epithelium (Fig. 6). Lysis of basement membrane is known to accompany other inflammatory reactions and is due to release of proteolytic and lysosomal enzymes from various activated cells, e.g. neutrophils, macrophages or mast cells[42].

Fibrin(ogen) Deposition

Extravascular deposition of fibrin(ogen) is extensive throughout the lamina propria (Fig. 7), and, where the basement membrane has been lysed, within intra-epithelial blebs.

From this it must be concluded that there is a marked increase in vascular permeability, which allows extravasation of fibrinogen, and probable activation of the clotting system, which leads to fibrin formation: fibrin itself encourages tissue oedema and fluid retention. There are reasons for supposing that the coagulation system is locally involved. First, we must account for the extensive sub-epithelial deposition of fibrillary material that occurs within hours of a massive gluten challenge[41]. This material is not collagen; the only rational explanation is that these fibres are recently deposited fibrin. Second, fibrin forms within the evolving tuberculin skin reaction, causing its characteristic induration, a proven example of fibrin deposition occurring in a pure T lymphocyte-mediated, delayed-type reaction[37].

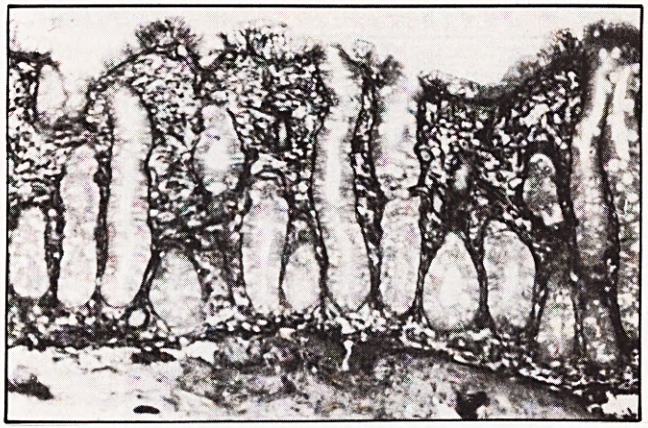


Fig. 7. *Peroxidase-labelled anti-fibrinogen monoclonal antibody reveals extensive extravascular deposition throughout lamina propria; epithelium is largely spared.*

Microvascular Damage

The extravasation of plasma leads to capillary sludging and compaction of erythrocytes which not only results in local thrombosis and platelet aggregation (Fig. 8) but also enhances tissue oedema and cellular destruction. Indeed, the rapid transition of a villus-bearing mucosa to a 'flat' lesion within a few hours of a massive gluten challenge[36] can only be due to tissue necrosis following small vessel occlusion, and is a further reflection of the intensity of the inflammatory reaction.

Vascular stasis leads to tissue anoxia[43], and anoxia after experimental arterial occlusion potentiates intra-epithelial bleb formation[44]. The latter, by lifting epithelium from its basal structures, removes enterocytes from sources of oxygen and energy derived by diffusion from subjacent capillaries: indeed, blebs usually occur in areas of vascular compaction and sludging (Fig. 8).

Anoxic Damage to Enterocytes

There seems little doubt that the vascular disturbances observed within the post-challenge mucosa give rise to focal ischaemia and anoxia of lamina propria and epithelium: the liberation of superoxide radicals from anoxic tissues and cells may also cause further impairment of the proper functioning of surface enterocytes involved in the inflammation[45,46].

The appearance of damaged coeliac enterocytes is identical to that resulting from controlled intestinal ischaemia in experimental animals and shows loss or disruption of microvilli, swelling of mitochondria and endoplasmic reticulum, and ultimate rupture of lysosomes, leading to cell death[47]. Earlier work on the coeliac enterocyte provides a detailed catalogue of the histological, cytochemical, ultrastructural and biochemical features of cells undergoing autolytic degeneration.

From these observations, there appears to be no *a priori* ground for supposing either that coeliac enterocytes lack certain peptidases, or that they are subject to lymphocyto-toxic or other forms of direct immunological attack.

Clearly, the high rate of loss of enterocytes (*see* Fig. 6)

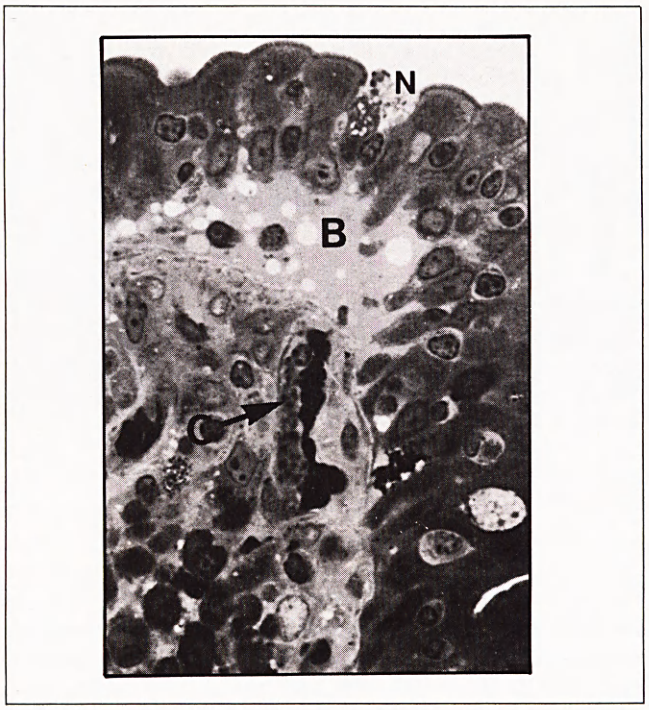


Fig. 8. *A 1μm epon section of bleb (B) with focal epithelial necrosis (N) overlying sub-epithelial capillary (C). The latter shows severe compaction and distortion of contained erythrocytes and adjacent platelet aggregate plugging lumen. Continuity of basement membrane is lost in vicinity of bleb. Note oedema of lamina propria.*

shown to be mediated by these inflammatory events is sufficient cause for crypt hypertrophy; it seems unnecessary to invoke special 'enteropathic enterokines' to account for these changes within the epithelial cell column, since they are merely secondary to other non-specific inflammatory processes.

The Role of the Mast Cell

The demonstration of lamina propria oedema and intra-epithelial blebs (Figs. 5, 8), increased vascular permeability leading to fibrin(ogen) deposition (Fig. 7) and erythrocyte compaction and sludging within the local microvasculature (Fig. 8) points to a prominent role for mast cells in the evolution of these changes. It should be noted that there is a major increase both of mast cells and eosinophils within the lamina propria (*see* Fig. 1).

Apart from specific IgE-mediated mast cell interactions in classical immediate-type anaphylaxis, an extensive literature testifies to the role of mast cells not only in late-phase cutaneous[48-50] and bronchospastic[51] allergic reactions, but also in pure T lymphocyte-dependent phenomena in man[52-54], including tuberculin skin hypersensitivity[37] and cutaneous basophil hypersensitivity states accompanying cell-mediated reactions to contact sensitisers, tumour and allograft rejection, and vaccinia infection[55-57]. In these latter circumstances it is clear that mast cells (and basophils) can only be activated and driven by T lymphocyte-derived lymphokines. More importantly, it has been amply demonstrated that the expression of delayed-type hypersensitivity is critically dependent on the release of vasoactive and other mediators of inflammation from mast cells. Also, isolated mast cell granules[50] evoke mononuclear infiltrates that appear 24 hours after intradermal injection.

It should not be forgotten that intra-epithelial bleb formation and increased vascular permeability regularly attend the expulsion of helminths[58], a process in which mast cells are deemed to play a major role. Mast cell products are also implicated in certain cutaneous blistering diseases, such as pemphigoid[59].

There is a close parallel between the alterations detailed for coeliac mucosae and those accompanying the evolution of cutaneous tuberculin reactions. The typical mature 'delayed' skin lesion is preceded by an influx of neutrophils, mast cells and eosinophils which leads to extensive microvascular damage, fibrin deposition, intra-epidermal oedema and, in more severe reactions, basement membrane lysis and central necrosis of epidermis around the site of inoculation[37].

While these changes are functions of activated T lymphocytes, a surprisingly similar series of cytological changes occurs within the bronchial epithelium of asthmatics as a result of IgE-mediated release of mast cell products[60]. Such reactions also lead to denudation of circumscribed clumps of epithelium ('Creola' bodies), destruction of basement membrane, tissue necrosis and oedema, and infiltrates of neutrophils, mast cells, eosinophils, macrophages and plasma cells. As is likely in coeliac mucosa, the antibody-dependent asthmatic lesion and/or the pure T lymphocyte-dependent tuberculin reaction are largely the result of mast cell discharge. This leads to, and perpetuates, epithelial or epidermal cell destruction, probably by superoxide release[61], and subsequent formation of other highly destructive radicals, in addition to microvascular injury, focal ischaemia and anoxia. The additional role of products of arachidonic acid metabolism are only just beginning to be evaluated in such circumstances: much still needs to be learned of their role in tissue inflammation and destruction.

The Immunopathology of the Coeliac Lesion

Thus, despite many current uncertainties, it is possible to assemble an immunopathological framework to account for the cytological abnormalities of the coeliac lesion.

Although the predisposition to coeliac disease is genetically controlled[62], other stimuli (infection, surgery and so on) may be necessary to trigger the innate susceptibility, both in adults and children. The late Dr Winifred Young showed that the minimal induction period between weaning and first onset of symptoms in infants is approximately six months[63].

At the cellular level, it is assumed from what has been discussed above that the single and specific interaction that can be envisaged at this time involves antigen (gluten), DR-compatible macrophage and T helper lymphocytes[64-66] within the lamina propria (Fig. 9). Once this small population of antigen-specific lymphocytes is activated, all subsequent events are entirely non-specific

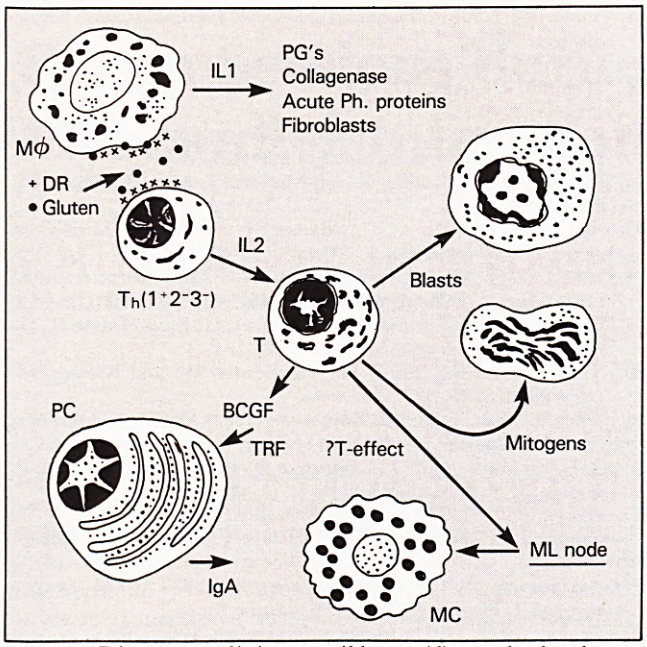


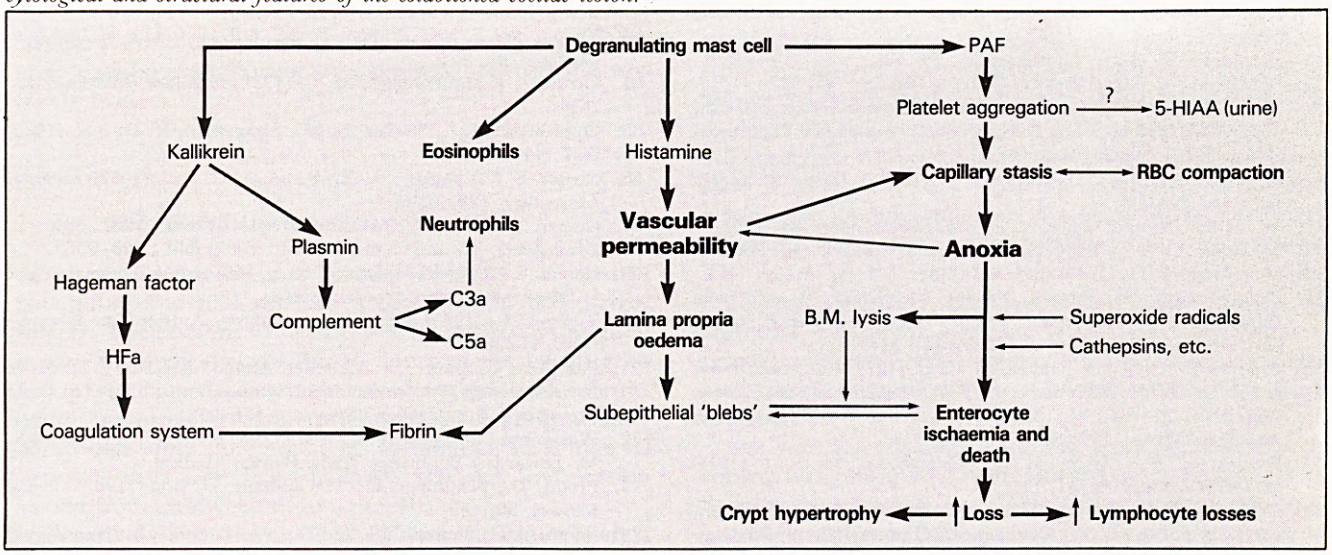
Fig. 9. *Diagram outlining possible specific, and related non-specific effects of gluten-induced, cell-mediated reactivity within coeliac mucosa. (IL1 = interleukin 1; IL2 = interleukin 2; BCGF = B lymphocyte growth factor; TRF = T lymphocyte replacing factor; MC = mast cell; MØ = macrophage; PC = plasma cell; T_h = T helper lymphocyte.)*

sinophils) augments the reaction and leads to the monotonous cytologic infiltrate that characterises so many unrelated inflammatory lesions (Fig. 10).

Polymorphs are frequently observed in the early stages of 'delayed' reactions[70] and thus do not necessarily imply the presence of complement-fixing IgA-gluten complexes[35]. Although such complexes may be formed within the mucosa, they are not seen to be fundamental to the pathogenesis of coeliac disease, although they may contribute to the promotion of inflammatory changes within the mucosa. Neither does the presence of C3 deposition[35,36] necessarily imply activation of the complement cascade by antigen-antibody complexes, as has been assumed. As shown above, the diffuse rather than perivascular distribution of fibrin is not entirely consistent with Arthus-type reactivity[37,71]. More probably, triggering of the alternate pathway is a manifestation of the inflammatory response, possibly by mast cell-derived enzymes that also lead to kallikrein formation[60,72,73] and subsequent activation of Hageman factor and the coagulation system, with fibrin production[37,48].

This scheme provides a comprehensive immunopathological framework to account for the major features of the mucosal lesion in coeliac disease, of which some are described here for the first time. Hitherto, no theory [62,74] has taken account of the increase in mast cells and eosinophils, the microvascular changes, intra-epithelial blebs, anoxic damage to enterocytes or other aspects of

Fig. 10. *Schematic framework of events following lymphokine activation of mast cell. Discharge of enzymatic, chemotactic, vasoactive and platelet-activating factor (PAF) from mast cells (and other inflammatory cells) could initiate inflammatory pathways that result in the cytological and structural features of the established coeliac lesion.*



and mediated through lymphokines which attract other lymphocytes, cause blast transformation (*see* Fig. 3) and mitosis (*see* Fig. 4), induction of B lymphocyte antibody production, and so on[67-69]. The recruitment of additional phlogistic pathways involving enzymatic, chemotactic and vasoactive agents derived principally from mast cells (and ancillary cells—macrophages, neutrophils, eo-

the inflammatory reaction involving the lamina propria that are discussed here, and in detail elsewhere[75]. Failure to do so means that all such previous proposals are too narrow in concept to be entirely valid.

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