Lymphocytic Oesophagitis Preliminary Ultrastructural Observations

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Abstract. *Background/Aim: Lymphocytic oesophagitis (LyE) is a newly described entity characterized by a high number of intraepithelial lymphocytes/ high power field (≥40 CD3+IELs/HPF) in the oesophageal epithelium. The aim of the study was to investigate possible ultrastructural changes taking place in LyE at the transmission electron microscopic (TEM) level. Materials and Methods: Oesopageal biopsies from seven patients were investigated: four were consecutive patients with LyE, one with reflux oesopagitis, one with eosinophilic oesopagitis (EoE) and one with histologically normal squamous epithelium. Results: In LyE, marked intercellular oedema (spongiosis) and a gamut of regressive changes were found in squamous cells, ranging from cytoplasmic oedema and vacuolization, to total cell disintegration. IELs also showed regressive changes ranging from ballooned, oedematous cytoplasm to signs of intracytoplasmatic disintegration. Conclusion: Besides hampered cell nutrition conveyed by spongiosis, putative noxious molecules contained in the intercellular spongiotic oedema might account for the dramatic TEM alterations found in LyE. The present findings provide, for the first time, "inside information" on the ultrastructural alterations taking place in LyE, both in squamous cells and in IELs.*

Dysphagia, that is difficulty in swallowing solid foods or liquids, is a common symptom to many oesophageal diseases. Dysphagia may be caused by a functional disorder of the smooth muscle layer and the lower oesophageal sphincter, by lumen narrowing, obstruction conveyed by inflammation, ulceration or other intraoesophageal or extraoesophageal causes (1).

In 2006, we reported a novel histologic phenotype of chronic oesophagitis in patients with dysphagia characterized by high numbers of intraepithelial lymphocytes (IELs) initially found gathering around peri-papillary fields in the squamous epithelium (2). In that publication, no occasional intraepithelial granulocyte was found. The histologic setting was called lymphocytic oesopagitis (LyE), a condition also recognized in non-human primates (3). In a more recent publication, cases with LyE were regarded as those having ≥40 IELs/ high power field (HPF) in the most affected area (4). Subsequently, several publications confirmed the identity of this condition (5-17).

The purpose of the present communication was to investigate, at the transmission electron microscopic (TEM) level, whether particular structural alterations take place in Lye.

Materials and Methods

Oesopageal biopsies from seven patients were investigated: four were consecutive patients with LyE, one with reflux oesopagitis, one with eosinophilic oesopagitis (EoE) and one with histologically normal squamous epithelium.

Aldehyde-fixed, paraffin embedded blocks were used. Semi-thin sections cut at 500 nm were de-paraffinized: 100% xylene for 1 hour, rehydrated in ethanol series of descending concentrations (15 minutes each) and final re-hydratation in 0.1M PBS pH7.4 (40 min) before placing the sections in 2.5% glutaraldehyde in 0.1M PBS pH7.4 (overnight). After rinsing with 2.5% glutaraldehyde in 0.1M PBS pH7.4 and with 0.1M PBS (30 min), sections were stained with OsO4 (1% OsO4 in Milli-Q water) for 90 min. Following rinsing with 0.1M PBS pH7.4, the preparations were dehydrated in 70%, 96% ethanol and absolute ethanol, followed by aceton-Agar100Resin (2 h) and 100% Agar100Resin (Agar Scientific, Essex, UK) overnight and, then, fresh 100% Agar100Resin (30 min). The material was finally poured into gelatin vials, from where ultrathin TEM sections were cut at 60 nm and examined under a JEOL 1230 transmission electron microscope (JEOL Scandinavia AB, Sollentuna, Sweden).

The Ethical Committee of the Karolinska Institute approved this study (No. 672-32/3013).

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Key Words: Oesophagus, lymphocytic oesophagitis, transmission electron microscopy, squamous epithelium, lymphocytes.

Results

Conventional histology in LyE. The oesopageal epithelium in the four cases with LyE exhibited marked intercellular oedema (spongiosis), widened intercellular spaces and basal-parabasal cell hyperplasia with diminished intermediate and superficial cell compartments. Nucleated squamous cells were seen at the luminal aspect of the epithelium, a witness of impaired surface maturation. The nuclei of the basal-parabasal cell compartment were enlarged, slightly hyperchromatic and the cytoplasm of some squamous cells was vacuolated. There was no elongation of the *lamina propia* papillae. The number of IELs in the most affected area was 43, 55, 63 and 87/HPF (10x objective and 40x objective/0.95 aperture, area 0.180 mm^2), respectively. Some IELs were stretched and convoluted, often referred to as "squiggle cells" (18).

TEM in LyE. In areas with marked spongiosis, many desmosomes were fragmented or absent (Figure 1). The cytoplasm of some squamous cells showed perinuclear vacuolization or haphazardly distributed vacuoles. Seriously injured squamous cells exhibited homogeneously pale, fibrillar and/or granular cytoplasm, cell shrinkage with "ghosts" nuclei or absence of nucleus. Other areas showed totally disintegrated squamous cells (Figure 2); only remnant desmosomes witnessed their squamous origin (Figure 3d). Clusters of jumbled desmosomes were also found "engulfed" in cytoplasmic vacules of some squamous cells (Figure 4c and d).

The increased "trafficking" of intra-spongiotic lymphocytes (ISLs) (19) was apparent (Figures 2, 3 and 4). At the advancing head of some "squiggle" ISLs, stretched desmosomes were found but no desmosomes were seen behind their "tail" (Figure 4 a). Less frequently, in "halo", lymphocytes were found in the cytoplasm of squamous cells, a phenomenon referred to as emperipolesis (20) (Figure 2 b). Other ISLs showed ballooned, oedematous, obviously damaged cytoplasm (Figure 5).

Controls biopsies.

Conventional histology in normal epithelium, in reflux oesopagitis and in eosinophilic oesopagitis. Hematoxylin and eosin (H&E) stained sections of the case with normal squamous epithelium of the oesopagus had predominantly intermediate and superficial cells. The basal-parabasal cells occupied 12% of the epithelial thickness. In the suprabasal cell compartment, two IELs with a round nucleus and one with a convoluted nucleus were seen.

H&E stained sections of the case with reflux oesopagitis showed basal-parabasal cell hyperplasia (35% of the epithelial thickness), elongation of the *lamina propia* papillae and nucleated squamous cells on top. Seven IELs were found; five were round and two were convoluted. In addition, two neutrophils and one eosinophil were present.

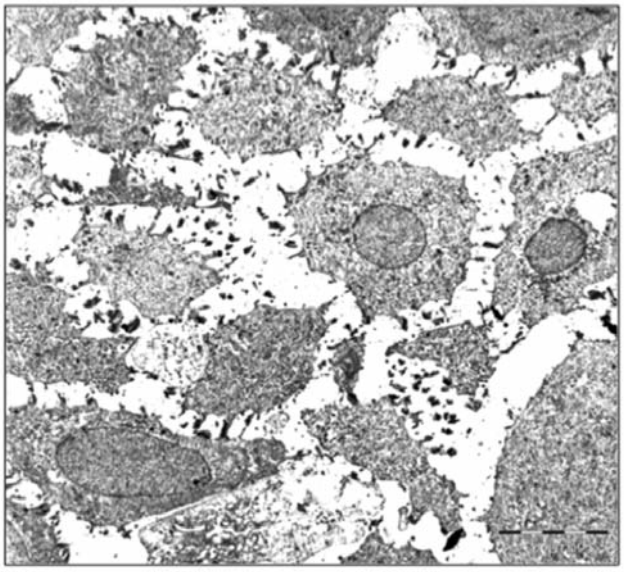

Figure 1. *Lymphocytic oesophagitis. Note spongiosis in the squamous epithelium, increased intercellular spaces, stretched or ruptured intercellular bridges (desmosomes), lack of desmosomes between many squamous cells and occasional remnant desmosomes connecting squamous cells. (Osmium tetroxide stain).*

H&E stained sections of the case with eosinophilic oesopagitis (EoE) showed marked spongiosis, basal-parabasal cell hyperplasia (42% of the epithelial thickness) with reduced intermediate and superficial cell compartments and nucleated squamous cells on top. The nuclei of the basal-parabasal cell compartment were enlarged, slightly hyperchromatic and the cytoplasm of some squamous cells was vacuolated. There was no elongation of the *lamina propia* papillae. Twenty-seven eosinophils/HPF were recorded in the most affected area. A Giemsa-stained section was observed under a fluorescent microscope (21) and, in addition to fluorescent eosinophils, innumerable fluorescent granules dispersed in the spongiotic oedema were found (21).

TEM in controls. At the TEM level, the normal epithelium of the oesopagus showed tightly packed squamous cells united by regularly distributed desmosomes (Figure 6). Occasional IELs were found. In reflux oesopagitis, the TEM images of examined areas were similar to those of their normal counterparts.

Segmented nuclei and coarse cytoplasmic granules identified eosinophils in eosinophilic oesopagitis. In addition, many free coarse granules, either isolated or in clusters, were observed in spongiotic areas (Figure 7).

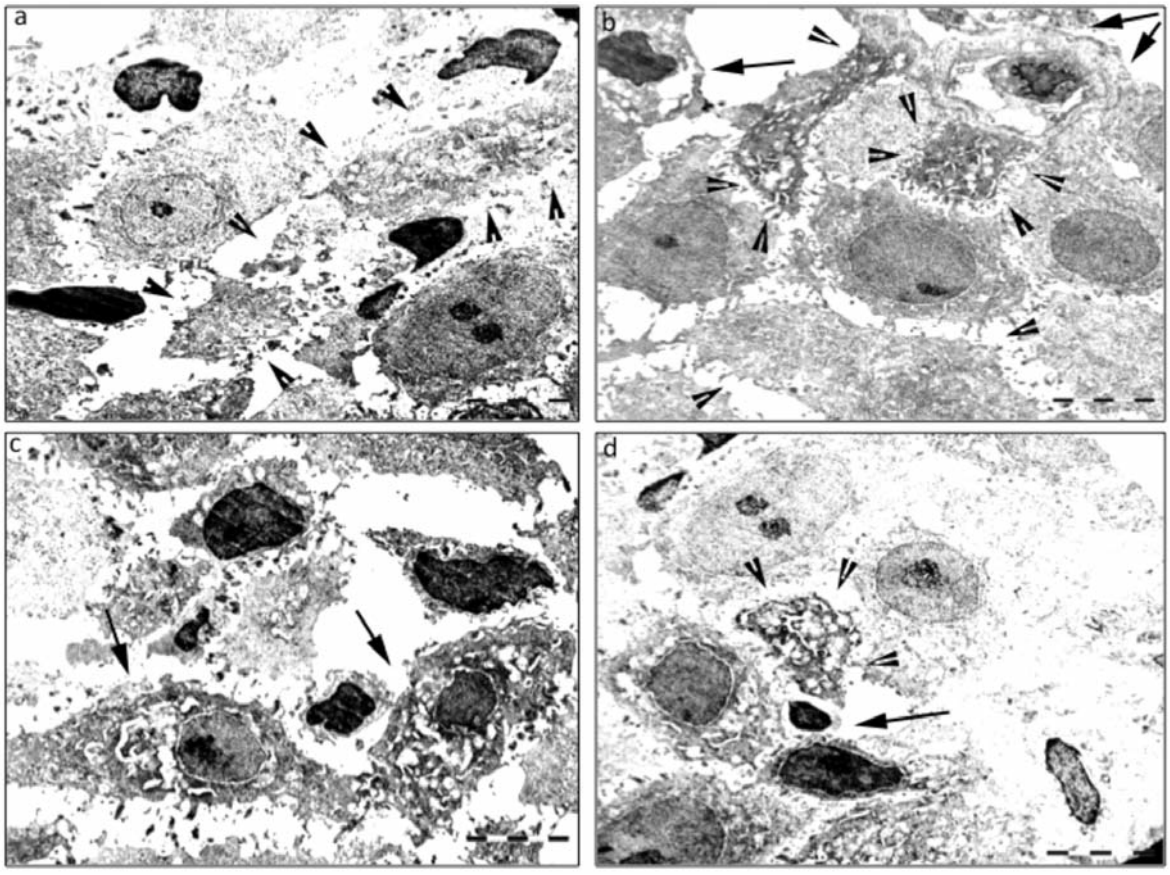

Figure 2. *Lymphocytic oesophagitis. a, b, d: Intra-spongiotic lymphocytes with nuclear deformation, some with cytoplasmic swelling. b: "Halo", lymphocyte within squamous cell (emperipolesis, double arrows). c: Squamous epithelium with irregular, confluent intracytoplasmatic vacuoles (arrows) or with total squamous cell disintegration (arrowheads). (Osmium tetroxide stain).*

Discussion

Despite the fact that this investigation was carried out in sections from paraffin blocks, the structures in the squamous epithelium of the oesopagus remained fairly well-preserved. It was assumed that the thin epithelial strip from oesopagus exhibiting widened intercellular spaces -due to oedematous spongiosis- were easily infiltrated by both formaldehyde and glutaraldehyde fixatives.

Four types of spongiosis are acknowledged in the squamous epithelium: (i) neutrophil spongiosis, (ii) eosinophilic spongiosis, (iii) milarial spongiosis (in the acrosyringium) and (iv) follicular spongiosis (in the follicular infundibulum) (22). In LyE, none of those phenotypes of spongiosis were found. Thus, the cause(s) for fluid extravasation –from papillar vessels at the base of the epithelium– leading to spongiosis in LyE remains unknown. The cytoplasm of the squamous cells in LyE showed considerable structural changes at the TEM level ranging from perinuclear vacuolisation and irregular cytoplasmic vacuoles to cytoplasmic disintegration. The nuclei of squamous cells also showed marked changes ranging from diminished osmium stainability, chromatic disruption, anucleation, to total cell disintegration. Spongiosis upsets squamous cell homeostasis, the result being hampered cell nutrition. The ongoing breakdown in the integrity of the squamous cells of the oesopagus demonstrated here in LyE at the TEM level might be the result of the aforementioned hampered cell nutrition.

In "halo", lymphocytes were found in the cytoplasm of some squamous cells. Humble *et al*. called this biological process emperipolesis, a term used to describe when a cell penetrates another cell (23). It has been demonstrated that lymphocytes under both physiological and pathological conditions are involved in emperipolesis (23). The exact machinery behind emperipolesis is unknown; however, it has been hypothesized that the natural killer-mediated lysosomal degradation pathway is involved in this process

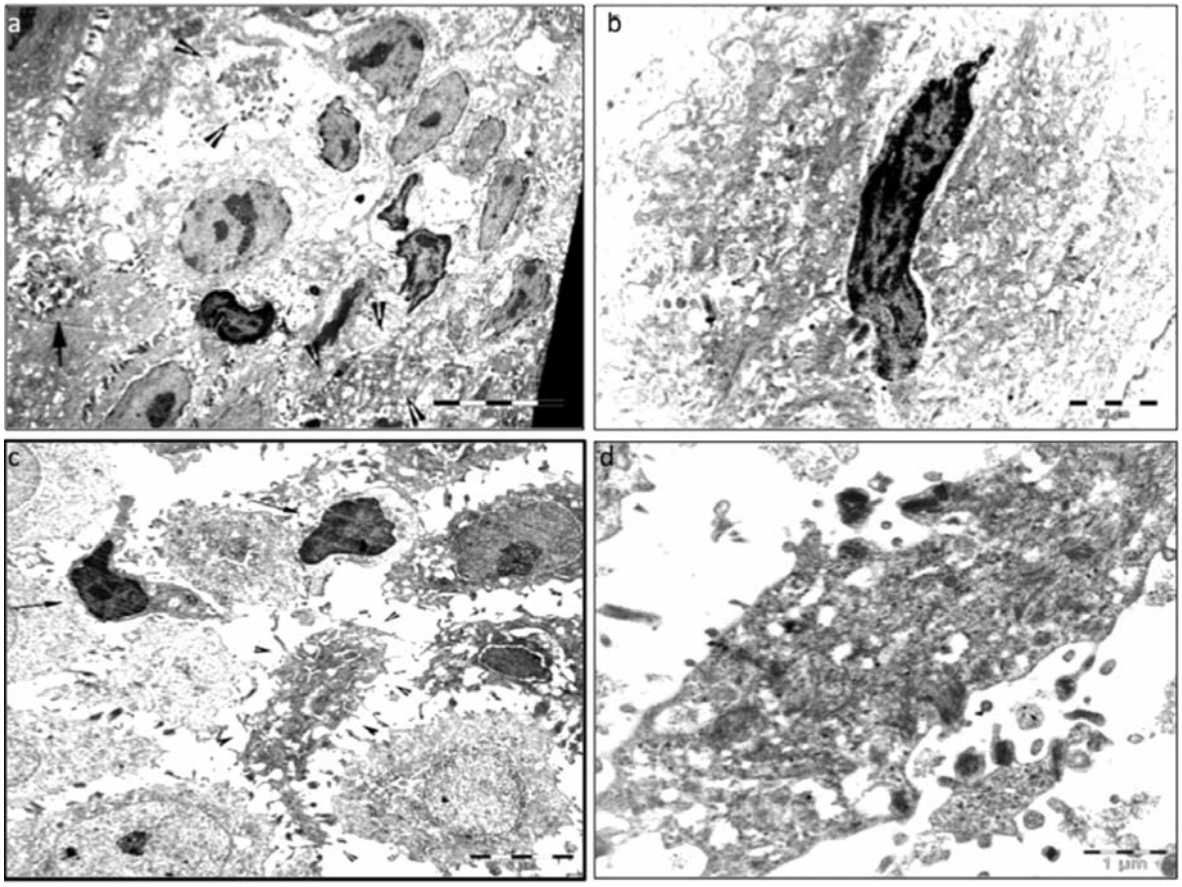

Figure 3. *Lymphocytic oesophagitis. a, b, c: Lymphocytes with nuclear deformation. a, c: The neighbouring squamous epithelium shows total disintegration (arrowheads). d: Disintegrated squamous cell with few remnants desmosomes (Osmium tetroxide stain).*

(24). Here we show, for the first time, that emperipolesis also occurs in LyE.

The trafficking of lymphocytes in the small intestine and colon has amply been documented (19). We found, in conventional microscopy and at the TEM level, a high number of IELs in LyE, strongly suggesting that this condition elicits an increased trafficking of lymphocytes in the squamous epithelium of the oesopagus. Many ISLs displayed various convoluted shapes as if they were searching for, or attracted by, an elusive antigen.

Profound structural changes also occurred in ISLs at the TEM level, ranging from cytoplasmic oedema, cytoplasmic vacuolisation, cytoplasmic disintegration, nuclear ballooning, nuclear vacuolisation, to nuclear disintegration.

Groups of disorderly arranged "free" desmosomes were found both in spongiotic areas and in intraepithelial vacuoles. These groups of desmosomes were most probably remnants from disintegrated squamous cells. The conundrum is why squamous cells engulfed (recruited?) detached desmosomes.

Apoptosis is a physiological auto-suicidal, genetically-induced cell deletion process of senescent effete normal cells (25). Apoptosis guarantees genetic fidelity, minimizes phenotypic variation and eliminates genotypic alteration. The auto-destruction is triggered by a cascade of caspases resulting in the breakdown of normal cells and the appearance of apoptotic bodies (nuclear fragments). Those DNA-containing bodies are rapidly phagocytized by macrophages and internalized by cells of the same type (25). The squamous epithelium in LyE showed neither nuclear pyknosis, nor apoptotic granules, nor macrophages, strongly suggesting that the regressive cellular changes found in LyE were not conveyed by the physiological caspase-dependent cell death paradigm (25).

Another mechanism of cell death occurs in dual-cell models, comprising of epithelial cells and lymphocytes. This dynamic process of cell destruction between two different cell systems (26) was called polemosis (from Greek polemos: war) (27). Polemosis is non-physiological, focal,

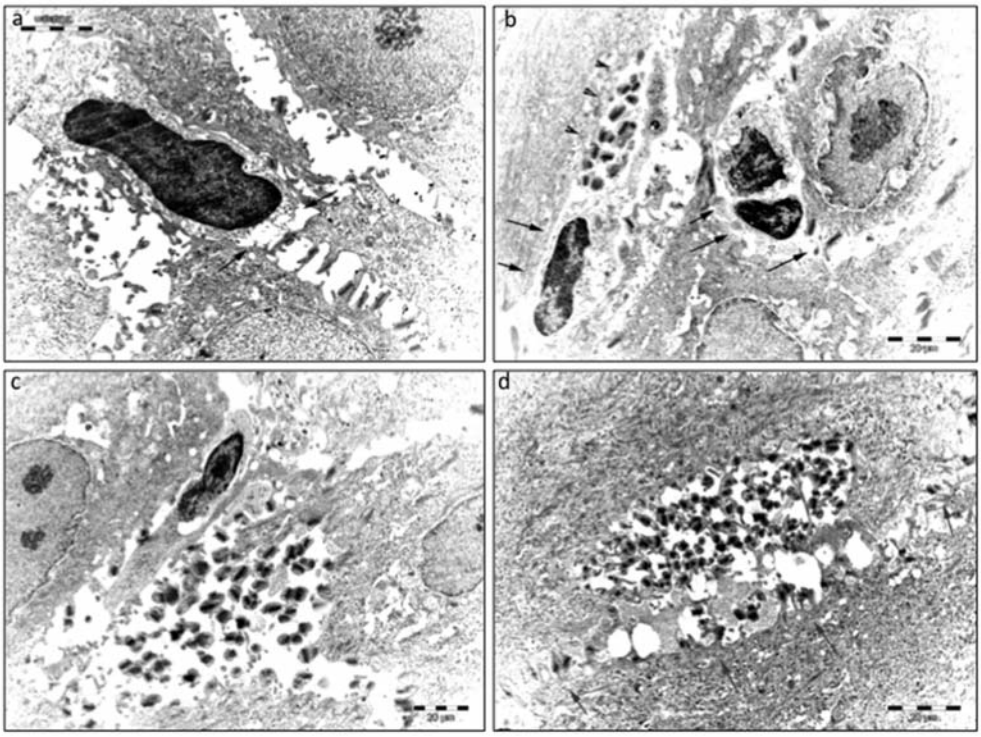

Figure 4. *Lymphocytic oesophagitis. a: Stretched lymphocytes pulling through between epithelial spaces. Note desmosomes at the advancing head (arrows) and lack of demosomes behind the tail. b: Lymphocytes with oedematous cytoplasmic swelling (double arrows) and desmosome-assembly "lagging" behind (arrowheads). c: stretched lymphocyte with sidewise assembly of "discarded" (?) desmosomes. d: Close view of assembled "discarded" (?) desmosomes within a squamous cell (Osmium tetroxide stain).*

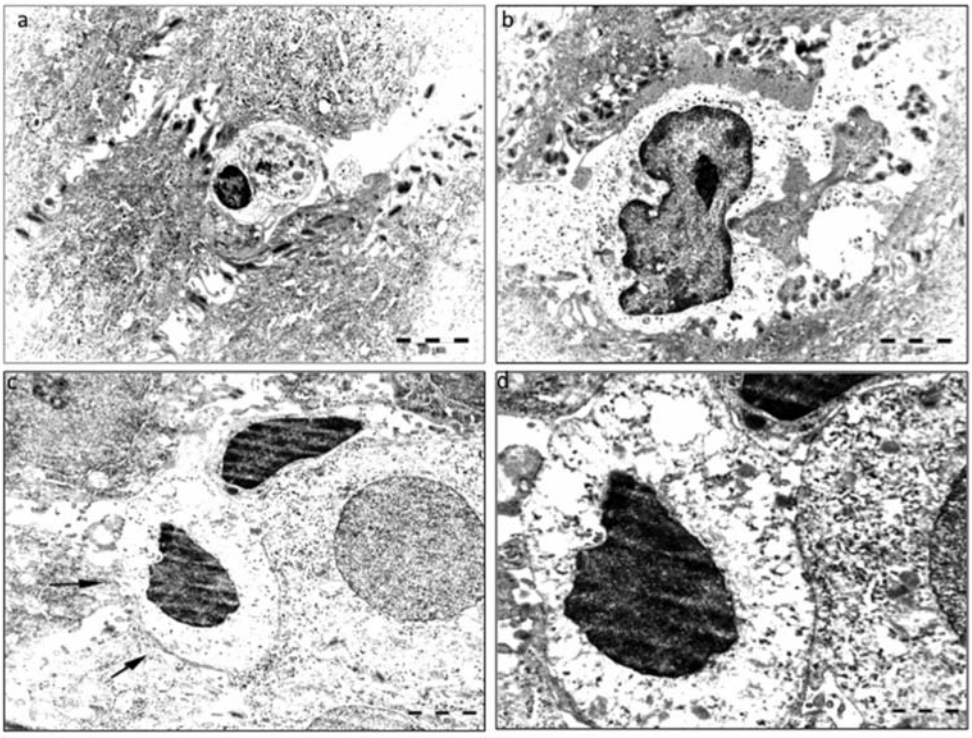

Figure 5. *Lymphocytic oesophagitis. a, b, c, d: Lymphocytes with oedematous cytoplasmic swelling. a: Beginning intracytoplasmatic disintegration (Osmium tetroxide stain).*

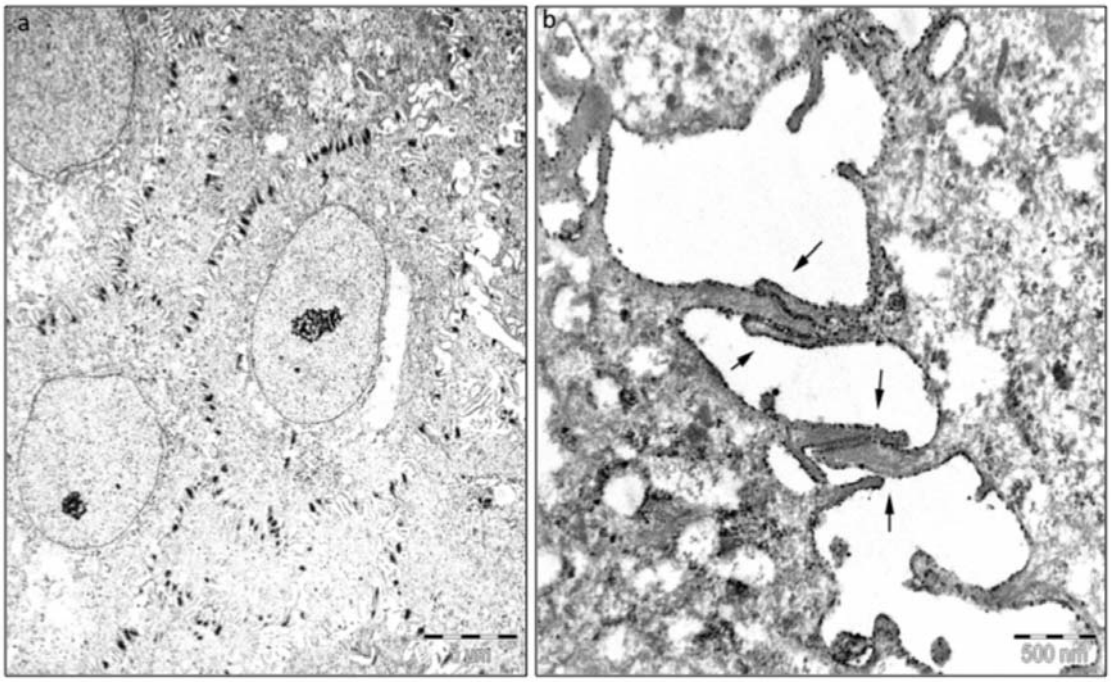

Figure 6. *Normal squamous epithelium of the oesophagus. a: Well-preserved, closely packed squamous cells united by distinct desmosomes. b: Desmosomes (macula adhaerens) in the normal squamous epithelium of the oesophagus. The extracellular domain of the desmosome or desmoglea is bisected by an electron-dense midline where desmoglein and desmocollin proteins bind to each other. (Osmium tetroxide stain).*

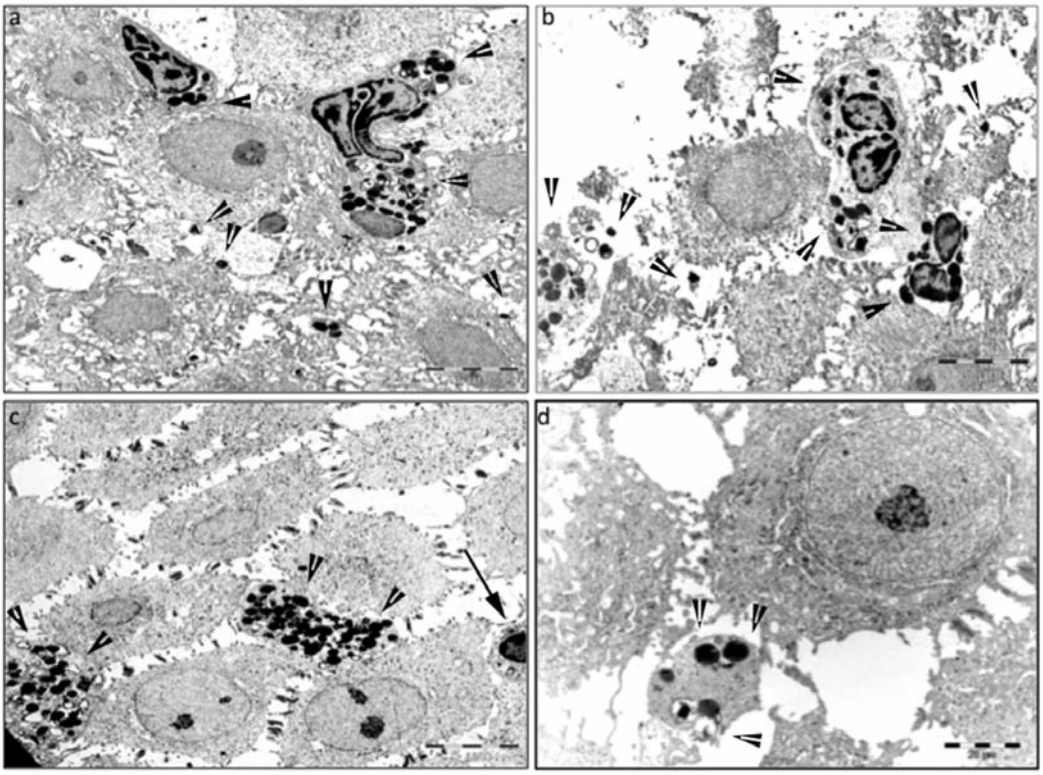

Figure 7. *Eosinophilic oesophagitis. a, c: coarse granules in the cytoplasm of eosinophils (arrowheads). a, b, c: Free intercellular coarse granules (arrowheads). d: One anucleated eosinophil (arrowheads) and one lymphocyte (arrow) are seen (Osmium tetroxide stain).*

haphazardly distributed at the time of observation, erratic and activated by committed lymphocytes, attracted by chemotaxis to epithelial cells. Polemosis is coordinated by the Fas-Fas ligand compulsory cellular system of self-defence (28). The end result of that struggle is manifested by the destruction of committed lymphocytes and the appearance of polemotic bodies (autologous T-cells' DNA) at the base of the epithelium (27). Polemotic bodies are not ingested by macrophages (27).

The absence of polemotic bodies in peripapillary areas -the gate of entrance for lymphocytes in LyE- militates against a possible Fas-FasL molecular mechanism of cell injury in this condition (28). Based on these considerations we are prone to speculate that a molecular pathway, other than the caspase cascade or Fas-FasL paradigms, might be responsible for cell injury, here demonstrated in LyE at the TEM level.

In conclusion, this study showed severe structural changes in LyE at the TEM level, in the cytoplasm and in the nuclei of both squamous cells and lymphocytes. The two-cell system displayed a gamut of regressive changes ranging from cytoplasmic oedema and vacuolisation to total cell disintegration (29). It is not inconceivable that besides hampered cell nutrition, conveyed by spongiosis, putative noxious molecules contained in the intercellular oedema were instrumental in the induction of the dramatic cellular alterations found in LyE at the TEM level. Some of these detrimental changes might be irreversible. On the other hand, endoscopic follow-up of patients with LyE (Rubio *et al*., in preparation) indicate that when the elusive agent(s) causing spongiosis and accumulation of ISLs subsides, then, the homeostasis in the squamous epithelium of the oesopagus (30) is eventually restored.

The present findings provide, for the first time, "inside information" on the alterations taking place in LyE, both in squamous cells and in IELs.

Conflicts of Interest

The Authors state that there are no conflicts of interest

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