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Morphometric analysis of small intestinal mucosa

III. The quantitation of crypt epithelial volumes and lymphoid cell infiltrates, with reference to celiac sprue mucosae*

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Summary. With the aid of computerised image-analysis, morphometric techniques were used to measure the volumes of crypt epithelium, with reference to a constant test area (10^4 μm^2) of muscularis mucosae, in untreated and treated celiac sprue mucosae in comparison with four other groups of control jejunal specimens. Crypt epithelial lymphocyte populations were also analyzed in terms of absolute numbers (N), mean nuclear ($\bar{D}_N$) and cytoplasmic ($\bar{D}_{CYT}$) diameters, and mean nuclear ($\bar{V}_N$) and cell ($\bar{V}_{CELL}$) volumes.

Untreated celiac sprue crypts, despite a 3–5 fold increase in volume over control mucosae, contained a markedly expanded population of lymphocytes which was localised predominantly to the upper crypt regions and comprised approximately 6% large lymphocytes ($\bar{D}_N > 6$ μm: $\bar{D}_{CYT} > 9$ μm). These changes were entirely reversed by dietary treatment and hence were considered to be gluten-driven.

The infiltrate might reflect the expression of gluten receptors on maturing upper crypt enterocytes: but why these lymphocytes do not cause any apparent injury to crypt epithelium (unlike surface epithelium) or influence its well-known compensatory response, remains unclear.

Key words: Morphometry – Jejunum – Celiac sprue – Crypt epithelium – Epithelial lymphocyte

Introduction

In previous papers we have described the theoretical basis for the quantitative analysis of small intestinal mucosa and shown how this permits comparisons between specimens irrespective of their mucosal contour or "shape".

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Furthermore, we have also demonstrated the extent to which count densities of cells are influenced by mucosal compartment volumes, with respect to inter-epithelial space lymphocytes, and plasma cell subsets within lamina propria (Niazi et al. 1984; Dhesi et al. 1984).

These analyses were performed relative to an arbitrary, but constant, test square (10^4 μm^2) of muscularis mucosae which provides a convenient reference structure to which all comparative mucosal compartment volumes, and cell counts contained therein, may be related. This procedure has now been adapted for use with an interactive image-analysis system (Reichert-Kontron MOP-Videoplan) which makes quantitative morphometric analysis of intestinal mucosa easy, rapid and accurate to perform.

In this paper, we describe volumes of crypt epithelium in several groups of subjects that were investigated for a variety of gastrointestinal symptoms, including subjects with celiac sprue (gluten-sensitive enteropathy). In addition, absolute counts of crypt epithelial lymphocyte populations were made and which, for the first time, are shown to be considerably raised in untreated celiac sprue mucosae.

In most recent authoritative commentaries on celiac sprue there is scanty reference to the crypt epithelial lymphocyte population (Watson and Wright 1974; Cooke and Holmes 1984; Trier 1983) so that these new findings are of considerable interest in evaluating what role epithelial lymphocytes may (or may not) play in the pathogenesis of this enteropathy.

Material and methods

1. Patients studied

Six groups of patients were studied:

a. Group I comprised 10 young healthy volunteers (HV) who agreed to take part in this investigation.

b. Group II was made up of 10 apparently healthy blood relatives of known celiac sprue patients. This group was designated by the term family member controls (FMC).

c. Group III consisted of 10 patients with gastrointestinal symptoms who were not considered to have celiac sprue disease and whose jejunal mucosae, on conventional grounds (Rubin et al. 1960) were judged to be histologically normal. These subjects were termed normal disease controls (NDC).

d. Group IV contained a group of 9 subjects with miscellaneous conditions accompanied by a "flat" jejunal mucosal biopsy but in whom celiac disease was not considered to be a cause. Among these individuals were patients with jejunal Crohn's disease (3), small-intestinal lymphoma (3), α-chain disease (1), common variable immunodeficiency (1) and untreated tropical sprue (1). These cases were termed flat disease-controls (FDC).

e. Group V consisted of mucosae obtained from 14 untreated celiac sprue patients presenting with diarrhoea, malabsorption or anaemia and who were shown to have a flat mucosa in which the percentage mitotic index of epithelial lymphocytes exceeded 0.2% (Marsh 1982). The percent mitotic index in FDC patients (Group IV) was $\leq 0.1\%$ (Marsh 1982; Marsh and Haeney 1983).

f. Group VI comprised jejunal mucosae obtained form 11 celiac sprue patients who had received a gluten-free diet between 6 months to 10 years, and in whom malabsorption was no longer evident, nutritional supplements were not used and histological evidence of regeneration was evident on subsequent mucosal sampling.

A total of 64 mucosal specimens was available for morphometric analysis.

2. Histological techniques

Mucosal specimens were obtained with a Watson capsule located fluoroscopically to a point just distal to the duodenal-jejunal flexure. After rapid retrieval, the mucosa was quickly oriented and spread out on thick card, flooded with cacodylate-buffered 2.5% ultrapure glutaraldehyde, embedded in araldite resin, sectioned at 1 μm thickness with a Reichert OMU-3 ultramicrotome and stained with toluidine blue, as described previously (Marsh 1980).

Five to six sections were mounted per slide and 10 μm steps of tissue were discarded between successive sections. Selected sections (one per slide) that were perfectly oriented perpendicular to the mucosal surface, were observed through a ×100 oil-immersion objective with an Olympus BHS-2 research microscope. Photographs as desired were taken on Ilford Pan F 35 mm film and appropriately enlarged.

For quantitative microscopy, sections were projected through a high-resolution colour television camera to a MOP-Videoplan (Reichert-Kontron) image-analysis system. The following measurements were made, and which were related, directly or indirectly to a constant test area (10^4 μm^2) of muscularis mucosae (Dhesi et al. 1984; Niazi et al. 1984).

3. Quantitative methods

A. Crypt epithelial volume (V_{CR}). For the determination of V_{CR} per specimen crypt profiles in well-oriented 1 μm plastic sections were outlined with the scribing cursor, and in order to quantitate total crypt epithelial volume per specimen, profiles overlying a total length of 100 × 100 μm (10 mm) muscularis mucosae were included.

The boundary between crypt and surface epithelium is imprecise and comprises a zone of transitional cells characterised by evolving structural, cytochemical and functional properties (Padykula et al. 1961; Smith 1985).

The procedure adopted in this study for the measurement of crypts was based on conventional histological criteria (Fig. 1). In flat untreated CS mucosa all crypt profiles, whether in communication with surface epithelium or not were measured (Fig. 1a). In "normal" villus-bearing mucosae, crypts whose sectioned profiles communicated with the circumvillar basin (Cocco et al. 1966) were included while uninterrupted intervillous epithelium was not. All other crypt profiles beneath villus bases were also included (Fig. 1b). For the remaining mucosae, with variable degrees of villous flattening or regeneration, all crypt profiles whether communicating with the surface, or not, were measured (Fig. 1c, d). The coefficients of variation for successive measurements per 100 μm length of muscularis mucosae per specimen were always less than 10%: these statistical calculations are automatically included in the print-out by the computer programme supplied by Kontron.

B. Morphometry of crypt epithelial lymphocytes. The nuclear and cytoplasmic perimeters of crypt epithelial lymphocytes were traced with the cursor, from which crude areas, diameters and volumes were computed. Sufficient observations were made to obtain a constant mean ± SD which, on average, required a total sample of 80–100 lymphocyte profiles per mucosal specimen.

The crude profile diameters were then sequentially adjusted (a) graphically in order to include "lost profiles" (Giger and Riedwyl 1970; Niazi et al. 1984) from which (b) the new mean was further corrected for imperfect (non-sagittal) sectioning by multiplying by $^4/\pi$ (Weibel 1979). In this way, the true nuclear ($\bar{D}_N$) and cytoplasmic (cellular) ($\bar{D}_{CYT}$) diameters were obtained, from which nuclear ($\bar{V}_N$) and cell volumes ($\bar{V}_{CELL}$) were also calculated..

C. Determination of crypt epithelial lymphocyte population (N). In counting the total number of cells within a defined tissue component, their true diameter must be calculated since each sectioned "profile" represents only a fragment of the whole cell. It has been shown that all particles (nuclei of mean diameter, $\bar{D}$) whose profiles appear in any finite section (of thickness, $t = 1$ μm) are contained within a 'superslice' whose overall thickness (or effective section thickness, EST) is $(t + \bar{D})$ μm) (Niazi et al. 1984; Marsh et al. 1983).

Since $\bar{D}_N$ for each specimen had been calculated, the total number of crypt lymphocytes (N) overlying the 10^4 μm^2 test area of muscularis mucosae was obtained by accumulating

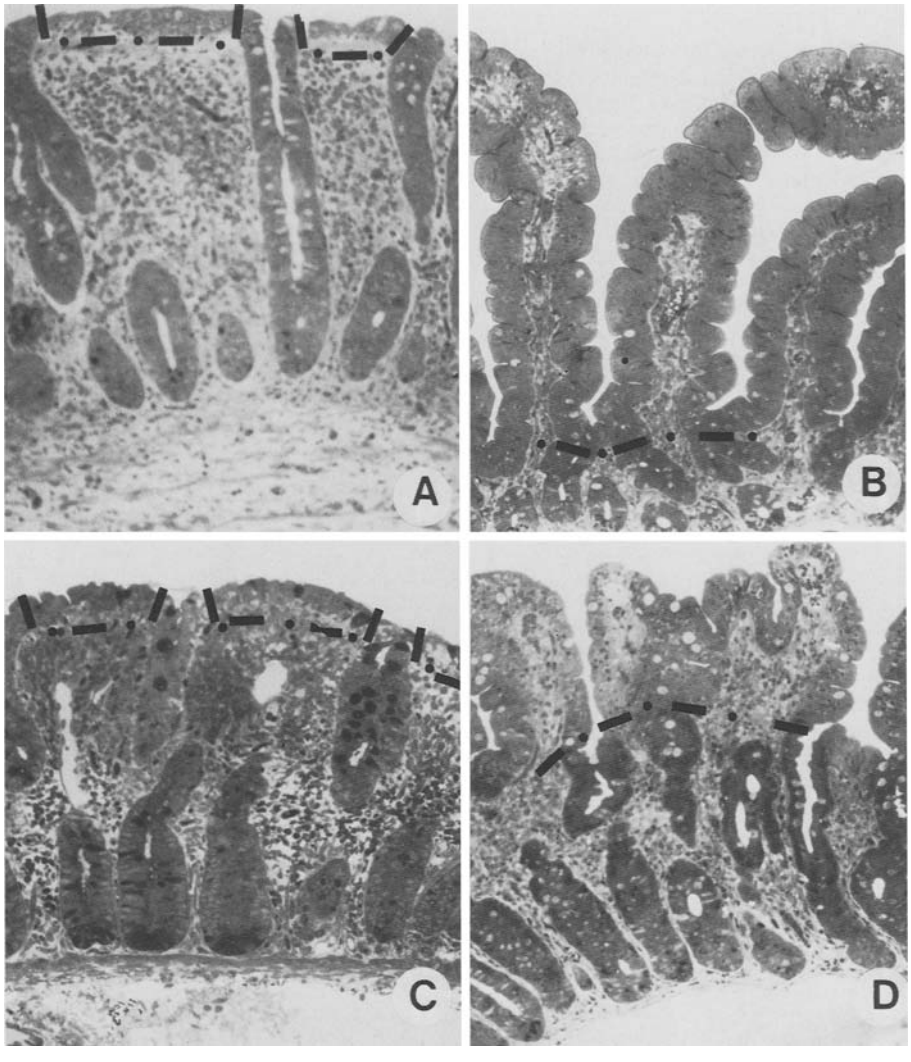

Fig. 1 a–d. These are representative sections of mucosae from subjects with untreated celiac sprue, **a** a normal volunteer, **b** a flat-disease control (lymphoma), **c** and partially-recovered celiac sprue, **d** The lines indicate arbitrary distinction between surface and crypt epithelium: all epithelial profiles below the lines were included in completing the measurements of crypt epithelial volume. (Magnification: ×110)

all crypt epithelial nuclei observed above a total length of muscularis mucosae = [100 × (^{100}/EST)] μm. Nuclear profiles were employed in calculating EST because they are more nearly circular in sectioned profile and hence may be more accurately measured (Niazi et al. 1984).

D. Proportional volumes. The proportion of total crypt epithelial volume (V_{CR}) occupied by N lymphocytes, of mean cell volume ($\bar{V}_{CELL}$ μm^3), was calculated for each mucosal specimen, and group means obtained. These latter means were expressed as percentage volume proportion.

Results

1. Crypt epithelial volumes (V_{CR}). There was no significant difference between the crypt volumes, per 10^4 μm^2 muscularis mucosae, of the three control groups ($0.5-0.6\times10^6$ μm^3) (Fig. 2). V_{CR} for untreated celiac sprue mucosae was increased three-fold above control levels (1.7×10^6 μm^3; $p<0.0001$), while that of the flat disease control mucosae was increased by a factor of two over controls (1.2×10^6 μm^3; $p<0.05$). There was also a slight difference between flat disease controls and untreated celiac sprue ($p=0.05$). There was a significant drop in crypt epithelial volume during treatment with gluten restriction, which now approximated that of flat disease controls but which was still significantly increased ($p<0.001$) above other control groups with normal mucosae.

2. Crypt EL/10^4 μm^2 muscularis mucosae (N). There was a highly significant difference in the absolute lymphocyte population in untreated celiac crypts (173 ± 26) compared with N for the three control groups with normal mucosae (30–39; $p<0.0005$) and with flat mucosae (65 ± 18; $p<0.006$) (Fig. 3). After treatment, there was no difference between the number of lymphocytes in celiac crypts and disease controls, although these were somewhat increased above the normal control specimens.

There were approximately five-fold, and threefold, increases in the size of the celiac crypt lymphocyte population compared with normal, and flat control specimens, respectively.

3. Size characteristics of crypt EL. There was a marked difference in the size of crypt epithelial lymphocytes, expressed either in terms of mean corrected nuclear diameter ($\bar{D}_N$), nuclear volume ($\bar{V}_N$), cytoplasmic diameter ($\bar{D}_{CYT}$) or total cell volume ($\bar{V}_{CELL}$) between untreated celiac sprue mucosae

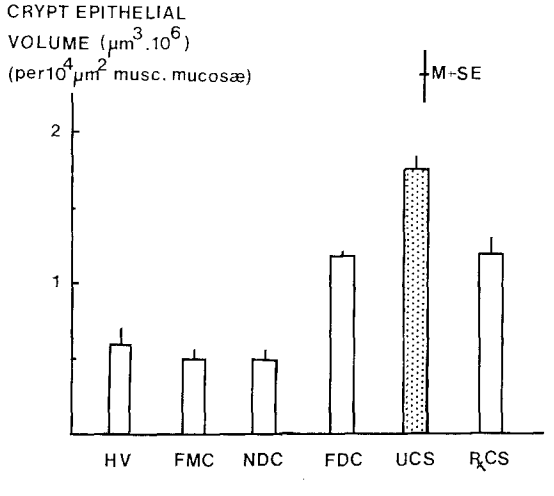

Fig. 2. Crypt epithelial volumes, expressed here as mean±SE per 10^4 μm^2 muscularis mucosae, are shown for each group of subjects: HV, human volunteers; FMC, family member controls; NDC, normal disease controls; FDC, flat disease controls; UCS, untreated celiac sprue; R_xCS, treated celiac sprue

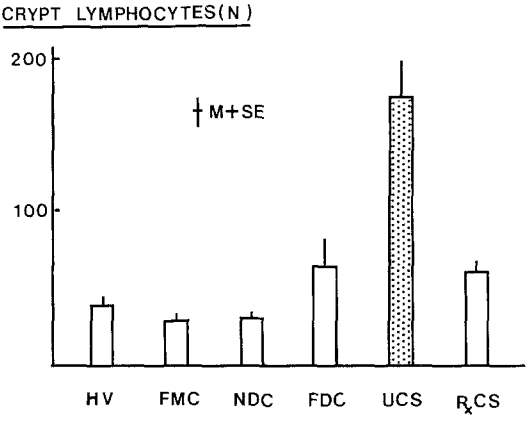

Fig. 3. This diagram illustrates the mean ± SE absolute lymphocyte counts (per 10^4 μm^2 muscularis mucosa) in crypts for each group of mucosal specimens. Abbreviations as in Fig. 1

Table 1. Compared with all other groups, epithelial lymphocytes in untreated celiac sprue crypts are considerably larger in respect of the dimensions given

	Mean nuclear diameter $\bar{D}_N$ ($\mu m \pm SE$)	Nuclear volume $\bar{V}_N$ ($\mu m^3 \pm SE$)	Mean cytoplasmic diameter $\bar{D}_{CYT}$ ($\mu m \pm SE$)	Cell volume $\bar{V}_{CELL}$ ($\mu m^3 \pm SE$)
Volunteers (10)	4.9 ± 0.3	64 ± 2	7.0 ± 0.05	183 ± 4
Family members (10)	4.9 ± 0.03	62 ± 2	7.0 ± 0.09	182 ± 7
Normal disease Controls (10)	4.8 ± 0.04	60 ± 2	6.9 ± 0.08	171 ± 6
Flat disease Controls (9)	4.9 ± 0.2	67 ± 7	6.9 ± 0.25	180 ± 18
Untreated celiac Sprue (14)	5.7 ± 0.07 ($p < 0.001$)	98 ± 4 ($p < 0.001$)	8.0 ± 0.07 ($p < 0.0001$)	265 ± 7 ($p < 0.0001$)
Treated celiac Sprue (11)	4.9 ± 0.04	65 ± 2	7.0 ± 0.07	180 ± 5

and the four other control groups ($p \ll 0.001$) (Table 1). This difference was no longer demonstrable with dietary gluten exclusion.

The 'shift-to-the-right' in the frequency distribution curves for each four measurements was due to an increased proportion of large, lymphoid cells within crypt epithelium of untreated celiac sprue mucosae.

Morphologically, these large, crypt epithelial lymphocytes revealed an expanded cytoplasm and large, euchromatic nuclei (Fig. 4) often containing several prominent nucleoli: they thus resembled those described earlier within the surface epithelium of celiac mucosae (Marsh 1980). In quantitative terms, these cells exceeded ± 2 SD the means of control mucosal lymphocytes, and hence largely comprised cells with mean corrected nuclear diamteters ≥ 6 μm and mean cytoplasmic diameters ≥ 9 μm, as indicated in other studies (Marsh 1980; Marsh et al. 1983) (Fig. 5). From Fig. 5, it is evident that nuclear, rather than cytoplasmic, diameters are more accurate in assessing lymphocyte size. This follows from the fact that sectioning artefacts increase with the size of the particle: thus, as diameter increases the chances

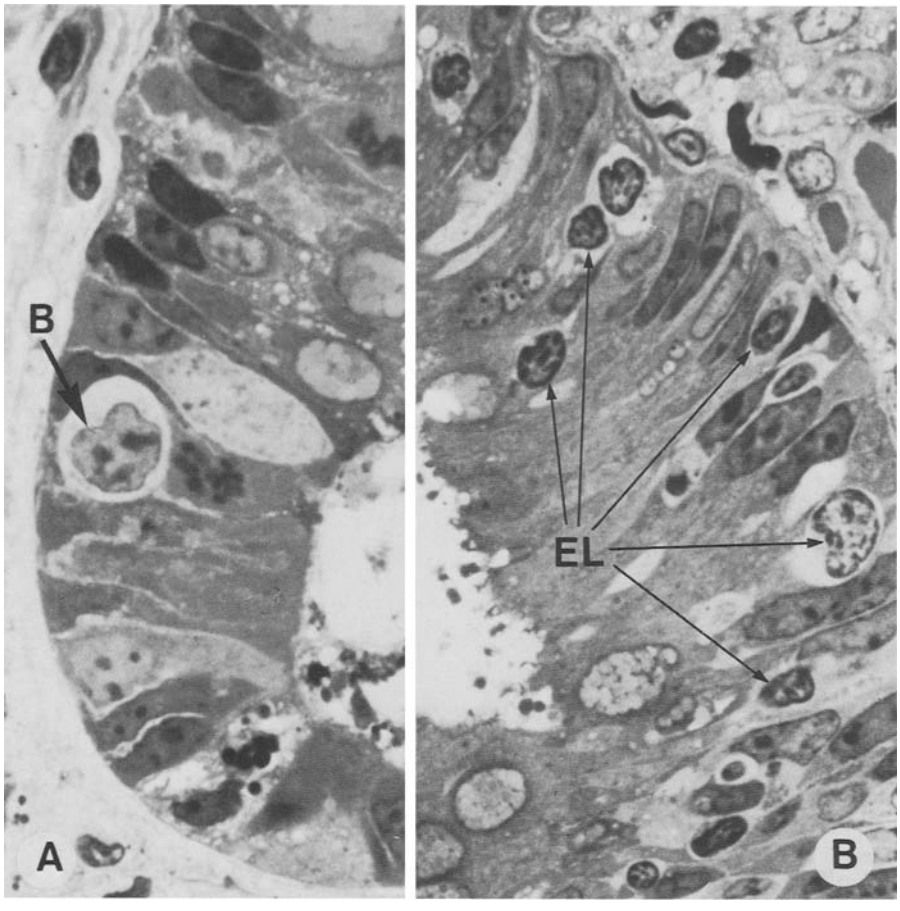

Fig. 4a, b. Each panel depicts toluidine blue-stained 1 μm plastic sections of CS crypts. In **a** crypt epithelium contains a large, immature lymphocyte, *B*. In **b** numerous epithelial lymphocytes, *EL*, exhibiting marked structural heterogeneity are clustered in this area of crypt epithelium. (Magnifications: **a** ×1600: **b** ×1340)

of any random section coinciding with the maximal (equatorial) diameter of the particle progressively falls. This bias in underestimating the correct diameter obviously affects overall cell diameters to a greater extent than their corresponding nuclei.

Despite this, there was a good correlation between nuclear and cytoplasmic diameters for each of the six populations studied (Table 2).

4. Volume proportions ($\bar{V}_{CR}/\bar{V}_{CELL}$). This is the proportion of total crypt volume (V_{CR}) per 10^4 μm^2 muscularis mucosae occupied by N lymphocytes of mean volume, $\bar{V}_{CELL}$. The proportional volume for untreated celiac sprue mucosae was 2.54% compared with approximately 1% for other normal disease-control mucosae ($p<0.05$). There was no significant difference in volume proportion between flat celiac, and flat disease-control, mucosae (Fig. 6).

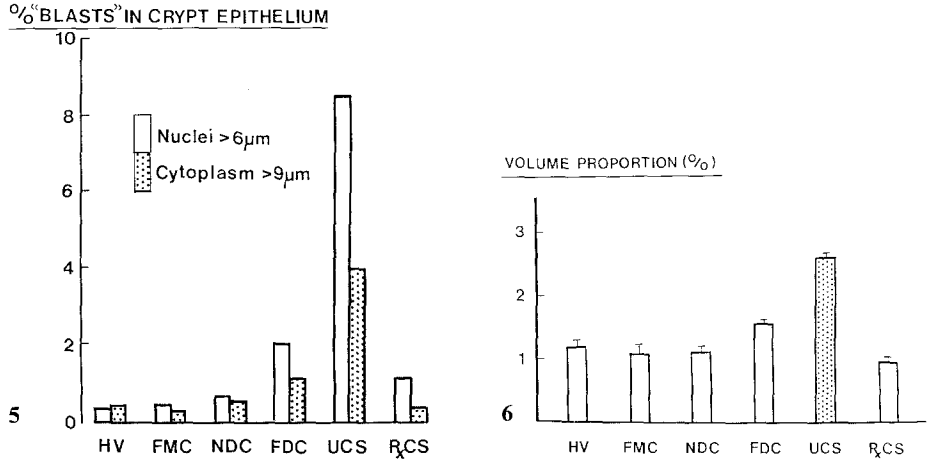

Fig. 5. This diagram shows the number of large, crypt-epithelial lymphocytes (expressed as percentage of either nuclei $>6\,\mu m$, or cytoplasmic diameter $>9\,\mu m$ of total lymphocytes (N)) per group of specimens. In general, lymphocyte nuclear diameters give a more accurate assessment of the size of these cells compared with cytoplasmic diameters, which are subject to greater sectioning errors

Fig. 6. This diagram illustrates the volume of crypts occupied by their respective lymphocyte populations. Total lymphoid volume is expressed as $N \times \bar{V}_{CELL}$. Despite the hypertrophy of crypts in untreated celiac sprue (*UCS*) the % lymphoid occupancy is considerably greater than that in all other mucosal groups

Table 2. Correlations between nuclear and cytoplasmic diameters for crypt epithelial lymphocytes in each group of mucosal specimens

	HV	FMC	NDC	FDC	UCS	R_xCS
Correlation coefficient (r)	0.81	0.79	0.75	0.79	0.79	0.78
Significance value (P)	0.001	0.001	0.001	0.001	0.001	0.001

If the mean volume ($0.54 \times 10^6\ \mu m^3$) for all "normal" control crypts (ie Groups I-III) is scaled up to that of untreated celiac sprue mucosae ($1.7 \times 10^6\ \mu m^3$), the proportional increase in N (~ 100 EL of mean volume 180 μm^3) still results in a 1% volume occupancy, compared with the 2.5% volume proportion of EL in celiac crypts.

Discussion

These data illustrate the application of a computerised image analysis technique to the morphometric study of small intestinal mucosa. Here we describe results obtained for crypt epithelial volumes and their lymphocyte populations in four groups of control individuals in comparison with untreated, and treated, celiac sprue patients.

From the data presented (Fig. 3) it is evident that the crypts in untreated

celiac spure mucosae contain an expanded population of lymphocytes that is increased approximately 5-fold over 'normal' control mucosae and 3-fold over non-celiac "flat" disease-control mucosae. The increase in celiac lymphocytes is not explicable solely in terms of an expanded crypt volume, because if the mean volumes of the control mucosae are scaled up, proportionally, to that of untreated celiac crypts, the latter still contain a marked excess of lymphocytes which is therefore presumably gluten-dependent. This conclusion is consistent with the marked fall in crypt lymphocytes during gluten withdrawal (Fig. 3).

In addition, our data provide other grounds for supposing that the crypt lymphocytes in celiac sprue patients are influenced by gluten (Table 1). Such lymphocytes were markedly larger than those in the other four control groups, when assessed in terms of nuclear or cytoplasmic diameter, or nuclear and total cell volume. Following gluten withdrawal, the size of the celiac lymphocytes became indistinguishable from those of other mucosae. The higher percentage of large lymphocytes in epithelium of untreated celiac sprue mucosae may be related to their increased rate of "turnover".

When all groups were examined (Table 2) a highly significant correlation was found between nuclear and cytoplasmic diameters. This confirms our previous experience that evaluating nuclear size is a sensitive measure of the overall dimensions of epithelial lymphocytes. Furthermore, lymphocyte nuclei are easier to measure, because of their denser staining and more nearly-circular profiles observed in 1 μm tissue sections. They are probably also less subject to sectioning bias, simply because they are smaller than overall cytoplasmic diameters (Marsh 1980). The latter are also considerably more distorted by the presence of adjacent epithelial cells, so that in sectioned profile, their cytoplasmic borders are rarely circular. For these reasons, we have found the estimation of corrected nuclear profile diameters to accurately reflect the overall size of any lymphoid cell population (Marsh et al. 1983).

The demonstration that crypt lymphocytes are enlarged runs parallel with previous findings for surface epithelial lymphocytes in celiac sprue (Marsh 1980). Morphologically, the shift-to-the-right in the mean cell diameter is caused by an increased fraction of probably immature large lymphocytes, which reveal larger euchromatic nuclei containing multiple nucleoli (Fig. 4). From our observations it has become apparent that the nuclear, and cytoplasmic, diameters of such cells exceed 6 μm, and 9 μm, respectively. These values are approximately +2 SD above the means of lymphocytes within the normally-structured control mucosae (HV:FMC:NDC). The number of cells exceeding these dimensions, expressed as a percentage of the total (N), was calculated for each group (Fig. 5) the average for the normal control groups being approximately 0.5% large lymphocytes. In comparison, there were 4% based on cytoplasmic morphology, and 8.5% when related to the more accurate determination of lymphocyte nuclear profile diameters. These figures are comparable to those determined for celiac surface epithelial lymphocytes where, on average, a mean increase of 5–6% immature lymphocytes was observed.

It may seem paradoxical that a marked rise in lymphocytes occurs within crypt epithelium without apparently affecting its functional capacities. There is considerable evidence for the "fitness" of crypt epithelium in its ability to match the 6-fold increase in the rate of enterocyte desquamation (Pink et al. 1970; Jones and Peters 1977) with a vast hypertrophic response occasioned by (i) an upward expansion of the growth fraction (ii) a shortening of the actual duration of mitosis (iii) a diminution of the inter-mitotic interval and (iv) an increased rate of cell migration (Trier and Browning 1970; Watson and Wright 1974). Furthermore, crypt differentiative capacity towards production of other cell types, such as enterochromaffin, goblet and Paneth cells, appears to be unaffected by gluten.

The view is widely held that the celiac lesion results from damage to surface epithelium by a gluten-induced lymphocytic infiltrate, although its validity still awaits formal proof. Since the phenotypic characteristics both of surface and crypt epithelial lymphocytes (Selby et al. 1983; Cerf-Bensussan et al. 1983) are identical, it is perhaps difficult to envisage why such lymphocytes apparently injure surface epithelium only, while leaving the crypts unscathed (Marsh 1985).

Our observations here shown that lymphocytes occur predominantly in the upper one-half to one-third of crypts, a location that could conceivably be related to receptors expressed by maturing enterocytes in these regions of the crypts. This interpretation is hardly consistent with the proposal that oligomannosyl-glycoprotein gluten receptors are largely expressed by immature crypt cells (Köttgen et al. 1982).

On the other hand, the localisation of lymphocytes coincides strikingly with the distribution of Ia (DR-like) surface membrane proteins in flat celiac mucosae (Scott et al. 1981). Possibly the lectin-like properties of gluten (Douglas 1976) either by altering enterocyte HLA determinants or associating with displayed DR proteins (Berke et al. 1983), render enterocytes recognisable by the predominantly $T8^+$ (cytolytic/suppressor) effector lymphocytes within epithelium. Even so, the selective damage to surface enterocytes alone would have to be explained, perhaps, by suggesting that an insufficiently critical density of Ia molecules is expressed by crypt cells to permit their effective lysis.

Such speculative ideas must nevertheless be viewed in the context of our other studies of controlled gluten challenges whereby dose-dependent, time-related infiltrates, both of surface and crypt, epithelium by small lymphocytes occurred in the absence of demonstrable mucosal damage (Leigh et al. 1985a, b). These observations, together with the additional results presented herein, argue that epithelial lymphocytes may not be the sole, nor predominant, agents of mucosal destruction in celiac sprue disease.

References

Berke G, Rosen D, Moscovitch M (1983) T lymphocyte-mediated cytolysis. III – Delineation of mechanisms whereby mitogenic and non-mitogenic lectins mediate lymphocyte-target interaction. Immunology 49:585–502

Cerf-Bensussan N, Schneeberger E, Bhan AK (1983) Immunohistologic and immunoelectron

microscope characterization of the mucosal lymphocytes of human small intestine by the use of monoclonal antibodies. J Immunol 130:2615–2622
Cocco AE, Dorhmann MJ, Hendrix TR (1966) Reconstruction of normal jejunal biopsies: three-dimensional histology. Gastroenterology 51:24–31
Cooke WT, Holmes GKT (1984) Coeliac Disease. London, Churchill Livingstone
Dhesi I, Marsh MN, Kelly C, Crowe P (1984) Morphometric analysis of small intestinal mucosa. II – Determination of lamina propria volumes; plasma cell and neutrophil populations within control and coeliac disease mucosae. Virchows Arch [Pathol Anat] 403:173–180
Douglas AP (1976) The binding of a glycopeptide component of wheat gluten to intestinal mucosa of normal and coeliac human subjects. Clin Chim Acta 73:357–361
Giger H, Riedwyl H (1970) Bestimmung der Größenverteilung von Kugeln ans Schnittkreisadien. Biometr Zeitschr 12:156–165
Jones PE, Peters TJ (1977) DNA synthesis by jejunal mucosa in responsive and non-responsive coeliac disease. Br Med J 1:1130–1131
Köttgen E, Volk B, Kluge F, Gerok W (1982) Gluten, a lectin with oligomannosyl specificity and the causative agent of gluten-sensitive enteropathy. Biochem Biophys Res Commun 109:168–173
Leigh RJ, Marsh MN, Crowe P, Kelly C, Garner V, Gordon D (1985a) Studies of intestinal lymphoid tissue. IX – Dose-dependent, gluten-induced lymphoid infiltration of coeliac jejunal epithelium. Scand J Gastroenterol 20:715–719
Leigh RJ, Loft DE, Marsh MN (1985b) The lymphocytic infiltration of crypt epithelium in celiac sprue. Gastroenterology 88:A1471
Marsh MN (1980) Studies of Intestinal Lymphoid Tissue. III – Quantitative analysis of epithelial lymphocytes in the small intestine of human control subjects and of patients with celiac sprue. Gastroenterology 79:481–492
Marsh MN 1982) Studies of intestinal lymphoid tissue. IV – The predictive value of raised mitotic indices among jejunal epithelial lymphocytes in the diagnosis of gluten-sensitive enteropathy. J Clin Pathol 35:517–525
Marsh MN (1983) Immunocytes, enterocytes and the lamina propria: an immunopathological framework of coeliac disease. J R Coll Physicians Lond 17:205–212
Marsh MN (1985) Functional and structural aspects of the epithelial lymphocyte, with implications for coeliac disease and tropical sprue. Scand J Gastroenterol, 20 (Suppl 114): 55–75
Marsh MN, Haeney MR (1983) Studies of intestinal lymphoid tissue. VI – Proliferative response of small intestinal epithelial lymphocytes distinguishes gluten- from non-gluten-induced enteropathy. J Clin Pathol 36:149–160
Marsh MN, Mathan M, Mathan VI (1983) Studies of intestinal lymphoid tissue. VII – The secondary nature of lymphoid cell "activation" in the jejunal lesion of tropical sprue. Am J Pathol 112:302–312
Niazi NM, Leigh RJ, Crowe P, Marsh MN (1984) Morphometric analysis of small intestinal mucosa. I – Methodology, epithelial volume compartments and enumeration of inter-epithelial space lymphocytes. Virchows Arch [Pathol Anat] 404:49–60
Padykula HA, Strauss EW, Ladman AJ, Gardner FH (1961) A morphological and histochemical analysis of the human jejunal epithelium in non-tropical sprue. Gastroenterology 40:735–765
Pink IJ, Croft DN, Creamer B (1970) Cell loss from the intestinal mucosa: a morphological study. Gut 11:217–222
Rubin CE, Brandborg LL, Phelps PC et al. (1960) Studies of coeliac disease. I – The apparent identical and specific nature of the duodenal and proximal jejunal lesion in coeliac disease and idiopathic steatorrhoea. Gastroenterology 38:28–49
Scott H, Brandtzaeg P, Solheim BG, Thorsby E (1981) Relation between HLA-DR-like antigens and secretory component (SC) in jejunal epithelium of patients with coeliac disease or dermatitis herpetiformis. Clin Exp Immunol 44:233–238
Selby WS, Janossy G, Jewell DP (1983) Lymphocyte subpopulations in the human small intestine. The findings in normal mucosa and in the mucosa of patients with adult coeliac disease. Clin Exp Immunol 52:219–228
Smith MW (1985) Expression of digestive and absorptive function in differentiating enterocytes. Annu Rev Physiol 47:247–260

Trier JS (1983) Celiac Sprue: In: Sleisenger MH, Fordtran JS (eds), Gastrointestinal Disease, Third Edition. Saunders, Philadelphia, pp 1050–1069

Trier JS, Browning TH (1970) Epithelial cell renewal in cultured duodenal biopsies in celiac sprue. New Engl J Med 283:1245–1250

Watson AJ, Wright NA (1974) Morphology and cell kinetics of the jejunal mucosa in untreated patients. In: Cooke WT, Asquith P (eds), Coeliac Disease: Clinics in Gastroenterology, London, Saunders 3(1) pp 11–31

Weibel ER (1979) Stereological Methods, vol 1. New York, Academic Press

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