

Studies of the rectal mucosa in coeliac sprue: the intraepithelial lymphocyte

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SUMMARY The dynamics of the rectal surface epithelial lymphocyte and leucocyte response to wheat, gluten, and gliadin enema challenges in control individuals and in patients with coeliac sprue in remission is shown. There is a clear increase in intraepithelial lymphocytes and polymorphonuclear (PMN) leucocytes in response to these enemas in coeliac sprue, but not in controls. The peak response was at eight hours and cleared within 24 hours. There was no change in the crypt epithelium. These data add further support to the role of wheat, gluten, and gliadin in the pathogenesis of coeliac sprue, at least in the rectal mucosa.

Twenty five years ago, we showed that wheat, gliadin, and gluten enemas, given to individuals with coeliac sprue in remission while on a gluten free diet, resulted in a sigmoidoscopic, histologic, and sometimes a clinical reaction.¹ Normal individuals did not react to such enemas. These observations indicated that the rectal mucosa, as well as the intestinal mucosa, was involved in the disease process. Our histological observations were confined to changes seen in epithelial cells and to leucocyte infiltration of the lamina propria and epithelium. At that time we did not note, or appreciate, the role of intraepithelial lymphocytes (IEL) in the disease process.¹ We now recognise that IEL may have a role in the putative immunopathology of coeliac sprue.² There should, therefore, be caution in suggesting a diagnosis of coeliac sprue, if the intestinal biopsy of the patient while on a gluten containing diet fails to show an increase in IEL when expressed as a ratio to epithelial cells.^{2,3} The availability of the tissue sections from our original study makes it possible for us to further report the effect of wheat, gluten, and gliadin enemas on the rectal epithelial lymphocyte population in normal subjects and in individuals with coeliac sprue.

Methods

PATIENTS

One hundred and thirty five rectal suction biopsies were taken during the 13 experiments comprising this study. Wheat enemas were given to four coeliac sprue patients whose diets had been gluten free for three to 24 months; several months later, gliadin, oat, and corn enemas were administered to one each of the same patients. The diagnosis of coeliac sprue was established on the basis of the finding of a severe mucosal lesion in individuals with a malabsorption syndrome, and in whom there was a clinical response, and morphological response on intestinal biopsy, after the introduction of a gluten free diet. The morphological response was characterised as improvement in mucosal appearance to at least that of a mild or non-specific lesion. Clinical and morphological relapse after gluten challenge was shown in one of the patients. Gluten challenge was not given to the other three patients. The following enemas were given to five normal controls: wheat 2; gliadin 1; gluten 1; isotonic saline 1; and blood 1.

Each patient was given two enemas with an interval of one hour between enemas. The patient was instructed to retain each enema for half an hour. Two or three biopsy specimens were obtained before the enemas and at four, eight, and 24 hour intervals thereafter. Baseline biopsy specimens were omitted

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(regrettably) before the blood enema study. All patients were clinically well at the time of the studies and had no known acute illnesses during the preceding week.

Wheat, oat, and corn enemas were prepared by placing 50 g of the grain flour in 200 ml water and administering it immediately after thorough mixing in a Waring Blender. The pH of these enemas was approximately 6 – that is, the same as tap water in Seattle, WN. Two and one-half grams of gliadin and 5 g gluten in 200 ml water were used for each of these enemas. Two hundred millilitres of isotonic saline were used for each of the saline enemas. Twenty five millilitres of heparinised blood obtained from a normal control with an additional 25 ml saline were administered to this same control. All enemas were administered *via* a standard enema bag and were of a watery consistency.

The suction biopsy specimens were obtained at 10–15 cm from the anal verge and were fixed in Bouin's solution and processed routinely. Serial paraffin sections were cut at 4 μ m and stained with haematoxylin and eosin. Every third section was observed and the number of intraepithelial lymphocytes (IEL) and other non-epithelial cells was counted in relation to a total of 500 surface and 500 crypt epithelial cells including goblet cells. (Goblet cells are generally excluded when performing IEL counts in the small intestine²).

Counts were only made in those areas in which all of the epithelial and crypt cells were sectioned parallel to their length and in which none of the cells were tangentially sectioned.^{2,4} Areas in which there was trauma were not counted. Observations were made blind and all counts and observations were done at a magnification of 400 \times .

Epithelial lymphocytes were generally basally located, contained dense nuclei surrounded by a pale cytoplasm, and had no other distinguishing features. Lymphocytes that straddled the basal lamina were called transmigrating lymphocytes and were tabulated separately from IEL. Other intraepithelial cells included PMN leucocytes, eosinophil leucocytes, mast cells, and very rarely, plasma cells. These latter cells were easily identified by standard morphological criteria. Occasional intraepithelial monocytes and macrophages were also observed in the counted areas. These cells were larger than lymphocytes (which range in size from 5–9 μ m), contain a small indented nucleus with abundant cytoplasm, and often had apparent cytoplasmic inclusions. If there was doubt in classifying a cell as a lymphocyte *versus* a monocyte, it was considered to be a lymphocyte.

Mitotic counts were generally made in crypts that were transected their entire length, from surface to muscularis mucosae, and in which the lumen was

Table 1 *Surface epithelial lymphocyte response to enemas in normal controls*

Subject	Enema	Baseline	4 Hour	8 Hour	24 Hour
HW	Wheat	6.2 (0.3)	4.2 (1.4)	3.2 (1.1)	4.5 (1.5)
TT	Wheat	6.7 (1.7)	5.4	10 (3.1)	7.2
GS	Gluten	3.3 (0.7)	4.5 (0.7)	4.8 (0.8)	5.7 (3.6)
RJ	Gliadin	4.3 (1.3)	7.2 (4.2)	5.1 (3.3)	7.7 (0.7)*
CB	Saline	10.7 (2.9)	9.9 (4.6)	12.5 (4.0)	7.1 (1.8)
CB	Blood	—	18.5 (1.5)	16.5 (1.0)	12.3 (2.7)
Number specimens		14	14	16	16
Mean (SD) (all expts)		6.2 (3.1)	9.0 (6.0)	9.1 (5.3)	7.5 (3.2)

Data are reported as number of intraepithelial lymphocytes/100 epithelial cells and is expressed as mean (SD) for each time interval (usually two or three biopsy specimens) and also as mean (SD) for all data combined. *The response at each time interval was compared with baseline (individual and all experiments) for statistical significance and only the 24 hour response to gliadin was significantly different from baseline ($p < 0.02$).

centrally placed. Epithelial cells in mitosis were counted in these well oriented crypts and were counted by two methods. In the first, all mitoses per 100 well oriented crypts were tabulated. In the second, all mitoses per 1000 crypt epithelial cells were tabulated; the counts were done in 10 separate sections, 100 crypt epithelial cells being counted in each section. In the second method, counts were made in both well oriented and tangentially sectioned crypts. Mitotic counts were done on only a single biopsy specimen from each time interval (baseline, 4, 8, and 24 h). Very rarely an epithelial lymphocyte in mitosis was observed but these were too few to count. Statistical analysis of the data was done by Student's *t* test. Values are expressed as mean (SD).

Results

The number of IEL/100 surface epithelial cells in normal subjects is shown in Table 1. There was a statistically significant increase in IEL only after gliadin enemas and only at 24 hours. When all data were analysed collectively, enemas in normal subjects did not provoke an epithelial lymphocyte response. In contrast, wheat and gliadin enemas (Table 2) evoked a significant increase in surface epithelial IEL counts at eight hours in four of the five patients with coeliac sprue in remission. When the response to wheat enemas in four patients, or to wheat and gliadin enemas in five patients, was analysed collectively, there was a significant response at eight hours. Oat and corn enemas in two of the sprue patients did not result in increased numbers of IEL but resulted in a significant decrease in surface epithelial IEL in one of the subjects at 24 hours.

The number of IEL in the surface epithelium of baseline biopsy specimens of individuals with sprue

Table 2 Surface epithelial lymphocyte response to enemas in patients with coeliac sprue in remission

Subject	Enema	Baseline	p	4 Hour	p	8 Hour	p	24 Hour
GF	Wheat	20.7 (4.5)	NS	21.9 (7.9)	<0.01	54.4 (6.0)	NS	14.3 (1.2)
DK	Wheat	13.6 (0.6)	NS	16.4 (9.4)	<0.05	29.4 (3.5)	NS	16.6 (3.2)
JS	Wheat	8.8 (0.7)	NS	9.7 (3.4)	<0.01	18.1 (2.0)	NS	9.3 (1.9)
PH	Wheat	14.2 (4.0)	NS	18.4 (8.0)	NS	15.4 (5.0)	NS	14.6 (6.2)
GF	Gliadin	21.2 (1.9)	<0.05	45.9 (8.9)	<0.02	48.7 (8.5)	NS	26.9 (4.6)
JS	Oats	6.0 (0)	NS	6.9 (0.9)	NS	8.5 (0.8)	NS	9.3 (3.5)
PH	Corn	12.9 (3.8)	NS	5.0 (2.6)	NS	3.8 (0.8)	<0.05	5.5 (1.2)
All specimens 17*		14 (5.8)						
Wheat enemas 11*		14.4 (5.4)	NS	16.4 (7.7)	<0.02	29.2 (17.3)	NS	13.6 (3.9)
Wheat and gliadin 13*		15.5 (5.5)	NS	23.2 (15)	<0.02	33.5 (17.6)	NS	16.5 (6.8)

Data are reported as number of epithelial lymphocytes/100 epithelial cells and are expressed as means (SD) for each time interval (usually two or three biopsy specimens) and also as mean (SD) for all data combined. * = total numbers of biopsy specimens. NS = not significant.

14 (5.8) was significantly higher ($p < 0.01$) than that of baseline biopsy specimens of control subjects 6.2 (3.1).

The number of IEL/100 crypt epithelial cells before enemas was 4.0 (2.4) in control and 4.4 (2.0) in coeliac sprue and there was no significant change in numbers of crypt IEL in control or sprue at any time interval. In controls there were 4.2 (2.3), 4.0 (1.8), and 3.7 (1.5) IEL/100 crypt epithelial cells at four, eight, and 24 hours, while in coeliac sprue there were 4.1 (1.4), 4.1 (2.1), and 4.7 (1.8) at four, eight, and 24 hours after the wheat and gliadin enemas.

Other cells observed in the surface epithelium in coeliac sprue included one mast cell and two plasma cells. Similar cells were not seen in the epithelium of controls. Few leucocytes were seen in the crypt epithelium and these consisted of one PMN leucocyte at eight hours, and eight eosinophils and one PMN at 24 hours in coeliac sprue, and only a single eosinophil at four hours in control.

Mitotic counts (Table 3) were not significantly different after enemas in both control and coeliac sprue. The data were similar whether counts were made per 100 crypts, or per 1000 crypt epithelial cell nuclei, and thus only the first set of data is given. There were some interesting trends in mitotic counts

– that is, there was a transient early decrease in number of mitoses after wheat enemas in coeliac sprue but not in control, and the mitotic count was doubled at 24 hours in all groups.

In the surface epithelium, the number of transmigrating lymphocytes (cells crossing the epithelial basal lamina) increased in coeliac sprue and control (Figure, Table 4). There were far more transmigrating IEL in coeliac sprue baseline biopsy specimens than there were in control. The number of transmigrating IEL observed at each time interval were too few to analyse statistically. No lymphocytes were seen crossing the basal lamina of crypt epithelium in control or in coeliac sprue.

Occasional transmigrating leucocytes were observed in the surface epithelium. In coeliac sprue there was one monocyte at 0 hours, and one eosinophil and one monocyte at four hours while in control there was one monocyte and two eosinophils at eight hours, and one eosinophil at 24 hours. In the crypt epithelium, transmigrating leucocytes in coeliac sprue included one eosinophil at 0 hours, one PMN leucocyte at eight hours, and one PMN leucocyte and four eosinophils at 24 hours, whereas in control there were only two eosinophils at 24 hours. In all our observations (counted and uncounted areas) only a

Table 3 Number of epithelial cell mitoses/100 crypts in coeliac sprue and control

Subject	Baseline	4 Hour	8 Hour	24 Hour
Coeliac sprue				
All experiments (7)	99 (37)	109 (81)	123 (77)	248 (125)
Wheat only (4)	99 (39)	72 (30)	90 (40)	204 (140)
Wheat and gliadin (5)	92 (38)	65 (31)	103 (45)	245 (151)
Controls (6)	107 (85)	163 (109)	190 (84)	212 (102)

Data are expressed as mean (SD) for each time interval. Number in parentheses represents number of biopsy specimens counted for each interval. There was no significant difference when the data for each time interval were compared with baseline.

Table 4 Transmigrating lymphocytes in surface epithelium of coeliac sprue and control

Subject	Baseline	4 Hour	8 Hour	24 Hour
Coeliac sprue				
All experiments	2.6	3.8	3.4	2.4
Wheat only	2.3	4.2	3.7	2.0
Wheat and gliadin	2.7	4.4	3.9	3.1
Controls	0.7	3.2	2.7	2.3

Data are expressed as mean number of lymphocytes traversing the basal lamina in the count area (500 epithelial cells) per biopsy specimen. Transmigrating lymphocytes were not observed in the crypt epithelium.

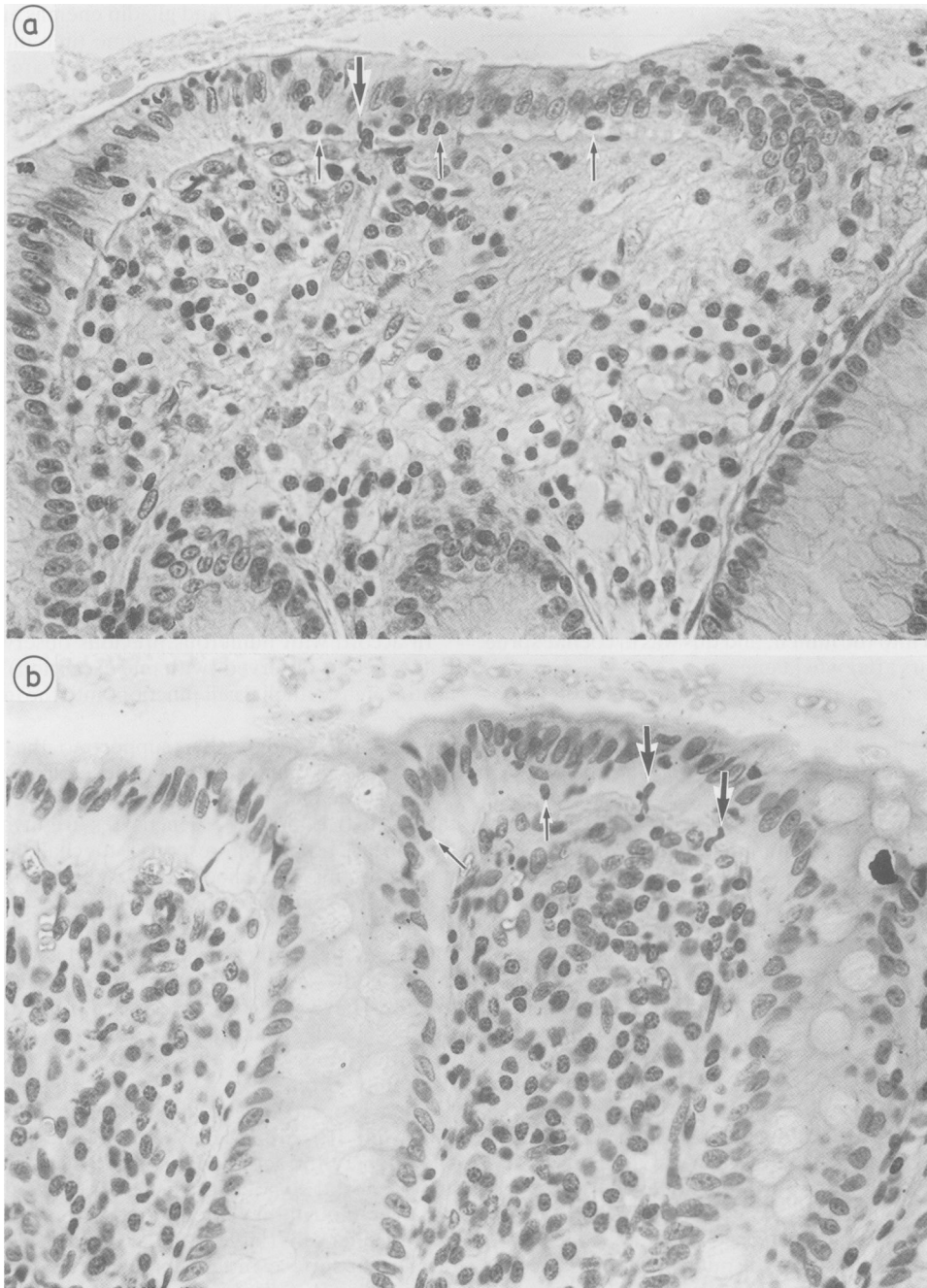


Figure (A) Light microscopic photomicrograph showing intraepithelial lymphocytes (small arrows) and transmigrating lymphocytes (large arrow) at 8 hours after wheat enemas given to a patient with coeliac sprue in remission. H & E. (B) Light microscopic photomicrograph showing transmigrating lymphocytes (large arrows) and intraepithelial lymphocytes (small arrows) at 8 hours after gliadin enemas in a normal individual. H & E.

Table 5 Other leucocytes observed in surface epithelium in coeliac sprue and control

Coeliac sprue	Baseline	4 Hour	8 Hour	24 Hour
All enemas				
PMN leucocytes	0.7	4.5	14.0	2.4
Eosinophils	1.2	2.6	2.8	1.8
Monocytes	0.1	0.3	0.1	0.1
Wheat enemas				
PMN leucocytes	0.1	6.6	21.3	2.2
Eosinophils	1.6	3.8	3.7	1.1
Monocytes	0.2	0.6	0.1	0.2
Oat and corn				
PMN leucocytes	2.8	0.4	3.6	3.3
Eosinophils	1.0	1.6	1.6	3.2
Monocytes	0	0	0	0
Control enemas				
PMN leucocytes	0	1.7	0.5	0.1
Eosinophils	0.4	1.7	1.5	2.4
Monocytes	0	0	0.1	0.1

Data are expressed as the mean number of individual leucocytes in the count area (500 epithelial cells) per biopsy specimen. Few leucocytes were present in the crypt epithelium.

single lymphocyte was observed apparently being extruded into the lumen, and this was in coeliac sprue eight hours after wheat enemas.

The surface epithelial PMN and eosinophil leucocyte response (Table 5) paralleled the lymphocyte response in both coeliac sprue and control. We emphasised this aspect, while ignoring the IEL, in our original paper.¹ The peak response was at eight hours and had largely cleared in 24 hours in coeliac sprue, and there were minimal changes in control.

Discussion

We report the dynamics of the epithelial lymphocyte in response to wheat, gluten, and gliadin enema challenges in patients with coeliac sprue in remission by using rectal biopsy specimens obtained for studies done 25 years ago. We have shown that there was a clear increase in intraepithelial lymphocytes and PMN leucocytes in response to these enemas in coeliac sprue, but not in control. The peak response was at eight hours, and it cleared within 24 hours. Other grains (oat and corn) did not evoke a response in coeliac sprue, or in controls. Because crypt hyperplasia with an increased epithelial cell mitotic index is characteristic of the intestinal mucosal lesion of coeliac sprue, the number of epithelial cell mitoses were also counted. The mitotic response, when expressed as number of mitoses per 100 crypts, doubled at 24 hours after enemas in both coeliac sprue and control individuals. Although the differences were not significant, the enemas in control

individuals appeared to stimulate mitotic activity at four hours while wheat and gliadin enemas appeared to inhibit such activity at the same time interval in individuals with coeliac sprue. There are limited studies of crypt mitosis in the jejunum in the one to two days after gluten challenge, most studies being carried out in organ culture.^{5,6} These studies do show an increased mitotic response to gluten challenges.

The number of lymphocytes traversing the basal lamina, presumably entering or leaving the epithelial compartment, was three to four times that of control in baseline biopsy specimens of coeliac sprue. In contrast, the number of lymphocytes traversing the basal lamina was proportionately greater after enemas in control than in coeliac sprue. Because there was an increase in IEL counts in coeliac sprue only, presumably more of the lymphocytes entered than departed the epithelial compartment in coeliac sprue than in control, but additional studies using radio labelled lymphocytes are necessary to answer this question. Marsh has shown in mouse, using radioautography of tissue sections containing tritiated thymidine labelled lymphocytes, that there was clearly bidirectional traffic of lymphocytes in the epithelium.⁷

In accord with numerous previous observations,² IEL were not observed (with one exception) extruding through epithelial cell junctions into the intestinal lumen.

These data add further support to the role of wheat, gluten, and gliadin in the pathogenesis of coeliac sprue (at least in the rectal mucosa) and show that both IEL and PMN leucocytes are involved in the surface epithelial response to challenge with these proteins in coeliac sprue in remission. There was no change in the crypt epithelium.

The function role of IEL is still not defined.³ Intraepithelial lymphocytes have been studied mainly in the small intestine and theories as to their function have been largely based on studies in rodents.^{8,9} Cell separation techniques have recently permitted functional studies of IEL obtained separately from other mucosal lymphocytes.^{3,10} Lamina propria T cells are predominantly helper cells while IEL are T cells largely of the suppressor phenotype. Intraepithelial lymphocytes are not activated, and do not express class II MHC antigens, and even though they often have the morphology of natural killer cells (large granular lymphocytes), they rarely bear surface antigens characteristic of natural killer cells. B cells are not usually found in the epithelial compartment. We did observe three plasma cells in the rectal epithelium of coeliac sprue. Macrophages, mast cells, PMN, and eosinophil leucocytes constituted a distinct minority of cells found in the normal and coeliac sprue epithelium, while there was

a clear PMN leucocytic response to the wheat enemas in coeliac sprue.

Intraepithelial lymphocytes when expressed as a ratio to intestinal epithelial cells, are clearly increased in number in coeliac sprue.^{2,3} In coeliac sprue, the helper to suppressor ratio of IEL is unchanged when compared with that of normal intestine although the proportion of suppressor IEL expressing phenotypic differences in T cell markers is significantly different when compared with normal.¹⁰⁻¹² Intraepithelial lymphocytes may play an immunoregulatory role possibly by suppressing the mucosal immune response (lamina propria) which is predominantly helper in direction. In coeliac sprue, one may speculate that the IEL inhibits the helper immune response to gluten gliadin antigenic challenge, a response that may be injurious to the host epithelium in the form of autoimmunity.

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