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Histology and Histopathology

Warthin's tumor as a hamartomatous dysplastic lesion: a histochemical and immunohistochemical study

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Summary. The etiology of Warthin's tumor was sought by histochemical and immunohistochemical methods using 7 surgically extirpated samples and normal salivary glands as a control for the epithelial component. All the samples exhibited a variety of amyloid deposition in the interfollicular area of the lymphoid component. The interfollicular lymphoid cells were both T-cells and cells of B-cell lineage with an almost 1 to 2 population ratio. Most antigen-positive B-cells were plasma cells that exhibited polyclonality of immunoglobulin. B-cells were also present in the lymphoid mantles and a few were found in the germinal centres. The epithelial component exhibited mucinous and proteinaceous fluid in the lumen and varied immunohistological reactions; being particularly positive to carcinoembryonic antigen, S-100 protein, and B-cell antigen; quite similar to that of normal salivary duct cells. The results suggest that Warthin's tumor may not be a hamartomatous neoplasm at all but a hamartomatous dysplastic lesion.

Key words: Warthin's tumor, Salivary gland, Immunohistochemistry

Introduction

The outstandingly characteristic histology of papillary cystadenoma lymphomatosum, better known as Warthin's tumor (Warthin, 1929), with dual epithelial and lymphoid components, has generated much interest. In particular, there is great interest in its histopathogenesis, which has yet to be clarified. The characteristically abundant lymphoid stroma is supposed to be either residual lymph node tissue (Thompson and Bryant, 1950; Bernier and Bhaskar, 1958; Azzopardi and Hou, 1964; Dietert, 1975; Diamond and Braylan, 1979; Foulshman et al., 1984; Masuda, 1988) or lymphoid tissue caused by autoimmune reaction (Allegra, 1971; Cossman et al., 1977; Tubbs et al., 1980).

In an attempt to establish the origin of the lymphoid component, immunohistochemical studies have been carried out and have demonstrated that it is a reactive lymphoid cellular proliferation to the epithelial component, or exhibited that it has the same characteristics as normal lymph-nodes (Diamond and Braylan, 1979; Foulshman et al., 1984; Masuda, 1988). The immunohistochemical identification of antigens in various histological types of salivary gland tumor has also been reported (Caselitz et al., 1981; Hara et al., 1983; Foulshman et al., 1984; Saito et al., 1984; Nakazato et al., 1985; Regezi et al., 1985). S-100 protein has been observed in the epithelial component of Warthin's tumor (Hara et al., 1983; Nakazato et al., 1985). In addition, the presence of IgA and minimal IgG in the epithelial component has been noted (Hsu et al., 1981; Foulshman et al., 1984).

In this study, we examined the histochemical and immunohistochemical characteristics of 7 cases of Warthin's tumor in an attempt to clarify its histopathogenesis.

Materials and methods

Paraffin-embedded tissue blocks from 7 cases of Warthin's tumor were obtained from the Department of Pathology, Ehime University School of Medicine, and the Division of Histopathology of the Matsuyama Adult Disease Center. Serial sections 5 μm in thickness from each tumor were deparaffinized and stained with haematoxylin and eosin (HE), mucicarmin for epithelial mucin, methyl-green-pyronin (MGP) for identification of immunoblasts and plasma cells, and Congo red stain for amyloid. Congo-red-positive tissue slides were further examined using polarized light to confirm whether it was genuine or not through its apple-green polarization.

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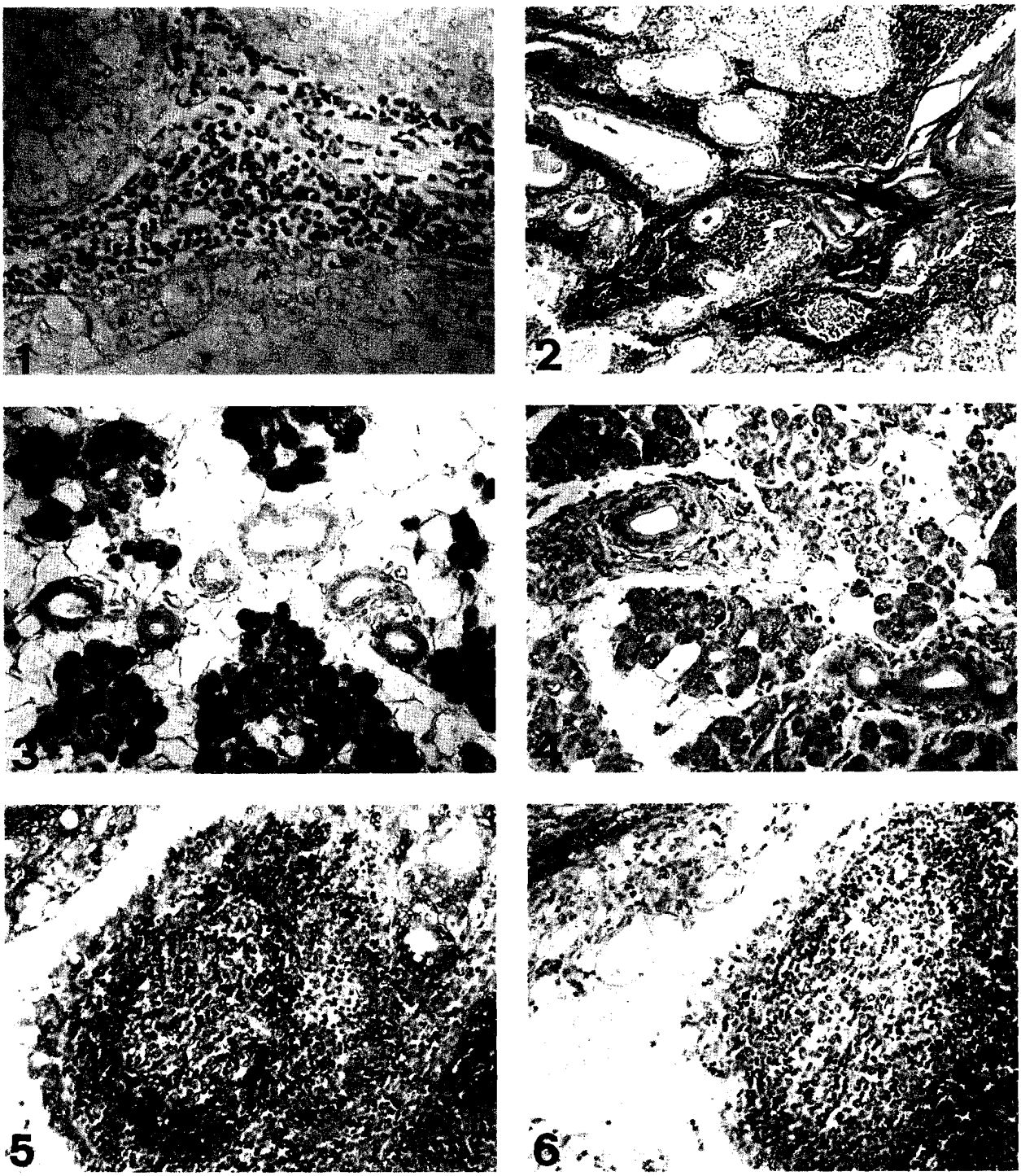

Fig. 1. Interfollicular area of the lymphoid component showing numerous methylgreen-pyronin-positive cells. MGP. × 200

Fig. 2. Area of the hyaloid interfollicular matrix showing positive antigen reaction. Congo-red. × 40

Fig. 3. Normal salivary gland showing intensely positive reaction of the serous acinar cells and ordinary positive reaction with B-cell antibody. ABC method-haematoxylin. × 100

Fig. 4. Normal salivary gland showing negative to trace reactions with T-cell antibody, ABC method-haematoxylin. × 100

Fig. 5. Mantle zone cells, a few follicular centre cells, numerous interfollicular cells and epithelial cells are intensely to moderately positive to B-cell antigen. ABC method-haematoxylin. × 100

Fig. 6. Numerous interfollicular cells are only positive to T-cell antigen. ABC method-haematoxylin. × 100

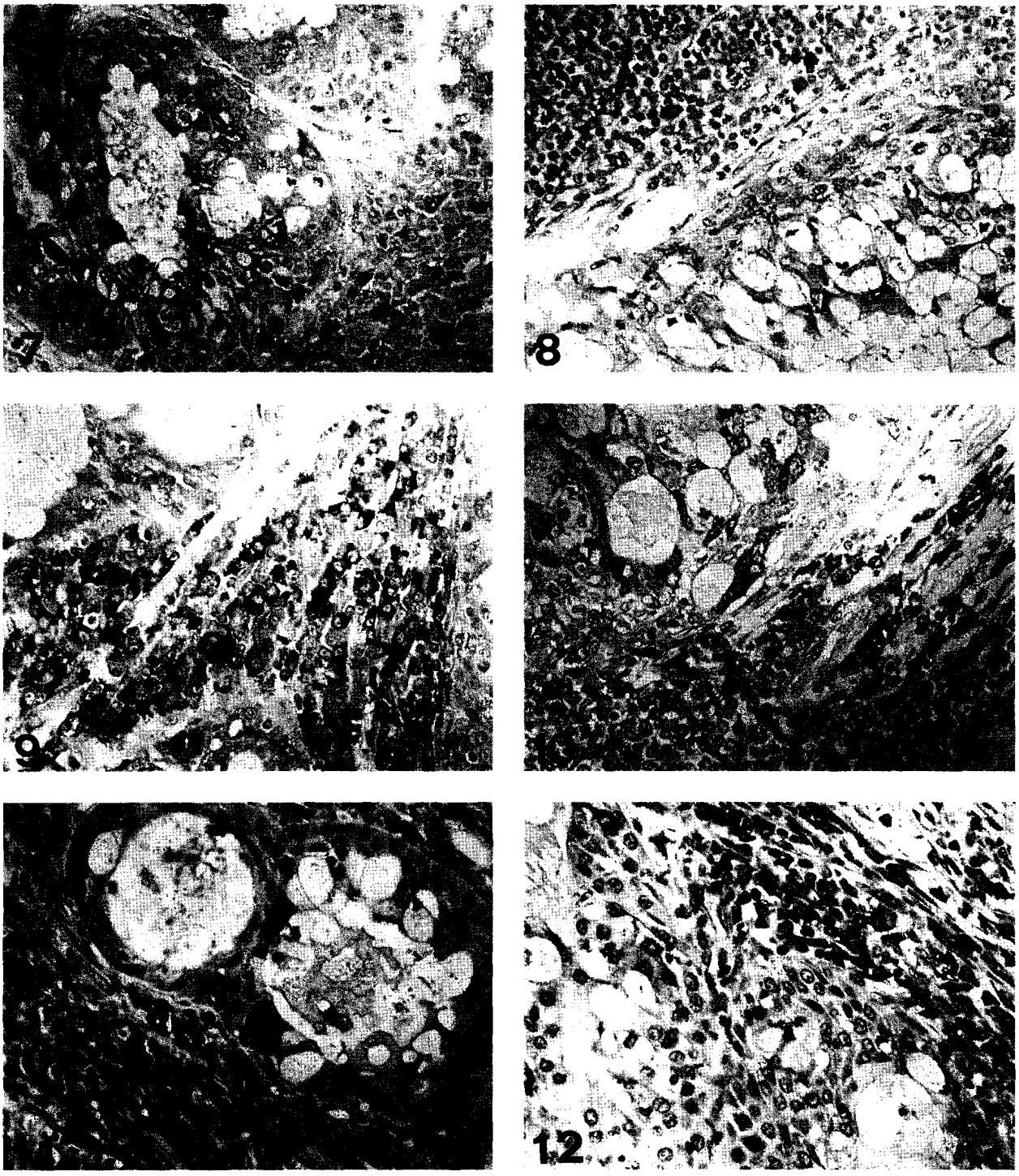

Fig. 7. Most of the outer layer cells and also the squamous metaplastic epithelial cells are intensely positive to CEA. ABC method-haematoxylin. × 200

Fig. 8. Epithelial cells except mucous cells are slightly positive and a few interfollicular cells are positive with S100. ABC method-haematoxylin. × 200

Fig. 9. Numerous plasma cells in the interfollicular area are intensely positive and epithelial cells are moderately positive to K-chain. ABC method-haematoxylin. × 200

Fig. 10. Numerous plasma cells in the interfollicular area are intensely positive and epithelial cells are moderately positive to L-chain. ABC method-haematoxylin. × 200

Fig. 11. Epithelial cells except mucous cells and numerous plasma cells in the interfollicular area are moderately positive to IgG. ABC method-haematoxylin. × 200

Fig. 12. A few plasma cells in the interfollicular area are intensely positive and epithelial cells are slightly to moderately positive to IgA. ABC method-haematoxylin. × 200

Other serial sections were prepared for immunohistochemical staining using avidin-biotin peroxidase complex (ABC) method staining kits produced from various sources, and the following 12 different types of monoclonal antibody obtained from diverse animals were used: antibody to carcinoembryonic antigen (CEA; rabbit, Biomeda), lysozyme (Ly; rabbit, Biomeda), lactoferrin (LF; rabbit, DAKO), S-100 protein (S100; rabbit, Biomeda), neuron specific enolase (NSE; rabbit, BioGenex), B-cell antibody (mouse, BIO-science), T-cell antibody (mouse, BIO-science), IgG (Goat, Biomeda), IgA (goat, Biomeda), IgM (goat, Biomeda), κ-light chain (K-chain; goat, Biomeda), and λ-light chain (L-chain; goat, Biomeda). Each of the stains was provided together with approved sections for positive control, and the primary antibody was omitted for negative control.

Results

Pathological and histochemical findings (Table 1)

All the tumors were well encapsulated and sharply circumscribed from the neighbouring salivary glands. No swelling of the draining lymph nodes was detected. In one tumor, residual lymph node-like tissue was found within the capsule (case 7).

Histologically, the epithelial component was composed of double-layered (inner and outer) cells forming a glandular or cystic structure surrounded by a few myoepithelial cells. Epithelial inner cells showed occasional mitotic figures in cases 2, 4, 6, and 7. Squamous metaplasia was also found in cases 3 and 7. Mucous production was found in all cases to some degree, being especially marked in case 7. The lymphoid component showed broad variations in the development of the germinal centre, especially in cases 3, 4 and 7. In the interfollicular area, particularly in the vicinity of the epithelial component or embedded in hyaline material, numerous MGP-positive cells (plasma cells) were observed in all cases, and markedly so in case 7 (Fig. 1). The hyalinization of the interfollicular area and the blood vessel walls of the capsule was conspicuous in all cases, most particularly in cases 3, 4 and 7, showing positive reactions with Congo-red stain (Fig. 2) and with apple-green reflection using polarized light.

Immunohistochemical findings

Normal salivary gland secretory unit (Table 2)

The immunohistochemical reaction of a normal parotid gland to 12 different types of antibody was studied using 3 specimens extirpated along with tumors (cases 2, 4 and 5). Concerning B- and T-cell antigens, serous acinar cells showed intensely positive reactions; mucous acinar cells and intercalated- and striated ductal cells exhibited moderate positive to trace reactions to B-cell antibody (Fig. 3), but trace to negative to T-cell antibody (Fig. 4). Concerning the other 10 types of antibody, acinar cells showed negative reactions to all. Ductal cells were moderately positive to CEA, LF, NSE and S100, but trace to Ly and IgM. Reaction to IgA was trace in the intercalated- and striated cells, but negative in the secretory cells; to IgG, negative in both acinar and ductal cells; to K- and L-chains, trace to negative in the striated and excretory cells, and negative in the intercalated cells.

Epithelial component of Warthin's tumor (Table 3)

The immunohistochemical reactions of the double-layered epithelial cells, excluding the mucous cells, are shown in Table 3. The intensity of the reactions was generally stronger than that of normal salivary duct cells, and the outer layer cells were stained more strongly than the inner layer cells. In addition, the intensity of the reactions exhibited some variation in each of the cases. All cases showed trace to intensely positive reaction to B-cell antibody (Fig. 5), but trace to negative reaction to T-cell antibody (Fig. 6). Reaction to the CEA and S100 antibodies was slightly to intensely positive across broad areas in 5 of the 7 cases (Figs. 7, 8).

Most of the cases showed trace to negative reactions to LF, Ly and NSE. Two cases for LF and one for NSE were positive across a broad area. Concerning the immunoglobulin, 5 cases for K-chains (Fig. 9) and 4 cases for L-chains (Fig. 10) were positive across a broad area. Two cases for IgG (Fig. 11) and one case each for IgA (Fig. 12) and IgM were positive across a broad area. Proteinaceous fluid in the epithelial lumen also revealed positive reactions with the immunoglobulin antibodies.

Lymphoid component of Warthin's tumor (Table 4)

In all cases, generally numerous interfollicular cells showed intensely positive reactions with B-cell antibody (Fig. 3), whereas T-cell antigen was found only in the focally numerous cells around the lymphoid follicles (Fig. 4). The population ratio of B- and T-cells in the interfollicular area was approximately 2 to 1. Antigen-positive B-cells in the interfollicular area showed a varied population of positive cells to several antigens: IgA, IgG, IgM, K-chain and L-chain. In most of the cases, a few or numerous K- or L-chain-positive cells were also found in the dense amyloid deposits (Figs. 9, 10). There were numerous IgG-positive cells in case 3 only (Fig. 11), and a few in case 4. There were a few IgA-positive cells in cases 6 and 7 (Fig. 12), and a few IgM-positive cells in case 3 only.

Discussion

The histopathological and immunohistochemical findings in this study shed some light on the

Table 1. Pathological and histochemical findings of 7 cases of Wartin's tumor.

Cases				Epithelial component		Lymphoid component	
Case No.	Age/Sex	Location	Size (cm)	Mitosis	Mucous secr.	Germ. ctr.	Amyloid
1	53 M	lt. submandibular	4.0 x 3.0	none	moderate	seen	slight
2	85 M	rt. submandibular	2.5 x 1.8	occasional	slight	rare	slight
3	60 F	rt. parotic	3.0 x 2.5	none	moderate	frequent	focally massive
4	70 M	lt. parotic	2.5 x 2.5	occasional	slight	seen	focally massive
5	52 M	lt. parotic	4.5 x 3.5	none	slight	seen	focally little
6	80 F	rt. parotic	3.5 x 3.5	occasional	moderate	frequent	focally little
7	40 M	lt. submandibular	6.0 x 4.0	occasional	intense	frequent	generally massive

Table 2. Immunohistochemical reactions of salivary (parotid) gland secretory unit

		CEA	LF	Ly	NSE	S100	B-cell antigen	T-cell antigen	IgG	IgA	IgM	K-chain	L-chain
Acinar cells	mucous	–	–	–	–	–	+	–	–	–	–	–	–
	serous	–	–	–	–	–	++	–	–	–	–	–	–
Ductal cells	inter-calated	+	+	±	+	+	+	±	–	±	±	–	–
	striated	+	+	±	+	+	+	±	–	±	±	±	±
	excretory	±	+	±	+	+	±	–	–	–	±	±	±

– : negative, ± : trace, + : positive, ++ : intensely positive, CEA : carcinoembryonic antigen, LF : lactoferrin, Ly : lysozyme, S100 : S-100 protein, K-chain : kappa light chain, L-chain : lambda light chain.

Table 3. Intensity of immunohistochemical reactions of epithelial component in Warthin's tumor

Case No.	CEA	LF	Ly	NSE	S100	B-cell antigen	T-cell antigen	IgG	IgA	IgM	K-chain	L-chain
1	+	+	±	±	+	++	–	+	–	–	++	–
2	+	–	–	±	+	++	±	±	±	±	±	+
3	±	±	–	+	++	+	–	±	–	±	+	±
4	+	+	±	±	+	+	±	–	±	+	+	±
5	+	±	–	–	±	±	–	–	–	–	±	+
6	±	–	–	±	±	+	±	–	+	–	+	+
7	++	±	–	±	+	++	–	++	±	–	++	++

– : negative, ± : trace in focal area, + : positive across broad area, ++: intensely positive across broad area, CEA : carcinoembryonic antigen, LF : lactoferrin, Ly : lysozyme, S100 : S-100 protein, K-chain : kappa light chain, L-chain : lambda light chain.

Table 4. Number of cells showing positive immunohistochemical reaction in lymphoid componente of Wartin's tumor

Case No.	B-cell antigen	T-cell antigen	IgG	IgA	IgM	K-chain	L-chain
1	++	+	–	–	–	++	+
2	++	+	–	–	–	±	±
3	++	+	+	–	±	+	±
4	++	+	±	–	–	±	±
5	++	+	–	–	–	–	±
6	++	+	–	±	–	±	±
7	++	+	–	±	–	±	±

– : none, ± : a few, + : focally numerous, ++ : generally numerous, K-chain : kappa light chain, L-chain : lamda light chain.

histopathogenesis of Warthin's tumor. The most interesting finding is that the interfollicular cells of the lymphoid component were composed of both T- and B-cell lineages and a number of plasma cells in the area produced polyclonal immunoglobulin, largely para-immunoglobulin with amyloid deposition. Furthermore, the epithelial component produced not only mucus but also proteinaceous materials that exhibited positive reactions to para-immunoglobulin and/or immunoglobulin secreted into the lumen. In addition, CEA and S100 were also detected in the epithelial component in all cases. These data may reflect cross reactions due to the structural similarity of the proteins, but they raise a new question about the histopathogenesis of Warthin's tumor; is it a hamartomatous neoplastic lesion or a hamartomatous dysplastic lesion?

S100 has been demonstrated in the epithelial tumor cells of various types of salivary gland tumor, including Warthin's tumor, and also in the myoepithelial cells, the intercalated ductal cells, and the serous acinar cells of normal salivary glands (Nakazato et al., 1985). CEA has been demonstrated not only in colon cancer but also in tumors of the major and minor salivary glands (McDicken and Scott 1981; Caselitz et al., 1982; Saito et al., 1984) and has also been considered a significantly important feature of tumors belonging to the group of oncofetal antigens. These data support the supposition that Warthin's tumor is a hamartomatous dysplastic lesion not a hamartomatous neoplasm.

Amyloid deposition was found in the tumor matrix with a close relation to the para-immunoglobulin and immunoglobulin production by the interfollicular plasma cells. It is known that neoplastic plasma cells produce monoclonal immunoglobulin and/or para-immunoglobulin, but non-neoplastic plasma cells found in plasma cell granulomata (Azar, 1973), rheumatoid disease, or lymphnode hyperplasia of a plasma cell type (Keller and Castleman, 1972; Yu and Carson, 1976) produce polyclonal immunoglobulin and/or para-immunoglobulin. The amyloid has been considered to be derived from some precursor proteins under various pathological conditions. Immunoglobulin and para-immunoglobulin may be the most likely cause of amyloid deposition in Warthin's tumor, causing the pathogenesis of amyloid proposed by Glenner et al. (1973). Thus, it is significant that the interfollicular plasma cells of the lymphoid component of most of the cases studied showed the polyclonal immunoglobulin and/or para-immunoglobulin production that is typical of non-neoplastic lymphoid lesions. And, the fact that in most of the cases the epithelial component showed a positive reaction to B-cell antibody deserves special attention. Therefore, both the epithelial and the lymphoid components of Warthin's tumor may possess an unknown immunological function.

Concerning the type of immunoglobulin, although a few IgA-positive cells were found in only two cases in both the epithelial and lymphoid components, in previous studies the predominance of IgA-positive cells in the epithelial cells (Hsu et al., 1981) and interfollicular plasma cells in Warthin's tumor (Hsu et al., 1981; Masuda, 1988) has been reported. Some researchers consider the lymphoid component in a reactive immune lesion to the epithelial component (Allegra, 1971; Tubbs et al., 1980; Hsu et al., 1981). Allegra (1971) postulated that the lymphoid stroma of Warthin's tumor is a lesion caused by delayed hipersensitivity as best exemplified in Hashimoto's disease of the thyroid. Tubbs et al. (1980) suggested that the lymphoid component in Warthin's tumor represents a reactive cellular proliferation because of the observations of polyclonal follicular centre cell staining with K-chain and L-chain and an apparent reduction in the number of T-cells. And, Hsu et al. (1981) postulated that the lymphoid stroma represents an exaggerated secretory immune response but not an anti-neoplastic response because of the predominance of IgA-positive plasma cells in Warthin's tumor in contrast to the lymphoid reaction observed in autoimmune disease or the usual host-tumor reaction.

While we comprehend Hsus'postulation, we cannot agree with some of the other researchers's explanation that Warthin's tumor is a heterotopic salivary gland lesion arising in a lymph node (Bernier and Bhaskar, 1958; Azzopardi and Hou, 1964) or a tumor arising from ductal inclusions entrapped during embryonic development in lymph nodes adjacent to, or within, the salivary gland (Thompson and Bryant, 1950). As shown in the present study, the epithelial component showed somewhat different immunohistochemical findings from those of normal ductal cells of the salivary gland and also a close relationship to that of the lymphoid component. Thus, we think that Warthin's tumor may be induced as a hamartomatous dysplastic lesion during the development of the salivary gland as a result of an altered developmental process.

Most recently, Takeya et al. (1989) reported an interesting case of multiple myeloma producing both salivary-type amylase and the α-chain of immunoglobulin (IgA), and suggested the possibility that the amylase gene is activated by the oncogene. In two of the seven cases in the present study, a few plasma cells in the lymphoid component and some epithelial cells reacted with IgA antibody. These data may suggest an unknown biological relationship between the plasma cells of the lymphoid component and the epithelial cells of the epithelial component in Warthin's tumor. Furthermore, the present data on Warthin's tumor may partially correspond to an accessory tissue of salivary gland as an inheritance of an apparatus consisting of lymphoid follicles with germinal centres related to the minor salivary gland found in the monkey *Maccaca Fascicularis*, as an organ involved in the local antigen recognition site studied by Schroeder et al. (1983).

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