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Studies of intestinal lymphoid tissue

XV. Histopathologic features suggestive of cell-mediated reactivity in jejunal mucosae of patients with dermatitis herpetiformis*

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Summary. Peroral jejunal mucosae from 32 patients with untreated DH were quantitated by computerized image-analysis in terms of surface (villous) and crypt epithelial volumes and their corresponding lymphoid infiltrates, together with lamina propria volumes, neutrophils, mast cells and basophils. Three distinctive patterns of mucosal abnormality were identified: (a) the "infiltrative" lesion in which normal villus epithelium was infiltrated by small, non-mitotic lymphocytes: (b) the "hyperplastic" type, in which crypt hyperplasia and hypertrophy together with lymphoid infiltration of crypt epithelium was additional to the "infiltrative" lesion, and in which lamina propria was swollen and contained modest neutrophilic and basophilic infiltration: and (c) the "destructive" lesion, identical to the classic celiac sprue appearances with effacement of villi, crypt hypertrophy and more intensive polymorph infiltration of lamina propria. These progressive lesions parallel those seen in experimental graft-versus-host reactions, so that the entire spectrum of changes described here in DH appear consistent with a cell-mediated mucosal response to gluten. The extent of mucosal abnormality was unrelated to individual HLA status.

Key words: Jejunal mucosa – Epithelial lymphocyte – Gluten – Cell-mediated immunity – Dermatitis herpetiformis

Introduction

Previous studies in dermatitis herpetiformis (DH) have established a firm relationship between intestinal damage, gluten-sensitivity and a genetic predisposition in which >90% patients are of HLA A1, B8, DR3$^+$ haplotype (Marks et al. 1966; Brow et al. 1971; Weinstein et al. 1971; Katz et al. 1972; Scott et al. 1976). In contrast to the classic celiac sprue (CS) patient, no major structural abnormality of small intestinal mucosa is seen in 20–40% DH patients, while non-specific to moderate changes occur in 25% and a severe ("flat") lesion in approximately 35–40% only (Marks 1977; Katz and Strober 1978).

Immunologically, the severe DH lesion appears identical to CS in terms of lymphoid elements within villous epithelium and lamina propria, increased production of IgA-anti-gliadin antibody, surface epithelial cell damage and reductions in enzyme activity (Gebhard et al. 1974). Conversely, the minimal DH lesion, comprising lymphocytic infiltrates into relatively normal villi is not associated with increased antibody production or enterocyte dysfunction, although the lymphocytic infiltrate is reduced by dietary gluten restriction (Fry et al. 1972, 1974). It has not been established why two such distinct lesions occur in DH and whether, or how, they might be related (Marks 1977). Furthermore in the majority of studies, performed several years ago, mucosal damage was assessed by arbitrary visual gradings or by stereomicroscopy with reference to villi, while crypts were never analyzed.

The mechanism(s) whereby villous flattening occurs in CS and some DH patients is not entirely understood. Studies of allograft rejection and graft-versus-host reactions (GVHR) in experimental animals indicate a central role for T lymphocytes (Ferguson and Parrott 1973; Ferguson and Jarrett 1975; MacDonald et al. 1977), probably acting via lymphokines, in the production of mucosal damage (Ferguson 1987). However, more recent experimental manipulations of GVHR have been successful in eliciting changes of less florid

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degree, thereby revealing a spectrum of T cell-mediated mucosal responses more varied and subtle in expression than was appreciated heretofore (Mowat and Ferguson 1982; Guy-Grand and Vasalli 1986): importantly, some of these lesions resemble the milder jejunal abnormalities of DH (Fry et al. 1972, 1974).

It was upon this new experimental background that the present study was performed. The aim was to seek a more detailed analysis of mucosal lesions from a large group of DH patients in order to draw parallels and permit some inferences to be made about the human intestinal immune response to presumptive T cell-mediated influences in gluten-sensitivity. At the same time, advantage was taken of recently-developed techniques in computerized image-analysis (Niazi et al. 1984; Dhesi et al. 1984; Marsh and Hinde 1986) in order to accurately quantitate the mucosal biopsies available for study.

Methods

Patients. These were referred to the Out-Patient Service of the University Department of Medicine at Hope Hospital by dermatologists within the Greater Manchester region. The group comprised 32 patients (18 males, age range 15–64 and 14 females, age range 26–77) who were not on dietary restriction at time of study. The diagnosis was based on (i) typical eruptive pruritic blisters involving face, shoulders, elbows and knees, (ii) the presence of IgA deposits in papillary dermis of uninvolved skin and (iii) response to sulfapyridine or dapsone. As detailed below, all jejunal biopsies in this group revealed abnormalities, either structural or involving lymphoid cell populations, that were responsive to dietary gluten restriction, thus affirming that all patients studied were indeed gluten-sensitive. Furthermore, HLA typing revealed high concordance rates for B8 (>90%) and DR3 (95%) consistent with previous series. Individual haplotypes did not correlate, however, with the severity of the mucosal lesion.

Histologic technique

Mucosal tissues, obtained under fluoroscopic control from the first loop of jejunum by Watson capsule, were quickly retrieved within 0.5 min, gently spread on card, flooded in 2.5% ultrapure glutaraldehyde buffered with 0.1 M sodium cacodylate to pH 7.2, post-fixed in 1% osmium tetroxide and processed to plastic (Araldite, Ciba-Geigy, Cambridge, UK) by routine procedures.

Sections 1 μm thick were cut from the cured blocks on a Reichert OMU-3 Ultramicrotome and stained with toluidine blue. Six consecutive sections were mounted per slide, and 10 μm steps were discarded between successive slides. One section only per slide was subsequently analyzed, the choice dependent on presence of least technical artefact. The remaining sections served for verification of cytologic detail if required, while the 10 μm step ensured that no cell was counted or analyzed more than once. Micrographs, as appropriate, were recorded on FP3 fine grain film (Ilford, Ilford, Essex, UK) through an Olympus BH2-S research microscope.

Morphometric analysis of mucosal specimens

1 μm sections were examined through an ×100 planapochromat objective and analyzed with a MOP-videoplan (Kontron, FRG) image-analysis system, as previously detailed (Niazi et al. 1984; Marsh and Hinde 1986; Marsh et al. 1983). All measurements were related, directly or indirectly, to a constant test area (10^4 μm^2) of muscularis mucosae, thus providing valid, comparable results between different specimens irrespective of the surface mucosal contour.

1. *Mucosal compartment volume.* In determining the absolute volumes of surface epithelium (V_{SE}), crypt epithelium (V_{CR}) and lamina propria (V_{LP}) per specimen, appropriate profiles in 1 μm epon sections were outlined with the scribing cursor, and individually cumulated relative to a total length of muscularis mucosae equal to 1 mm, thereby approximating the (100 × 100) μm^2 test area: Coefficients of variation were <10% per specimen.

2. *Mean epithelial lymphocyte diameters (D_N).* Nuclear profiles of 100 EL per specimen were traced with the cursor, from which the crude diameters for each population of lymphocytes was computed. Previous work had established that 100 cells were sufficient to achieve a constant mean ± SD. To this initial distribution were added "lost profiles", determined by scaling existing values in the left-hand 25% of the distribution up to a line joining zero origin to half-value of the mode (Geiger and Riedwyl 1970): the new mean was then multiplied by $^4/\pi$ to correct for non-sagittal sectioning (Weibel 1979), thus permitting the true mean nuclear diameter (D_N) of surface EL per specimen to be obtained.

3. *Calculation of absolute EL population sizes (N_V).* Calculation of the true size of EL populations either within surface epithelium ($N_{V,SE}$) or crypt epithelium ($N_{V,CR}$) requires knowledge of their mean diameter (D_N) and their volume of distribution (V_{SE}, V_{CR}). It has been shown that particles, of mean diameter D, whose profiles appear in any finite section of t μm thickness occupy a superslice of tissue whose overall thickness ('effective section thickness', EST) is given by the relationship, EST = $(t+D)$ μm (Marsh et al. 1983; Marsh 1980).

In determining $N_{V,SE}$ and $N_{V,CR}$ respectively, the actual number of EL profiles in surface, or crypt, epithelium were counted relative to (100 × 100) μm muscularis mucosae, from which respective values of $N_V = [100 \times (100 \div EST)]$. Nuclear profiles were used in calculating EST because they are approximately circular in sectioned profile, easy to define, and hence more accurately measured. Data in this category are expressed logarithmically.

4. *Percentage mitotic index of EL.* This index was calculated from the total number of metaphases observed in a total sample of 3000 EL per specimen. Only EL in surface epithelium were counted in order to prevent confusion with mitotic precursors in generative crypt epithelium (Marsh 1982; Marsh and Haeney 1983).

Results

Surface (villous) epithelial volumes (V_{SE}) are ranked in descending order (Fig. 1) with their corresponding crypt volumes (V_{CR}) and respective EL populations ($N_{V,SE}$ and $N_{V,CR}$), thereby illustrating the spectrum of immunopathological changes between villus-bearing (left-hand) and "flat" (right-hand) specimens. While V_{SE} and V_{CR} in the first seven mucosae (Group 1A) in this series of 32 specimens fell within the 95% confidence limits

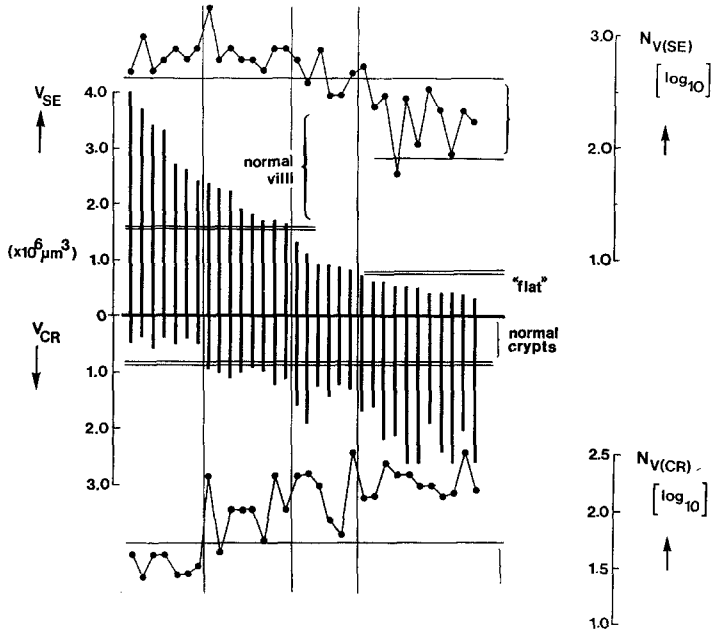

Fig. 1. Mucosae from 32 untreated DH patients are displayed (left to right) in terms of surface epithelial volume ($\times 10^6$ μm^3) (V_{SE}: vertical axis) and corresponding crypt epithelial volume (V_{CR}). Horizontal paired lines represent lower range for control villi (V_{SE}), upper range for "flat" untreated celiac sprue mucosae (V_{SE}), and upper range for control crypts (V_{CR}). Values for lymphoid populations within surface ($N_{V,SE}$) and crypt ($N_{V,CR}$) epithelium are displayed on right hand axes, and expressed logarithmically: horizontal lines represent lower and upper range for villi, and upper range for crypts, respectively. First seven mucosae (up to first vertical line) (Group 1 A) are normal except for lymphocytic infiltration of villous epithelium. The next eight mucosae (up to second vertical line) (Group 1 B) reveal crypt hypertrophy exceeding upper reference range, and are now infiltrated with small lymphocytes. Flat mucosae (beyond third vertical line) (Group 3) reveal marked crypt hypertrophy and sustained lymphoid infiltrates: however the flattened surface epithelium contains a reduced number of lymphocytes which fall within the control reference range (bracketed)

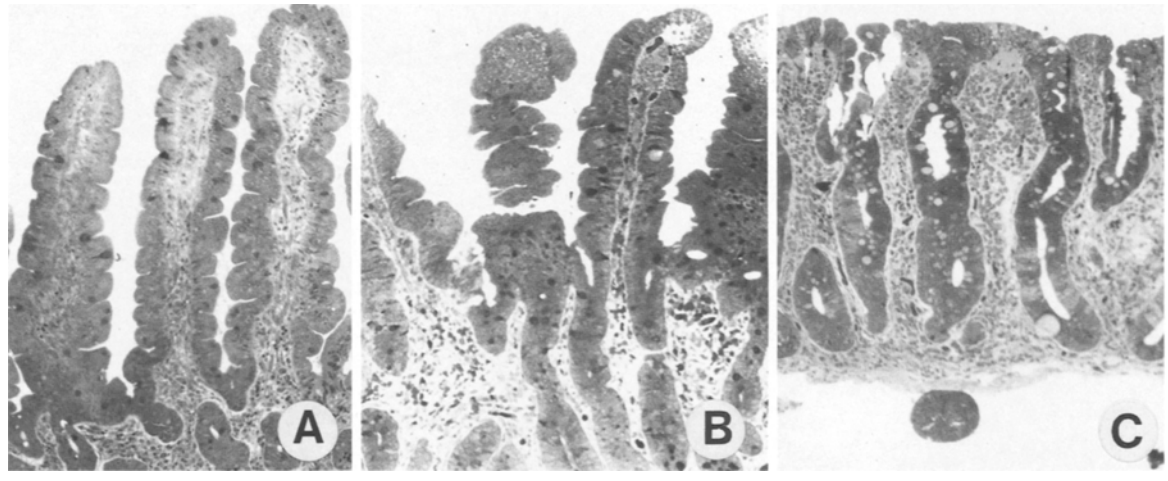

Fig. 2 A–C. These three 1 μm toluidine blue-stained sections illustrate progressive degrees in mucosal flattening. The type 1 lesion comprises normal villi with either normal (type 1: "infiltrative") **A** or enlarged (type 2: "hyperplastic") **B** crypts. Villous epithelium is infiltrated by small, non-mitotic IEL (see Fig. 5 A). In panel **C** there is a typical flat ("destructive", type 3) lesion (Magnifications: $\times 90$)

for control mucosae (Niazi et al. 1984; Marsh and Hinde 1986), villous epithelium contained elevated populations of EL that exceeded the upper control reference range. Crypt epithelium was not infiltrated (Fig. 2), while crypt mitotic activity (Fig. 3) was normal. Furthermore, volumes of lamina propria (Fig. 4) were essentially normal as was mast cell content, while neutrophils and basophils were not detected.

The succeeding eight specimens (Group 1 B) revealed marked increases in crypt epithelial volume despite the presence of normal villi (Fig. 2). Crypt epithelium was also associated with a rise in EL ($N_{V,CR}$) (Fig. 1): crypt cell mitotic activity was also increased (Fig. 3), both sets of values lying outside their respective control ranges. In addition, lamina propria volumes were increased in these mucosae (Fig. 4) and infiltration by neutrophils and some basophils was now observed. EL, although increased in absolute number throughout the entire epithelial cell column, were of normal size (D_N) and contained few large ($D_N > 6$ μm) or mitotic cells (Figs. 3, 5).

The next 6 specimens (Group 2) occupied an intermediate position in which V_{SE} lay below the lower reference range for control villi, but exceeded

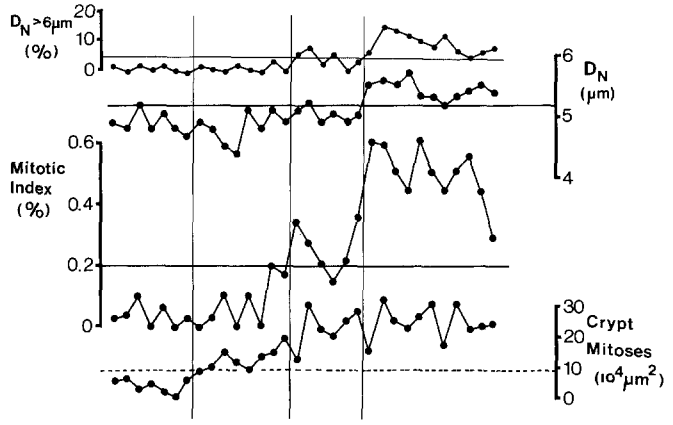

Fig. 3. This diagram contains data for percentage large epithelial lymphocytes ($D_N > 6$ μm), and their mean nuclear diameter (D_N) and mitotic index. Lower graph represents crypt mitotic activity, expressed as crypt mitoses per 10^4 μm^2 muscularis mucosae. Horizontal lines represent upper reference ranges for control mucosae. Crypt cell mitotic activity is increased in Group 1 B mucosae (between first and second vertical bars) and increases still further as mucosal flattening occurs. The mitotic activity of intra-epithelial lymphocytes is increased in Group 2 mucosae (between 2 and 3 vertical lines) and rises steeply in the 11 flat mucosae (beyond third vertical bar). It is only in these latter mucosae that the average size (D_N) and percentage of larger epithelial lymphocytes ($D_N > 6$ μm) is increased. Thus, there is a dissociation between these latter indices of lymphocyte 'activation', in that mitotic activity is raised without any accompanying increase in size or percentage of large 'immunoblastoid' epithelial lymphocytes

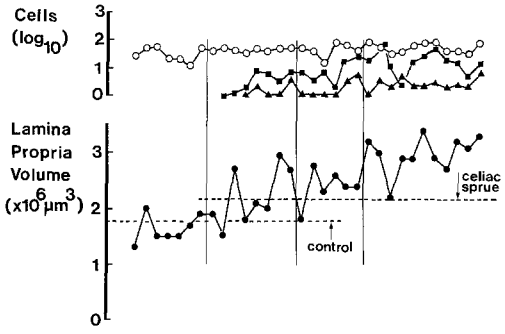

Fig. 4. This diagram illustrates lamina propria volumes ($\times 10^6$ μm^3 per 10^4 μm^2 muscularis mucosae) together with mast cells (0), neutrophils (■) and basophils (▲) expressed in absolute population sizes on logarithmic axes, corresponding to the 32 mucosae displayed in Fig. 1. Group 1 B mucosae (between first and second vertical lines), in addition to crypt hypertrophy and lymphoid infiltration also show increased lamina propria volumes exceeding upper control range and, in some instances, lower range for flat celiac sprue mucosae (horizontal dotted lines) together with the presence of neutrophils and some basophils indicating occurrence of an inflammatory reaction within this region of these mucosae. As flattening progresses, there are further rises in neutrophils, and a slower rise in basophils, while lamina propria volumes also increase, reaching maximum as mucosae becomes flat (Group 3) (to right of third vertical line). Mast cell content slowly rises throughout

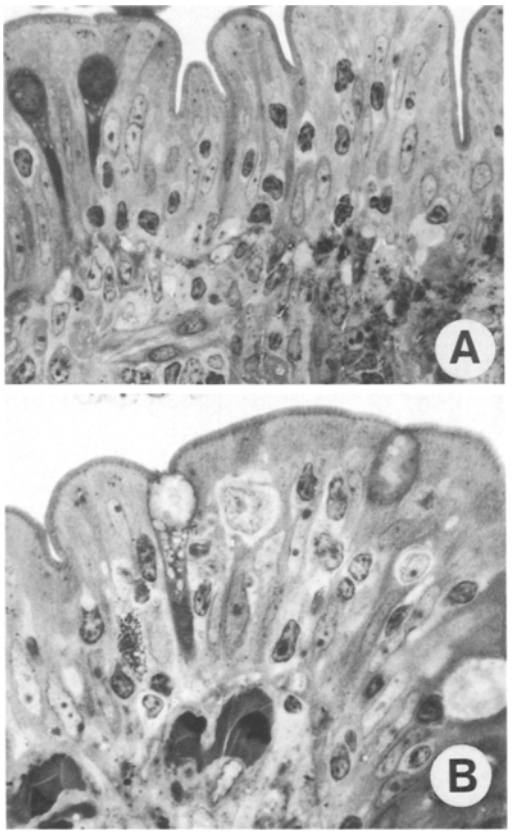

Fig. 5. Details from a Type 2 lesion, illustrating infiltration of epithelium with essentially small, non-mitotic IEL **A**. In **B**, from a flat, type 3 ("destructive") lesion, epithelium contains a more heterogeneous collection of IEL, including larger, 'blast'-like cells (Magnifications: × 750)

the upper range established for flat, untreated CS mucosae (Niazi et al. 1984). Crypt epithelial volumes (V_{CR}) remained elevated as did EL populations both within crypt, and surface, epithelium. The size (mean nuclear diameter, D_N) and proportion of large cells ($D_N > 6$ μm) remained constant. The mitotic activity of EL in these specimens was increased, and in 5 specimens exceeded the arbitrary value of 0.2% (Marsh 1982; Marsh and Haeney 1983) previously shown to be a feature of flat CS lesions only (Marsh and Hinde 1986; Marsh 1982). These specimens were also characterised by progressive rises in lamina propria volume (Fig. 4) together with increases in their absolute populations of neutrophils and basophils.

The remaining 11 specimens (Group 3) (Fig. 1) fulfilled criteria typical of flat CS mucosae with marked crypt hypertrophy (Fig. 2) and crypt cell proliferative activity. The mean EL diameter was also raised (Fig. 5) (D_N 5.43 ± 0.17 compared with D_N 4.84 ± 0.21 for Groups 1 A, 1 B specimens) of which 8% comprised "immunoblastoid" lympho-

cytes (compared with <2% in Groups 1 A, 1 B mucosae) with $D_N > 6$ μm: their mitotic activity was also elevated >>0.2% (Fig. 3). Although EL population in crypt was increased, the EL population in surface epithelium was reduced so that individual values lay within the control reference range. Similar decreases in the total population of EL in surface epithelium were previously demonstrated in flat CS mucosae (Marsh 1980) probably on account of the high rate of desquamation of surface epithelium compared with crypt epithelium which does not exfoliate. Lamina propria volumes for these flat specimens were highest compared with the other mucosae in this series: in addition, like untreated CS mucosae, lamina propria contained the largest populations of infiltrating neutrophils, mast cells and basophils (Fig. 4).

Discussion

These results provide details on the spectrum of histopathologic changes in the jejunal mucosae of DH patients. The proportion lacking normal villi (55%), or with entirely flat mucosa (42%) is consistent with other series (Brow et al. 1971; Weinstein et al. 1971; Katz et al. 1972; Scott et al. 1976; Marks 1977). The data also extend previous work in providing further quantitative data on crypt size, crypt cell mitotic activity and crypt lymphocyte populations, as well as examining lamina propria in terms of alterations in volume and in its populations of inflammatory cells such as mast cells, basophils and neutrophils.

All data were considered with respect to, and in descending order of, values for surface epithelial volume, since progressive decrements in villous height ultimately lead to a completely flat mucosa. However, such a display is static, each mucosal specimen representing a single event that is unrelated to a common variable, such as time or dose-response. Despite this, the measurements when evaluated together revealed progressive abnormalities as a greater degree of mucosal flattening occurred. Furthermore, they are consistent with other observations on the spectrum of gluten-induced changes as seen (i) in first-degree coeliac relatives (Marsh et al. 1989) (ii) after low-dose gluten challenge (Leigh et al. 1985) and (iii) during spontaneous in vivo development of the classical lesion (Marsh 1989).

Fry and colleagues (1972, 1974) first drew attention to lymphocytic-infiltration of normal villi in DH, and 15 of 32 mucosae in this study fulfilled those criteria. Such lymphoid infiltrates are gluten-dependent, since they are modified by either gluten restriction (Fry et al. 1974), or loading (Weinstein 1974; Leigh et al. 1985). However in 8 of these 15 DH mucosae, the simultaneous occurrence of crypt hypertrophy, increased crypt cell mitotic activity and infiltration of crypt epithelium by small, non-mitotic lymphocytes was also documented. An identical pattern of immunopathologic events, evoked by experimental graft-versus-host (GVH) reactions (Mowat and Ferguson 1982) is one characteristic form of a T lymphocyte-mediated reaction within small intestine. Because of the early appearance of mitotic crypt hypertrophy, despite preservation of normal villi whose epithelium carried raised lymphocytic infiltrates, this lesion has been termed the "hyperplastic' (or "type 2") lesion, as compared with the "infiltrative" lesion (type 1) described above (Fig. 6). Furthermore, the type 2 lesion can be produced by fractionated T cells, being more severe with $CD4^+$ (or murine L3T4) lymphocytes (Guy-Grand and Vassalli 1986; Mowat et al. 1986) than with $CD8^+$ cells.

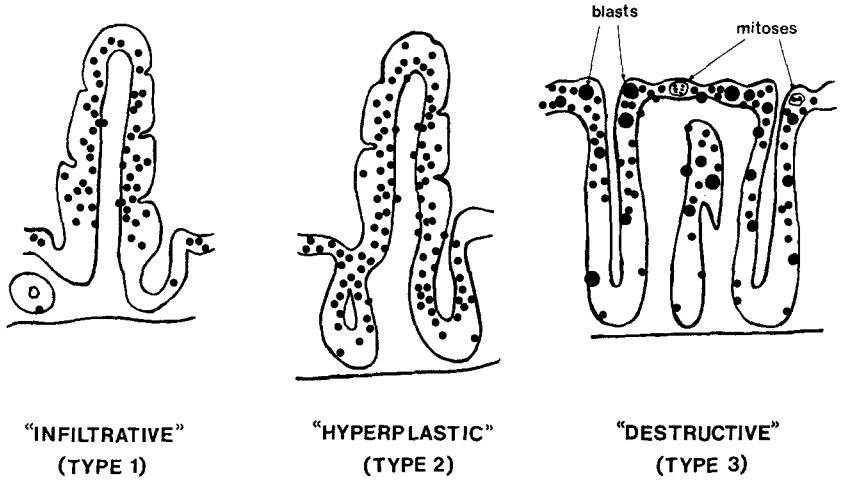

Fig. 6. Diagrammatic representation of the three distinctive phases in the evolution of a flat mucosal lesion. Similar steps have been observed in experimental models of T lymphocyte-mediated damage, suggesting that the changes observed in DH are representative of the entire spectrum of cell-mediated events associated with gluten sensitivity. In becoming flat, it appears obligatory for the mucosa to pass through the infiltrative (Type 1) and hyperplastic (Type 2) lesions

More cogent evidence for this view comes from recent experiments with explants of organ-cultured human fetal intestine (MacDonald and Spencer 1988) in which crypt hypertrophy was shown to be a rapid response to activation of lamina propria T lymphocytes (epithelial lymphocytes being absent in this model) by anti-CD3 monoclonal antibody. Furthermore, it was apparent (Monk et al. 1988) that activation of lamina propria T lymphocytes ($CD25^+$) also resulted in marked infiltration of epithelium by $CD3^+$ lymphocytes. Since such explants do not suffer the additional complication of infiltration by blood-borne cells, it is evident that local differentiation, mitotic expansion and migration of lamina propria T cells directly contributes to the size of the interepithelial lymphocyte pool. In our DH specimens with early crypt hypertrophy, the lamina propria was swollen and contained neutrophil and basophil infiltrates. Such observations invite the suggeston that inflammatory processes triggered within the lamina by gluten may also activate lamina propria T lymphocytes (Marsh 1983; Bjarnason et al. 1985) causing crypt hypertrophy and lymphoid infiltration of villous epithelium.

The most severely damaged mucosae exhibited identical pathologic features as untreated CS mucosae (Marsh 1980) and those experimentally-induced in allograft rejection and GVH reactions (Ferguson and Parrott 1973; MacDonald and Ferguson 1977). This "destructive" ("type 3") lesion (Fig. 5) appears to result from damage to surface enterocytes and their rapid rate of desquamation. Although in the experimental lesions there is nonspecific boosting of T- (Ferguson et al. 1978; Elves and Ferguson 1975), and NK- cell (Elves and Ferguson 1975; Mowat et al. 1985; Varkila et al. 1985) cytotoxicity, tissue damage does not result from this latter activity but rather from soluble mediators released by activated donor T cells reacting with host-derived lymphocytes and antigen-presenting cells. These T cells are $Lyt\ 2^+$ lymphocytes restricted by I-A (MHC class 2) gene products, or DTH effector cells (Guy-Grand and Vassalli 1986; Mowat et al. 1986) which are activated within the intestinal stroma and not villous epithelium (Ferguson 1987).

In the severe "destructive" lesions of gluten-sensitivity (CS and DH) it is still not entirely clear whether EL are the cause of enterocyte damage. However, architectural damage in the presence of large EL infiltrates is clearly absent in type 1 and 2 lesions (Mowat and Ferguson 1983), while the persistence of heavy crypt lymphoid infiltration throughout the entire spectrum of changes evidently does not inhibit crypt hypertrophy, cell proliferation or the capacity for continued differentiation of argentaffin, goblet and Paneth cells (Marsh 1987). Furthermore, the induction of MHC class 2 expression by crypt enterocytes in these circumstances (Scott et al. 1981), presumably by IFN-γ elaborated by EL (Barclay and Mason 1982; Cerf-Bensussan et al. 1984), clearly does not lead to their 'autoreactive' destruction. Indeed, only those cells actually covering the mucosal surface are damaged while cells immediately adjacent in the upper crypts, despite the presence of heavy EL infiltration, display the cytologic and ultrastructural features of normally-differentiated villus enterocytes (Padykula et al. 1961; Shiner 1974; Marsh 1972).

In summary, the observations contained herein document three well-defined patterns of mucosal injury in DH which are consistent with T cell-mediated lymphocyte responses (Mowat and Felstein 1989; Marsh 1989). The data also suggest that crypt hypertrophy is an early event in the progression to a flat mucosa and draw attention to the accompanying alterations in volume and cellular content of the lamina propria at each stage of evolution. This part of the mucosa deserves much further detailed study if the pathophysiology of the gluten-induced lesion is to be fully understood. These data provide a pathologic framework upon which future studies in this context may be most effectively based.

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