Virchows Arch [Pathol Anat] (1986) 408:603–609

Virchows Archiv A

'Epimyoepithelial' islands in lymphoepithelial lesions

An immunocytochemical study

Richard M. Palmer[1], John W. Eveson[1], and Barry A. Gusterson[2]

[1] Departments of Periodontology and oral Medicine and Pathology, United Medical and Dental Schools of Guy's and St. Thomas's, London Bridge, London SE1 9RT
[2] Ludwig Institute for Cancer Research, Royal Marsden Hospital, Sutton SM2 5PX, Great Britain

Summary. A panel of antibodies has been used in an immunoenzyme study in an attempt to characterize the cell types found in the 'epimyoepithelial' islands of lymphoepithelial lesions. Myoepithelial cells, which can be specifically stained with an anti-smooth muscle antibody were not found. The majority of cells stained with anti-prekeratin, suggesting a duct cell differentiation. A subpopulation of duct cells stainable with a monoclonal antikeratin (16a), previously described as occupying a basal location in normal salivary gland ducts, was clearly demonstrated. It is suggested that these cells may be important in the epithelial proliferation in these islands. The hyaline material within and surrounding the epithelial islands was positive for type IV collagen and therefore consists of basement membrane material.

Key words: Sjogrens – Myoepithelial – Cytokeratins

Introduction

Lymphoepithelial lesions of salivary glands, including Sjögren's syndrome, are characterized microscopically by infiltration and destruction of acini by masses of lymphoid tissue and proliferation of ducts which form islands of epithelium. The epithelial masses consist of closely packed, round or polyhedral cells which have prominent vesicular nuclei but often indistinct cytoplasmic outlines. Eosinophilic hyaline material is frequently present in and surrounding the epithelial islands, sometimes in large amounts.

These epithelial masses have been termed epimyoepithelial islands on the assumption that they have formed by proliferation of duct epithelium and myoepithelial cells (Morgan and Castleman 1953). However, the nature of the constituent epithelial cells is poorly defined and the role of myoepithelial cells in the formation of these islands is particularly uncertain (Boquist et al. 1970; Donath and Seifert 1972; Batsakis 1979; Takeda 1980; Saku

Offprint requests to: R.M. Palmer at the above address

and Okabe 1984). The object of this study, therefore, was to examine epimyoepithelial islands with a well characterized panel of antibodies to smooth muscle myosin, cytokeratins and type IV collagen in an attempt to define more fully the cell types involved in their formation.

Material and methods

Parotid tissue from normal glands and from four patients with Sjögren's syndrome was fixed in methacarn (60% methanol, 30% chloroform, 10% acetic acid) as this fixative is satisfactory for immunoenzyme staining of paraffin processed tissue with the range of antibodies used in the present study (Palmer et al. 1985). Specimens were dehydrated through ascending concentrations of alcohol, cleared in chloroform and impregnated with paraffin wax. Rehydrated sections were equilibrated in phosphate buffered saline. Immunocytochemical staining was undertaken using the appropriate peroxidase or alkaline phosphatase conjugated secondary antibodies (Sigma, 1:120 dilution) with the following primary antibodies:

1. Antibody to smooth muscle myosin raised in rabbits (1:100 dilution). This antibody has been shown to stain myoepithelial cells in normal glands on methacarn fixed/paraffin processed and cryostat sections with equal reliability (Palmer et al. 1985; Palmer 1986).
2. Antibody to human callus prekeratin raised in rabbits (1:120 dilution). This antibody has been shown to stain duct cells in normal glands (Gusterson et al. 1982; Palmer et al. 1985).
3. Monoclonal antibody 16a (1:100 dilution) to a 45/46K keratin doublet which stains a subpopulation of basally located duct cells in normal salivary glands (Knight et al. 1985; Palmer et al. 1985).
4. Monoclonal antibody to type IV collagen (1:1000 dilution) which has previously been shown to stain basement membranes (Gusterson et al. 1984; Palmer et al. 1985).

Control sections were incubated with preimmune sera and it was also demonstrated that staining was extinguished by prior absorption of the antibodies with the appropriate antigen. All controls were negative. Sections were counterstained with Mayer's haemalum.

Results

Normal glands

Myoepithelial cells surrounding the acini and intercalated ducts were stained with anti-smooth muscle myosin (Fig. 1). Acinar and duct cells were negative and the blood vessel media and endothelium were positive, thus providing a good internal positive control. The anti-prekeratin produced positive staining of all duct cells. The monoclonal anti-keratin 16a stained a sub-population of basally located cells in the striated and excretory ducts (Fig. 2). The anti-type IV collagen stained the basement membranes surrounding the acini, ducts and blood vessels.

Sjögren's syndrome

Cells in the 'epimyoepithelial' islands were negative with anti-smooth muscle myosin, but the majority of cells were positive with anti-prekeratin (Fig. 3). The monoclonal anti-keratin 16a produced staining of a subpopulation of epithelial cells which were found peripherally and as discrete strands throughout the epithelial islands (Fig. 4). The islands were surrounded by

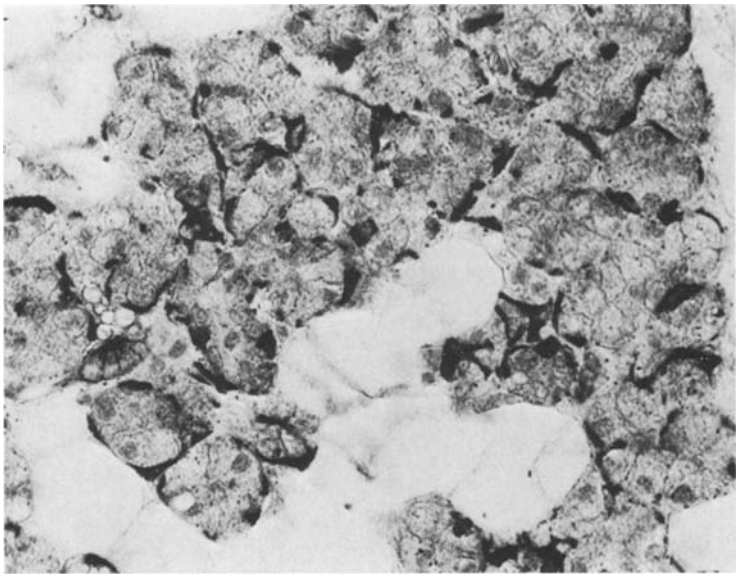

Fig. 1. Myoepithelial cells surrounding acini and intercalated ducts stained with anti-smooth muscle myosin. Acinar and duct cells are negative. × 300

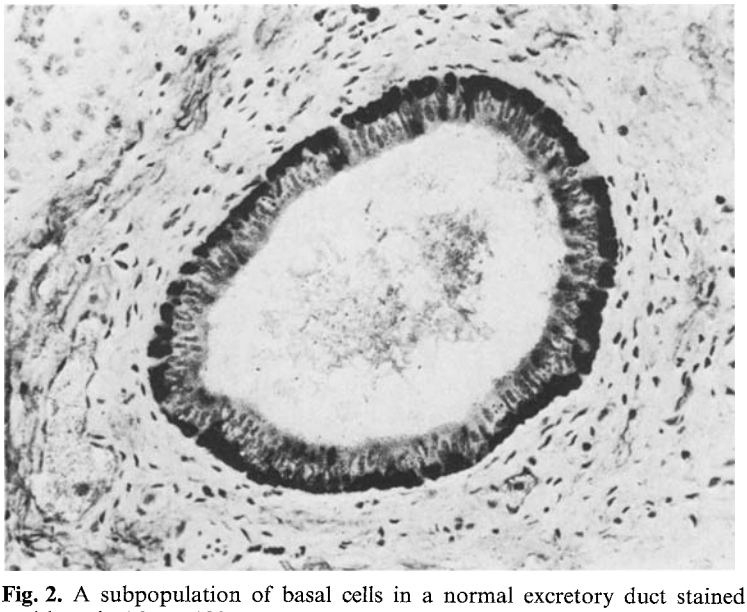

Fig. 2. A subpopulation of basal cells in a normal excretory duct stained with monoclonal anti-keratin 16a. × 190

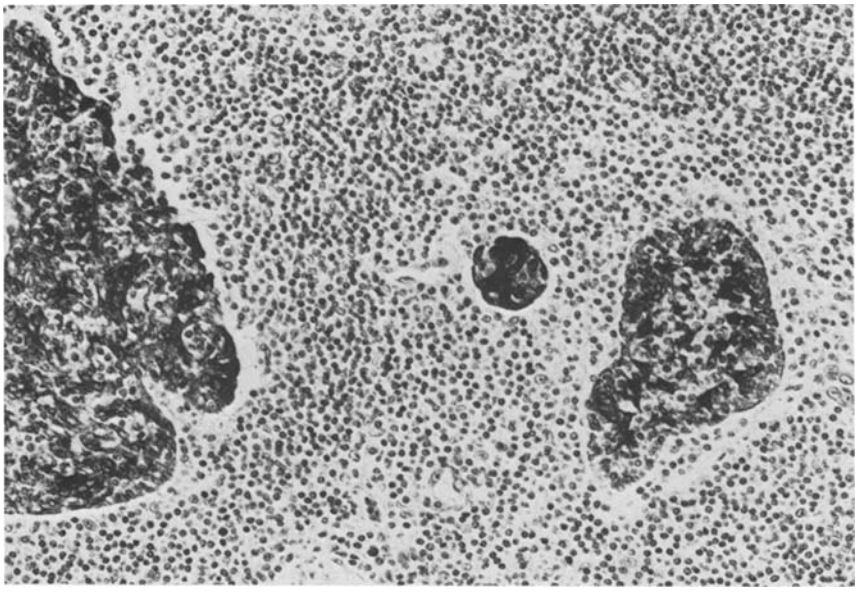

Fig. 3. The majority of cells in the epithelial islands stained with polyclonal antibody to human callus prekeratin. × 190

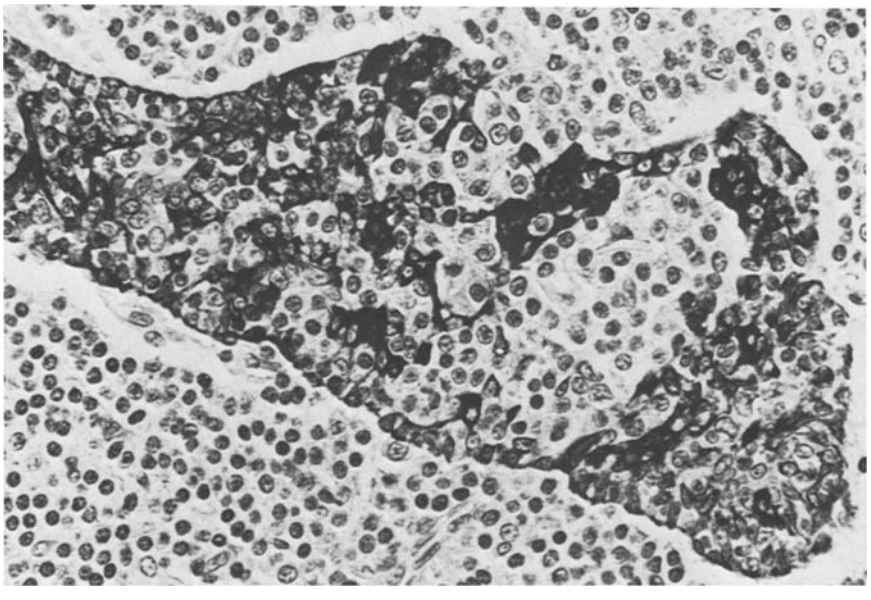

Fig. 4. A subpopulation of epithelial cells stained with monoclonal antikeratin 16a. × 400

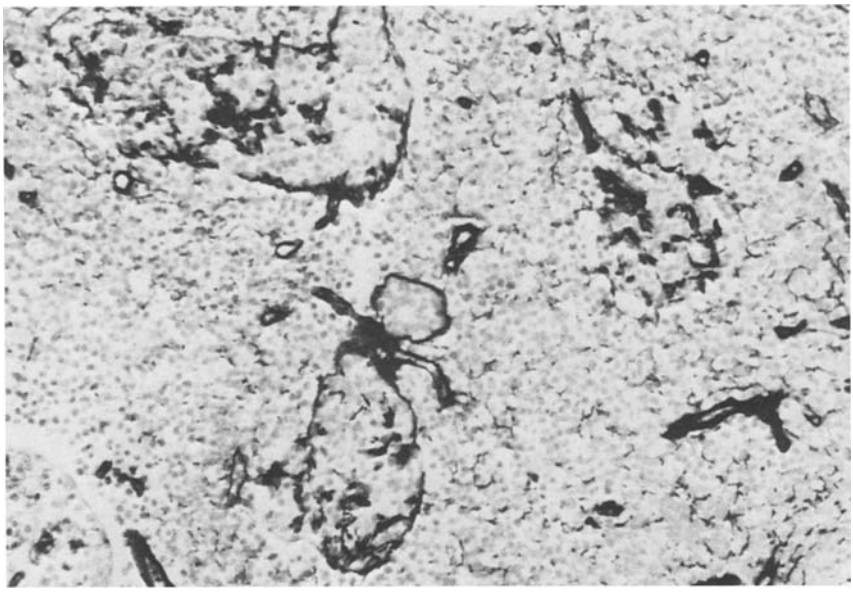

Fig. 5. Type IV collagen clearly delineates the epithelial islands. ×190

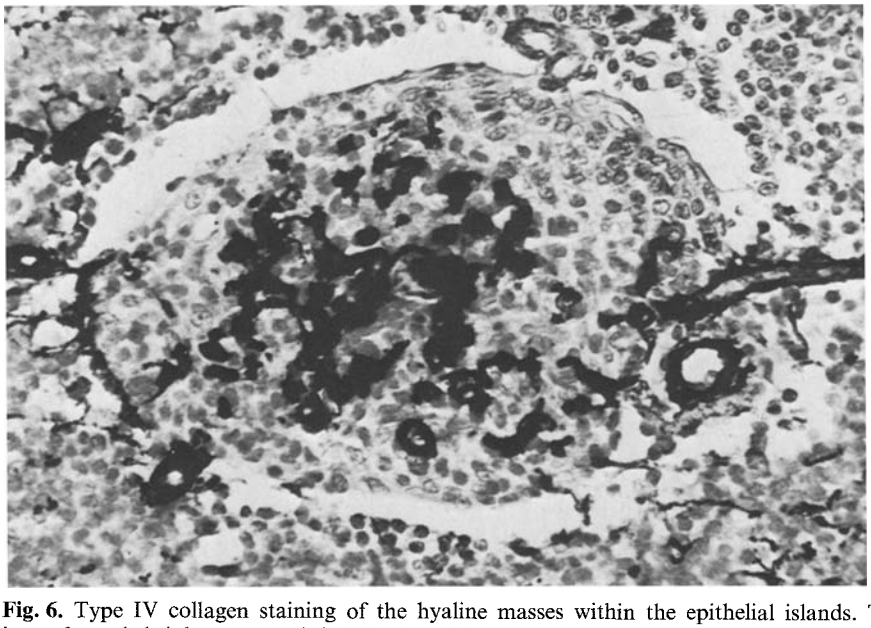

Fig. 6. Type IV collagen staining of the hyaline masses within the epithelial islands. There is artefactual shrinkage around the island. ×400

type IV collagen and in addition there were circumscribed aggregates of positive material within the cell masses (Figs. 5 and 6).

Discussion

The first comprehensive description of the histogenesis of Sjögren's syndrome was by Morgan and Castleman in 1953. The two co-existing fundamental changes were progressive infiltration and proliferation of lymphocytes within lobules and loss of salivary gland parenchyma, and intraductal proliferation with gradual loss of the duct lumen to form solid cellular islands in a lymphoid stroma. They called the altered ducts epimyoepithelial islands believing them to consist of both epithelial and myoepithelial cells. In a light and electron microscopic investigation Donath and Seifert (1972) showed these islands were derived from intercalated ducts. They also proposed that the islands consisted of duct cells, myoepithelial cells and inflammatory cells, at least in the early stages. However, the role of the myoepithelial cell in the histogenesis of these islands has been disputed on the basis of electronmicroscopic (Boquist et al. 1970) and immunocytochemical investigations (Saku and Okabe 1984).

In the present study myoepithelial cells were not found in any of the islands examined, which consisted mainly of cells positive for prekeratin and thus resembled the duct cells of the normal gland. This is in agreement with the recent work by Saku and Okabe (1984). The anti-smooth muscle myosin used in our study is a highly specific label of myoepithelial cells in salivary glands (Palmer et al. 1985; Palmer 1986) and breast (Gusterson et al. 1982) and is preferable to anti-actin. However, it must be appreciated that the material examined in the present study was typical of advanced disease. It is therefore possible that during the early development of these epithelial islands myoepithelial cells persist at the periphery of proliferating ducts as described in the ultrastructural study by Takeda (1980). Certainly, in the early stages of obstructive sialadenitis the myoepithelial cells in association with the ducts survive very well as shown by Emmelin et al. (1974) and Palmer (unpublished observation). It may be postulated that in benign lymphoepithelial lesions the myoepithelial cells are subsequently lost or undergo dedifferentiation to epithelial cells as suggested by Dardick et al. (1983) in benign neoplasia. Further immunocytochemical and ultrastructural study of early lesions is therefore required.

In the report by Takeda (1980) there was no evidence of myoepithelial proliferation in lymphoepithelial lesions, in direct contrast to the work by Donath and Seifert (1972). The present study does not support myoepithelial proliferation as an important factor in the development of these islands. However, many cells, both in a basal location and in strands within the islands, were positive with the monoclonal anti-keratin 16a. In normal salivary glands this antibody stains a subpopulation of basally located cells in the striated and excretory ducts which may represent a population of reserve cells (Palmer et al. 1985; Knight et al. 1985). Similar cells have been demonstrated in the outer layer of the double layered duct structures in pleomorphic adenoma (Palmer et al. 1985) and may be important in the

histogenesis of this tumour. It is possible that this duct cell population is also involved in the proliferation of duct epithelium in the islands of lymphoepithelial lesion rather than the previously suggested myoepithelial proliferation.

In addition, a characteristic feature of the epithelial islands in lymphoepithelial lesion is hyaline material surrounding the islands and between the epithelial cells. Electron microscopic studies have shown the hyaline material to consist of collagen fibres which are separated from the epithelium by an electronlucent zone (Kahn 1979). This appearance was identical to basement membrane at the periphery of the islands. Therefore, the finding in the present study that both the delimiting membrane and the hyaline material stain strongly for type IV collagen, a well characterized basement membrane component, confirms the ultrastructural observations. Furthermore, it is possible that this type IV collagen is produced by the subpopulation of 16a positive cells situated at the periphery and within the epithelial islands.

Acknowledgements. The authors thank Alex Woodman for the photomicrography.

References

Batsakis JG (1979) Tumors of the head and neck. 2nd ed. The Williams and Wilkins company. Baltimore, pp 106–112

Boquist L, Kumlien A, Östberg Y (1970) Ultrastructural findings in a case of benign lymphoepithelial lesion (Sjögren's syndrome). Acta Otolaryngol 70:216–226

Dardick I, Van Nostrand AWP, Jeans MTD, Rippstein P, Edwards V (1983) Pleomorphic adenoma. I. Ultrastructural organization of "epithelial" regions. Hum Pathol 14:780–797

Donath K, Seifert G (1972) Ultrastruktur und Pathogenese der myoepithelialen Sialadenitis. Über das Vorkommen von Myoepithelzellen bei der benignen lymphoepithelialen Läsion. Virchows Arch [Pathol Anat] 356:315–329

Emmelin N, Garrett JR, Ohlin P (1974) Secretory activity and the myoepithelial cells of salivary glands after duct ligation in cats. Archs Oral Biol 19:275–283

Gusterson BA, Warburton MJ, Mitchell D, Ellison M, Neville AM, Rudland PS (1982) Distribution of myoepithelial cells and basement membrane proteins in the normal breast and benign and malignant breast disease. Cancer Res 42:4763–4770

Gusterson BA, Warburton MJ, Mitchell D, Kraft N, Hancock WW (1984) Invading squamous cell carcinoma can retain a basal lamina: an immunohistochemical study using a monoclonal antibody. Lab Invest 61:82–87

Kahn LB (1979) Benign lymphoepithelial lesion (Mikulicz's disease) of the salivary gland: an ultrastructural study. Hum Pathol 10:99–104

Knight J, Gusterson BA, Russell Jones R, Landells W, Wilson P (1985) Monoclonal antibodies specific for subsets of epidermal keratins: biochemical and immunocytochemical characterization – applications in pathology and cell cultures. J Pathol 145:341–354

Morgan WS, Castleman B (1953) A clinicopathologic study of "Mikulicz's disease". Am J Pathol 29:471–503

Palmer RM, Lucas RB, Knight J, Gusterson B (1985) Immunocytochemical identification of cell types in pleomorphic adenoma, with particular reference to myoepithelial cells. J Pathol 146:213–220

Palmer RM (1986) The identification of myoepithelial cells in human salivary glands. A review and comparison of methods. J Oral Pathol (In press)

Saku T, Okabe H (1984) Immunohistochemical and ultrastructural demonstration of keratin in epi-myoepithelial islands of autoimmune sialadenitis in man. Arch Oral Biol 29:687–689

Takeda Y (1980) Histopathological studies of the labial salivary glands in patients with Sjögren's syndrome. Part II: Electron microscopic study. Bull Tokyo Med Dent Univ 27:27–42

Accepted December 10, 1985