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Verruciform xanthoma of the oral mucosa: a clinicopathological study with immunohistochemical findings relating to pathogenesis

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Abstract. Verruciform xanthoma is an uncommon benign lesion with unknown aetiology and pathogenesis. In this study, we report ten cases of verruciform xanthoma and document their clinical and histopathological findings. An immunohistochemical investigation was performed using antibodies to macrophage, leukocyte common antigen, T lymphocytes, B lymphocytes, S-100 protein, lysozyme and alpha-1-antichymotrypsin. Our results were similar to the other reported cases. Eighty percent of our cases were found on the gingiva. Candidal hyphae were found in the superficial parakeratotic layers in five cases. The clinical diagnosis of the lesion ranged between papilloma and squamous cell carcinoma. It is important for clinicians to take into consideration the possibility of verruciform xanthoma in the differential diagnosis of papillary and granular lesions of oral mucosa. Immunohistochemically, all foam cells were strongly stained with antimacrophage antibodies. T lymphocytes were the predominant infiltrating lymphocytes in the lesion. Langerhans cells in the epithelia were fewer than those in corresponding normal tissue. Our immunohistochemical findings suggest that verruciform xanthoma is may be a local immunological disorder, with a cell mediated mechanism.

Key words: Verruciform xanthoma – Macrophage – Immunohistochemistry – Pathogenesis – Cell mediated immune response

Introduction

Verruciform xanthoma (VX) is a relatively uncommon benign lesion of the oral cavity. Since the first description by Shafer (1971), more than 120 cases have been reported in the English literature (Neville and Weathers 1980; Nowparast et al. 1981). Although most VX were found to occur on the gingiva or alveolar mucosa, several cases have been seen on the floor of the mouth, buccal mucosa and tongue (Miller and Elzay 1973; Nowparast et al. 1981; van der Waal et al. 1985). The lesion has also been reported to occur in nose, vulva and penis (Santa-Cruz and Martin 1979; Kraemer et al. 1981; Duray and Johnston 1986).

Although the lesion does not seem to be associated with a systemic abnormality of lipid metabolism (Zegarelli et al. 1974; Miyake et al. 1988), the aetiology and pathogenesis are still unclear. Whether it is reactive or neoplastic remains to be determined. To our knowledge, the only paper based on an immunohistochemical study of this lesion was published by Rowden et al. (1986). They suggested that VX belongs to a new category of "non-X histiocytosis" in which the presence of Langerhans cells (LCs) suggests an immunological pathogenesis.

The main purpose of the present study is to document the clinical, histopathological and immunohistochemical findings of ten cases of VX with review of the possible aetiological factors. Since thorough immunohistochemical investigation of VX has not been done, it was hoped that this approach would contribute to a better understanding of the mechanism underlying the lesion.

Materials and methods

Ten cases of VX were retrieved from the files of the Oral Pathology Department, Hiroshima University School of Dentistry. All tissue specimens were fixed in 10% neutral buffered formalin and embedded in paraffin according to routine histological procedures.

New sections were cut at 5 μm from the paraffin blocks and stained with haematoxylin and eosin for routine histological examination and periodic acid schiff reagent for demonstration of candidal hyphae.

For immunohistochemistry, all tissue sections were attached to glass slides coated by aminoalkylsilane. Briefly, after rehydration, the endogenous peroxidase activity was blocked by incubation in 0.3% hydrogen peroxide/methanol solution for 20 min. A panel of commercially available antibodies against macrophage (two dif-

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Table 1. Technical data of antibodies used

Antibody to	Clonality	Dilution	Incubation time (h)	Temp. (°C)	TD	Source
Macrophage (CD 68, KPI)	Monoclonal	1 : 200	12	4	No	Dakopatts
Macrophage (CD 68, PG-M1)	Monoclonal	1 : 300	12	4	No	Dakopatts
T lymphocyte	Monoclonal	1 : 200	12	4	No	Dakopatts
B lymphocyte	Monoclonal	1 : 100	12	4	No	Dakopatts
LCA	Monoclonal	1 : 200	12	4	Yes	Dakopatts
ACh	Polyclonal	1 : 1500	1	20	Yes	Dakopatts
Lysozyme	Polyclonal	1 : 150	1	20	Yes	Dakopatts
S-100 Protein	Polyclonal	1 : 1000	2	20	No	Dakopatts

TD, Trypsin digestion; 0.01% trypsin/phosphate buffer solution (pH 7.6) 15 min, 37 °C

Table 2. Clinical findings of verruciform xanthoma

Pat.	Age	Sex	Duration	Surface texture	Pain	Location		Size (mm)	Clinical diagnosis
1[a]	66	M	2 weeks	Papillary	no	Gingiva	67	10 × 10	Papilloma
2	44	M	2 months	Ulcerative	yes	Gingiva	8	9	SCC
3[b]	78	M	?	Papillary	no	Tongue		10 × 5	Tongue tumour
4	78	F	1 year	Papillary	no	Gingiva	11	10 × 6	Papilloma
5	25	M	3 months	Granular	no	Tongue		3 × 3	Tongue tumour
6	49	F	2 weeks	Papillary	no	Gingiva	56	2.5	Papilloma
7	57	F	3 months	Granular	no	Gingiva	45	4 × 2	Papilloma
8	41	M	3 months	Granular	no	Gingiva	6	8	SCC
9[c]	65	F	?	Ulcerative	yes	Gingiva	56	6 × 9	SCC
10	34	M	2 years	Granular	no	Gingiva	78	10 × 10	Papilloma

[a] Lesion observed within the covering mucosa overlying an osteoma
[b] The lesion appeared under chemo- and radiotherapy of malignant lymphoma
[c] Patient with a history of chemo- and radiotherapy of malignant lymphoma four years ago
SCC, Squamous cell carcinoma

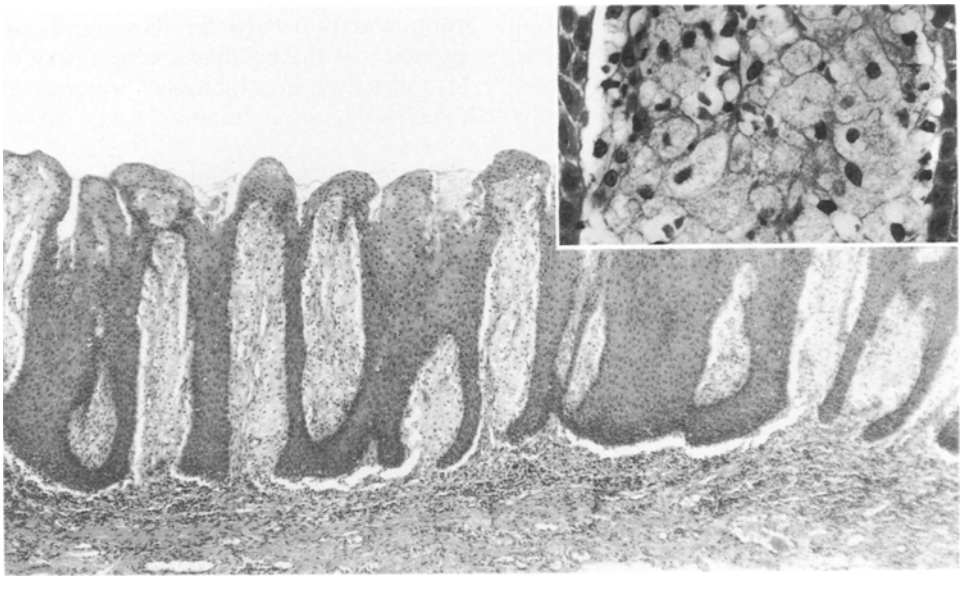

Fig. 1. Photomicrograph of verruciform xanthoma (VX) showing a verrucous surface with a relative uniform depth of epithelial rete ridges with accumulation of huge number of foam cells between them. Note the subepithelial lymphocytic infiltration (H & E, ×40). Inset; a higher magnification illustrating large xanthoma cells containing foamy cytoplasm (H & E, ×100)

ferent antibodies; CD 68, KP1; stains strongly macrophages and weakly LCs and CD 68, PG-M1; stains only macrophages), leucocyte common antigen (LCA), T lymphocytes, B lymphocytes, S-100 protein, alpha-1-antichymotrypsin (ACh) and lysozyme, was applied to the sections using the indirect enzyme labelled streptavidin biotin technique through the commercially available peroxidase or alkaline phosphatase labelled streptavidin kits. The data relative to the primary antibodies used was shown in Table 1. The substrate for development of peroxidase activity was 3,3 diaminobenzidine activated by hydrogen peroxide for 3 to 5 min. The substrate for development of alkaline phosphatase was naphthol AS-BI phosphoric acid and hexazotized new fuchsin as a coupler (Nanba et al. 1987). Sections were weakly counterstained with Mayer's haematoxylin. Negative control was obtained by substitut-

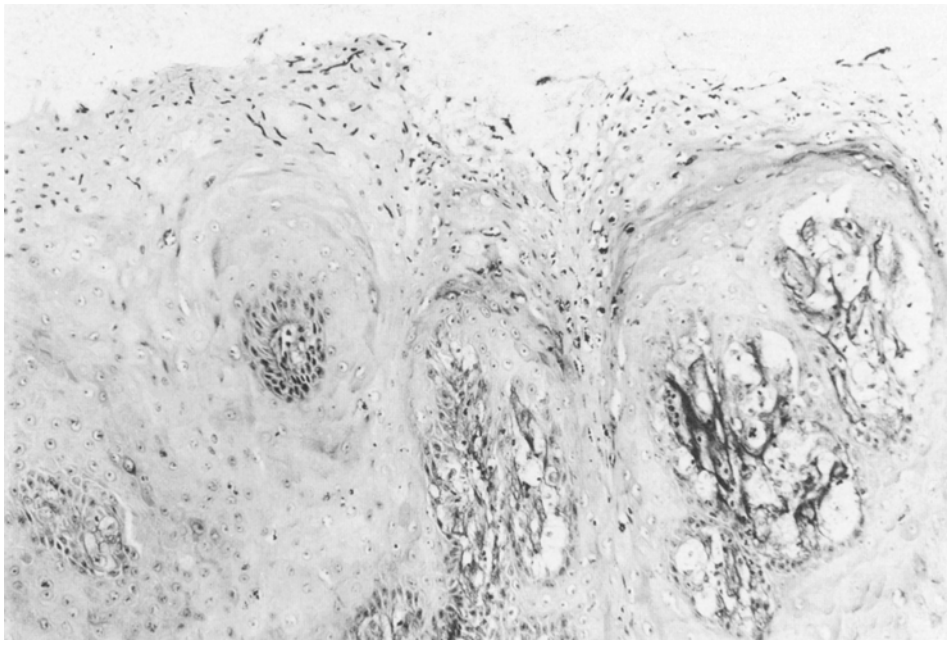

Fig. 2. Photomicrograph of VX illustrating colonization of candidal hyphae in the upper layer of epithelium (PAS, × 40)

ing primary antibodies with non-immune sera. Sections of normal gingiva and palatal mucosa were used as positive controls.

Results

The clinical findings in these ten cases are summarized in Table 2. The six male and four female patients were between 25 to 78 years of age on admission with an average of 53 years. The diameter of the lesion ranged from 2.5 mm to 10 mm in the greatest dimension. The gingiva was the predominant site of involvement; in eight of the ten cases. Of these gingival lesions, seven cases occurred on the posterior gingiva and one on the anterior region. The two remaining lesions involved the lateral surface of the tongue. Interestingly, two of our cases were seen in patients with a history of malignant lymphoma and another case was found within the mucosa covering an osteoma. Generally, the lesion was asymptomatic and flat or slightly raised with a granular to papillary surface. The clinical diagnosis of the lesion was ranged between papilloma and carcinoma.

Histologically the lesion was generally flat or somewhat elevated with a papillomatous or verrucous parakeratinized surface showing parakeratin plugging. The rete ridges were thin, extremely elongated and extended into the lamina propria at a relatively uniform level (Fig. 1). No epithelial dysplastic changes were encountered in our series. The presence of numerous large foam cells in the connective tissue papillae between the epithelial ridges constituted one of the most characteristic features of the lesion (Fig. 1). A moderate subepithelial chronic inflammatory cell infiltrate, mostly of lymphocytes, was a constant feature of all cases. Neutrophils were seen infiltrating the surface epithelia of the two cases showing surface ulceration. Hyalinization of collagen fibres as well as dilatation of the blood vessels were occasionally seen in the underlying lamina propria. Candidal hyphae were found in the superficial parakeratotic layers in five cases (Fig. 2).

The immunohistochemical findings of VX are summarized in Table 3. All foam cells in the ten cases were stained intensely with both monoclonal antimacrophage antibodies (Fig. 3), moderately with anti-LCA, faintly with anti-lysozyme and ACh antibodies and negative with the others (Fig. 4b). Using antimacrophage antibodies, the presence of intra-epithelial macrophages and the occasional extension of foam cells beneath the rete ridges were easily encountered (Fig. 3). Xanthomas, particularly those showing candidal infection, contained fewer S–100

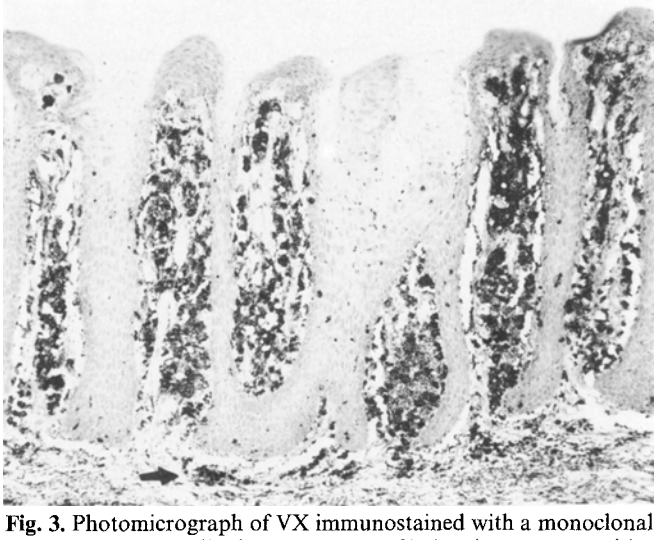

Fig. 3. Photomicrograph of VX immunostained with a monoclonal antimacrophage antibody CD 68, PG–M1 showing strong positivity of all foam cells. Note the extension of foam cells beneath rete ridges *(arrow)* Intra-epithelial macrophages were also stained (ABA, × 33)

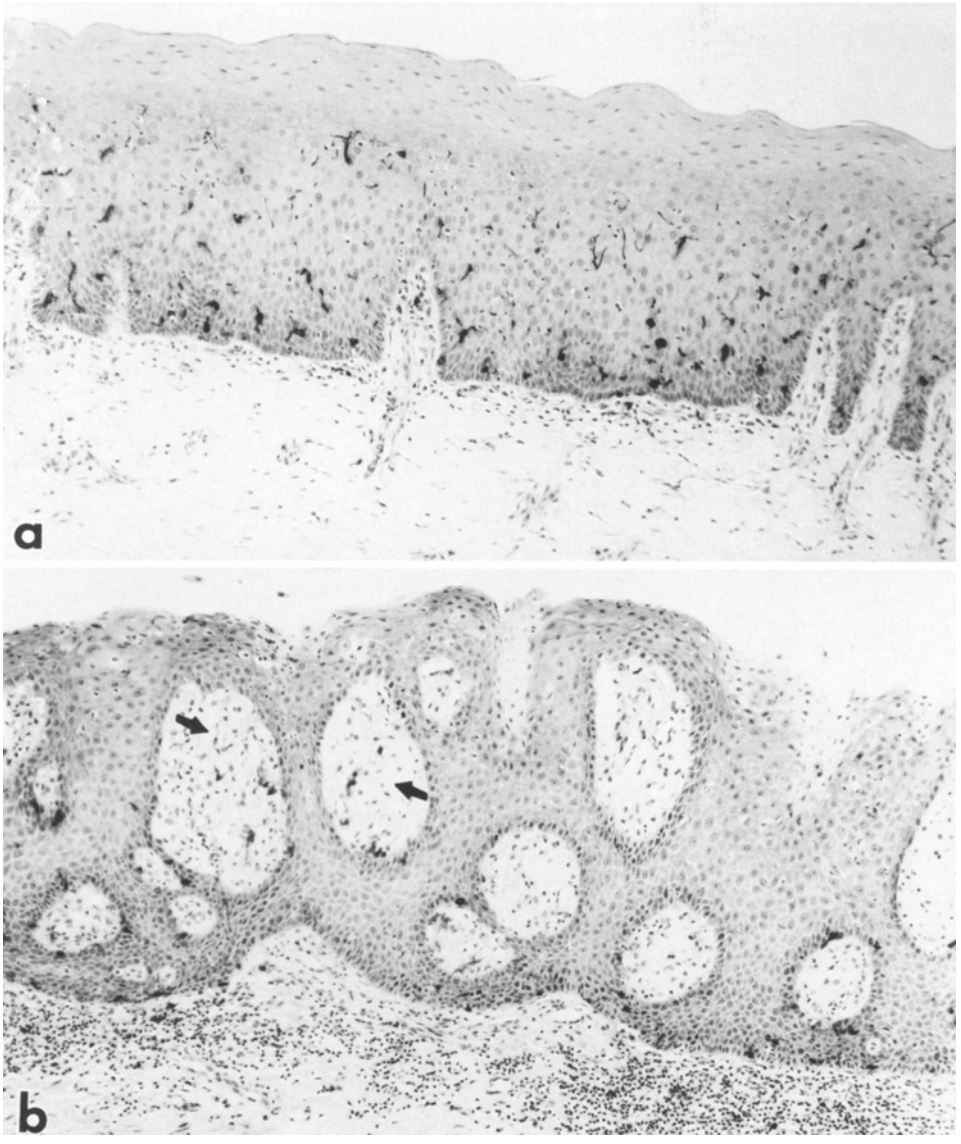

Fig. 4a, b. Photomicrograph of normal gingiva (**a**) and VX (**b**) immunostained with a polyclonal anti-S–100 protein antibody illustrating fewer S–100 protein positive Langerhans cells in VX than those in normal control section. Note all foam cells are negative for S–100 protein *(arrows)* (a-ABA ×40, b- ABP, ×40)

Table 3. Immunohistochemical findings in verruciform xanthoma

Antibody to	Foam cells	Inflammatory cell infiltration	Langerhans cells
Macrophage (CD 68, KP1)	+ + +	−	−/+
Macrophage (CD 68, PG-M1)	+ + +	−	−
T Lymphocyte	−	+ + +	−
B Lymphocyte	−	−/+	−
LCA	+ +	+ + +	−
ACH	+	−/+	−
Lysozyme	+	−/+	−
S–100 protein	−	−	+ + +

−, Negative; −/+, occasional positive; +, faint stain; + +, moderate stain; + + +, strong stain

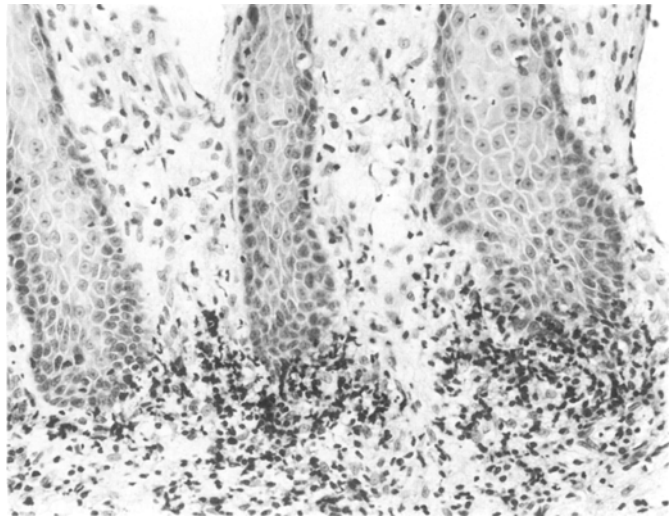

Fig. 5. Photomicrograph of VX immunostained with a monoclonal anti-T lymphocyte antibody. Note the numerous T lymphocytes around the tips of the rete ridges (ABP, ×66)

protein positive cells (designated as LCs), than did the normal control tissue, which showed diffuse suprabasal distribution both in more superficial layers and in the deeper parts of the epithelium (Fig. 4a, b). The subepithelial lymphocytic infiltrate was predominantely of T lymphocytes which tended to surround the tips of the elongated rete ridges (Fig. 5). B lymphocyte were, very sparse. In all specimens T lymphocytes were seen infiltrating the surface epithelium, more than were observed in normal control tissue. Massive perivascular infiltration of T lymphocytes was occasionally seen around the dilated blood vessels of the lamina propria.

Discussion

Analysis of the clinical findings in our series revealed similarity with the other described cases (Shafer 1971; Nowparast et al. 1981). Sex predilection nor age prevalence could not be evaluated in our cases.

From the practical point of view, certain xanthomatous, granular and verrucous lesions such as dystrophic xanthoma (Rosen 1978), cutaneous lipidoses, granular cell myoblastoma, seborrhoeic keratosis, verruca vulgaris, condyloma accuminata and condyloma lata, may resemble VX (Lever and Schaumburg-Lever 1983). Biopsy is required for definitive diagnosis. Kraemer et al. (1981) reported a case of VX on the penis which had been misdiagnosed clinically as verrucous carcinoma resulting in a wide excision circumcision. In our series, about half of the cases were diagnosed clinically as papilloma while the other clinical diagnoses favored were papillary hyperplasia and squamous crell carcinoma. Therefore, it is very important for the clinician to take into consideration the possibility of VX in the differential diagnosis of papillary and granular lesions of the oral mucosa.

With regard to the unique histological feature of the lesion: the thinning and elongation of rete ridges at a relatively uniform level, we suggest that this elongation is illusory and is not a proliferation of epithelial cells with downgrowth of the rete ridges into the underlying connective tissue, but rather results from the upward pushing effect of the accumulated macrophages toward the epithelium. This is reflected in the thinning of the epithelium overlying the macrophages accumulated in the connective tissue papillae. The side pressure exerted by this huge number of macrophages will in turn lead to the thinning of the rete ridges through compression of their epithelial cells which display a relatively spindled cell appearance, rather than a polygonal one.

Although there is a consensus based on histological as well as ultrastructural studies, that foam cells of VX are macrophage in nature immunohistochemical staining for xanthoma cells of VX has not been reported. Formerly, lysozyme, ACh and alpha-1-antitrypsin have been employed as markers for histiocytes. Howevere, they are also found in a large number of other cell types, and as a result their diagnostic reliability is rather limited (Mason and Taylor 1975; Leader et al. 1987) and so we used the most recent two monoclonal antibodies against human macrophages. We found very strong stainability in the cytoplasm of all foam cells of VX with both antibodies; the first demonstration of this. Using these antibodies, the presence of intra-epithelial macrophages and the extension of foam cells beneath the epithelial rete ridges was easily demonstrated.

The aetiology and pathogenesis of the lesion are still debatable. Zegarelli et al. (1975) suggested that the lesion may be inflammatory in nature and that the lipid resulting from degeneration of the epithelial cells is ingested by macrophages that form the most striking feature of the lesion. Ultrastructurally, Kakaraniza-Angelopoulous et al. (1991) failed to demonstrate such degenerating epithelial cells. In our series, no degeneration of epithelial cells could be demonstrated. Interestingly, seven of the eight cases that occured in the gingiva were seen on the posterior part, a site liable to the trauma of mastication as well as the sensitizing agents of foodstuffs. Drummond et al. (1989) reported a case of VX within carcinoma in situ. Based on their report and the other reported cases associated with various epithelial diseases such as snuff dipper's keratosis (Neville and Weathers 1980), pemphigus vulgaris (Gehrig et al. 1983) and epithelial nevus (Grosshans and LaPlanch 1981), they suggested that conditions other than trauma that affect epithelial turnover could play an aetiologic role. In addition, candidal hyphae were demonstrated in the superficial parakeratotic layer of the epithelium in five cases, a finding which may be added to the possible aetiologic factors for the lesion.

The antibody to S–100 protein may have a value in the differential diagnosis of certain reactive and neoplastic conditions. In histiocytic lesions, S–100 protein is considered to be a useful marker in distinguishing histiocytes of the histiocytosis X group of diseases which stained positive, from those of the non-X histiocytosis group which are negative (Winkelmann 1981). In our series, all xanthoma cells were negative for S–100 protein which is in accordance with result obtained by Rowden et al. (1986). The strong staining of all xanthoma cells of VX with the monoclonal antimacrophage antibody CD 68, PG–M1 which stains only histiocytes added strong support to the suggestion that VX belongs to a new category of non-X histiocytosis. Since S–100 protein has also been expressed in granular cells of granular cell tumour (Regezi et al. 1989), it is considered to be a preferential marker in differential diagnosis between foam cells of VX from those of granular cell tumour.

S–100 protein is a useful marker for LCs, in retrospective studies. It may expressed by melanocytes but this problem has been excluded by avoiding considering all S–100 protein positive basal cells as LCs. Rowden et al. (1986) speculated that the presence of LCs in VX suggests an immunological pathogenesis. Although LCs were seen in the epithelia of our specimens, their numbers showed marked decrease when compared with those of control sections. This decrease was marked in those cases showing candidal hyphae in their superfacial parakeratotic layers. The relationship between LCs and candidal hyphae has been examined in oral mucosa biopsy specimens by Daniels et al. (1985). They proposed that diminished LCs number and/or function contributes to

the persistence of fungi in candidal leukoplakia. Since both macrophages and LCs are known to be antigen presenting cells, we suggest that the migration of such a large number of macrophages toward the epithelium may be a compensatory phenomenon.

The exact mechanism for the accumulation of macrophages in VX is unclear. Immunohistochemically, our results demonstrated that the predominant infiltrated lymphocyte in the lesion was of the T cell type. Such predominance of T lymphocytes may be reflected in immunological response to various exogenous antigens continuously present in the oral cavity. In addition, it is well-known that T lymphocytes can produce lymphocyte mediators or lymphokines leading to their accumulation at the site of antigen (Raviola 1986).

Our immunohistochemical findings suggest that VX is a local immunological disorder, most probably of cell mediated mechanism, although the exact cause of this reaction is still unclear. The very poor immune response observed in the oral mucosa due to the binding of memory T lymphocytes to bacterial and food antigens in a low affinity cross-reactive fashion, without the threshold required for cell activation and proliferation (Colasante et al. 1992) might explain the uncommon occurrence of the lesion, although a variety of antigens are continuosly present in the oral cavity.

References

Colasante A, Rosini S, Piattelli A, Artese L, Aiello F, Musiani P (1992) Distribution on phenotype of immune cells in normal human gingiva: active immune response versus unresponsiveness. J Oral Pathol Med 21:12–16

Daniels TE, Schwartz O, Larsen V, Dabelsteen E, Pindborg JJ (1985) Langerhans cells in candidal leukoplakia. J Oral Pathol 14:733–739

Drummond JF, White DK, Damm DD, Cramer JR (1989) Verruciform xanthoma within carcinoma in situ. J Oral Maxillofac Surg 47:398–400

Duray PH, Johnston YE (1986) Verruciform xanthoma of the nose in an elderly male. Am J Dermatopathol 8:247–253

Gehring RD, Baughman RA, Collins JF (1983) Verruciform xanthoma in a young male patient with a past history of pemphigus vulgaris. Oral Surg 55:58–61

Grosshans E, LaPlanche G (1981) Verruciform xanthoma or xanthomatous transformation of inflammatory epidermal nevus. J Cutan Pathol 8:382–385

Kakaraniza-Angelopoulous E, Nicolatou O, Anagnostopoulous S (1991) Verruciform xanthoma of the palate: case report with electron microscopy. J Oral Maxillofac Surg 49:409–414

Kraemer BB, Schmidt WA, Foucar E, Rosen T (1981) Verruciform xanthoma of the penis. Arch Dermatol 117:516–518

Leader M, Collins M, Patel J, Henry K (1987) Antialpha-1-antichymotrypsin staining of 194 sarcoma, 38 carcinoma, and 7 malignant melanoma. Its lack of specificity as a tumor markers. Am J Surg Pathol 11:133–139

Lever WF, Schaumburg-Lever G (1983) Histopathology of the skin (6th edn) J.B. Lippincott, Philadelphia

Mason DY, Taylor CR (1975) The distribution of muramidase (lysozyme) in human tissues. J Clin Pathol 28:124–132

Miller AS, Elzay RP (1973) Verruciform xanthoma of the gingiva: report of six cases. J Periodontol 44:103–105

Miyake M, Dateoka Y, Yasui R, Nomura M, Tanaka K, Ikemoto K, Fukai N, Ishikawa T, Shimosato T, Ogawa I, Takata T (1988) Verruciform xanthoma, case report and a clinicopathologic review of our four cases and 151 references (in Japanese). Jpn J Oral Maxillofac Surg 34:2430–2437

Nanba K, Aoki J, Sasaki N (1987) A new enzyme immunohistochemical technique using alkaline phosphatase-labelled avidine and new fuchsin (in Japanese). Byori to Rinshou (Pathol Clin Med) 5:333–339

Neville BW, Weathers DR (1980) Verruciform xanthoma. Oral Surg 49:429–434

Nowparast B, Howell FV, Rick GM (1981) Verruciform xanthoma. A clinicopathologic review and report of 54 cases. Oral Surg 51:619–625

Raviola E (1986) The immune system. In: Fawcett DW A textbook of histology (11th edn), W.B. Saunders Philadelphia, pp. 406–35

Regezi JA, Zarbo RJ, Coutney RM, Crissman JD (1989) Immunoreactivity of granular cell lesions of skin, mucosa, and jaw. Cancer 64:1455–1460

Rosen T (1978) Dystrophic xanthomatosis in mycosis fungoides. Arch Dermatol 114:102–103

Rowden G, Lovas G, Shafer W, Sheikh K (1986) Langerhans cells in verruciform xanthoma: an immunoperoxidase study of 10 cases. J Oral Pathol 15:48–53

Santa-Cruz DJ, Martin SA (1979) Verruciform xanthoma of the vulva. Am J Clin Pathol 71:224–228

Shafer WG (1971) Verruciform xanthoma. Oral Surg 31:784–789

Waal I van der, Kerstens HC, Hens CJ (1985) Verruciform xanthoma of the oral mucosa. J Oral Maxillofac Surg 43:623–626

Winkelmann RK (1981) Cutaneous syndromes of non-X histiocytosis: a review of the macrophage-histiocyte diseases of the skin. Arch Dermatol 117:667–672

Zegarelli DJ, Zegarelli-Schmidt EC, Zegarelli EV (1974) Verruciform xanthoma. Oral Surg 38:725–734

Zegarelli DJ, Zegarelli-Schmidt EC, Zegarelli EV (1975) Verruciform xanthoma: further light and electron microscopic studies with the addition of a third case. Oral Surg 40:246–256