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# Lymphocytes and Langerhans cells in the human oesophageal epithelium

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**Summary.** Using monoclonal antibodies on fresh frozen endoscopically obtained oesophageal biopsies the distribution of Langerhans cells, B lymphocytes, and various subpopulations of T lymphocytes was studied in the normal human oesophageal mucosa and in oesophagitis.

Identification of the lymphocytes was carried out by an immunoperoxidase technique using $OKT_3$ (antihuman T cell antibody), $OKT_4$ (antihuman helper T cell antibody), $OKT_8$ (antihuman cytotoxic T cell) and $OKT_{10}$ (antihuman null cell antibody). Identification of the Langerhans cells was carried out using an ATPase stain and OKIa (Ia like antigen) and $OKT_6$ (antihuman thymocyte).

In the normal oesophageal epithelium cytotoxic T lymphocytes are found as well as Ia positive Langerhans cells. Helper T lymphocytes and B lymphocytes are present mainly in the lamina propria. In oesophagitis an increase in Langerhans cells and cytotoxic T lymphocytes within the epithelium is found. From these findings it can be concluded that the oesophagus contains a reticuloepithelial system as well as a lymphocytic population which are a part of the gut-associated lymphoid tissue.

**Key words:** Oesophageal epithelium – Oesophagitis – Normal biopsies – langerhans cells – Intraepithelial lymphocytes – Monoclonal antibodies

## Introduction

Intraepithelial lymphocytes (I.E.L.) have been described in the mucosa of the normal human stomach, the small intestine, the colon and rectum (Toner and Ferguson 1971; Ferguson 1977; Selby et al. 1981). The I.E.L. population consists of both small and large lymphocytes lying above the basement membrane between epithelial cells but not forming specialised attachments to either of these structures. The role of the I.E.L. is not fully understood.

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Studies with surface markers and monoclonal antibodies show that the majority of the I.E.L. in the gut are T suppressor/cytolytic cells. There are considerable cytological, ultrastructural and immunohistological data indicating that I.E.L. are immunocompetent cells involved in local defence systems (Lyscom and Brueton 1982). The presence of lymphocytes in the oesophageal epithelium has occasionally been mentioned (Al Yassin and Toner 1976; Goldman and Antonioli 1982) but has not been studied systematically.

Langerhans cells have been described in various squamous epithelia including the skin (Birbeck et al. 1961), the buccal mucosa (Waterhouse and Squire 1967), and the female genital tract (Younes et al. 1968). In the human oesophagus, they have been identified by means of transmission electron microscopy (Al Yassin and Toner 1976). Their function has mainly been studied in the epidermis of the skin. The identification of the Ia antigen, Fc and $C_3$ receptors on the membrane of the epidermal Langerhans cell gave the cell a functional identity as a member of the monocyte-macrophage family. Using the mouse monoclonal antibody $OKT_6$ (Ortho) it has been found that the Langerhans cell has some characteristics in common with thymocytes and the interdigitating reticulum cells of lymph nodes (McMillan et al. 1982).

Langerhans cells have also been found to stain positively for ATPase and to be able to take up dopamine in vitro (Stangl et al. 1980). The aims of this study were: to compare I.E.L. in the normal human oesophagus and in oesophagitis; to characterise the various lymphocytic subpopulations in the oesophageal mucosa; and to describe the distribution of the Langerhans cells in the normal oesophageal epithelium and in oesophagitis and their relation with the I.E.L.

## Material and methods

Oesophageal biopsies were obtained from 20 adult patients undergoing upper gastrointestinal endoscopy (with a forward viewing Olympus GIF-K fibreoptic endoscope) for gastrointestinal symptoms, predominantly epigastric pain, and from 18 patients with evidence of gastroesophageal reflux. In the patients without reflux the biopsies were taken from endoscopically normal areas of oesophageal mucosa. In the patients with evidence of reflux the biopsies were obtained in the vicinity of erosive or ulcerative lesions. The diagnosis of reflux oesophagitis was based upon the following criteria: (1) a positive clinical history of reflux using a standard questionnaire (Johnson et al. 1978); (2) a barium oesophagogram; (3) endoscopy; (4) oesophageal histology showing the presence of polymorphonuclear leukocytes (Richter and Castell 1982); (5) prolonged pH monitoring indicating upright reflux in 9%, supine reflux in 37% and combined reflux in 54% of the patients; (6) a standard acid clearance test (Geboes et al. 1980).

The biopsies were obtained with a standard Martin biopsy forceps (7 Fr), immediately oriented, and snap frozen in liquid nitrogen cooled isopentane. Five micron frozen sections were cut, fixed in cold acetone and air dried for 18 h at room temperature. For the identification of the lymphocyte subpopulations an immunoperoxidase technique with mouse hybridoma monoclonal antibodies was applied. The characteristics of the various reagents we used are described in Table 1. After washing the sections in phosphate buffered saline (PBS), pH 7.4, they were incubated for 30 min at room temperature with the OKT reagents (Ortho Diagnostic Systems Inc., Raritan, NJ) as first layer. The second layer consisted of rabbit anti-mouse IgG at a dilution of 1:20. Before incubation, hydrogen peroxide, 3%, was added to block endogenous peroxidase activity. The sections were stained with the peroxidase-antiperoxidase

**Table 1.** Characteristics of monoclonal antibodies

| Monoclonal Antibody | Cellular distribution | Application |
|---|---|---|
| $OKT_3$ | Peripheral T lymphocytes<br>Thymocytes<br>Splenocytes | Identification of human<br>Peripheral T lymphocytes |
| $OKT_4$ | Peripheral T lymphocytes<br>Thymocytes<br>Splenocytes | Identification of human<br>Inducer/helper T<br>Lymphocyte subclass |
| $OKT_6$ | Thymocytes | Identification of human<br>"Common" thymocytes |
| $OKT_8$ | Peripheral T lymphocytes<br>Thymocytes<br>Splenocytes | Identification of human<br>Suppressor/cytotoxic T<br>Lymphocyte subclass |
| OKBA I | B lymphocytes and monocytes | Identification of B<br>Lymphocytes |
| OKIA | | Identification of Ia bearing macrophages |

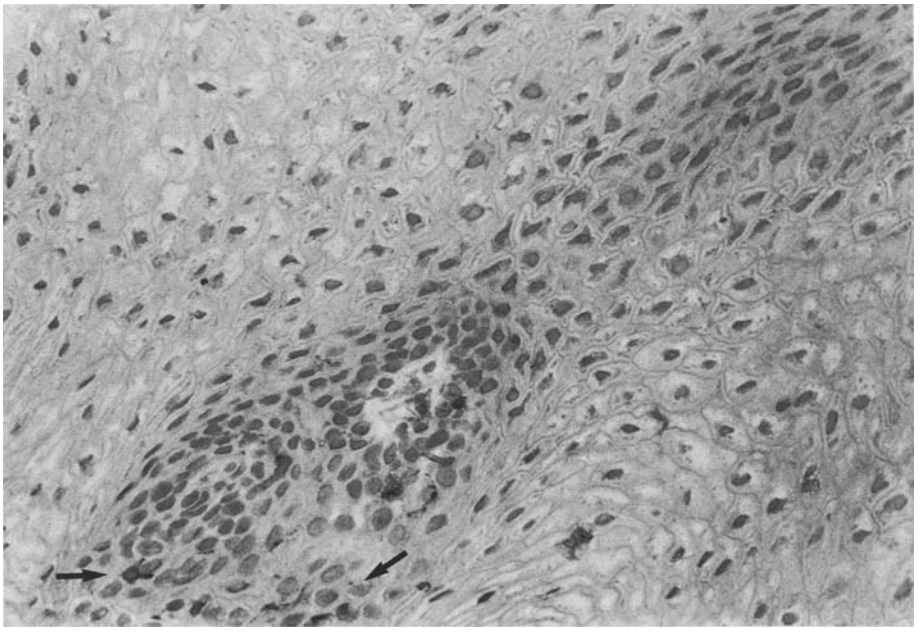

**Fig. 1.** $OKT_8$ positive cells in the human oesophageal epithelium. (Immunoperoxidase, $\times 100$); normal oesophagus

complex (Dako, Denmark) and counterstained with haematoxylin. Using an indirect immunoperoxidase staining technique serial sections were also stained for IgA, IgG, IgM and IgE. For the identification of the Langerhans cells we performed an Ia stain and an $OKT_6$ stain. For the Ia stain, which is a specific stain for an Ia histocompatibility antigen found on the surface of the Langerhans cell but not on epithelial cells or melanocytes we used also

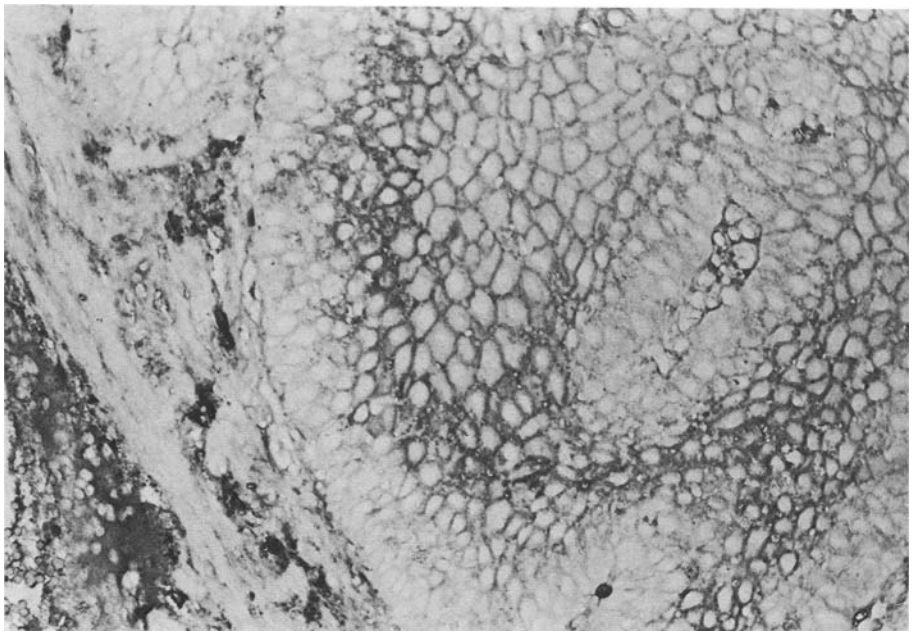

**Fig. 2.** Human oesophageal epithelium. IgA positive cells are present in the lamina propria in the subepithelial area. (Immunoperoxidase, × 125)

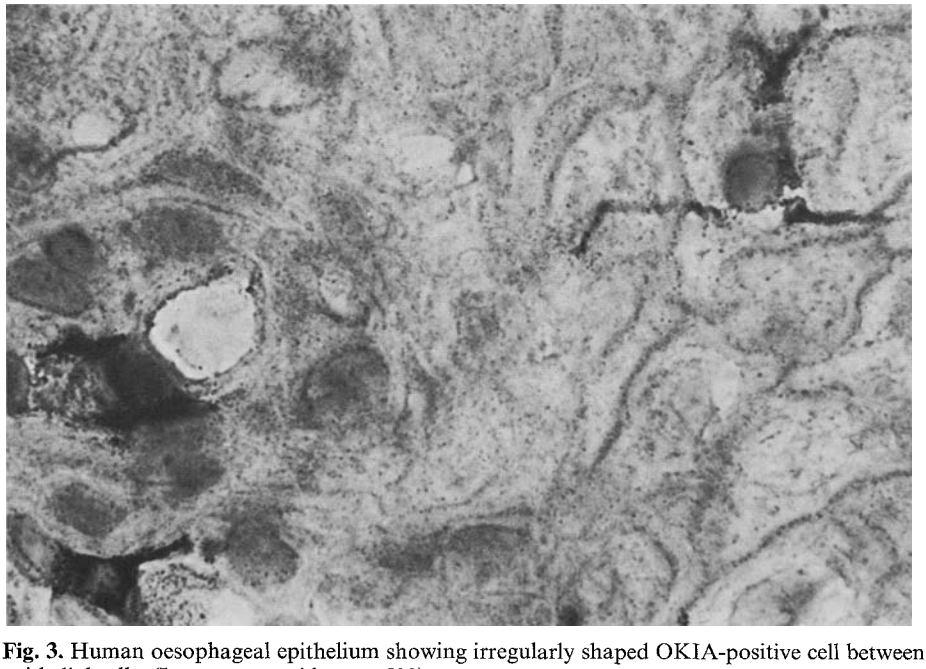

**Fig. 3.** Human oesophageal epithelium showing irregularly shaped OKIA-positive cell between epithelial cells. (Immunoperoxidase, × 500)

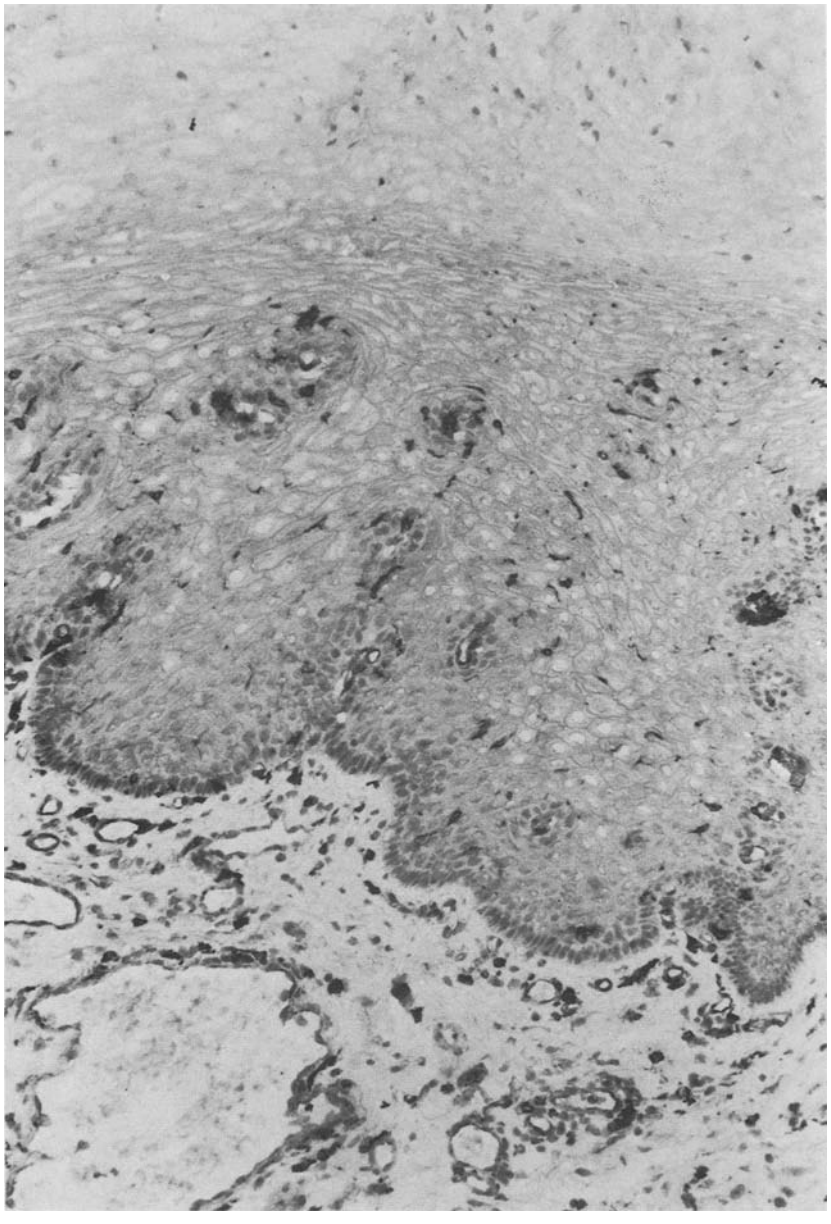

**Fig. 4.** Human oesophagus: oesophagitis. $OKT_6$-positive cells are present between the epithelial cells. (Immunoperoxidase, × 50)

a monoclonal antibody: OKIa (Ortho) with an immunoperoxydase technique. A similar technique was used to demonstrate $OKT_6$-positivity.

Method-controls consisted of omission of primary antiserum and substitution by PBS; and use of diaminobenzidine alone without antibodies to detect endogenous peroxidase activity. ATPase activity in the Langerhans cells was demonstrated using a lead salt method modified after Wachstein and Meisel (1957) and dopamine was stained according to a procedure de-

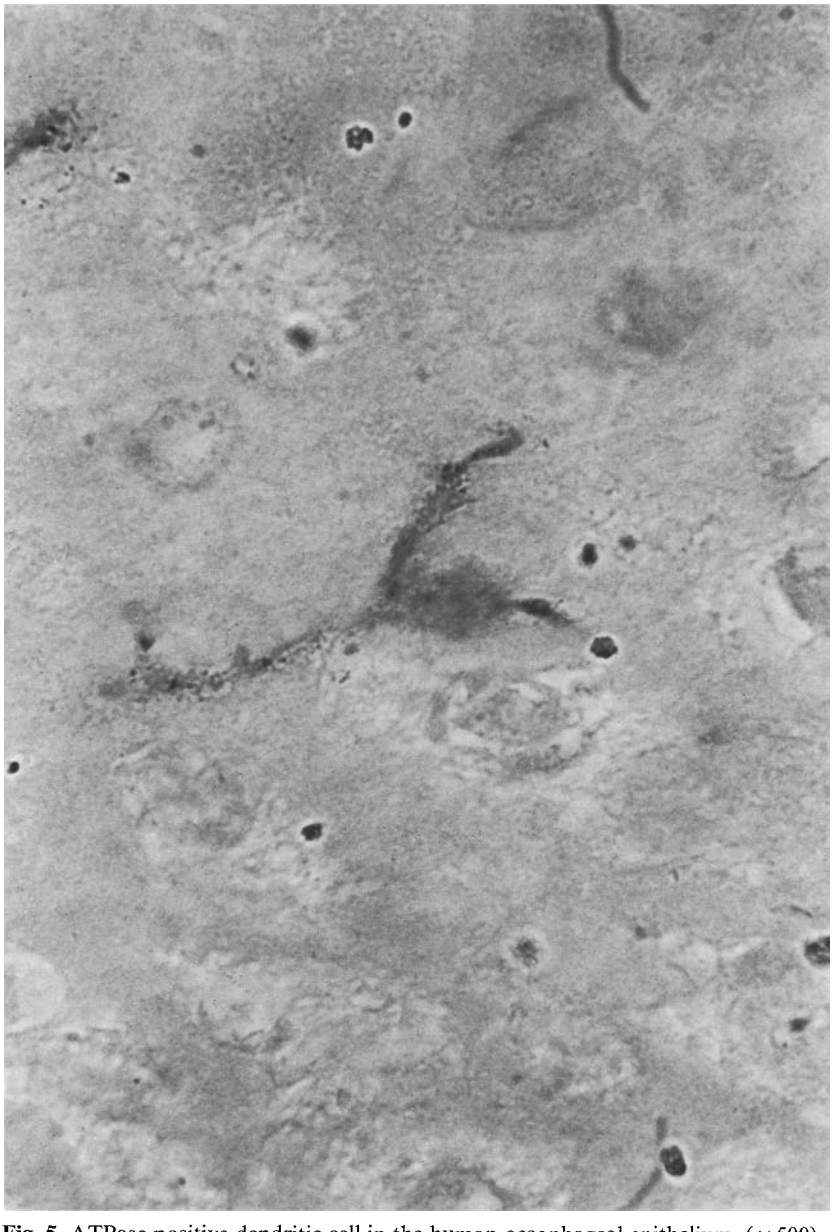

**Fig. 5.** ATPase positive dendritic cell in the human oesophageal epithelium. ( × 500)

scribed by Becker et al. (1935). The uptake of dopamine in vitro after incubation of the tissue was assessed according to the histofluorescence method of Falck and Hillarp (Axelsson et al. 1978).

## Results

In the normal human oesophageal epithelium a small number of lymphocytes, mainly localized in the suprabasal area, can be found. They are almost

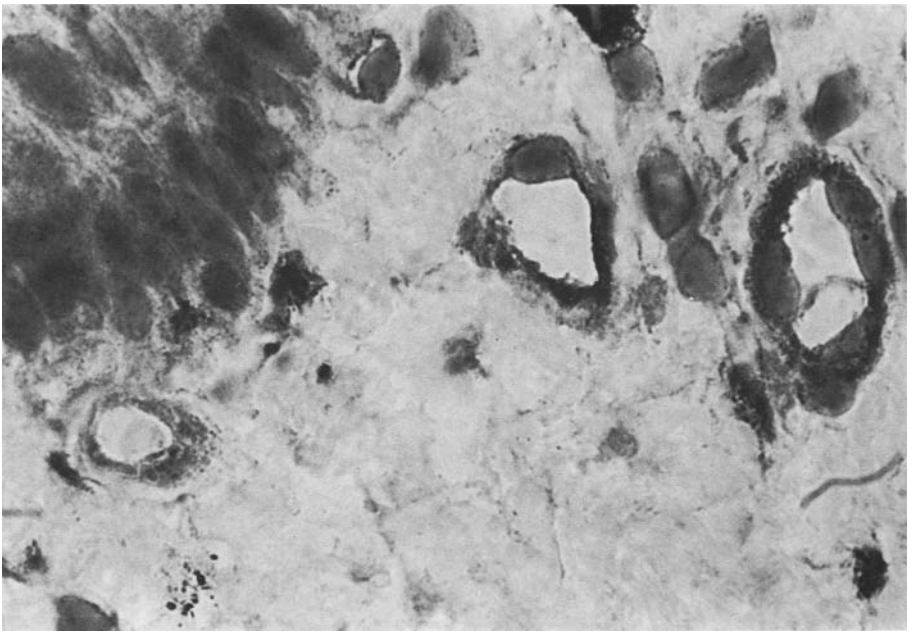

**Fig. 6.** Positive staining for Ia in the endothelial cells of the capillaries of the lamina propria in the human oesophagus. (× 200)

exclusively positive for $OKT_3$ and $OKT_8$. The positivity is seen as a dark brown membranous and cytoplasmic staining (Fig. 1). Occasional cells show a positive staining for $OKT_4$. In the lamina propria $OKT_3$-positive as well as BA1-positive cells are present. In this localization the $OKT_3$-positive, $OKT_4$-positive cells outnumber the $OKT_3$-positive, $OKT_8$-positive cells. The BA1-positive cells are mainly found in small lymphoid aggregates. Plasma cells are mainly found in the subepithelial area. IgA containing cells predominate while those containing IgG and IgM are present in a small minority (Fig. 2).

Irregularly shaped dendritic $OKT_6$-positive, OKIA-positive cells are found scattered in between the epithelial cells as well as in the lamina propria (Fig. 3). In the epithelium they are mainly present in the suprabasal area and along the papillae of the lamina propria (Fig. 4).

A few positive cells are found in the superficial layers of the epithelium. In serial sections a comparable number of dendritic cells with a similar distribution pattern are found to be strongly ATPase positive (Fig. 5). In vitro uptake of dopamine by dendritic intraepithelial cells can also be demonstrated by the histofluorescence method. Dopa-positive cells are however few in number and localized in the basal layer.

Positive staining for the Ia antigen is also observed in the endothelial cells of the capillaries of the lamina propria, especially in the stromal papillae (Fig. 6).

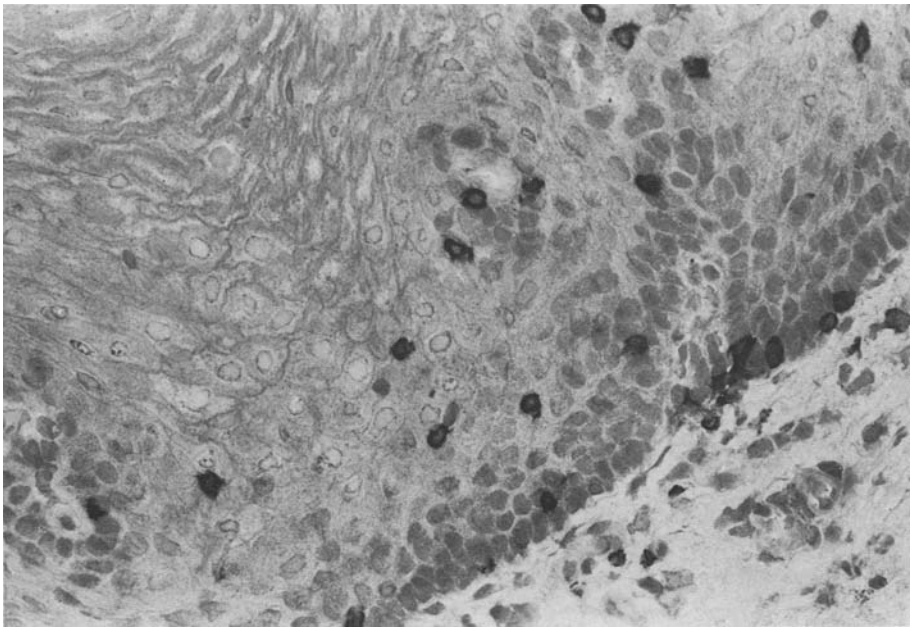

**Fig. 7.** Human oesophageal epithelium: numerous $OKT_8$-positive cells are present in the suprabasal area in this biopsy from a patient with oesophagitis. (Immunoperoxidase, × 125)

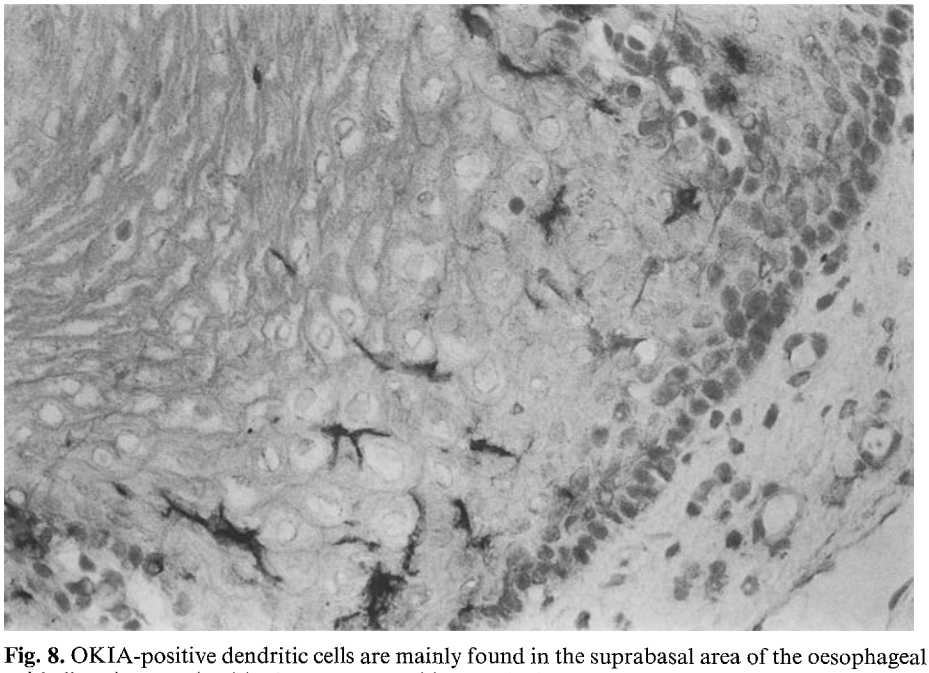

**Fig. 8.** OKIA-positive dendritic cells are mainly found in the suprabasal area of the oesophageal epithelium in oesophagitis. (Immunoperoxidase, × 125)

In oesophagitis an increase of I.E.L. is occasionally seen in routinely stained sections. Using monoclonal antibodies $OKT_3$-positive, $OKT_8$-positive cells are clearly increased within the epithelium (Fig. 7), as well as $OKT_6$-positive, OKIA-positive cells (Fig. 8). No obvious changes are found in the lymphocytic cell population of the lamina propria. No obvious increase of plasma cells is observed. The most important immunoglobulin-containing cell class remains IgA.

## Discussion

From our findings it appears that T-lymphocytes ($OKT_3$-positive), mainly of the suppressor/cytotoxic subtype ($OKT_8$-positive) are normally present in the oesophageal epithelium. This finding is in agreement with the results observed elsewhere in the gastrointestinal tract (Selby et al. 1981).

The presence of Langerhans cells in the oesophageal epithelium has already been established (Al Yassin and Toner 1976; Hopwood et al. 1978). It has been shown by transmission electron microscopy that these cells have similar morphological characteristics to the Langerhans cells present in the epidermis of the skin (Al Yassin and Toner 1976). From our findings it is apparent that the oesophageal Langerhans cells have functional and immunohistochemical characteristics comparable to the epidermal Langerhans cell, such as ATPase activity, in vitro uptake of dopamine, presence of the Ia antigen and positive staining for $OKT_6$. The earlier reports that Langerhans cells are Ia-like antigen positive is confirmed by our study (Rowden et al. 1977; Natali et al. 1981) although the expression of Ia-like antigens in the human oesophagus has not been found previously (Natali et al. 1981). Because of the functional similarities between oesophageal and epidermal Langerhans cells we suggest that in the oesophagus, as in the skin, the Langerhans cell can mediate the functional role of Ia-bearing mononuclear phagocytes: i.e. binding of an antigen so that it may be recognized by the T-lymphocytes. The Langerhans cell can thereafter, either in the epithelium or, after having migrated to a lymph node, trigger a helper T cell to proliferate and provide help to generate "cytotoxic" T cells for the given antigen. In this way its function should be compared with the role of the so-called "M" (microfold) cell, a human intestinal mucosal cell type described in the appendix (Bockman and Cooper 1975), the colon and the small intestine (Owen and Jones 1974).

In oesophagitis no increase of lymphocytes in the lamina propria has been found (Seefeld et al. 1977) and it has been said that no correlation exists between the presence and number of lymphocytes within the epithelium and the demonstration of acid reflux (Goldman and Antonioli 1982). It is however not so easy to recognize I.E.L. in routinely stained sections. An increase of intrusive cells, mostly lymphocytes, in the inflamed oesophageal epithelium has been described by means of transmission electron microscopy (Hopwood et al. 1978). Using monoclonal antibodies I.E.L. can easily be recognized in the normal oesophagus as well as in oesophagitis. From our findings it appears that a focal increase of I.E.L. can be found

in oesophagitis when compared with the normal oesophagus. The I.E.L. are distributed unevenly, however making counting and quantitative observations extremely difficult. Similar observations have been made for I.E.L. in other areas of the gastrointestinal tract (Husby et al. 1982) and for T-lymphocytes in the epidermis of the skin in various inflammatory conditions. The function of the I.E.L. is not precisely known but must be assessed in the light of the finding that they are predominantly T cells of the suppressor-cytotoxic subtype. These cells can be toxic for epithelial cells either directly or after activation through Langerhans cells and T helper lymphocytes (Stobo 1982). The increase of Langerhans cells in oesophagitis can also be explained by cellular immunological mechanisms. Whether the increase of $OKT_8$-positive cells and Langerhans cells in oesophagitis is a phenomenon primarily related to reflux or merely secondary to increased permeability can not be judged from our findings.

We can however conclude that the oesophagus contains a reticuloepithelial system (Langerhans cells) together with a lymphocytic population which are similar to the gut-associated lymphoid tissue already described in the other parts of the gastrointestinal tract.

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