Virchows Arch [Pathol Anat] (1982) 398:119–128

Virchows Archiv A

Identification of Langerhans Cells: Simultaneous Use of Sera to Intermediate Filaments, T6 and HLA-DR Antigens on Oral Mucosa, Human Epidermis and their Tumours

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Summary. Langerhans cells in oral mucosa and epidermis have been identified using antibodies to intermediate filament proteins in conjunction with antibodies to T6 and HLA-DR antigens. Langerhans cells, lymphocytes and melanocytes are positive when tested with antibodies to vimentin, and negative with antibodies to prekeratin. Langerhans cells are also positive with antibodies directed against HLA-DR and T6 antigens. In contrast keratinocytes are positive for prekeratin and negative for vimentin, HLA-DR and T6. These methods provide a useful tool for the identification of Langerhans cells at the level of the light microscope, and have been used to show changes in the number and arrangement of these cells in squamous cell carcinomas of skin and oral mucosa, and in inflammatory conditions.

Key words: Langerhans cells – Vimentin – Keratin – Squamous cell carcinoma – Histocompatibility antigens

The predominant cell type present in oral mucosa and human epidermis is the keratinocyte. The structure and biology of this cell type has been studied extensively (see for example Squier et al. 1976; Green 1980; Schroeder 1981).

Of particular interest is that keratinocytes contain keratins in line with the known epithelial origin of this cell type. Epithelial tissues also reveal other cell types in an interspersed manner (Breathnach 1980; Burkhardt 1980; Seelig and Billingham 1980; Friedmann 1981). These include melanocytes, Merkel-cells, lymphocytes and Langerhans cells. The relative numbers of each cell type depends on the particular epithelium under study. The distinction of these cells from keratinocytes as well as the differential diagnosis of each individual cell type is often difficult (Birbeck et al. 1961; Niebauer

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Supported by the Deutsche Forschungsgemeinschaft (Lo 285/2-1, Ca 93 1-1) and by the Hamburger Stiftung zur Förderung der Krebsbekämpfung

0340-1227/82/0398/0119/$02.00

1968; Lever and Schaumburg-Lever 1975), although recently two methods have seemed to offer promise. Firstly, conventional and monoclonal antibodies against products of the HLA-DR histocompatibility gene locus and against differentiation antigens of cells of the lymphoid and macrophage system allow a further characterization of the different cell types (Stingl et al. 1980; Murphy et al. 1981; Fithian et al. 1981).

Secondly, use of conventional antibodies to different intermediate filament types have shown that cells can be subdivided according to their intermediate filament content. This approach distinguishes cells of epithelial, muscle, neuronal, glial and mesenchymal origin (for review see: Osborn et al. 1982). Here we combine both approaches and analyze the different cell types, emphasizing the characteristics of Langerhans cells, in normal human oral mucosa, epidermis, and squamous cell carcinomas.

Material and Methods

Biopsies from normal human oral mucosa (5 cases), normal human epidermis (4 cases) and squamous cell carcinomas of the skin (2 cases) as well as of oral mucosa (6 cases) were frozen in liquid nitrogen and stored at 70° C until use.

Some of these cases (4 normal specimens; 3 carcinomas) were taken for double labelling procedures. The others were analyzed on serial sections.

The keratin and vimentin antibodies used have been extensively characterized in previous studies (see Osborn et al. 1982). *Keratin antibody* was raised in guinea pigs against keratin purified from cow hoof (Franke et al. 1978) and affinity-purified on the antigen covalently coupled to Sepharose 4B. This preparation of specific IgGs was used at a final concentration of 50 µg/ml. *Vimentin antibody* was raised in guinea pigs against the protein purified from mouse 3T3 cells (Franke et al. 1978) and purified on rabbit chondrocyte vimentin bound to Sepharose 4B. Specific antibodies were used at a concentration of 50 µg/ml. The second antibody was FITC-labelled goat anti-guinea pig IgGs (Cappell Labs. Cochranville, Pa, USA). Alternatively peroxidase labelled anti-guinea pig IgGs were applied (Medac, Hamburg, FRG).

The properties of a monoclonal antibody against human HLA-DR ($OKJa_1$) and T_6 (OKT_6) have been reviewed by Stingl et al. (1980), Reinherz and Schlossman (1980), Haynes (1981). They were purchased from Ortho Diagnostic Systems (Heidelberg) and used at a dilution of 1:5 in phosphate buffered saline. The second antibody in this case was FITC-labelled rabbit anti-mouse IgGs (Medac, Hamburg, FRG). In some experiments double label immunofluorescence microscopy was performed (for experimental details see Osborn et al. 1980). The following combinations were possible: vimentin or keratin versus T_6; vimentin or keratin versus HLA-DR.

In this case the second antibodies (either FITC-labelled anti-guinea pig IgGs, or rhodamine-labelled anti-guinea pig IgGs and FITC-labelled antimouse IgGs) were applied simultaneously. Second antibodies were used at concentrations equal or lower than 0.4 mg/ml. Cryostat sections were dried for 1–24 h before use. They were incubated with the first antibody for 45 min., washed and incubated with the appropriate second antibodies coupled to either FITC, TRITC or peroxidase. After a further wash samples were mounted in Mowiol 4–88. For further details of the fluorescence technique see Osborn and Weber (1982) and Becker et al. (1982).

Results

Oral Mucosa and Epidermis

Stratified epithelia of skin and oral mucosa are known to contain keratin (e.g. Sun et al. 1979). This protein was present in keratinocytes (Fig. 1a).

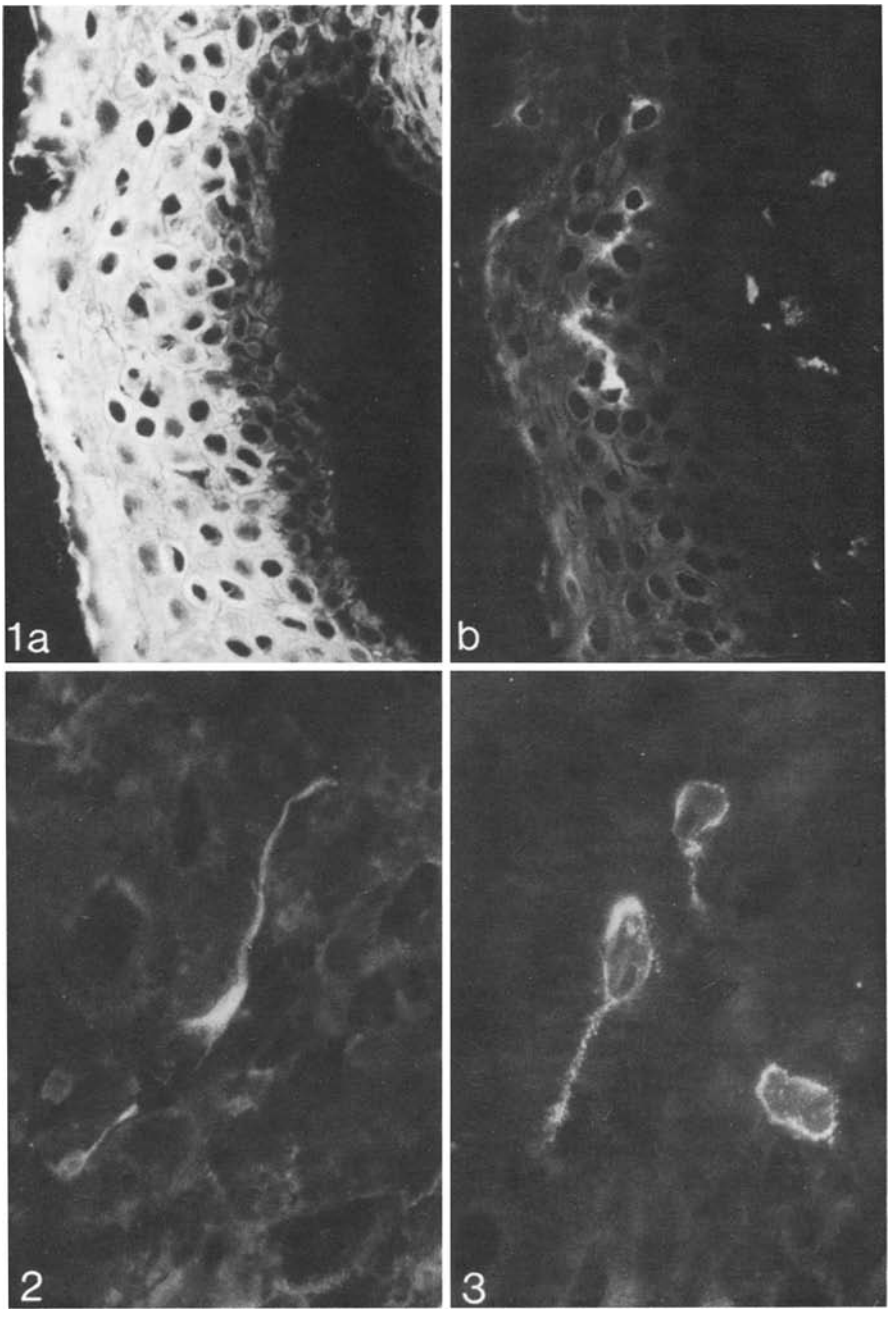

Fig. 1. a Normal squamous epithelium. Strongly prekeratin-positive epithelial cells. Staining for prekeratin. Magnification ×350. **b** Normal squamous epithelium, same section as **a**. In the center is one dendritic HLA-DR positive cell. Negative epithelial cells. Staining for HLA-DR Magnification ×350

Fig. 2. Normal squamous epithelium. In the center a vimentin-positive cell. Adjacent vimentin-negative epithelial cells. Staining for vimentin. Magnification ×1,370

Fig. 3. Normal oral mucosa. T_6-positive cells. Negative epithelial cells. Staining for T_6. Magnification ×1,370

Table 1. Markers of different cell types in stratified epithelia

	Intermediate filament		Cell surface marker	
	Prekeratin	Vimentin	HLA-DR	T_6
Langerhans-cells	−	+	+	+
Lymphocytes	−	+	+	−
Melanocytes	−	+	−	−
Keratinocytes	+	−	−	−

Careful observation of both oral and cutaneous epithelia showed that some cells were negative for this protein and that such cells were either small and round or had dendritic elongations. Use of the vimentin antibody revealed the presence of these nonepithelial cells more clearly. Highly branched cells, assumed to be Langerhans cells or melanocytes, and rounded cells of the lymphoid type were all positive when assayed with the vimentin antibody. As in previous work keratinocytes were not stained by vimentin antibody. When antibodies recognizing either the HLA-DR or T_6 antigens were used, individual cells positive for these antigens were detected within the stratified epithelia. Figure 1b shows cells within human skin stained with the $OKIa_1$ antibody. Figure 2 shows a vimentin positive cell in normal squamous epithelium. Figure 3 shows a section through normal oral mucosa stained with the T_6 antibody. In samples doubly labelled for either vimentin and HLA-DR, or vimentin and T_6, it became clear that not all vimentin positive cells were also positive for HLA-DR, or T_6. The HLA-DR-positive or T_6-positive cells usually displayed a characteristic dendritic morphology (e.g. Fig. 1b).

The staining patterns of Langerhans cells lymphocytes and melanocytes with the vimentin, the HLA-DR and T_6 antibodies is summarized in Table 1.

All three cell types are positive for vimentin, but only the Langerhans-cells are positive for HLA-DR and T_6.

Squamous Cell Carcinoma

Carcinomas of the mouth and the skin were analyzed at various stages using antibodies to keratin, vimentin, HLA-DR and T_6. All carcinomas were, as expected, positive for prekeratin (e.g. Fig. 4a).

Large numbers of non-epithelial inflammatory cells within the neoplastic epithelium and the adjacent stroma were a constant feature of these tumours. These cells contained vimentin filaments (see the immunofluorescence micrograph in Fig. 5 and the peroxidase label in Fig. 6a, b). Vimentin-positive cells could be divided into two types. First, the small lymphoid cells were vimentin-positive. Second, there was a considerable number of vimentin-positive dendritic cells. Again the expression of HLA-DR and T_6-antigens was observed almost exclusively in the dendritic cells (e.g. Figs. 4b, 7). An

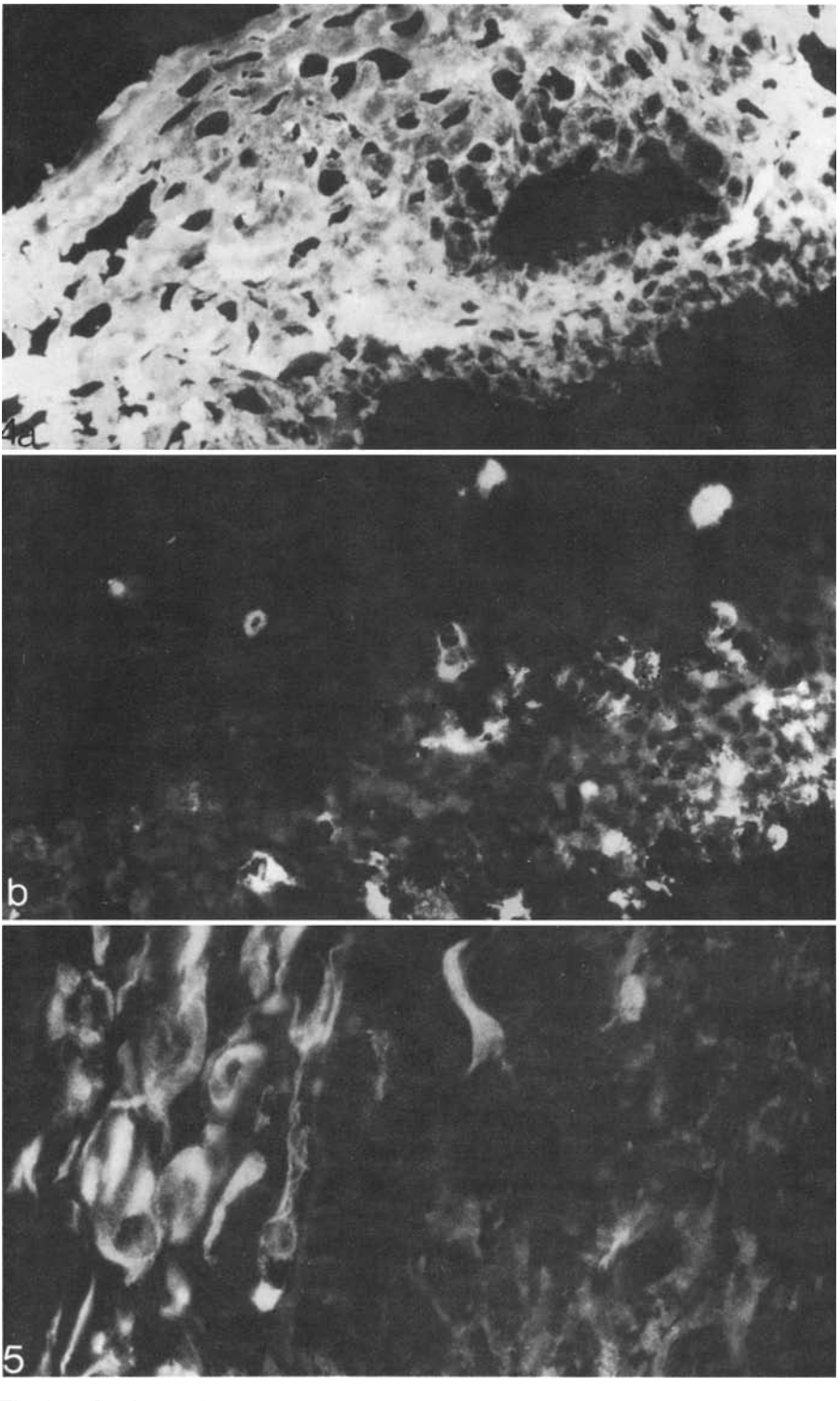

Fig. 4. **a** Carcinoma in situ of the oral mucosa. Intensely stained epithelial cells. Staining for prekeratin. Magnification ×400. **b** Carcinoma in situ of the oral mucosa. Same section as in Fig. 4a. High concentration of HLA-DR positive cells. Staining for HLA-DR. Magnification ×400

Fig. 5. Oral carcinoma. Epithelial mesenchymal interface (*left:* mesenchymal part). Vimentin-positive, partly dendritic non-epithelial cells. Staining for vimentin. Magnification ×1,370

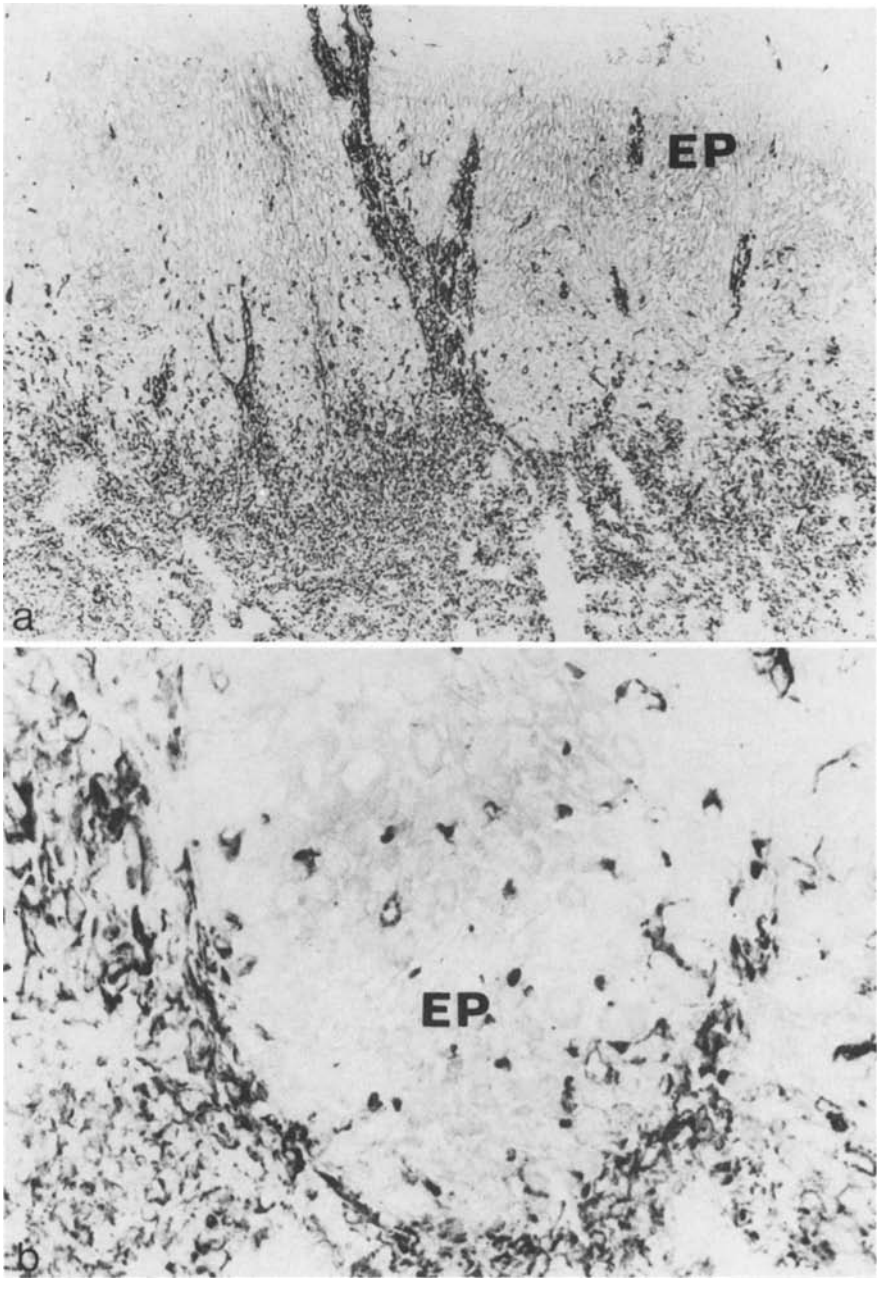

Fig. 6. a Invasive oral carcinoma. Strongly stained vimentin-positive cells in the stroma. Numerous dendritic vimentin-positive cells in the neoplastic epithelium. Staining for vimentin. Magnification ×90. **b** Same section as **a**. Vimentin-positive cells with distinct cellular branches. *EP* = epithelium staining for vimentin. Magnification ×550

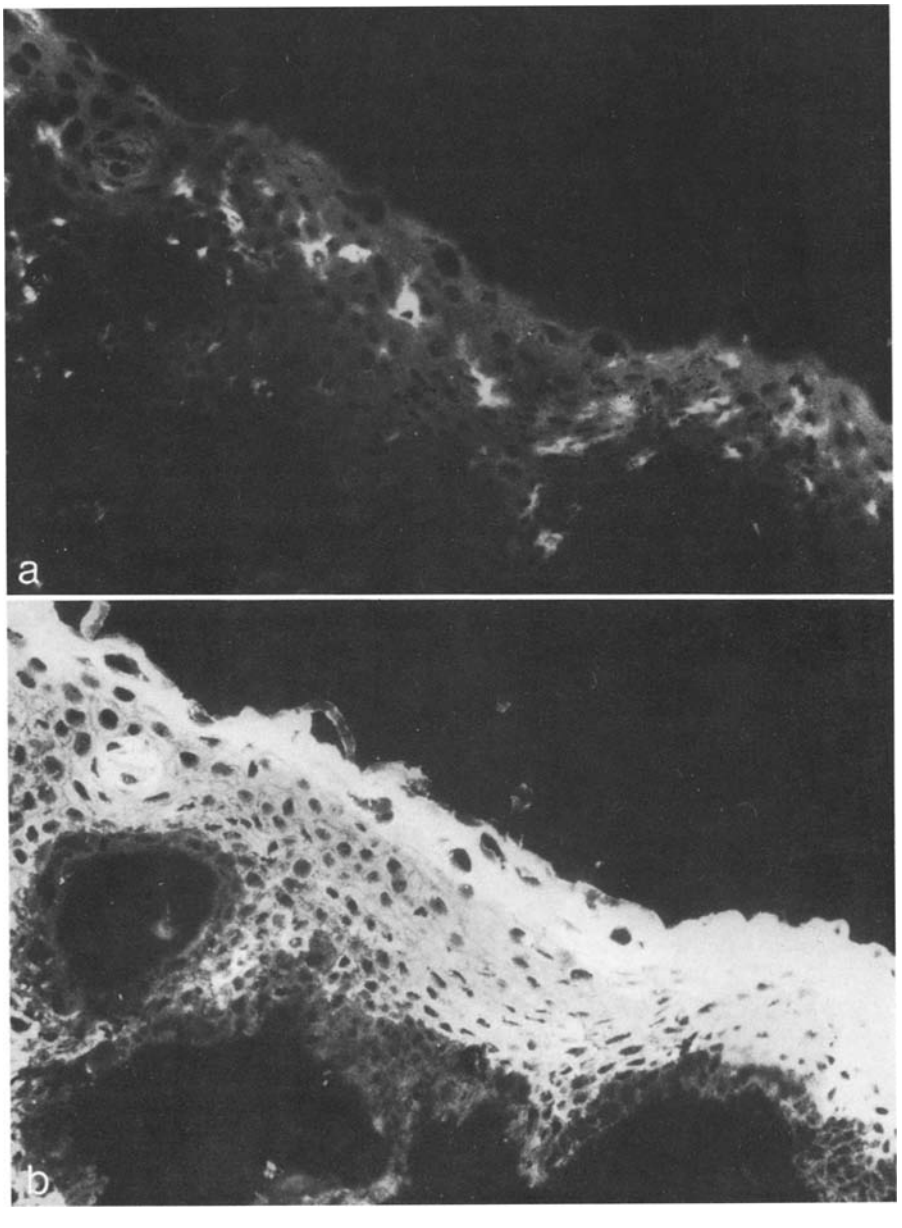

Fig. 7. **a** Atypical epithelium in a case of multifocal cancerization. T_6-positive dendritic cells within the epithelium. Staining for T_6. Magnification ×380. **b** Atypical epithelium in a case of multifocal concerization (same as **a**). Prekeratin-positive cells. Staining for prekeratin. Magnification ×380

atypical epithelium from a case of multifocal cancerization stained for T_6 is shown in Fig. 7. An unusually high number of T_6-positive dendritic cells are present within the epithelium.

Discussion

In conventional light microscopy it is difficult to distinguish epithelial and non-epithelial cells. To date the distinctions between non-epithelial cells in skin and oral mucosa i.e. the differentiation of Langerhans-cells, lymphoid-cells, melanocytes and Merkel cells have had to be drawn by histochemical and electron microscopical methods, both of which often fail to give a definitive answer.

For example, on the ultrastructural level Birbeck granules are specific for Langerhans cells but their absence does not exclude the possibility that a given cell may be a Langerhans- cell. (Breathnach 1980; Burkhardt 1980).

The methods used here provide further criteria for the typing of cells in stratified epithelia (see Table 1). Thus the use of antibodies to prekeratin and vimentin provides an easy way to distinguish keratinocytes from the other cell types present in oral mucosa and epidermis. This data shows that whereas keratinocytes are keratin-positive and vimentin-negative, Langerhans cells, melanocytes and lymphocytes are vimentin-positive and keratin negative. Vimentin positivity for melanocytes and lymphocytes in animal systems has already been reported (Franke et al. 1978).

The presence of vimentin filaments in Langerhans cells is consistent with the proposed mesenchymal origin of this cell type (see Stingl et al. 1980), since the class of intermediate filaments present in a cell can be used to type the cell of origin in both animal and human material (see e.g. Osborn et al. 1982). Antibodies against vimentin indicate the presence of this intermediate filament type in dendritic cells including Langerhans-cells, as well as in lymphoid cells. Therefore a further distinction of cell types requires the use of an additional selective marker for Langerhans cells. In the past markers such as IgG-Fc and complement receptors have been used for the identification of Langerhans cells within the squamous epithelia, but other cell types also carry these markers (Stingl et al. 1980; Burke and Gigli 1980). The introduction of conventional antibodies to Ia allowed the Langerhans cells and certain fraction of the lymphoid cells to be positively identified (Rowden et al. 1977; Klareskog et al. 1977; Katz et al. 1979; Frelinger et al. 1979; Rowden 1980).

A further refinement of this technique became possible with the introduction of monoclonal antibodies to Ia and T_6 which recognize different cell populations of the lymphocyte and macrophage system (Haynes 1981).

In this study monoclonal antibodies against HLA-DR gene products and against T_6 antigens provided a reliable tool with which to identify Langerhans cells (see also: Klareskog et al. 1977; Rowden et al. 1977; Stingl et al. 1980; Murphy et al. 1981; Fithian et al. 1981). Our study has the advantage that we have used a double immunofluorescence method to identify both the intermediate filament type as well as surface components of

these enigmatic dendritic cells within the squamous epithelia. Thus the absence of prekeratin filaments and the presence of vimentin filaments, HLA-DR and T_6 antigens identify Langerhans cells (see Table 1). Certain neoplastic epithelial disorders are characterized by an alteration in the number of Langerhans cells (see for instance Burkhardt 1980). So far we have studied only a small number of specimens. However our results show in particular that the number of vimentin positive non epithelial cells is very high in squamous cell carcinoma. This was true both for carcinoma in situ and for invasive carcinomas. In both tumours a high percentage of the vimentin positive dendritic cells expressed the HLA-DR and T_6 antigens, and could therefore be classified as Langerhans cells. Thus our data support previous quantitative ultrastructural studies which have demonstrated that up to 40% of intraepithelial inflammatory cells in oral squamous carcinoma are Langerhans cells (Löning and Burkhardt 1979; Burkhardt 1980). As expected from other studies (Löning et al. 1980; Altmannsberger et al.1981 and 1982; Caselitz et al. 1982) the tumour cells in the carcinomas were keratin-positive.

Immunocytochemical analysis of cell type characteristic cytoplasmic and membranous components improves not only our knowledge of the epidermal and mucosal microenvironment and its disturbances but is also an aid for the classification of mucocutaneous tumours of the histiocytic, lymphoid or melanocytic series.

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Accepted September 22, 1982