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Scanning Electron Microscopy of the Small Intestine during Gluten-Challenge in Celiac Disease

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Summary. **The celiac disease syndrome is characterized by structural and ultrastructural alterations of the small intestine mucosa. According to criteria by European Society of Paediatric Gastroenterology and Nutrition, the conclusive diagnosis of celiac disease in children depends on the demonstration of histological relapse of the mucosa after reintroduction of gluten in the diet, as this syndrome is a permanent condition of gluten intolerance. Under these diseased conditions, the structure of the intestinal villi has been studied by light microscopy; morphological alterations were revealed only when the gluten challenge induced a clinical relapse.**

Scanning electron microscopy analyses of the intestinal mucosa in celiac diseased patients showed a strikingly uniform destruction of the villi with changes in their dimensions and arrangement. At high magnification the enterocytes were irregular in size and shape with a decrease and disruption of the glycocalyx. Reductions in length and density of microvilli were also clearly identified.

Although these scanning electron microscopy findings could not demonstrate a relationship between the degrees of mucosal atrophy and the duration of the gluten challenge, they nevertheless revealed early stages of fine villous alterations that cannot be detected by the presently employed low resolution light microscopic techniques.

Celiac Disease syndrome (CD), also known as gluten-sensitive enteropathy, is a nutritional intolerance that is produced by the presence of gluten in foods ingested. It is one of the most common causes of chronic diarrhea, abdominal pain and growth retardation in infants and children.

CD is characterized by structural and ultrastructural lesions of the small intestine mucosa. These lesions consist of total, subtotal or partial villous atrophy and crypt hyperplasia (SHINER and DONIACH, 1960). When gluten is withdrawn from the diet, clinical features improve, and in 12-24 months the morphological alterations tend to disappear. As this syndrome is a permanent expression of gluten intolerance, the ultimate diagnosis, following criteria established by the European Society of Paediatric Gastroenterology and Nutrition (ESPGAN), is the histological confirmation of mucosal relapse after gluten has been reintroduced into the diet (WALKER SMITH et al., 1990).

At times the diagnosis may be difficult to determine in subjects with no classical signs, and CD may be ruled out for a long period (MAKI et al., 1990).

During gluten challenge, the structure of the intestinal villi has been studied by light microscopy (LM); unfortunately, the related alterations were mostly revealed only when they induced a clinical relapse.

Recently a series of simple and non invasive tests have been proposed as an alternative to jejunal biopsy, for ameliorating the diagnosis of CD during gluten challenge (UNSWORTH et al., 1983; GRECO et al., 1987). Nevertheless, at present, no test has shown sufficient effectiveness in substituting small intestinal biopsy in making the clinical diagnosis (WALKER-SMITH et al., 1990).

Some early scanning electron microscopy (SEM) observations of jejunal biopsies taken from celiac patients were found to be more effective than LM in revealing morphological lesions of the mucosal surface (HALTER et al., 1982; CARPINO et al., 1985).

The aim of this study was to evaluate the usefulness of SEM in determining the earliest lesions in the intestinal mucosa of patients undergoing gluten challenge.

PATIENTS AND METHODS

We took twenty-six jejunal intestinal biopsies from 13 children (5 boys and 8 girls), their ages ranging between 2 and 9 years with the average age being 5 years, 4 months. The biopsies were examined during gluten challenge in order to confirm the diagnosis of CD; informed consent by the parents was obtained in each case.

Gluten challenge was performed by the uncontrolled administration of gluten-containing food. For monitoring the duration of the challenge, we evaluated the increase of serum levels of IgA or IgG AGA; the serum AGA levels were assessed according to a standard immunoenzymatic method (VOLTA et al., 1985).

The patients were placed into 4 groups based upon the duration of gluten challenge. Two mucosal biopsies obtained from the ligament of Treitz were taken from each patient, one at the beginning of gluten challenge and the second at various time intervals during challenge. After examination by dissecting microscope, the mucosal specimens were divided into two portions, one processed for classical histology and the other for SEM.

The biopsies prepared for SEM were fixed in 2.5% glutaraldehyde (0.1 M cacodylate buffer at pH 7.4) for 48 to 72 h. The specimens were washed for four hours in the same buffer and post-fixed for two hours with 1.3% osmium tetroxide and rewashed in the same buffer. Tissues were dehydrated by gradually increasing concentrations of acetone or alcohol and then critical point dried in liquid CO_2. They were then mounted on aluminium stubs, coated with gold or gold-palladium and viewed under the Cambridge stereoscan 150 scanning electron microscope, using an accelerating voltage of 15-25 kV.

Parallel hematoxylin-eosin and periodic acid Shiff (PAS) sections were observed by LM.

RESULTS

In the biopsies of the patients included in this study, we observed a normal jejunal mucosa before the reintroduction of gluten into the diet. In fact, at low power, the observations made by SEM showed a mucosal pattern characterized by villi with finger-like and mitten-like appearance (Fig. 1). The mucosal ridges were very close to each other, and the glandular crypt openings were not identifiable because they were hidden by villi; the epithelial surface was free of mucus and debris (Fig. 2). At medium power the cell borders were clearly identifiable, the enterocytes were convex and assumed a cobblestone appearance, goblet cells were clearly seen (Fig. 3).

In all children that underwent gluten provocation the presence of several lesions on the mucosal surface was found, indicating a persistence of gluten intolerance. The results of the morphological study of the jejunal samples and the serum antibody levels to

Table 1. Structure of jejunal intestinal biopsies during gluten-challenge in celiac disease.

Group	Pts	Sex	Age years	Gluten challenge time	AGA IgG	AGA IgA	LM	SEM
A	1 L. P.	F	6, 2	58 days	7.8	4.5	Total atrophy	Parallel crests
A	2 Q. M.	F	3, 8	64 days	2.9	9.0	Total atrophy	Parallel crests
A	3 H. A.	M	5, 3	67 days	0.8	3.9	Normal mucosa	Convoluted crests
A	4 D. V.	M	8, 3	69 days	3.1	2.5	Total atrophy	Circular crests
B	5 D. D.	M	6, 1	89 days	2.9	3.1	Total atrophy	Semicircular crests
B	6 A. M.	F	6, 10	93 days	0.2	Absent	Partial atrophy	Convoluted crests
B	7 P. D.	M	2, 0	93 days	2.1	3.2	Total atrophy	Flat mucosa
B	8 G. S.	F	2, 11	101 days	6.2	2.5	Total atrophy	Circular crests
C	9 L. M.	F	3, 4	174 days	Absent	1.1	Total atrophy	Semicircular crests
C	10 P. S.	F	3, 1	183 days	1.0	1.3	Partial atrophy	Convoluted crests
C	11 D. G.	F	4, 0	187 days	Absent	Absent	Total atrophy	Flat mucosa
D	12 P. R.	M	8, 11	281 days	Absent	Absent	Total atrophy	Semicircular crests
D	13 T. A.	F	6, 1	852 days	Absent	0.9	Normal mucosa	Convoluted crests

AGA: Anti-gliadin antibody

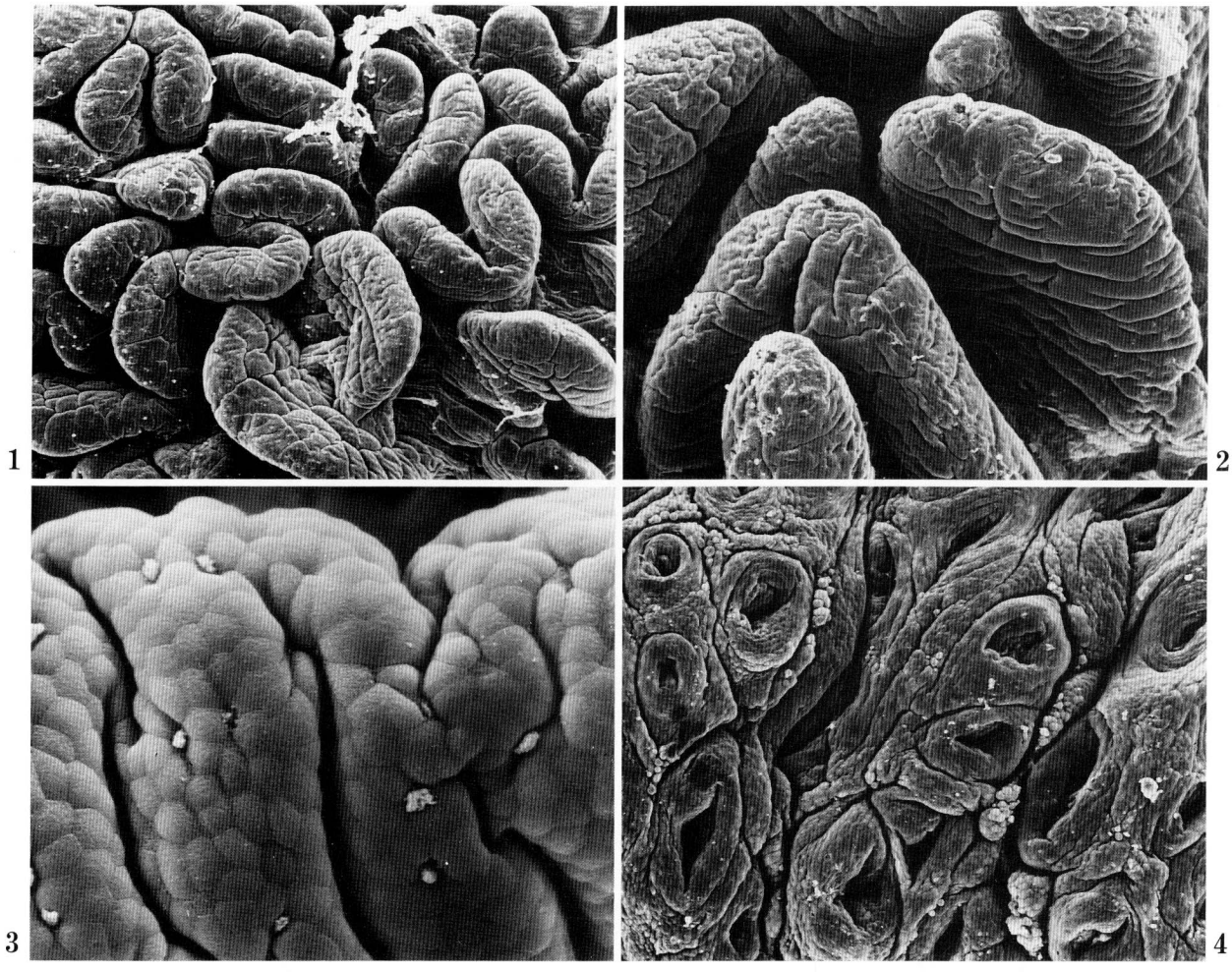

Fig. 1. Patient 4; gluten free diet: normal jejunal mucosa: the ridges are very close to each other. SEM, ×100

Fig. 2. Patient 10; gluten free diet: normal jejunal mucosa, finger-like and mitten-like villi are present. SEM. ×500

Fig. 3. Patient 7; gluten free diet: the enterocytes are polygonal and show a convex surface; goblet cells are also present. SEM, ×2,000

Fig. 4. Patient 4; gluten challenge for two months: total absence of jejunal villi, only round collars surronding the crypt openings are evident. SEM, ×100

gliadin are given in Table 1.

According to the study design, the modifications of serum levels of IgA or IgG AGA were considered as markers for monitoring the patients before performing the biopsy. Elevated IgA and IgG AGA levels were observed respectively in ten patients and in nine patients of thirteen children. Two children (patients 11 and 12) were both AGA negative at the time of biopsy although the mucosal specimens showed typical lesions of CD.

By LM, jejunal histology was grossly altered in eleven children, but two patients, numbers 3 and 13 respectively, had a normal villous architecture and epithelial surface as well.

SEM observations during gluten provocation showed various degrees of intestinal villous atrophy with changes in their dimensions and arrangement. In all cases, there was an alteration of the villi, although the degree of relapse did not always corresponded to the length of challenge. For example, patient 4 was rebiopsied two months after starting gluten ingestion, and had a flattened mucosal surface exposing large

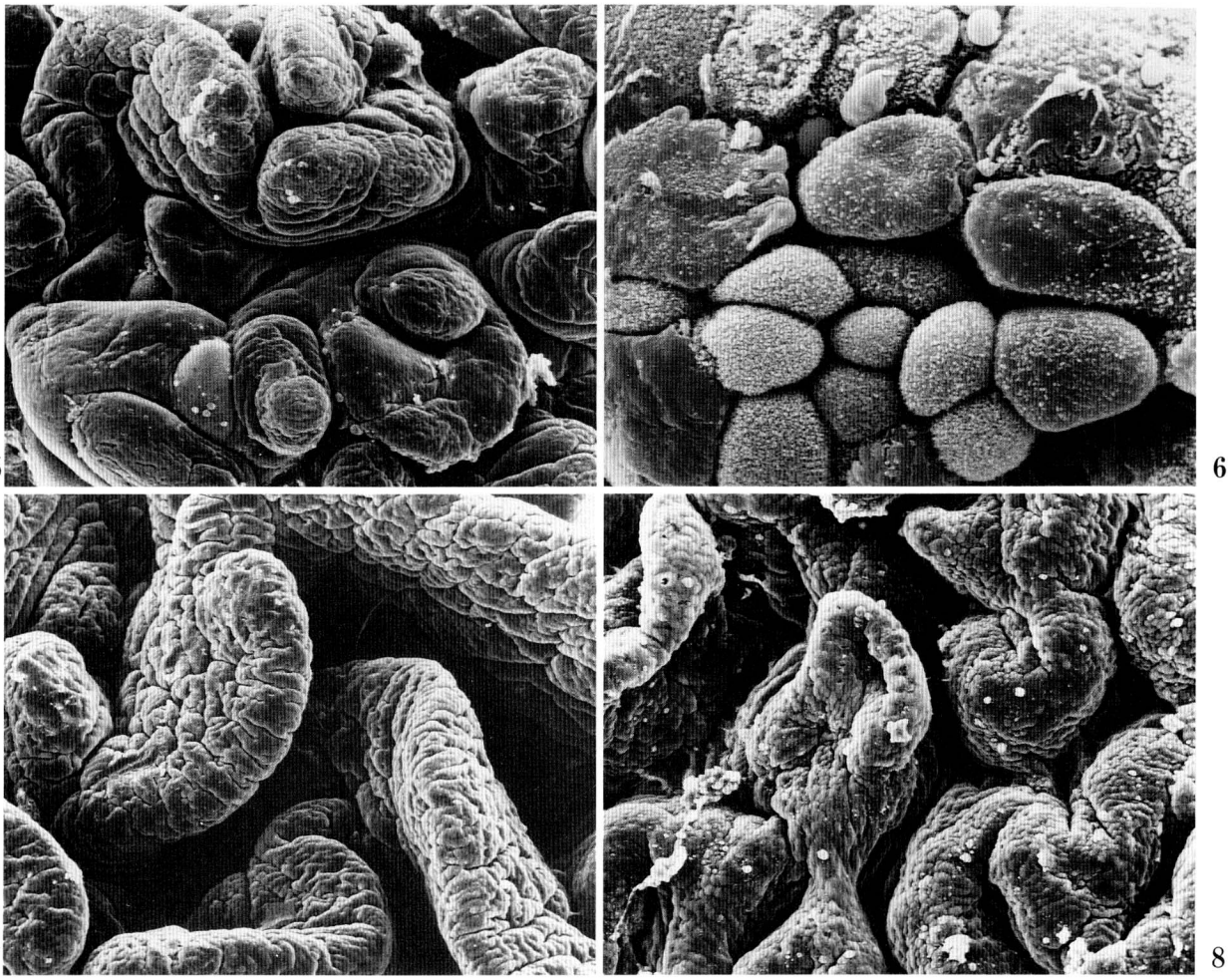

Fig. 5. Patient 10; gluten challenge for six months: the jejunal mucosal surface shows a cerebriform aspect. SEM. ×300

Fig. 6. Pateint 7; gluten challenge for three months: extensive alterations of the jejunal mucosa epithelium; surface shows enterocytes irregularly sized and shaped. SEM. ×5,000

Fig. 7. Patient 13; gluten free diet: normal mucosal pattern. SEM. ×500

Fig. 8. Patient 13; gluten challenge for twenty-eight months: the jejunal villi are low in height. SEM. ×300

and prominent crypt openings (Fig. 4). Patient 10, rebiopsied at six months, showed cerebriform ridges, with low and often fused villi; the crypt openings were not easily identified (Fig. 5). At high power and after challenge, SEM showed extensive alterations of the epithelium consisting of irregularly sized and shaped enterocytes and a conspicuous loss and disruption of the glycocalyx (Fig. 6).

In the last patient, after long term gluten challenge which lasted about 28 months, the IgG AGA serum levels were negative and the IgA AGA had borderline titers; in this patient a normal villous aspect was seen by LM. However, when checked by SEM, the mucosa was arranged in more regular ridges, but the villous morphology was lower when compared with a normal mucosa (Fig. 7, 8).

The diagram (Fig. 9) summarizes schematically the major structural changes recognizable in three consecutive small intestinal biopsies: 1) during gluten ingestion, 2) after a gluten free diet, and 3) after gluten challenge.

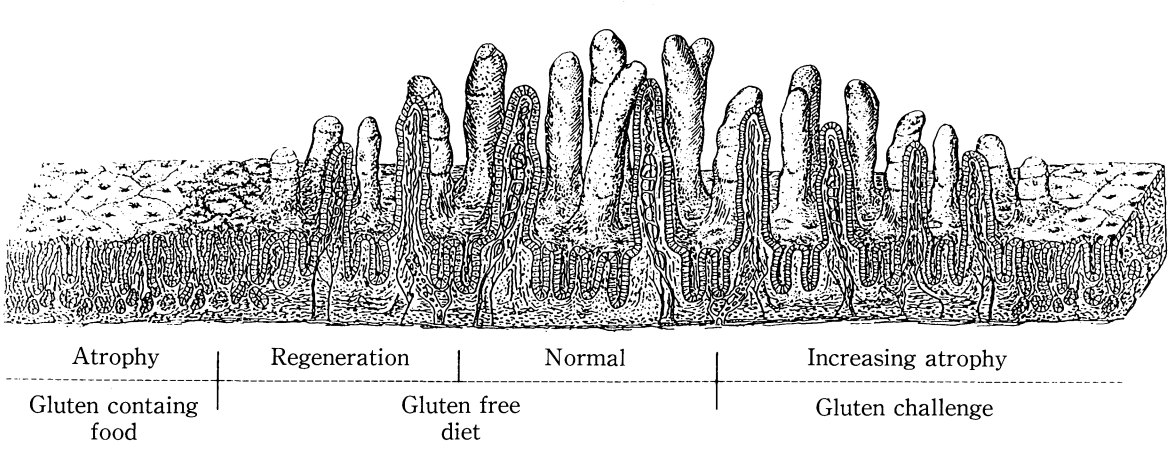

Fig. 9. Diagram of morpho-dynamic events present in the jejunal intestinal biopsies taken from CD patients.

DISCUSSION

At present, two methods have been widely used to achieve a conclusive diagnosis of persistent gluten intolerance: the dosage of serum levels of AGA (GRECO et al., 1987; MAYER et al., 1989; VALLETTA et al., 1990; BURGIN-WOLFF et al., 1991), and the observation of small intestinal biopsies by conventional histology (JUTO et al., 1985; KHOSOO et al., 1988; MONTGOMERY et al., 1988).

The sensitivity and specificity of the AGA test is usually more than 80% and shows wide individual variation in regard to time, type and quantity of the antibody produced (BRANSKI and LEBENTHAL, 1989; BURGIN-WOLFF et al., 1991). The serum IgA and IgG AGA titers shown in our study are in disagreement with these results, as we observed both IgA and IgG class of AGA in less than 65% of our patients after the reintroduction of gluten into the diet, although the biopsy examination showed clear morphological signs of gluten induced damage.

Only a few reports regarding the study of surface morphology by SEM during gluten provocation in patients with suspected CD have been published (STENLING et al., 1984; MAGAUDDA et al., 1989).

In this study we evaluated the usefulness of SEM in assessing intestinal biopsies taken during gluten challenge in order to confirm the diagnosis of CD. The finding of structural alterations in the small intestinal mucosa demonstrated by SEM after the ingestion of gluten-containing food was fundamentally the same as described in celiac patients during the mucosal improvement process secondary to gluten withdrawal (HALTER et al., 1982; CARPINO et al., 1985; MAGLIOCCA et al., 1987). Furthermore, we found no relationship in our patients between the duration of gluten challenge and the degree of intestinal mucosa atrophy; these data suggest a different individual reactivity of the mucosal surface to gluten provocation.

When the results from the jejunal biopsy are correlated in our study, SEM was found more sensitive than LM in documenting early morphological relapse. This observation supports previous works by indicating that at times the diagnosis of CD has been ruled out for long periods when biopsies were examined by LM (MAKI et al., 1990).

In conclusion, our results suggest that the use of AGA testing alone cannot replace small intestinal biopsy; however, it is effective in monitoring the time for the third and final biopsy necessary for the definitive diagnosis. In addition, SEM makes it possible to reveal early pathological changes in villous morphology prior to their detection by the presently used low resolution LM techniques.

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