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Lymphoepithelial carcinoma (Schmincke type) as a derivate of the tonsillar crypt epithelium *,**

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Summary. Ten tumours of tonsillar or epipharyngeal localization showing the histological picture of "lymphoepithelial carcinoma" (Schmincke 1921) were examined immunohistochemically using Peanut lectin, Ulex europaeus lectin-I and an antiserum to S-100 protein. The findings suggest a close relationship of this type of carcinoma to the normal tonsillar crypt epithelium. The majority of tumour cells are UEA-I-positive and PNL-negative, as is the crypt epithelium, while oral mucosa is both PNL- and to a lesser extent UEA-I-reactive. Tumour areas expressing this pattern contain a large number of asteroid-shaped PNL-positive histiocytes and arachnoid-shaped histiocytes reacting with anti-S-100 protein; both cell types being probably identical and representing typical elements of the normal tonsillar crypt epithelium. Consequently, the WHO-term "nasopharyngeal carcinoma, undifferentiated type" seems to be inadequate for this type of tumour.

Key words: Lymphoepithelial carcinoma – Peanut lectin – Ulex europaeus lectin – S-100 protein

In his original paper of 1921 Alexander Schmincke described a special radiosensitive tumour of the naso- and oropharynx characterized by an intimate contact of epithelial tumour cells and lymphocytes; he called it "lymphoepithelial carcinoma". Whether this neoplasm represents an entity is still a matter of debate. The World Health Organisation (WHO) classifies it as being a variant of squamous cell or transitional cell carcinoma (Wahi et al. 1971) or as a "undifferentiated carcinoma" of the nasopharyngeal type (Shanmugaratnam and Sobin 1978). Nevertheless, its relationship to the Epstein-Barr-Virus first demonstrated by Zur Hausen et al. (1970) claims

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a special place for it in nosology. We would like to draw attention to some new aspects of this tumour, favouring an origin from tonsillar crypt epithelium.

Ulex europaeus lectin-I, a lectin with a high affinity to α-L-Fucose has prooved to be a very reliable endothelial cell marker (Borisch et al. 1983), whereas only some epithelial cells express UEA-I-receptors (Yonezawa et al. 1982; Mazzuca et al. 1982; Hsu and Ree 1983; Franklin 1983). Tissue receptors for Peanut lectin, a lectin specific for non-reducing terminal β-D-Galactosyl residues, are widespread but not ubiquitous among epithelial and carcinoma cells (Mazzuca et al. 1983; Kuhlmann et al. 1983; Lehmann et al. 1984; Böcker et al. 1984; Teshima et al. 1984).

With regard to tonsillar epithelium only two papers describe the normal binding patterns of UEA-I and PNL (Hsu and Ree 1983; Wirbel et al., unpublished work) and lymphoepithelial carcinoma has not been examined by this method.

S-100 protein is an acidic Ca^{2+}-binding protein originally isolated from bovine brain (Moore 1965). Besides its occurrence in nervous tissue (Cocchia and Michetti 1981; Ferri et al. 1982) it has been found within intraepidermal histiocytic cells with the morphological features of Langerhans cells (Cocchia et al. 1981) and in interdigitating reticulum cells in human thymus (Ushiki et al. 1984), thymomas (Lauriola et al. 1984) and lymph nodes (Takahashi et al. 1981) and therefore has prooved to be a suitable marker for these special histiocytic cells. Consequently we used it to study their occurrence within the tonsillar crypt epithelium and in Schmincke tumours.

Material and methods

This study is based on ten cases of lymphoepithelial carcinomas (Schmincke-type) from which formalin fixed and paraffin embedded material was available.

There were 5 females and 5 males. In 7 cases the primary site was the epipharynx. In two cases the tumour arose in the pharyngopalatine arch and the tonsilla lingualis, respectively. In one case data on the primary site were not available.

In 6 cases cervical lymph node metastases were present. The age range was between 14 and 77 years with a mean of 51.5 (±15.9 SD) years. 15 hyperplastic infantile tonsils served as controls and were examined as frozen sections as well as formalin/paraffin preparations.

Ulex europaeus lectin-I (UEA-I) (Lot. no. 0813E), Peanut lectin (PNL) (Lot. no. 0111E) as well as the corresponding rabbit derived antisera (Lot. no. 0990D, 051F resp.) were purchased from Medac (Hamburg, GFR). The specifically inhibiting sugars (D-Galactose for PNL and L-Fucose for UEA-I) were produced by Medac and Merck (Darmstadt, FRG). The serum (produced in rabbit) anti-S-100 protein was a Dako (Copenhagen, Denmark, Lot. no. 014A) product as were the porcine anti-rabbit-immunoglobulin serum and the rabbit horseradish derived peroxidase-anti-peroxidase complex. To avoid the artifacts of demonstrating endogenous peroxidase in frozen sections an alkaline phosphatase method was chosen and an alkaline phosphatase conjugated goat-anti-rabbit-immunoglobulin serum from Tago (Burlingame, CA, USA) was used. The staining procedures have been described in detail elsewhere (Möller 1982; Borisch et al. 1983; Wirbel et al., unpublished work), principally the lectin receptors and the S-100 protein were demonstrated via an indirect immunoenzymatical color reaction. Controls revealed negative results whenever the lectin or one of the antisera were omitted while the inhibition of the lectin binding by monosaccharides was complete for PNL and D-Galactose and incomplete, i.e. generating as weak but specific reaction for UEA-I and L-Fucose.

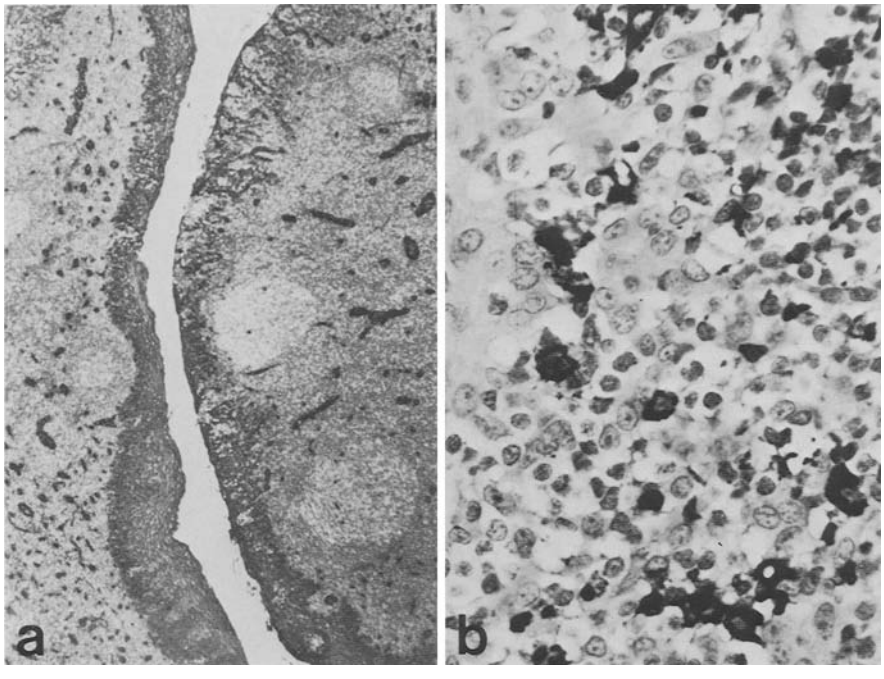

Fig. 1. a Normal infantile tonsil. Binding of Ulex europaeus lectin-I (UEA-I) restricted to the epithelium and the vessels. The reticulated crypt epithelium is depicted on the top right (diaminobenzidine/haematoxilin, × 40). **b** Normal tonsillar crypt epithelium showing no affinity to Peanut lectin (PNL). Dense cytoplasmic PNL-affinity of intraepithelial asteroid shaped histiocytic cells is present (× 400)

Results

Ulex europaeus lectin-I

The normal tonsillar crypt epithelium shows a pronounced cytoplasmic UEA-I-affinity in frozen and in paraffin sections, while in the oral mucosa the reaction is only weak and is restricted to the intermediate and superficial layers (Fig. 1 a). Apart from this aspect there are no other UEA-I-reacting structures except endothelial cells. In Schmincke-tumours there are areas showing a binding intensity corresponding to the crypt epithelium (Fig. 2). At some sites, the cytomembrane is more intensely stained than the cytoplasm. In 6 cases there are non-reacting tumour-areas, which sometimes collide with reacting areas (Fig. 3 b). Among primary lesion and in the few corresponding lymph node metastases available there is no remarkable difference in the phenotype; there are positive and negative tumour-areas in both the primary lesion and in the metastases.

Peanut lectin

Apart from a single exception the normal crypt epithelium is non-reactive for PNL in both, frozen and paraffin sections (Fig. 1 b), whereas the tonsillar

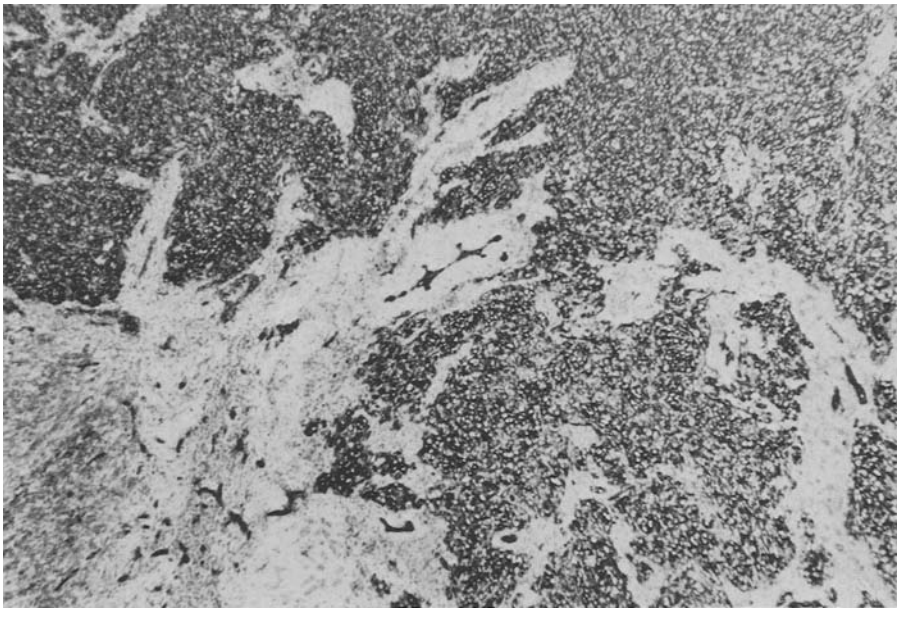

Fig. 2. Schmincke tumour infiltrating a cervical lymph node; typically the tumour expresses cytoplasmatic location of UEA-I-receptors, permitting a clear distinction from malignant lymphomas which are always UEA-I-negative (× 39)

surface epithelium and the oral mucosa are PNL-positive. Other cells with PNL-receptors on their cell surface are: plasma cells, centrocytes and centroblasts. Histiocytic cells are cytoplasmatically stained.

Among the latter cells a particular cell type shows a strongly expressed cytoplasmic PNL-affinity, emphasising its asteroid cytoplasmic shape. This cell is situated between the follicles and is characteristically surrounded by small lymphocytes. A comparable cell is situated within the meshes of the crypt epithelium (Fig. 1 b), showing, in some cases, rosetting of lymphocytes.

The lymphoepithelial carcinomas are mostly PNL-negative (Fig. 3 a, bottom). Such "typical" areas contain numerous "asteroid" histiocytes as described above (Fig. 3 c). Small foci of the tumour mass are cytoplasmatically PNL-positive. The border between the reactive and non-reactive parts is sometimes linear (Fig. 3 a) but in most cases it is ill defined. The content of PNL-positive "asteroid" histiocytes in positive carcinoma areas seems to be reduced but can not be quantitated. The epithelial binding pattern is strong and intracytoplasmic and can clearly be differentiated from the faint surface binding of PNL by follicular centre cells. It can also be distinguished from the vesicular cytoplasmic localization of the reaction product in tingible body macrophages in preserved follicles. Again, there is a mosaic-like phenotype in the primary sites and in the metastases.

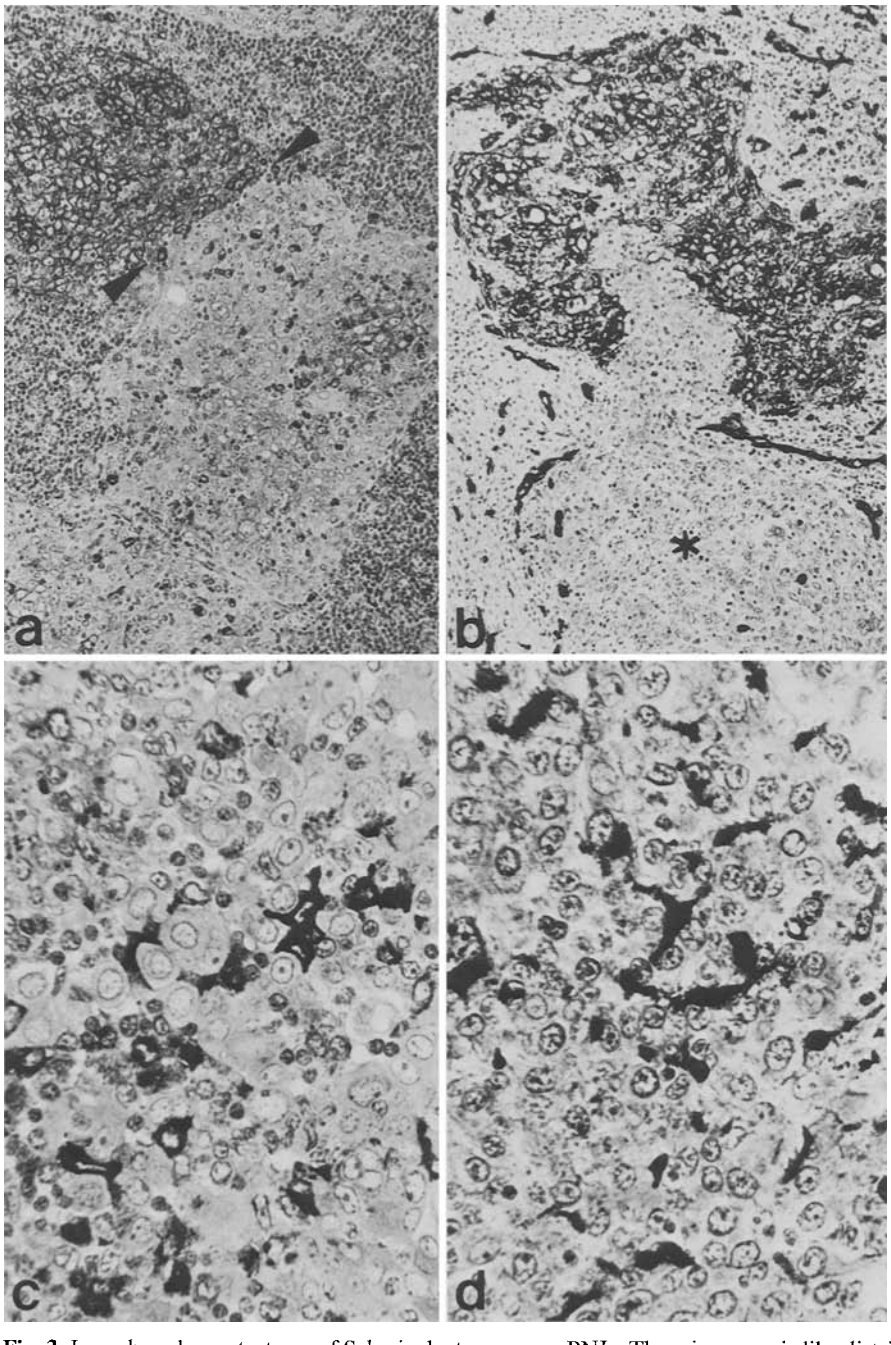

Fig. 3. Lymph nodes metastases of Schmincke tumours. **a** PNL: There is a mosaic like distribution of epithelial PNL-receptors, sometimes with distinct local limitation (*arrows heads*) suggesting a subcloning within the tumour cell population. Note the numerous intra-tumour PNL-positive histiocytes readily visible in the non-reactive area (×100). **b** UEA-I: A non-reactive part of the tumour (bottom, asterix surrounded by vessels) protruding into a typically reacting UEA-I-positive tumour area (×100). **c** PNL: Asteroidal intra-tumour PNL-positive histiocytes. Note the short cytoplasmic processes (×400). **d** S-100-protein: Intermingeled with negative tumour cells are S-100-protein-positive histiocytes with long arachnoid cytoplasmic projections, showing a distribution pattern comparable to that of the PNL-positive intraepithelial histiocytes (×400)

S-100 protein

S-100 protein containing cells are arachnoid shaped, located in the epithelium and to a much lesser degree among the follicles (Fig. 3d). Some cells with long extending projections can be visualized within the folliclar centres. In the carcinomas those cells can be detected in a distribution pattern which is comparable to that of the intratumour PNL-histiocytes, but in contrast to the latter, their cytoplasmatic processes seem to be longer and thinner than of those stained by lectin. Nevertheless, local differences in number per area are evident. The tumour cells do not contain any detectable S-100 protein.

Discussion

Normal human epidermis does not possess UEA-I-receptors (Reano et al. 1982; Schuler et al. 1982, own unpublished results) and only Nemanic et al. (1983) have detected them in the stratum granulosum. UEA-I recognizes the H-blood group antigen (Kent et al. 1964). Using monoclonal antibodies Dalbesteen et al. (1982) described the H-antigen on the cells in the basal part of the stratum spongiosum of the buccal mucosa and detected it within the cytoplasm in the dysplastic cells of oral pre-cancerous lesions (Dalbesteen et al. 1983). In the present study the normal tonsil showed an consistent binding pattern concerning its epithelial component with the exception of one case which was different in its PNL-receptor: the crypt epithelium being cytoplasmatically UEA-I-positive/PNL-negative (vd. Hsu and Ree 1983). The superficial epithelium and the oral mucosa reacted differently, that is to say, inversely with respect to its PNL-receptor expression and with a weaker (especially in the basal layer) UEA-I binding. Thus, the crypt epithelium seems to be a specialized structure presently demonstrated by its differing sugar equipment of cellular glycoconjugates. Being classified by us as typical lymphoepithelial carcinoma of the Schmincke type (1921), this nasopharyngeal tumour revealed the lectin phenotype of the tonsillar crypt epithelium in most areas. This finding supports the origin of this tumour from the tonsillar crypt epithelium and refutes the statement expressed by the WHO (Shanmugaratnam and Sobin 1978) that it is derived from the squamous or transitional epithelium but should be classified as an 'undifferentiated carcinoma'. A practical aspect of the UEA-I affinity of the tumour is that using the lectin reaction the otherwise difficult differential diagnosis between lymphoepithelial carcinoma and centroblastic or immunoblastic lymphoma can readily be solved, as lymphomas never express UEA-I-receptors (Möller and Lennert 1984). The apparent individual instability of the lectin phenotype in this series merits a comment. It appears in the primary lesion as well as in the lymph node metastases. Gupta (1983) however, who examined the expression of blood group isoantigens in nasopharyngeal carcinomas, described a complete loss of these antigens in all 10 lymph node metastases in contrast to their presence in the primary site and therefore discusses clonal selection. Our own findings either favour

the concept of mosaic-like gene-expression or an intrinsic lability of the phenotype, rather than the emergence of definite subclones. Hockey et al. (1984) came to the same conclusion when studying their data on polymorphism of gastric carcinoma in CEA-expression. The reticulated crypt epithelium contains PNL-positive histiocytes in slightly higher density than those containing S-100 protein. In the interfollicular areas these cells are scattered and surrounded by small lymphocytes. The PNL-positive "asteroid" histiocyte has already been described in detail (Möller 1982; Wirbel et al. unpublished work) and is interpreted as an antigen-presenting histiocyte of the T-cell branch of the immune system (Poulter 1983; Faure et al. 1984; van der Valk et al. 1984). The question of whether PNL specifically indicates the same cell as anti-S-100 protein can only be clarified by double staining techniques. We feel that with this technique there would be some overlapping but no identity of the binding spectra. The differences seem to be the higher density of PNL-positive cells and the fact that the S-100 protein containing cells have longer cytoplasmic processes and can be detected in follicular centres especially at the base (Wirbel and Möller unpublished work). Against the background of the orthological situation within the tonsillar crypt epithelium, we tend to interpret the conspicuous but regionally different density of these histiocytes among the tumour cells as quasi-physiological tropism. Thus, the tumour cells express the specific ecotactic signal attracting the Langerhans cells or their equivalents or precursors at least locally. The histiocytic component seems to be higher in tumour areas showing the "typical" lectin receptor phenotype (i.e. PNL-negative/UEA-I-positive) – a situation that was originally described as "lymphoepitheliale Durchdringung" (Schmincke 1921; Doerr 1976; Döhnert 1977). Such areas probably constitute the "well-differentiated" part of the tumour. A corresponding situation was recently observed in epithelial thymomas (Lauriola et al. 1984).

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