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Immunohistochemical localization of αl-antitrypsin and αl-antichymotrypsin in salivary pleomorphic adenomas

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Summary. Immunohistochemical identification of αl-antitrypsin (αl-AT) and αl-antichymotrypsin (αl-ACh) in pleomorphic adenomas of salivary glands is reported in order to compare their distribution profiles with those of lysozyme and lactoferrin, already described elsewhere.

Normal salivary glands indicated positive αl-AT staining in ductal segments and had no αl-ACh in any glandular cell. Pleomorphic adenomas displayed moderate positivity to αl-AT staining in duct-like, tubular and glandular epithelia which was particularly intense in luminal cells. The limited number of tumour cells which showed duct-like structures with a single cellular layer arrangement, displayed the highest staining to αl-ACh. Strongly αl-AT positive tumour cells located on the inner side of luminal cavities were also markedly positive to αl-ACh. Spindle shaped tumour cells existed outside tubular and ductal structures and were negative to αl-AT and αl-ACh.

Distribution of αl-AT in salivary glands was similar to that of lysozyme as is usual in ductal segments or their transformed cells, and occurrence of αl-ACh localization rather resembled that of lactoferrin, with occurrence in acinar compartments and changed epithelia within acini.

The biological role of a specific immunohistochemical distribution of αl-AT and αl-ACh in pleomorphic adenomas may be associated with a self regulating mechanism which inhibits degradation by tissue proteinases.

Key words: αl-antitrypsin – αl-antichymotrypsin – Salivary gland – Pleomorphic adenoma – Immunohistochemistry

Introduction

αl-antitrypsin (αl-AT) and αl-antichymotrypsin (αl-ACh) are major proteinase inhibitors which are distributed in the pancreas, liver, gall bladder,

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gastrointestinal mucosa, and inflammatory cells (Gauldie et al. 1980; Kittas et al. 1982 b; McElrath et al. 1979; Morse 1978; Travis et al. 1978 a). It has been reported that αl-AT and αl-ACh exist biochemically in many body fluids such as tears, saliva, lymph, milk, duodenal, semen and aminiotic fluid, and gall bladder bile. The two proteinase inhibitors affect trypsin, chymotrypsin, elastase, collagenase, renin, kallikrein, urokinase and Hagemam-factor, thrombin, and plasmin as inhibitors of their functions (Kittas et al. 1982 a; Papadimitriou et al. 1980; Travis et al. 1978 b).

The biological significance of the occurrence of αl-AT and αl-ACh in tumour tissues is uncertain, however several theses have demonstrated that they appear on absorption epithelia such as duodenal mucosa and that their existence suggest a control mechanism for preventing degradation of involuting cells, including those in inflammatory conditions and in neoplastic growth. It is reported that duct cells of the submandibular gland (SMG) contain αl-AT and not αl-ACh, whereas, in obstructive adenitis in SMG, αl-AT decreases gradually and finally gives a negative response. This is contrast to αl-ACh which appears quickly in acinar or transformed acinar elements (Murase et al. 1985).

The present immunohistochemical investigation demonstrates αl-AT and αl-ACh in pleomorphic adenomas of salivary gland origin and then compares the results with those obtained in obstructive adenitis in the submandibular glands and in normal salivary glands.

Materials and methods

Material. 33 cases of pleomorphic adenoma from salivary glands and associated normal salivary gland tissue were used. The tumour specimens were fixed in 10% formalin at 12 hours and embedded in paraffin. Paraffin sections 4μm thick were made to detect αl-AT and αl-ACh immunohistochemically, as well as for HE staining.

Immunohistochemical Detection of αl-AT and αl-ACh. Deparaffinized sections were rinsed with 0.01 M Phosphate buffered saline (PBS) (pH 7.0) and immersed in 0.3% H_2O_2/methanol solution for 30 minutes to inactivate endogenous peroxidase. The sections were rinsed with PBS. The following steps were then done:

1) Immersion in normal swine serum (1:20 dilution) for 30 min.
2) Reaction with rabbit anti-human αl-AT or αl-ACh (1:100) for 2 hours.
3) Reaction with swine anti-rabbit Immunogloblins (1:20) for 30 min.
4) Reaction with rabbit peroxidase antiperoxidase complexes (PAP) (1:100) for 60 min.

Finally, they were rinsed well and made to react with 0.05 M Tris buffer containing 0.3% diaminobenzidine (DAB) and 0.05% H_2O_2 for 10 min. All reactions were done at room temperature (20 °C).

Control sections with anti-human αl-AT and αl-ACh deleted from these procedures, gave negative results.

Normal swine serum, Rabbit anti-human αl-AT and αl-ACh, swine anti-rabbit IgG antiserum and PAP were purchased from Dakopatts, Copenhagen, Denmark.

Results

αl-antitrypsin (αl-AT)

Normal major salivary glands indicated positive αl-AT staining in ductal segments: in striated and excretory ducts at moderate levels, and in intercalated duct at slight levels only (Fig. 1 a, b). Minor salivary glands

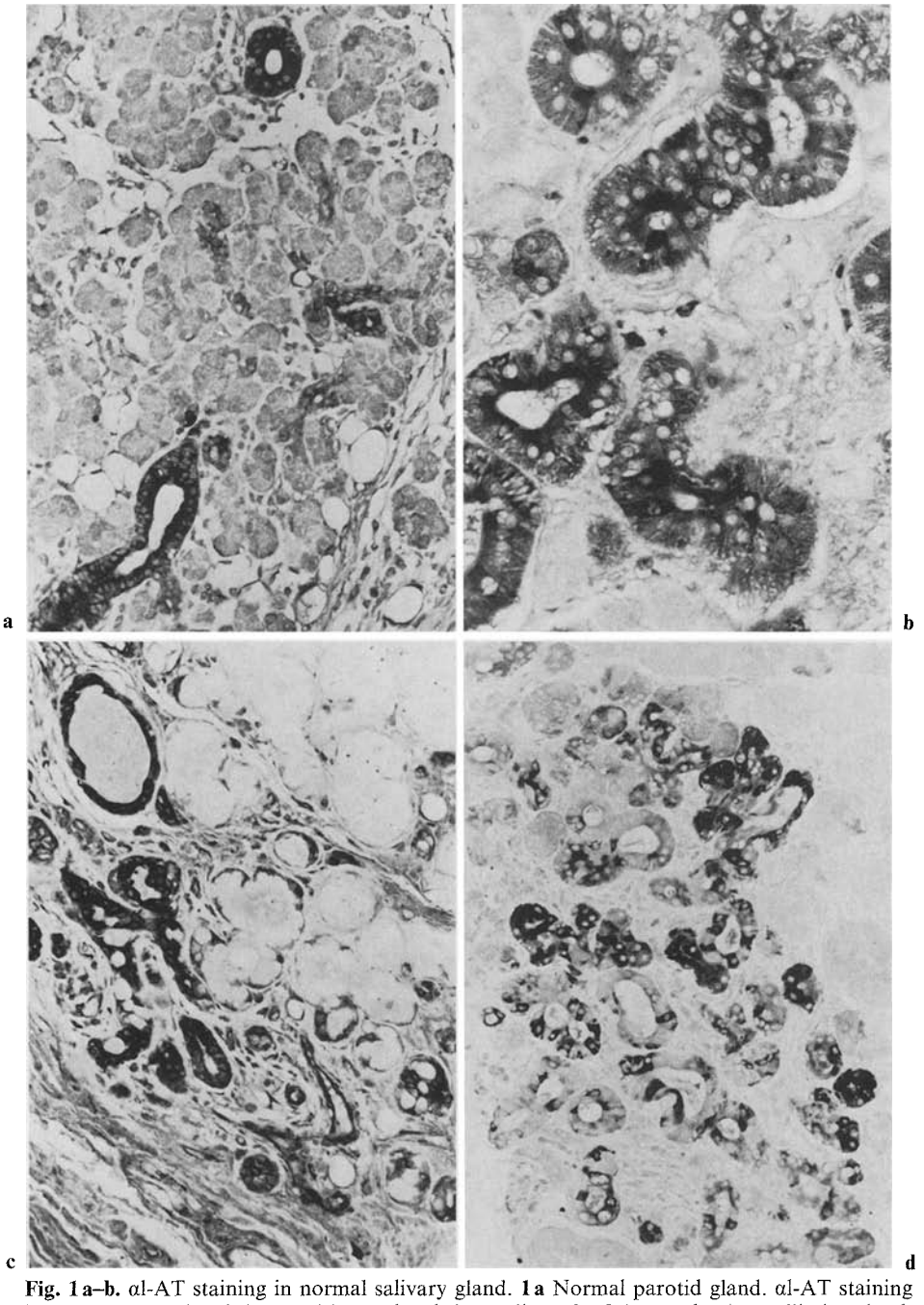

Fig. 1 a–b. αl-AT staining in normal salivary gland. **1 a** Normal parotid gland. αl-AT staining is confined to striated duct and intercalated duct cells. ×85. **b** Normal submandibular gland. αl-AT staining is strong in ductal epithelia and particularly intense on the luminal side of ductal cells. ×170. **1 c** Mucous glands of palatal mucosa.
αl-AT staining of duct is strong and demilune cells are moderate. ×85. **d** Atrophic glands of submandibular gland adjacent to pleomorphic adenoma. There are numerous αl-ACh positive cells in the atrophic glandular structure. ×85

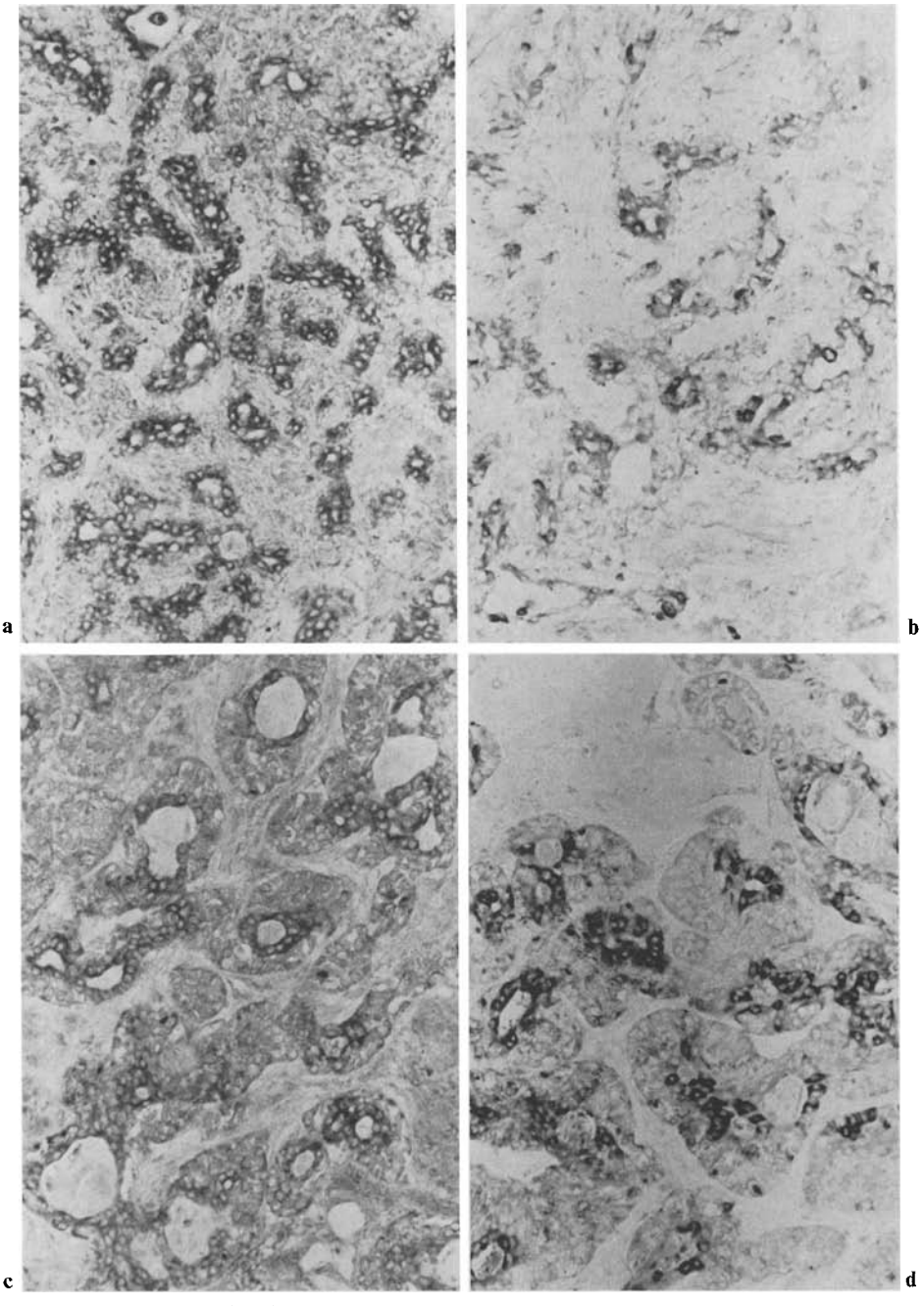

Fig. 2a–d. Pleomorphic adenoma in parotid gland. **a** α1-AT staining exists at the inner side of glandular and duct-like structures. ×85. **b** Similar area to Fig. 2a. Small amounts of α1-ACh positive tumour cells are distributed on the inner side of the structure. ×85. **c** Slight α1-AT staining is found in glandular cells, and intense in luminal side. ×85. **d** Similar area to fig. 2c. α1-ACh staining is strong in luminal side of glandular structures and intense positive cells are scattered in neoplastic foci. ×85

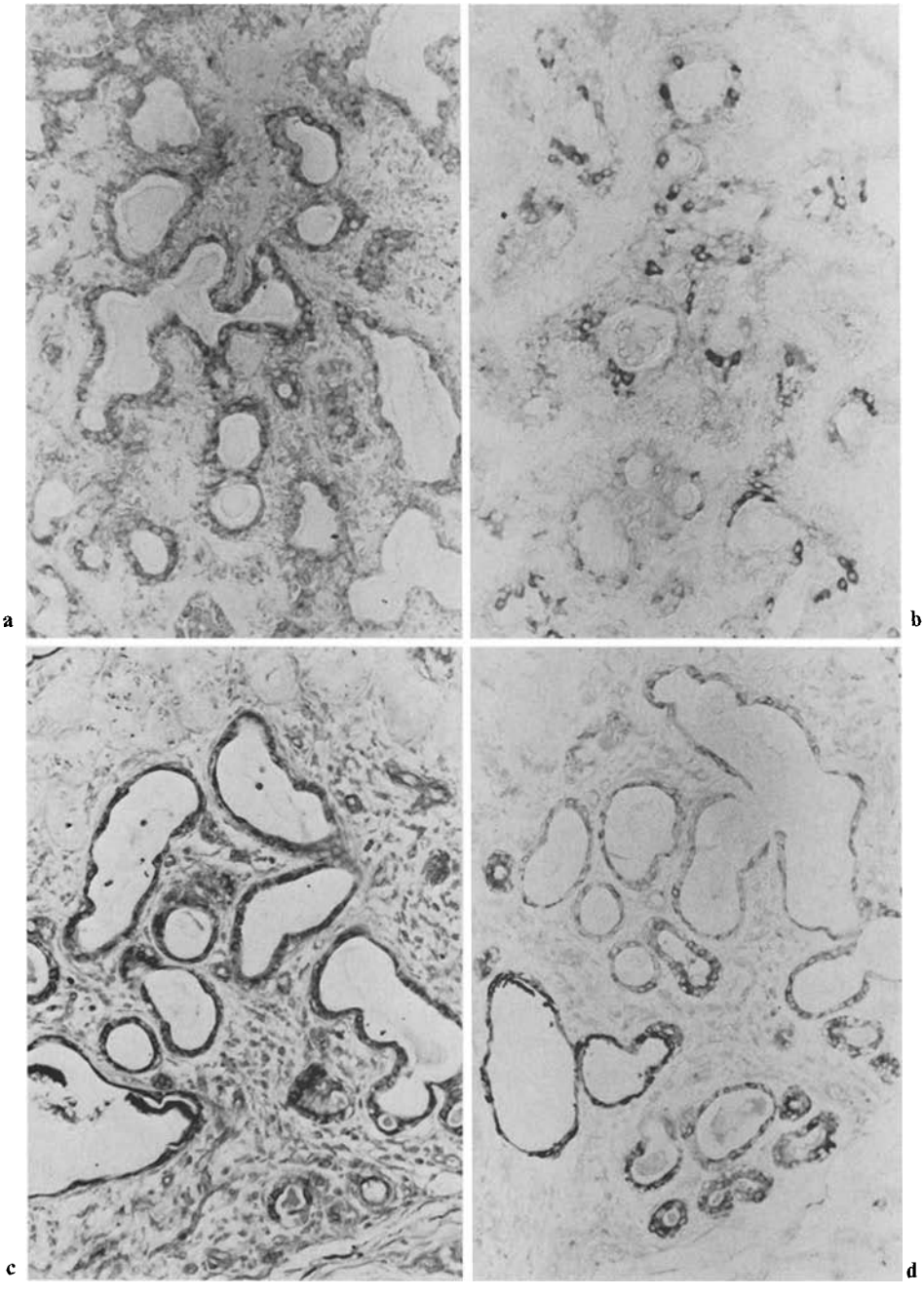

Fig. 3a–d. Pleomorphic adenoma in submandibular gland. **a** Slight αl-AT staining is located on a single cellular layer of a multiple cystic structure. ×85. **b** Similar area to Fig. 3a. αl-ACh positive cells are scattered on the inner side of a duct-like structure. ×85. **c** Relativly marked αl-AT staining is found in ductal tumour epithelium. ×85. **d** Similar area to Fig. 3c. Varyingly positive αl-ACh cells are distributed in a ductal tumour structure. ×85

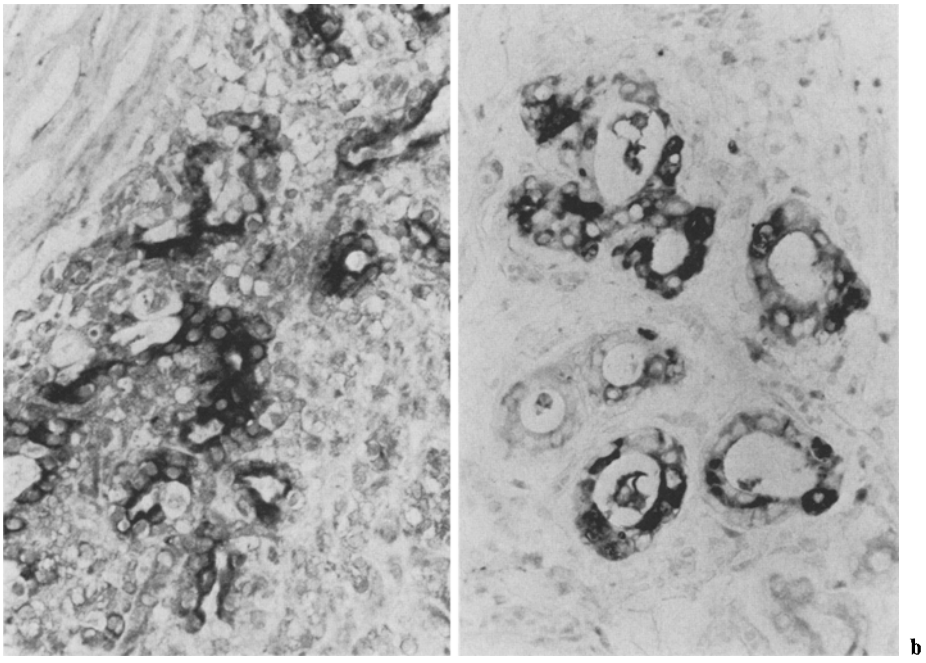

Fig. 4a–b. Pleomorphic adenoma of parotid gland. **a** Strong αl-AT staining is located on the luminal side of tumour cells. × 85. **b** Strongly αl-ACh positive cells exist in a duct-like structure. There are intermingled strongly positive αl-ACh cells and negative cells in a duct-like tumour structure. × 170

indicated moderate staining to αl-AT in ductal cells and some serous or demilune cells surrounding mucous acinar compartments (Fig. 1c).

Pleomorphic adenomas of salivary glands showed variable histological findings, that is, they usually consisted of tumour strands and nests that grew as duct-like structures with single, double or multiple cellular layers. The inner layers of the lumen revealed cuboidal, flattened or thin epithelial cells. Outer layer cells showed spindle and long amastamosing cells. αl-AT staining usually existed at slight levels in glandular, tubular and duct-like structures and was negative in spindle shaped cells, amastamosing cells or solid structures. The reaction products of αl-AT were particularly intense at the inner lining cells of duct-like or glandular tumour structures from pleomorphic adenomas (Figs. 2a, 2c, 3a, 3c). The staining intensities in neoplastic epithelial cells varied independently of their histological structure (Fig. 4a). No reaction was obtained in connective tissue fibers or hyaline and myxomatous tissues. Mast cells in the tumour stroma displayed strong αl-AT staining.

αl-antichymotrypsin (αl-ACh)

Normal salivary glands were devoid of αl-ACh staining in both acinar and duct cells. In the histologically normal parts of submandibular gland which

are associated with pleomorphic adenoma, small regional acinar cells displayed strong αl-ACh staining (Fig. 1d). In such regions, the epithelia which consisted of single acinar or duct-like components had varying positive αl-ACh staining from negative to the strongly positive. In pleomorphic adenoma, almost all tumour cells showed a negative reaction to αl-ACh and slightly positive tumour cells were very rare (Fig. 2b). Strongly positive tumour cells were grouped and scattered in neoplastic foci and were always at the inner-lining of duct-like structure as a single row. There were glandular and/or tubular structures with double or multiple arrangements of epithelial cells (Fig. 2d). In single arrangements of cuboidal or flattend tumour epithelial cells, the strongest αl-ACh positive cells and negative or tracable staining cells were intermingled in the same ductal or duct-like epithelia (Figs. 3b, d). In double or multiple layered arrangements, αl-ACh reaction usually developed on the inner-side of cells of the structure and the αl-ACh strongly cells located the luminal side were always positive to αl-AT staining (Fig. 4b). αl-AT positive tumour cells were always positive to αl-ACh reaction, but αl-ACh positive tumour cells were not always positive to αl-AT staining. Histiocytes in the stroma as well as mast cells showed strongly positive staining to αl-ACh.

Discussion

Immunohistochemical detection of αl-AT and αl-ACh has been established in gastrointestinal neoplastic lesions including the stomach (Tahara et al. 1984) and large intestine (Kittas et al. 1982a, b) and gall bladder (Aronio et al. 1984). αl-AT has been found in small intestine (Geboes et al. 1982), in hepatic cells and tumours (Palmer and Wolfe 1976; McElarth et al. 1979; Carlson et al. 1981; Gauldie et al. 1980) and in pancreatic islet cells and their tumours (McElrath et al. 1979; Ordonez et al. 1983). It is suggested that the biological roles of αl-AT and αl-ACh in intestinal epithelia may be related to a self-protective mechanism against the pancreatic proteolytic enzymes (Kittas et al. 1982b), Aroni et al. (1984) stated that in gall bladder adenocarcinoma, positive reactions to αl-AT and αl-ACh supports the possibility of local production from epithelial cells rather than absorption from bile.

Immunohistochemical investigations of αl-AT and αl-ACh reactions in normal salivary glands and their obstructive lesions have been examined. A recent immunohistochemical study has pointed out that αl-AT reactions occurred in normal salivary gland ducts, but αl-ACh was non-existent in normal glandular cells. αl-ACh in obstructive adenitis increased abundantly and quickly in acinar elements, but αl-AT decreased in ductal segments (Murase et al. 1985). It is likely that αl-AT and αl-ACh are present in salivary glands as they are found in tears, saliva, lymph and intestinal secretions (Caselitz et al. 1982; Takata et al. 1984). Histochemical findings in obstructive adenitis suggest that the striking reaction of αl-ACh might be

explained as a self-controlling mechanism to prevent cellular damage from proteolytic enzymes. The self-defense mechanism in the oral and nasal cavities is related to the secretion of IgG, lysozyme and lactoferrin from salivary gland and nasal glands, as an antibacterial function (Korsrud and Brandtzaeg 1984; Miyauchi 1984; Moro et al. 1984; Ogawa et al. 1984; Saito et al. 1984; Sato et al. 1984; Tsukitani et al. 1985). Immunohistochemical detectable lysozyme and αl-AT show a similar distribution in ductal segments of normal salivary glands, and lactoferrin and also αl-ACh showed similar localization in acinar components in obstructive conditions. These combined effects of lysozyme and αl-AT (duct), and lactoferrin and αl-ACh (acinar) in different parts of the same salivary glands may indicate that one purpose is a role for simultaneous antibacterial defense and antidegradation in the salivary gland cells.

In salivary pleomorphic adenoma the origins of the neoplastic cells are probably duct cells and myoepithelium (Erlandson et al. 1984). Intercalated duct cells displayed slight positive staining to αl-AT in normal glands; benign tumour epithelia appearing as duct-like structures also displayed positive αl-AT staining in general. Such findings may suggest that one histogenesis of pleomorphic adenoma is that it arises from intercalated duct cells. In contrast to this, the immunohistochemical localization of αl-ACh in pleomorphic adenoma was found only in limited epithelia distributed at the inner-lining of the lumena in duct-like structures, those αl-ACh positive tumour cells may participate in transformation from acinar origin cells. However, the histological figures do not show whether this is from ductal or acinar origins. Spindle tumour cells which showed growth in ductal, tubular and glandular structures were devoid of αl-AT and αl-ACh reactions. In the present study, no positive reaction for either αl-AT or αl-ACh was shown in these spindle shaped tumour cells.

Pleomorphic adenoma is characterized by a wide distribution of αl-AT at moderate intensity, in contrast to the limited distribution of αl-ACh with strong staining. Inner lining tumour cells in duct-like structures and tubular and glandular structures were generally characteristically strongly stained for both αl-AT and αl-ACh. This coexistense of αl-AT and αl-ACh reactions may also be associated with inhibition of control of degradation of proteinase in pleomorphic adenoma.

It has been pointed out that aminopeptidase and varying kinds of β-naphthylamidases are distributed in many gland components, ducts, acinar cells and stroma (Kawakatsu et al. 1960; Monis et al. 1959; Murata and Miyaji 1966). Aminopeptidases using β-naphthyl amide derivatives of *L*-leucin or *DL*-alanin as substrates were usually localized in proteolytic areas and fibroblastic regions; they may develop simultaneously in the tumours. However, using azo coupling methods for αl-AT, αl-ACh distribution was not compatible. This problem may be solved if specific antisera against proteinase, trypsin and chymotrypsin are purified immunologically. Further studies should be made involving comparative observations of αl-AT and αl-ACh and of trypsin and chymotrypsin in salivary gland tissues.

References

Aroni K, Kittas C, Papadimitrou CS, Papacharalampous NX (1984) An immunocytochemical study of the distribution of lysozyme, αl-antitrypsin and αl-antichymotrypsin in the normal and pathological gall bladder. Virchows Arch [Pathol Anat] 403:281–289

Carlson J, Eriksson S, Hagestrand I (1981) Intra- and extra-cellular alpha-l-antitrypsin in liver disease with special reference to Pi phenotype. J Clin Pathol 34:1020–1025

Caselitz J, Seifert G, Jaup T (1982) Tumor antigens neoplasms of human parotid gland. J Oral Pathol 11:374–386

Erlandson RA, Cardon-Cardo C, Higgins PJ (1984) Histogenesis of benign pleomorphic adenoma (mixed tumor) of the major salivary glands. An ultrastructural and immunohistochemical study. Am J Surg Pathol 8:803–820

Gauldie, J, Lamontagne L, Horsewood P, Jenkins E (1980) Immunohistochemical localization of αl-antitrypsin in normal mouse liver and pancreas. Am J Pathol 101:723–735

Geboes K, Ray MB, Rutgeerts P, Callea F, Desmet VJ, Vantrappen G (1982) Morphological identification of alpha-l-antitrypsin in human small intestine. Histopathology 6:55–60

Kawakatsu K, Mori M, Mizushima T, Murakami M (1960) Histochemical observation aminopeptidase activity in normal salivary gland. Arch Histol Jpn 19:509–517

Kittas C, Aroni K, Kotsis L, Papadimitriou CS (1982a) Distribution of lysozyme, αl-antichymotrypsin and αl-antitrypsin in adenocarcinomas of the stomach and large intestine. Virchows Arch [Pathol Anat] 398:139–147

Kittas C, Aroni K, Matani A, Papadimitriou CS (1982b) Immunocytochemical demonstration of αl-antichymotrypsin in human gastrointestinal tract. Hep Gastroenterol 29:275–277

Korsrud FR, Brandtzaeg P (1984) Immunoflourescence study of secretory epithelial markers in pleomorphic adenomas. Virchows Arch [Pathol Anat] 403:291–300

McElrath MJ, Galbraith RM, Allen RC (1979) Demonstration of alpha l-antitrypsin by immunoflourescence on paraffin-embedded hepatic and pancreatic tissue. J Histochem Cytochem 27:794–796

Miyauchi J (1984) Distribution and subcellular localization of lactoferrin in human tissues with special reference to the submandibular gland. Acta Histochem Cytochem 17:77–89

Monis B, Nachlas M, Seligman AM (1959) Study of leucine aminopeptidase in neoplastic and inflammatory tissues with a new histochemical method. Cancer 12:601–608

Moro I, Umemura S, Crago SS, Mestecky J (1984) Immunohistochemical distribution of immunoglobulines, lactoferrin, and lysozyme in human minor salivary glands. J Oral Pathol 13:97–104

Morse JO (1978) Alpha L-antitrypsin deficiency. N Eng J Med 299:1045–1048

Murase N, Kobayashi K, Mitani Y, Mori M (1985) Immunohistochemical demonstration of αl-antitrypsin and αl-antichymotrypsin in obstructive adenitis of submandibular glands. J Oral Pathol (in press)

Murata I, Miyaji T (1966) Histochemical evaluation of enzymatic activities in pleomorphic salivary adenoma. Oral Surg 22:82–90

Ogawa I, Takata T, Nikai H, Ogawa M, Ijuhin N, Ito H (1984) Immunohistochemical studies on salivary gland tumors. II Localization of secretory epithelial markers in various benign tumors. Jpn J Oral Biol 26:1200–1209

Ordonez NG, Manning JT, Hanssen G (1983) Alpha-l-antitrypsin in islet cell tumors of the pancreas. Am J Clin Pathol 80:277–282

Palmer PE, Wolf HJ (1976) αl-antitrypsin deposition in primary hepatic carcinomas. Arch Pathol Lab Med 100:232–236

Papadimitriou CS, Stein H, Papacharalampous NX (1980) Presence of αl-antichymotrypsin and αl-antitrypsin in haematopoietic and lymphoid tissue cells as revealed by the immunoperoxidase method. Pathol Res Practerial 169:287–297

Saito I, Teratani K, Inoue A, Saito K, Funatsu K, Moro I (1984) Immunohistochemical characterization of functional markers in human minor salivary gland tumors. J Oral Pathol 13:525–534

Sato M, Hayashi Y, Yoshida H, Yanagawa T, Yura Y, Nitta T (1984) Search for specific

markers of neoplastic epithelial duct and myoepithelial cell lines established from human salivary gland and characterization of their growth in vitro. Cancer 54:2959–2967

Tahara E, Ito H, Taniyama K, Yokozaki H, Hata J (1984) Alphal-antitrypsin, alphal-antichymotrypsin, and alpha2-macroglobulin in human gastric carcinomas: A retrospective immunohistochemical study. Hum Pathol 15:957–964

Takata T, Ogawa I, Nikai H, Ogawa M, Ijuhin N , Ito H (1984) Immunohistochemical studies on salivary gland tumors. I Localization of various secretory epithelial markers in the normal salivary gland tissues. Jpn J Oral Biol 26:1190–1199

Travis J, Bowen J, Baugh R (1978a) Human α1-antichymotrypsin: Interaction with chymotrypsin-like proteinases. Biochemistry 17:5651–5656

Travis J, Garner D, Bowen J (1978b) Human α1-antichymotrypsin: Purification and properties. Biochemistry 17:5647–5651

Tsukitani K, Nakai M, Tatemoto Y, Hikosaka N, Mori M (1985) Histochemical studies on obstructive adenitis of human submandibular salivary glands. 1. Immunohistochemical demonstration of lactoferrin, lysozyme, and carcinoembryonic antigen. J Oral Pathol (in press)

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