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Mast cell degranulation after a single dose of gliadin in the jejunum of patients with coeliac disease

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The exact pathomechanism of jejunal damage caused by gliadin in coeliac disease has not been clarified. 0.3 g of gluten per kg bodyweight was administered to 14 children affected by coeliac disease, being on gluten-free diet, five hours before jejunal biopsy. There was no change in the number of intraepithelial lymphocytes and eosinophils while cellular infiltration of the lamina propria exhibited a marked increase and the number of mast-cells per tissue unit significantly decreased as compared with controls. It is concluded that mast-cells may play an important role in the mechanism of gluten induced jejunal damage.

The central event in coeliac disease is a jejunal damage elicited by gliadin in sensitive individuals. While on gliadin containing diet they develop villous atrophy, epithelial flattening, increased synthesis of epithelial cells and increased cellular infiltration of the mucosa. Since the exact pathomechanism of gliadin sensitive enteropathy is not clear, the large number of hypotheses is not surprising. Recently, the eventual role of mast-cells has been put forward [6].

Mast cells, originating from the mesenchyma, play an outstanding part in tissue growth and regeneration and they participate in inflammatory and immunological processes as well. They are found in great numbers in organs exposed to the external environment. They contain granules which markedly influence adjacent tissues if released from the cell. A decrease of the mast-cell number has been observed in the jejunum of patients affected by coeliac disease if continuously exposed to gliadin [4, 5], and increased tissue levels of histamine have been observed under such circumstances [2].

This has prompted us to investigate the effect of a single dose of gliadin on the number of jejunal mast-cells in children with coeliac disease keeping a gliadin free diet and undergoing routine rebiopsy.

Material and Methods

Study group. This comprised 14 children ranging in age from 7 to 15 (mean, 10) years in whom persistence of gliadin sensitivity in clinical remission induced by gliadin-free diet had been tested by reintroduction of gluten, without performing a second biopsy (this was the case before the publication of ESPGAN criteria [7]) and all patients had shown exacerbation of coeliac disease symptoms after the test. The second biopsy prescribed by the ESPGAN

principles was now performed in order to test the effect of gliadin-free diet. The children had been keeping the diet for 2 to 6 years.

Control group with coeliac disease. 13 patients with confirmed coeliac disease were selected on the basis of histological findings observed at their second jejunal biopsy, comparable with the findings of the study group in respect of main parameters, villus/crypt height ratio and intraepithelial lymphocyte count. Their mean age was 5.4 years, with a range from 3 to 12 years.

Healthy control group. 11 children admitted for evaluation of stunted growth in whom jejunal biopsy demonstrated normal findings. Their mean age was 6.6 years ranging from 1 to 13 years.

The children of the study groups received 0.3 g/kg bodyweight gluten (Aleuronat, Blattmann Co). Jejunal biopsy was performed by a paediatric Watson capsule 5 hours later. The specimen was taken from the duodeno-jejunal junction, fixed in formol and embedded in paraffin. 5μm sections were stained with methylene blue and examined by microscopy, using an ocular lens provided by a rectangular grid, at 640-fold magnification. Mast-cells and eosinophils were counted in 1 mm^2 areas. Cells sited in both the villi and between the crypts were counted. The number of intraepithelial lymphocytes per 100 epithelial cells was calculated, and the ratio of villus and crypt height was also measured.

Results

Figure 1 shows the number of intraepithelial lymphocytes. There was no difference in this respect between the study group and the control group of patients affected by coeliac disease. Similarly, there was no difference in the villus/crypt height ratio (mean

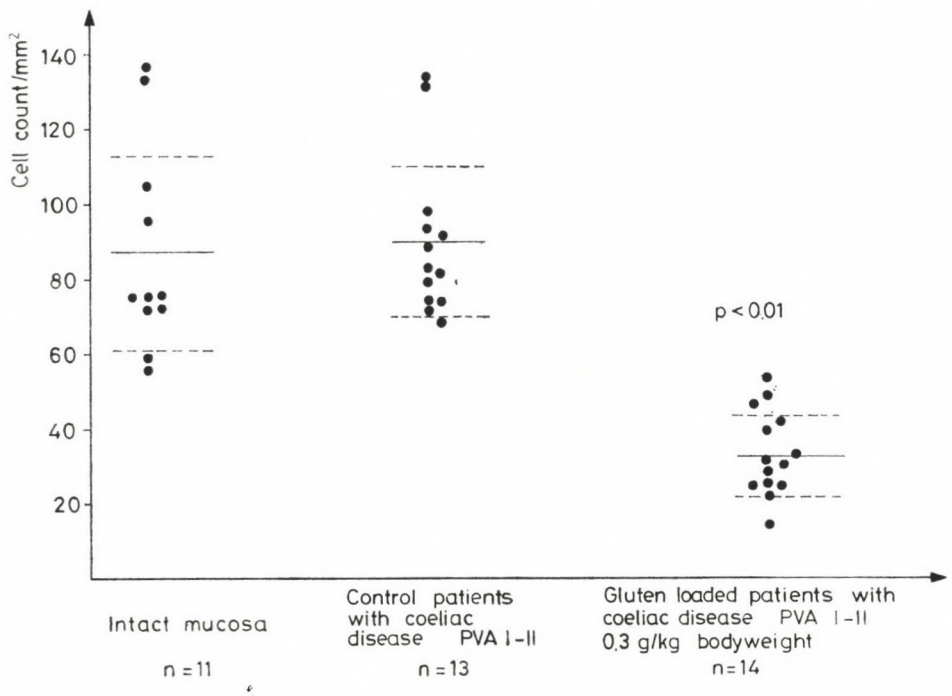

Fig. 1.

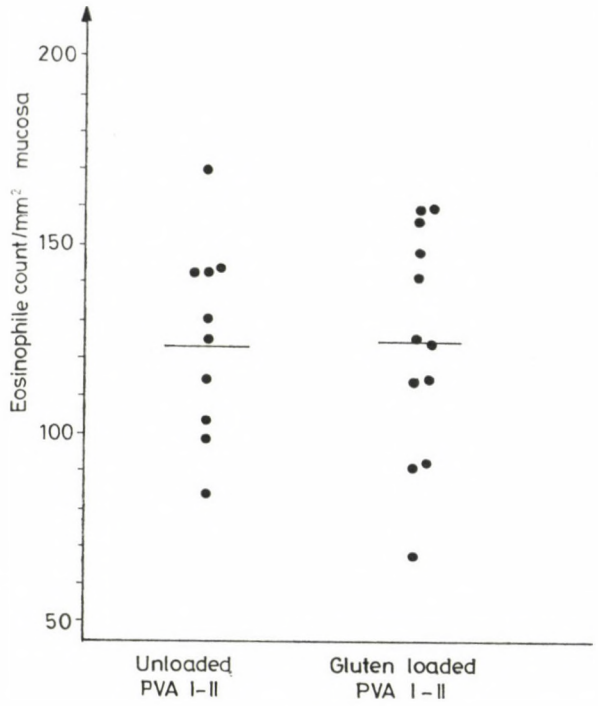
200
150
100
50
Eosinophile count/mm² mucosa
Unloaded
PVA I-II
Gluten loaded
PVA I-II

FIG. 2.

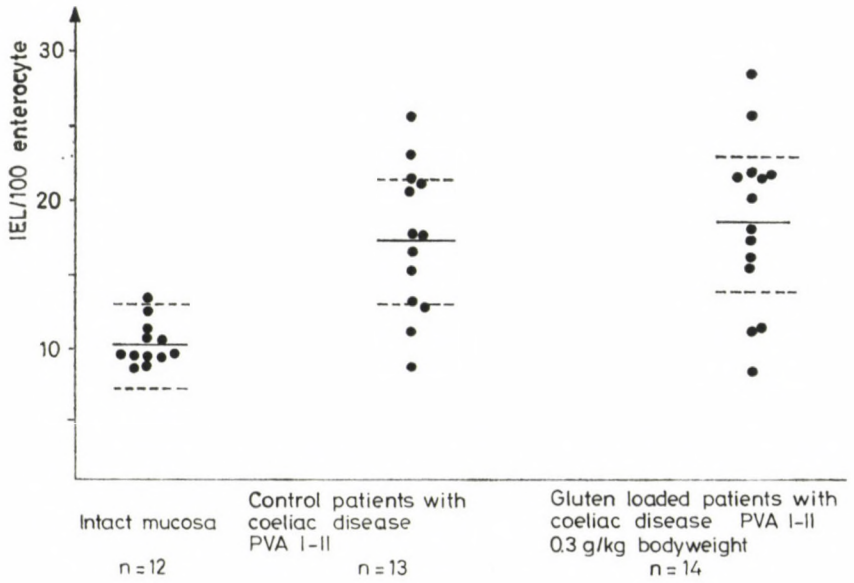
30
20
10
IEL/100 enterocyte
Intact mucosa
n = 12
Control patients with coeliac disease PVA I-II
n = 13
Gluten loaded patients with coeliac disease PVA I-II 0.3 g/kg bodyweight
n = 14

FIG. 3.

ratio = 2.45 in coeliac controls and 2.6 in the study group).

There was, however, a difference of cell count per 1 mm² lamina propria. In the coeliac control group this value was 1.23 times while in the study group 1.92 times higher than in the healthy controls. This means that in sensitive patients the single gliadin load provoked an increase in cellular infiltration of the lamina propria (Fig. 2).

In addition, significant changes in the mast-cell counts were encountered (Fig. 3). This figure was identical in healthy and unloaded children with coeliac disease (mean, 89.7 ± 20 SD and 87 ± 26 SD, respectively) while 5 hours after gliadin loading markedly lower values were obtained (mean, 32.4 ± 10.5 SD); this represents a significant difference ($p < 0.01$).

The findings are summarized in Table I.

TABLE I

All values are expressed as means ± SD

	n	IEL per cent	Height ratio villus/crypt	Cell count per mm²	Eosinophil count/mm²	Mast-cell per mm²
Healthy controls	11	10.4 ± 3.2	3.4	1800	131 ± 27.6	89.7 ± 20
Control patients with coeliac disease	13	17.2 ± 4	2.45	1990 ± 499	125 ± 24.4	87 ± 26
Gluten loaded patients with coeliac disease	14	18.5 ± 4.3	2.6	3460 ± 843	124.6 ± 29	32.4 ± 10.5
p. between coeliac loaded patients and controls		N.S.	N.S.	$p < 0.01$	N.S.	$p < 0.01$

IEL: intraepithelial lymphocyte
All values are expressed as means ± SD

DISCUSSION

Serial biopsies performed in patients with gluten sensitive enteropathy after a single load of gliadin showed pronounced changes in the jejunal mucosa within a few hours [1, 3]. These observations have made us to perform a single biopsy five hours after the ingestion of gluten.

The dose and nature of the gliadin fraction used in loading is not indifferent. Anand et al. [1] applied a large dose (40 g) of fraction B while Marsh et al. [6] used a relatively small dose of digested gluten (100—1500 mg). In this study gluten, containing all gliadin fractions, was used in a dose of 0.3 g/kg body weight, recommended for combined gliadin-xylose loading tests [9] and the degree of pathological changes is probably influenced by the dose of gliadin. Dose-dependence was encountered in the intraepithelial lymphocyte count 12—48 hours after loading [6], but in this study no similar change was demonstrated 5 hours after ingestion of the provoking agent.

A characteristic change induced by

gluten loading is an increased cellular infiltration of the lamina propria [1, 3]. In this study the increase was attributable to an increase in the number of lymphocytes and polymorphonuclear granulocytes but not of eosinophils. Kósnai et al. [5] observed a significant increase in eosinophil count in untreated children with gluten sensitive enteropathy; this has been confirmed in the present study. In order to determine the exact time of onset of an increase in tissue eosinophilia, serial biopsies would have been necessary but we did not perform biopsies before loading for ethical reasons. Similarly, serial biopsy after loading does not seem justified in children. Moreover, serial biopsies performed by the blind method may furnish misleading results since it may happen that a subsequent specimen is taken from the zone of reaction of the preceding biopsy. For these reasons we preferred to compare the findings of the study group with those obtained in unloaded children with treated coeliac disease exhibiting comparable histology.

Marsh [6] suggests that oedema of the lamina propria, damaged basal membrane, epithelial detachment and necrosis ("intraepithelial bleb"), fibrinogen deposition due to increased vascular permeability and erythrocyte aggregation all point to the role of mast-cells in the pathomechanism of gliadin induced jejunal damage. Dollberg et al [4] showed mast-cell degranulation following gluten loading while Challacombe and Dawkins [2] found increased tissue histamine levels. It is well known that the bulk of histamine in the intestinal wall is present in the mast-cells [8]. Kósnai et al [5] have shown that the number of mast-cells is significantly lower in untreated patients than in children with treated coeliac disease. Strobel et al [11], however, obtained results contradicting those of Dollberg et al. [4] and Kósnai et al [5]: they demonstrated increased mast-cell counts in the jejunum of untreated patients with gluten sensitive enteropathy. Strobel et al [11] explained this difference by the fixation technique being different in these studies [10] and in addition there are more immature mast-cells with a low proteoglycan content if the condition is untreated and fixation and staining properties of these young mast-cells may be quite different. The high histamine level in untreated patients is more compatible with an increased number of mast-cells. In our opinion, there may be an additional factor: mast-cells exhibit a refractory period after degranulation and during this period the granules are resistant to staining. It is not beyond imagination that some authors happened to fix their specimens during the refractory period while others did that thereafter.

In this study, fixation of the specimen was carried out 5 hours after loading, i.e. certainly during the refractory phase following degranulation. The role of mast-cells has been confirmed once again since their number — more correctly, the number of mast-cells susceptible to staining — appeared to be markedly reduced

after the single dose of gliadin. In our study we applied a traditional fixation and staining method subjecting the specimens to staining at the same time; materials from all groups were fixed by the same method. At the moment we cannot decide whether degranulation is performed via IgE mediation or by non-immune reactions. This would need additional studies.

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