HISTOCHEMICAL EXPRESSION OF LECTIN BINDING IN PAPILLOMAS

KYUNG-HWA HYUN, NORIYASU MURASE, HIROSHI MITANI
AND MASAHIKO MORI

*Department of Oral Surgery, Gifu College of Dentistry, Hozumi,
Hozumi-cho, Motosu-gun, Gifu 501-02*

Received for publication March 26, 1984 and in revised form December 17, 1984

The lectin-binding activity in papillomas arising from the skin, oral cavity, nasal, paranasal, and pharyngeal mucosa was examined by the use of the horseradish peroxidase (HRP)-conjugated lectin method. Keratinized epithelia in the skin and hyperkeratinized papillomas showed a marked regional or zonal distribution in binding of the lectins used; on the contrary, non-keratinized epithelia such as pseudo-stratified columnar epithelia had no zonal lectin distribution. The hyperortho-keratinized layer gave negative binding for the various lectins; the hyperpara-keratinized layer, slightly positive; the granular cell layer containing keratohyalin granules and the upper spinous layer, the most prominent binding; and the basal cell layer, mostly negative. These lectin-binding patterns in the homologous epithelium were similar to that of keratinized papillomas. Cytochemical localization of such lectin binding was limited to intercellular materials and cellular surface in keratinized squamous epithelium and pseudo-stratified columnar epithelium. PA/Con A-HRP staining was positive in granular materials located in the spinous layer cells, corresponding to glycogen-positive cells. Malignant or dysplastic cells in papillomas lesions were reduced in lectin binding and irregular in their distribution pattern.

A common histological feature of papillomas is their continuous growth of a benign nature from various epithelial structures. Homologous tissues of papillomas are generally keratinized squamous-cell epithelia of skin and oral mucosa origin, non-keratinized epithelia from the oropharynx, and columnar and cylindrical epithelia from the nasal and paranasal cavities (1, 8, 14, 17, 20, 21, 24, 26, 28). Recently, histochemical properties of the binding of various lectins have been described in surface epithelia and glandular structures. It is well known that lectins isolated from plant and animal sources interact with specific sugar residues such as D-galactose (Gal), L-fucose (Fuc), N-acetyl-galactosamine (GalNAc), N-acetyl-glucosamine (GlcNAc) and N-acetyl-neuraminic acid (NANA) (7, 13, 23). The cell coats, cellular surfaces, and intercellular materials of epithelial cells contain polysaccharides, or PAS-positive substances. Thus, lectins bind to specific sugar residues of the polysaccharide coat of the epithelial cells. For histochemical procedures for detecting sugar residues, the lectins, concanavalin A (Con A) and RCA-1 (Ricinus communis-1), have been used, and also blood group antigens A, B, and H, have been studied by the use of FITC-conjugated techniques in the oral

epithelium (3-6, 9). The preceding paper has described lectin-binding activities in keratinized lesions including oral leukoplakia, carcinoma *in situ*, Paget's disease, keratoacanthoma, and condyloma and such lectin-bindings are usually restricted to cellular surfaces and intercellular materials of the lesions (10).

The present study describes the binding reaction of several lectins in epithelial cells of papillomas arising from the skin, oral, pharyngeal, and nasal mucosa as well as from that of the paranasal sinuses, since papillomas of different origin reveal a different pattern of keratinization as an expression of keratinocytic differentiation.

MATERIALS AND METHODS

Tumor materials

A total of 40 cases of papillomas were studied. 15 cases of skin papilloma were a highly keratinized squamous type, and 14 cases of oral papilloma were of the squamous cell type with varying degrees of keratinization. Papillomas of the tonsilla (1 case) and oropharyngeal mucosa (4 cases) were almost all of the non-keratinized type. Papillomas obtained from the upper respiratory tract consisted of 6 cases from the nasal and paranasal sinuses.

The tumors obtained by surgery were fixed in 10% formalin for 12 hr, and 4 μ m paraffin sections were made.

Lectin histochemistry

The Con A-HRP technique with a slight modification by Katsuyama and Spicer (11) was employed and consisted of the Con A-HRP method, periodic acid oxidation Con A-HRP (PA/Con A-HRP) method, and periodic acid oxidation, reduction with borohydrate (NaBH_4), and Con A-HRP (PA/Red/Con A-HRP) method.

In addition to the Con A binding method, Gal-binding lectin such as RCA-1 (from castor bean, *Ricinus communis*-1), GalNAc-binding lectin such as DBA (from horse gram, *Dolichos biflorus*), Gal and GalNAc-binding lectins such as PNA (from peanut, *Arachis hypogaea*) and SBA (from soybean, *Glycine max*), and the Fuc-binding lectin such as UEA-1 (from gorse seed, *Ulex europeus*-I) and GlcNAc-, NANA-binding lectins, WGA (from wheat germ, *Triticum vulgare*), were used. Deparaffinized sections were reacted with HRP-conjugated lectin solutions for 40 min at 20°C. The lectin concentrations were 50 μ g/ml for SBA, DBA, RCA-1 and PNA, 100 μ g/ml for WGA and UEA-1, and 250 μ g/ml for Con A. After being incubated with the lectins, sections were rinsed with phosphate buffered saline (PBS) for 10 min and immersed in 0.05 M Tris buffer (pH 7.6) containing 0.03% diaminobenzidine (DAB) and 0.3% H_2O_2 . These lectins were purchased from E.Y. Laboratories. Details of the lectin binding procedures have already been reported (10).

Competition control

0.1 M and 1.0 M Gal, GalNAc, GlcNAc, and NANA and 0.1 M α -methyl-D-mannose and D-mannose (Sigma Chemical USA) were employed as competitive inhibitors of their binding reactions. The substrate solution of Con A (250 μ g/ml) was added with α -methyl-mannoside or D-mannose. 0.1 M of Gal, GalNAc, GlcNAc, and NANA were mixed solutions of RCA-1 (50 μ g/ml), SBA (50 μ g/ml),

DBA (50 $\mu\text{g/ml}$), PNA (50 $\mu\text{g/ml}$), and WGA (100 $\mu\text{g/ml}$). These lectin mixed solutions were kept for 6 hr at 20°C. The sections were immersed in the mixture solutions for 1 hr.

RESULTS

Papillomas of the skin

Papillomas from the skin were a histologically well-keratinized type with intense exophytic proliferation. The superficial keratinized zone showed hyperortho-keratosis as eosinophilic staining and occasionally hyperpara-keratosis with a few basophilic cells having pyknotic nuclei. Tumorous spinous cells corresponding to the spinous layer cells of normal epithelia were generally well-developed and projected into the subepithelial connective tissue (Figs. 1a, 2a). Malignant papillomas indicated dysplasia and dyskeratosis in the spinous layer and had intact basement membranes.

Staining due to lectin binding showed an intense regional distribution in the neoplastic epithelia. Con A-HRP staining was conspicuous in keratohyalin granule cells and spinous cells and slightly weaker in the peripheral cells corresponding to the basal cells (Fig. 1b). Staining by the PA/Con A-HRP method was most prominent in the upper spinous cells and slight in the lower spinous and peripheral cells. The cytoplasmic distribution of PA/Con A-HRP staining was granulae-like in the cytoplasm (Fig. 1c). PA/Red/Con A-HRP staining was similarly distributed and also less positive as compared to the PA/Con A-HRP method.

Lectin binding was characterized by the existence of a regional or zonal distribution in the papilloma epithelia. The most prominent staining was found in the cells having keratohyalin granules and in the upper spinous zone, with a strong level for RCA-1 binding and moderate for PNA, DBA, SBA, and WGA (Figs. 1d, 1e). Lectin binding in the lower spinous cells was gradually reduced and that in peripheral cells (basal cells) even lower. The staining patterns in the hyperkeratinized layer of papillomas indicated a different configuration; that is, the hyperortho-keratinized layer (eosinophilic zone) was negative for the lectins used. On the contrary, the hyperpara-keratinized layer (containing pyknotic nuclei) was slightly positive for the lectins and the intercellular materials in this layer showed prominent staining. The distribution of lectin binding in papilloma cells included the cellular surface and intercellular materials which were strongly positive (Fig. 1f) and the cytoplasm, particularly the perinuclear region. The staining intensity among the lectins used was strong for RCA-1 and UEA-1 and moderate to slight for PNA, DBA, SBA and WGA (Figs. 2b, 2c, 2d). The basement membrane of skin papillomas bound many of the lectins.

Papillomas with malignant changes showed a decreased staining for all the lectins used, and irregular binding occurred in malignant transformed or dysplastic cells. Malignant papillomas had transformed spinous cells showing dysplastic and dyskeratotic figures. The intercellular materials surrounding such transformed cells showed markedly reduced lectin binding (Figs. 3a, 3b) (Table 1).

Papillomas of the oral mucosa

Keratinization in the oral papillomas was not so intense as that of skin papil-

TABLE 1. *Lectin Binding Histochemistry in Papillomas*

	SKIN					ORAL MUCOSA				TONSILS AND PHARYNX	NASAL AND PARANASAL SINUSES	
	HPZ	HOZ	GUZ	LZ	BZ	HPZ	GUZ	LZ	BZ	PC	PSZ	BZ
Con A-HRP	1-2	±-1	3	1	±-1	2-3	2-3	1	±	2	2	±
PA/Con A-HRP	1	0	2-3	1	±	2	2	1	±	1-2	1	±
PA/Red/Con A-HRP	±	0	1	±	±	1	1	±	±	±-1	±	±
SBA	1-2	0	2-3	1	±-1	2-3	2-3	1	±	1	2-3	±-1
DBA	±	0	2-3	1	±-1	2	1	1	±-1	1	1	±
PNA	1-2	0	2-3	1-2	±-1	2-3	2	1-2	±-1	1	1	±
UEA-1	±	0	2	1	±	1	1	1	±	1	1	±
RCA-1	1-2	±-1	3	2	1	2-3	2-3	2	1	2-3	2-3	±-1
WGA	1	±	2-3	2	1	2-3	2	2	1	1	2	±

HPZ; Hyperpara-keratinized zone, HOZ; Hyperortho-keratinized zone, GUZ; Granular and upper spinous cell layer zone, LZ; Lower spinous cell layer zone, BZ; Basal cell layer zone. PSZ; Pseudo-stratified cell zone, PC: papilloma cells. The grade of lectin intensity is arbitrarily divided into 0-3; 0: negative, ±: trace, 1: weak, 2: moderate, 3: strong.

lomas. Normal oral mucosa generally had a parakeratinized epithelium, and the palatal mucosa had the highest degree of keratinization (parakeratinization) among the oral mucosa. Histologically, papillomas arising in the palate, gingiva, and tongue showed a parakeratinized or hyperpara-keratinized epithelia, and those from the mucosa of buccal, lip, and floor of the mouth regions showed no keratinization.

Staining by the Con A-HRP reaction was markedly localized in the intercellular materials of the hyperpara-keratinized layer, moderate to strong in the

Figs. 1a-1f. Squamous papilloma of the skin.

Fig. 1a. Hematoxylin-eosin staining. Note intense ortho-keratinization with abundant keratohyalin granules. × 80

Fig. 1b. Serial section of Fig. 1a. Con A-HRP staining is positive in basal cell layer to upper spinous and granular cell layers and negative in totally keratinized layer. × 80

Fig. 1c. PA/Con A-HRP staining. Positive reaction is found in upper spinous and granular cell layers of papilloma. × 80

Fig. 1d. Lectin, PNA-binding is confined to the cellular surface and intercellular materials in papilloma epithelia, and binding reaction in the basal cells is less than that in upper spinous and granular cell layers. × 80

Fig. 1e. Lectin, RCA-1-binding. RCA-1 staining is found in upper spinous and lower spinous cell layers. × 100

Fig. 1f. Higher magnification of Fig. 1e. Marked RCA-1 staining is confined to the cellular surface and intercellular materials which are probably desmosomes. Well-keratinized cells show no RCA-1 binding. × 400

Figs. 2a-2d. Squamous papilloma of the skin. × 80

Fig. 2a. Hematoxylin-eosin staining. Note intense keratinization of papilloma.

Fig. 2b. Lectin, SBA-binding. The SBA staining reaction is found in upper spinous and granular cells, and absent in basal layer cells of papilloma.

Fig. 2c. Lectin, RCA-1-binding. The RCA-1 staining is strong in upper spinous and granular cells and slight in basal and lower spinous cells of papilloma.

Fig. 2d. Lectin, UEA-1-binding. The UEA-1 staining is strongly positive in upper spinous and granular cells and moderately positive in lower spinous and basal cells.

Figs. 3a-3b. Lectin, RCA-1-binding in dysplastic epithelium from skin papilloma. RCA-1-binding of epithelial structure shows irregular staining in intercellular materials and desmosomes among dysplastic spinous cell. Fig. 3a $\times 100$, Fig. 3b $\times 400$

5d

5c

upper spinous cells, and weak in the peripheral cells corresponding to the basal cells. PA/Con A-HRP staining of oral papillomas was moderately positive in the upper spinous cells and less so in the peripheral cells. Lectin binding in papilloma epithelia varied. RCA-1 and SBA bindings were most conspicuous; PNA and WGA, moderate; and DBA and UEA-1, weak. Intercellular materials in hyperpara-keratotic layer indicated prominent staining for RCA-1, SBA, and WGA. The spinous cell layer of the papillomas displayed moderate binding to PNA, RCA-1, SBA, and WGA, and slight to DBA and UEA-1. The peripheral cell layer indicated negative or very slightly positive staining for all the lectins (Table 1).

Papillomas of the tonsilla and pharynx

The papilloma epithelium in the oropharynx and nasopharynx consisted of non-keratinized squamous cells. Papilloma presented in the tonsilla and in Waldyer's ring revealed a undifferentiated squamous cell epithelial surface with outgrowth and occasionally invagination into lymphoid tissue (Fig. 4a).

Staining by Con A-HRP technique was positive in the intercellular materials or cellular membranes of the tumor epithelium (Fig. 4b). Staining by the PA/Con A-HRP method revealed irregular granular materials in the neoplastic cells and was also positive for the cellular surface and intercellular elements. Also, inclusion materials located in the apical portion of the tumor cells were strongly stained. PA/Red/Con A-HRP staining was similar to that of PA/Con A-HRP staining. Lectin binding in the papilloma cells was strong for RCA-1 (Figs. 4c, 4d), and rather slight or trace for PNA, DBA, SBA, UEA-1, and WGA. The cytochemical distribution of lectin binding was usually intercellular and at the cellular surface with slight degrees in the papilloma cells (Table 1).

FIGS. 4a-4d. Squamous papilloma of the oropharynx (tonsil).

FIG. 4a. Hematoxylin-eosin staining. Note non-keratinizing epithelium covered in the oropharyngeal papilloma. $\times 80$

FIG. 4b. Con A-HRP staining. Weaker Con A-binding in epithelial structure of oropharyngeal papilloma. $\times 80$

FIG. 4c. Lectin, RCA-1-binding. Epithelial cells of papilloma show weaker RCA-1-binding activity and lymphocytes in the stroma intense binding. $\times 80$

FIG. 4d. Higher magnification of Fig. 4c. Irregular RCA-1-binding shows in cellular surface and desmosomes in epithelial cells. $\times 160$

FIGS. 5a-5d. Transitional epithelial papilloma of nasal cavity. $\times 100$

FIG. 5a. Hematoxylin-eosin staining. Papilloma cells show no keratinization and vacuolization at surface area.

FIG. 5b. PA/Con A-HRP staining. Strongly positive staining coincides with vacuolated cells of papilloma. Basal layer cells show not so prominent staining.

FIG. 5c. Lectin, SBA-binding. Staining of SBA conjugate is strong in cellular surface and intercellular materials of middle layer cells and weak or negative in basal layer cells.

FIG. 5d. Lectin, WGA-binding. Staining of WGA conjugate is uniformly positive in cellular surface and intercellular materials of middle layer cells.

FIGS. 6a-6c. Ciliated columnar epithelium of upper respiratory tract (nasal mucosa). $\times 400$

FIG. 6a. Lectin, PNA-binding. Some cells of ciliated columnar epithelium show marked PNA staining, but almost all epithelial cells no staining.

FIG. 6b. Lectin, RCA-1-binding. Apical parts of ciliated epithelium including cilia show an intense RCA-1 staining.

FIG. 6c. Lectin, WGA-binding. WGA-binding is limited to cilia of high columnar epithelial cells and weak staining granules of WGA conjugate is found apical area of the epithelial cells.

Papillomas of the nasal and paranasal sinuses

The epithelial structure of the upper respiratory tract was composed of pseudo-stratified ciliated or high columnar cells. Papillomas which developed in this region consisted of pseudo-stratified cells and loose connective tissue stroma with higher vascularity (Fig. 5a) and are termed cylindrical papillomas, Schneiderian papillomas, transitional cell papillomas, and so on.

Lectin binding in normal ciliated epithelia showed weaker stainings to the Con A-HRP and PA/Con A-HRP methods, strong to RCA-1 binding, and moderate to weak to DBA, SBA, UEA-1, and WGA. Some of the ciliated cells exhibited an intense PNA staining and the cilia of tall columnar cells displayed a positive WGA binding (Figs. 6a, 6b, 6c).

Staining by the Con A-HRP method was weakly or moderately positive in the tumor cells, and that by the PA/Con A-HRP method was moderate, appearing as fine granules, and more intensely positive for the intercellular materials and inclusions in the apical portion of the papilloma epithelial cells (Fig. 5b). Staining by the PA/Red/Con A-HRP method was reduced as compared to that of the PA/Con A-HRP method and was distributed mainly in the middle layer of the tumor cells; it was absent in the basal tumor cells. No regional distribution of Con A binding was found for the nasal papilloma epithelium. RCA-1 and SBA bindings were the most prominent in the papilloma epithelia (Fig. 5c). Stainings for RCA-1 and SBA were strong to moderate in the ciliated columnar cells at the surface. The staining intensities for PNA, DBA, UEA-1 were slight in the papilloma cells, and that of WGA was moderate in the cellular membranes and ciliated cells. The staining due to WGA binding was decreased in the basal papilloma cells as compared with the upper cells (Fig. 5d). The basement membrane of nasal papillomas was moderately to strongly positive for RCA-1, DBA, SBA, and WGA binding. The inclusion materials located near the surface of papilloma epithelium contained substances which strongly bound RCA-1 and SBA. Nasal and paranasal papilloma exhibited no regional distribution of lectin binding staining (Table 1).

Results of competitive inhibition test

The lectin solutions with RCA-1, PNA, SBA and DBA added with 0.1 M Gal and GalNAc showed decreased staining as compared to the original staining. Those with 1.0 M sugars showed completely blocked in the staining. The incubation of WGA added with 0.1 M GlcNAc and NANA revealed a negative staining. The substrate solution of Con A added with 0.1 M α -methyl-mannoside and D-mannose showed negative response.

DISCUSSION

Keratinocytic stratification in the squamous cell epithelium involves keratinocytic or epithelioblastic cell division in the basal cell layer, proliferation of size in the spinous cell layer, transformation to keratohyalin granule-containing cells in the granular cell layer, and finally desquamation following hornification. These phenomena are well-known as morphologic expressions of normal keratinocytic stratification. The histochemical expression of squamous cell epithelial differentiation, including keratinized and non-keratinized epithelia, involves a three-layer pattern as detected by enzyme histochemistry (12, 16), and regional distribution in

the individual cell layers as observed by filamentous protein histochemistry (15, 19, 22, 25). The preceding paper has shown that in keratinized lesions including malignancy, lectin binding in the stratified squamous epithelium of human skin and oral mucosa indicates a zonal distribution of sugar residues in the normal state and a marked reduction in binding in malignant epithelia (10).

Lectin binding has been widely used as a histochemical approach for detecting sugar residues, with a given lectin linking specifically to its corresponding sugar residues. The present experiment employed HRP-conjugated lectins, with Con A binding to glucose, mannose, and fructose (found in polysaccharide complexes such as glycogen), RCA-1 binding to Gal residues, DBA to GalNAc, PNA and SBA to Gal and GalNAc, UEA-1 to Fuc, and WGA to GlcNAc and NANA (7, 13, 23). The cytochemical distribution of lectin binding in the epithelial cells was usually restricted to the cellular surface or cellular membrane and intercellular materials in normal and papilloma epithelia (2, 18, 20). A regional or zonal distribution of lectin binding was distinguishable in highly keratinized squamous epithelium and hyperkeratinized papillomas; for example, no lectin staining occurred in the hyperortho-keratinized cell layer, and intercellular binding was found in the hyperpara-keratinized cell layer. In the spinous cell layer, lectin binding was located at the cellular surface and in intercellular materials, being conspicuously strong mostly in the upper spinous cells, and slight or traceable lectin binding occurred in the peripheral tumor cells corresponding to the basal cells in the homologous epithelium. No regional lectin binding pattern was found in epithelia from nasal, paranasal, and pharyngeal papillomas, because those epithelia were devoid of strong keratinization. The regional lectin-binding pattern in highly keratinized squamous epithelia suggests the presence of specific sugar residues in individual cell layer as an expression of keratinocytic stratification.

Papillomas arising from the nasal and paranasal sinus mucosa are called transitional papillomas or Schneiderian papillomas and are composed of pseudo-stratified columnar epithelial cells (14, 17, 21, 24, 26, 28). Histochemical lectin binding by the upper respiratory epithelium has not been previously reported in detail. In the present study, the cilia of pseudo-stratified columnar cells were characterized by intense binding by RCA-1 and SBA, indicating Gal and GalNAc residues, respectively. The mucociliary mechanism in the air passage probably is responsible for the ciliar stroke with humidification by nasal mucosal secretions. Those motive functions related to cilia strokes may be obtained partly from metabolic activities in sugar containing cells of nasal epithelia. Papillomas from the nasal, paranasal, and nasopharyngeal cavities were composed histologically of pseudo-stratified columnar cells without keratinization. In pseudo-stratified epithelium and transitional cell papillomas which lack keratinocytic stratification, no zonal or regional distribution was found for the lectin-binding reaction.

The papilloma cells in which malignant transformation had occurred, or severe dysplastic cells, were particularly reduced in lectin-binding capacity. A similar trend toward decreased staining in malignant epithelial cells has been previously noted in the case of oral carcinoma *in situ* (4, 5, 10). Malignant cells of epithelial origin were characterized by the loss of intercellular aggregation due to a marked decrease in the number of desmosomes and hemidesmosomes (27). The lectin binding sites found in intercellular materials and cellular membranes containing

sugar residues in their cell coat materials, which have been reported to be positive for PAS-staining. Dabelsteen *et al.* (5, 6) have pointed out that blood type antigens A and B were lost in premalignant and malignant cells of oral mucosal lesions. Immunologically speaking, blood type antigen A contains Gal and GalNAc which are involved in its antigenicity; and antigen B, Gal and GlcNAc. This finding of the loss of blood type antigens linked to oral malignant lesions resembles the present results. Lack of the lectin-binding reaction in malignant cells of epithelial origin is one indication that malignancy has occurred in the cells.

REFERENCES

1. Abbey, L. M., Page, D. G. and Sawyer, D. R.: The clinical and histopathologic features of a series of 464 oral squamous cell papillomas. *Oral Surg.* 49; 419-428, 1980.
2. Brabec, R. K., Peters, B. P., Bernstein, I. A., Gray, R. H. and Goldstein, I. J.: Differential lectin binding to cellular membranes in the epidermis of the newborn rat. *Proc. Natl. Acad. Sci. U.S.A.* 77; 477-479, 1980.
3. Dabelsteen, E., Fejerskov, O., Noren, O. and Mackenzie, I. C.: Concanavalin A and Ricinus communis receptor sites in normal human oral mucosa. *J. Invest. Dermatol.* 70; 11-15, 1978.
4. Dabelsteen, E. and Mackenzie, I. C.: Expression of Ricinus communis receptors on epithelial cells in oral carcinomas and oral wounds. *Cancer Res.* 38; 4676-4680, 1978.
5. Dabelsteen, E., Vedtofte, P., Hakomori, S. I. and Young, W. W.: Carbohydrate chains specific for blood antigens in differentiation of human oral epithelium. *J. Invest. Dermatol.* 79; 3-7, 1982.
6. Dabelsteen, E., Vedtofte, P., Hakomori, S. I. and Young, W. W.: Accumulation of a blood group antigen precursor in oral premalignant lesions. *Cancer Res.* 43; 1451-1454, 1983.
7. Debray, H., Decout, D., Strecker, G., Spik, G. and Montreuil, J.: Specificity of twelve lectins towards oligosaccharides and glycopeptides related to N-glycosylproteins. *Eur. J. Biochem.* 117; 41-55, 1981.
8. Greer, R. O. and Goldman, H. M.: Oral papillomas. Clinicopathologic evaluation and retrospective examination for dyskeratosis in 110 lesions. *Oral Surg.* 38; 435-440, 1975.
9. Hashimoto, K., King, L. E., Yamanishi, Y., Beachey, E. H. and Maeyens, E.: Identification of the substance binding pemphigus antibody and concanavalin A in the skin. *J. Invest. Dermatol.* 52; 423-435, 1974.
10. Hyun, K. H., Nakai, M., Kawamura, K. and Mori, M.: Histochemical studies of lectin binding patterns in keratinized lesions, including malignancy. *Virchows Arch (Pathol Anat)* 402; 337-351, 1984.
11. Katsuyama, T. and Spicer, S. S.: Histochemical differentiation of complex carbohydrates with variants of the Concanavalin A-horseradish peroxidase method. *J. Histochem. Cytochem.* 26; 233-250, 1978.
12. Kawakatsu, K. and Mori, M.: Histochemical evaluation of enzymatic activities in human squamous-cell cancer. *Cancer Res.* 23; 539-545, 1963.
13. Kennedy, J.: Proteoglycans—Biological and chemical aspects in human life. Studies in organic chemistry 2, Elsevier, Amsterdam, 1979, p. 225-253.
14. Lasser, A., Rothfeld, P. R. and Shapiro, R. S.: Epithelial papilloma and squamous cell carcinoma of the nasal cavity and paranasal sinuses. A clinicopathological study. *Cancer* 38; 2503-2510, 1976.
15. Löning, T., Staquet, M. J., Thivolet, J. and Seifert, G.: Keratin polypeptides distribution in normal and diseased human epidermis and oral mucosa. *Virchows Arch (Pathol Anat)* 388; 273-288, 1980.
16. Mori, M., Mizushima, T. and Koizumi, K.: A comparative histochemical evaluation of various dehydrogenases in the oral squamous epithelium. *Histochemie* 3; 111-121, 1962.

17. Neumann, O. G., Klopp, L. and Franz, B.: Klinische und histologische Klassifizierung der Larynxpapillome und Papillomatosen. *Laryngol. Rhinol. Otol.* 59; 57-65, 1980.
18. Nieland, M. L.: Epidermal intercellular staining with fluorescein-conjugated phytohemagglutinins. *J. Invest. Dermatol.* 60; 61-66, 1973.
19. Oberle, L. K., Fukuyama, K., Daniels, T. E. and Greenspan, J. S.: Immunohistochemistry of keratin in normal and diseased human oral epithelium. *Arch. Oral Biol.* 24; 321-326, 1979.
20. Ookusa, Y., Takata, K., Nagashima, M. and Hirano, H.: Distribution of glycoconjugates in normal human skin using biotinyl lectins and avidin-horseradish peroxidase. *Histochemistry* 79; 1-7, 1983.
21. Quade, R. and Löbe, L. P.: Klinik und Therapie von Schleimhaut Papillomen im HNO-Bereich. *Laryngol. Rhinol. Otol.* 58; 490-494, 1979.
22. Schlegel, R., Banks-Schlegel, S. and Pinkus, G. S.: Immunohistochemical localization of keratin in normal human tissues. *Lab. Invest.* 42; 91-96, 1980.
23. Sharon, N.: Complex carbohydrates. Their chemistry, biosynthesis and functions, Addison-Wesley Pub. 1975. p. 19-71. (in Japanese)
24. Snyder, R. N. and Perzin, K. H.: Papillomatosis of nasal cavity and paranasal sinuses (inverted papilloma, squamous papilloma). A clinicopathologic study. *Cancer* 30; 668-690, 1973.
25. Sun, T. T., Shih, C. and Green, H.: Keratin cytoskeletons in epithelial cells of internal organs. *Proc. Natl. Acad. Sci. U.S.A.* 76; 2813-2817, 1979.
26. Vrabec, D. P.: The inverted Schneiderian papilloma: A clinical and pathological study. *Laryngoscope* 85; 186-220, 1975.
27. Weinstein, R. S., Merk, F. B. and Alroy, J.: The structure and function of intercellular junctions in cancer. *Adv. Cancer Res.* 23; 23-89, 1976.
28. Wilson, W. R., Carrol, E. D., Bentkover, S. H. and Schuknecht, H. F.: Inverted papilloma. (clinical conference) *Arch. Otolaryngol.* 106; 54-61, 1980.