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Tissue Binding Patterns of Lectins in Premalignant and Malignant Lesions of the Oral Cavity

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SUMMARY

Lectins from the seeds of Jackfruit (Artocarpus integrifolia) and winged bean (Psophocarpus tetragonolobus) were isolated using an immobilized N-acetyl D-galactosamine column and conjugated to type VI horse radish peroxidase. The purified conjugate was used for the study of tissue specificities using diaminobenzidine as the substrate on dewaxed tissue sections of normal, oral leukoplakia, oral submucous fibrosis, verucous carcinoma and squamous cell

ABBREVIATIONS USED :

JFL : JACK FRUIT LECTIN

WBL : WINGED BEAN LECTIN

OL : ORAL KEUKOPLAKIA

OSMF : ORAL SUBMUCOUS FIBROSIS

VC : VERRUCOUS CARCINOMA

OC : ORAL CANCER

PBS : PHOSPHATE BUFFERED SALINE

HRP : HORSE RADISH PEROXIDASE

DAB : DIAMINO BENZIDINE DIHYDROCHLORIDE

carcinoma of the oral cavity. In spite of having a common inhibitory sugar, winged bean lectin did not bind to any lectins whereas Jackfruit lectin showed varying degrees of binding towards the above tissues. The difference in the nature and intensity of binding of the Jackfruit lectin suggest the utilizing this lectin in the differential diagnosis of the premalignant and malignant lesions of the oral cavity.

INTRODUCION

Lectins are proteins or glycoproteins mainly of plant origin, the interactions of which can be inhibited or reversed by simple sugars (Pena et al 1981). Lectins have two or more binding sites and can agglutinate cells by binding specific carbohydrate molecule on cell surfaces (Sharon, 1980, Harrison and Chesteron, 1981). They have been used in the purification of proteins and polysaccharides, for studies on lymphoblastogenesis, and of blood group substances and in membrane studies of normal and cancerous cells (Navogrodsky et al 1975; Miller 1983; Sharon 1984). The difference in carbohydrate structures on cell surfaces have been studied by the use of plant lectins from peanut, soyabean and Helix pomatia (Brown and Williams, 1982; Sharon, 1984).

We have earlier reported the isolation and purification of the lectins from the seeds of the Jackfruit and winged bean (Vijayakumar and Forrester, 1986, 1987). Lymphoblastic transformation and mitogenic stimulation of human and rhesis monkey lymphocyte cultures by the extract of jackfruit seeds were reported by Seares et al (1982). The horse-radish peroxidase (HRP) conjugate of the agglutinin from the jackfruit were shown to possess selective staining towards murine and human tissues (Vijayan et al, 1982; Vijayakumar et al, 1985); however, the possibility of utilizing the tissue binding property of this lectin as a diagnostic tool for the differential diagnosis of premalignant and malignant lesions has not been explored. Hence, the present study was undertaken using tissues of oral leukoplakia, oral submucous fibrosis, verrucous carcinoma, or squamous cell carcinoma.

MATERIALS AND METHODS

Ten specimens each of histopathologically proved cases of oral leukoplakia (OL) oral submucous fibrosis (OSMF), verrucous carcinoma (VC) and squamous cell carcinoma (SCC) of the oral cavity were taken up for study. For comparison, ten normal punch biopsy specimens of the oral cavity were also included. Wax-embedded tissues were cut into 3-5/u size, dewaxed using 3 changes of xylene, and rehydrated using graded alcohol. The JFL and WBL, isolated using a column of immobilized N-acetyl-D-galactosamine as described by Vijayakumar and Forrester (1986), were conjugated to HRP by the method of Nakane and Kawaoi (1974). The purified conjugate was incubated with the rehydrated tissue sections for 30 minutes, washed in three changes of phosphate buffered saline (PBS), and finally incubated in a solution of 30 mg diaminobenzidine dihydrochloride (DAB) in PBS containing 40 ul of H_2O_2. The slides were washed in distilled water, counterstained with haemalum, dehydrated with alcohol, and mounted. Depending upon the intensity of staining, slides were graded + to +++. The HRP was purchased from Sigma Chemical Company and DAB, from BDH, England.

RESULTS

The physical and chemical properties of the lectins are listed in Table 1. In spite of having a common inhibitory sugar, WBL did not stain any of the tissues studied whereas JFL showed varying degrees of staining. The JFL binding patterns of the tissues are described in Figs 1 to 5 and in Table 2. Trials were carried out using HRP conjugated peanut lectin. The binding pattern was similar to JFL, but the intensity was less. Another important observation was that three of the premalignant lesions showed an irregular binding pattern similar to that of squamous cell carcinoma.

DISCUSSION

The ability of plant lectins to bind specific carbohydrate and the specificity with respect to the type of lymphocyte they stimulate had been

well explained (Lis and Sharon, 1977; Sharon, 1980). This high specificity of the lectins is being utilized for the identification and separation of cells (Sharon, 1984). In the present investigation, even though WBL and JFL have the same inhibitory sugar, they showed marked differences in their staining properties.

Walker (1985) demonstrated the selective staining of malignant cells by lectins, whereas Howard et al (1981) detected carcinoma associated antigenic alterations in breast cancer by lectin binding. Ree and Kadin (1985) reported that the lectin from Ricinus communis could differentiate benign from malignant histiocytes. The binding properties of the lectins used in the present study were not investigated earlier in detail. The studies on JFL were mostly on crude preparations (Soares et al, 1982; Vijayan et al 1982), whereas no reports are available on WBL. In this study, the binding shown by JFL towards the premalignant and malignant lesions of the oral cavity were different. The figures clearly demonstrate the binding patterns and are suggestive of their utilization as an immunohistochemical stain.

The malignant potential of OSMF is well documented (Pindborg and Zachariah, 1965). Rajendran et al (1986) have reported that OSMF can be an intermediary stage in the transformation process of a normal cell into malignancy. In our study, three out of ten OSMF tissues showed a binding pattern similar to that of SCC towards JFL. The similarity in the binding pattern shown by these three cases of OSMF is strongly suggestive of their malignant potentiality. Since no specific treatment is available for OSMF, we are following these cases to see if they turn into frank malignancies.

Results with murine thymus (Rose et al, 1980) and human skin (Vijayakumar et al, 1985) suggest that the lectin from the jackfruit recognizes incomplete, nonsialated forms of membrane glycoconjugates. Since these less than fully differentiated forms may be expressed at the surface of malignant cells than their normal counterparts, the JFL may be of use as a diagnostic aid in exfoliative cytology.

Figure 1 : Normal oral tissues stained with JFL-HRP conjugate.

Figure 2 : Oral leukoplakia tissues showing dense binding in the intercellular regions with JFL.

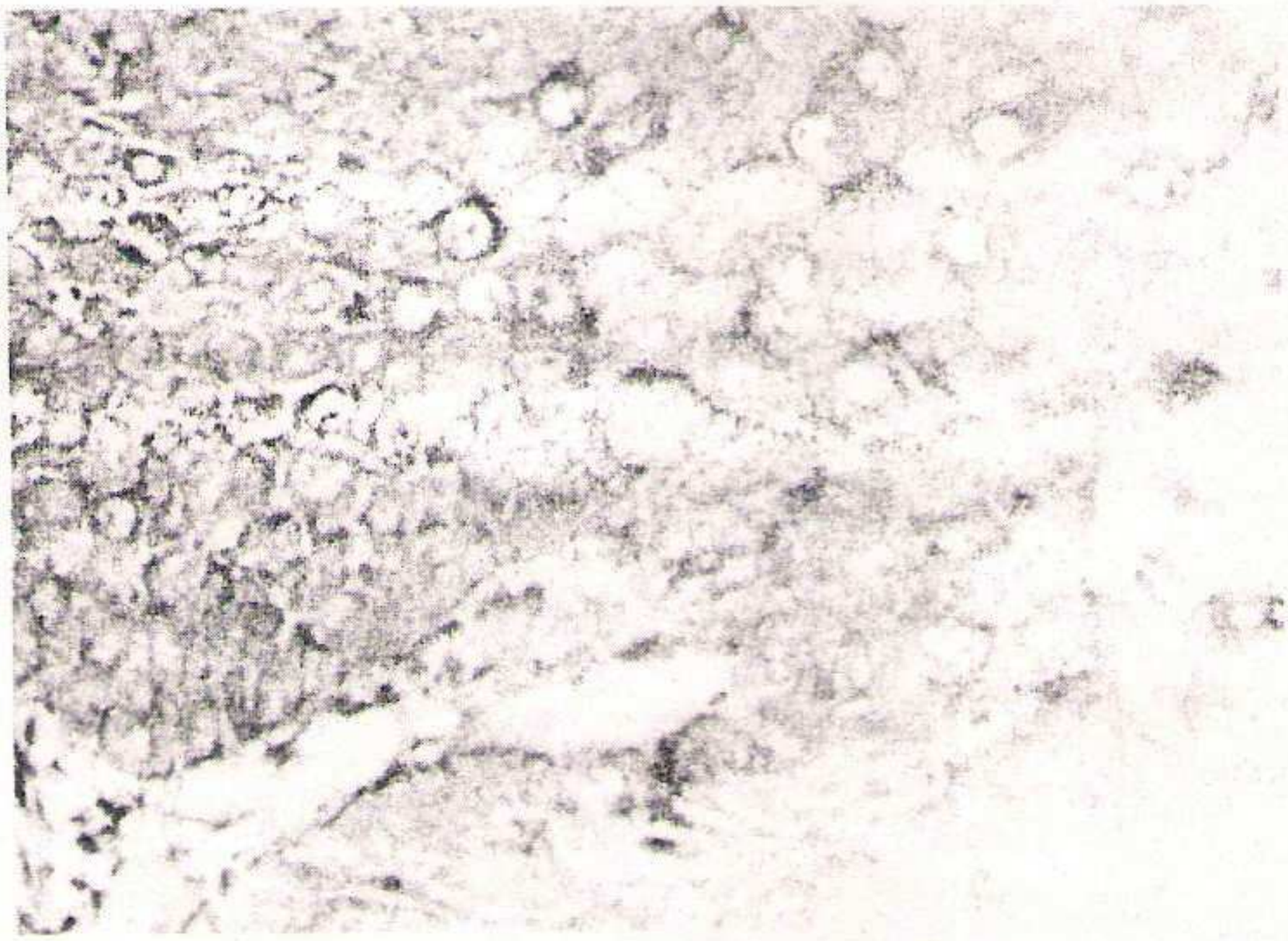

Figure 3 : OSMF tissues showing irregular dense binding of cytoplasm with JFL.

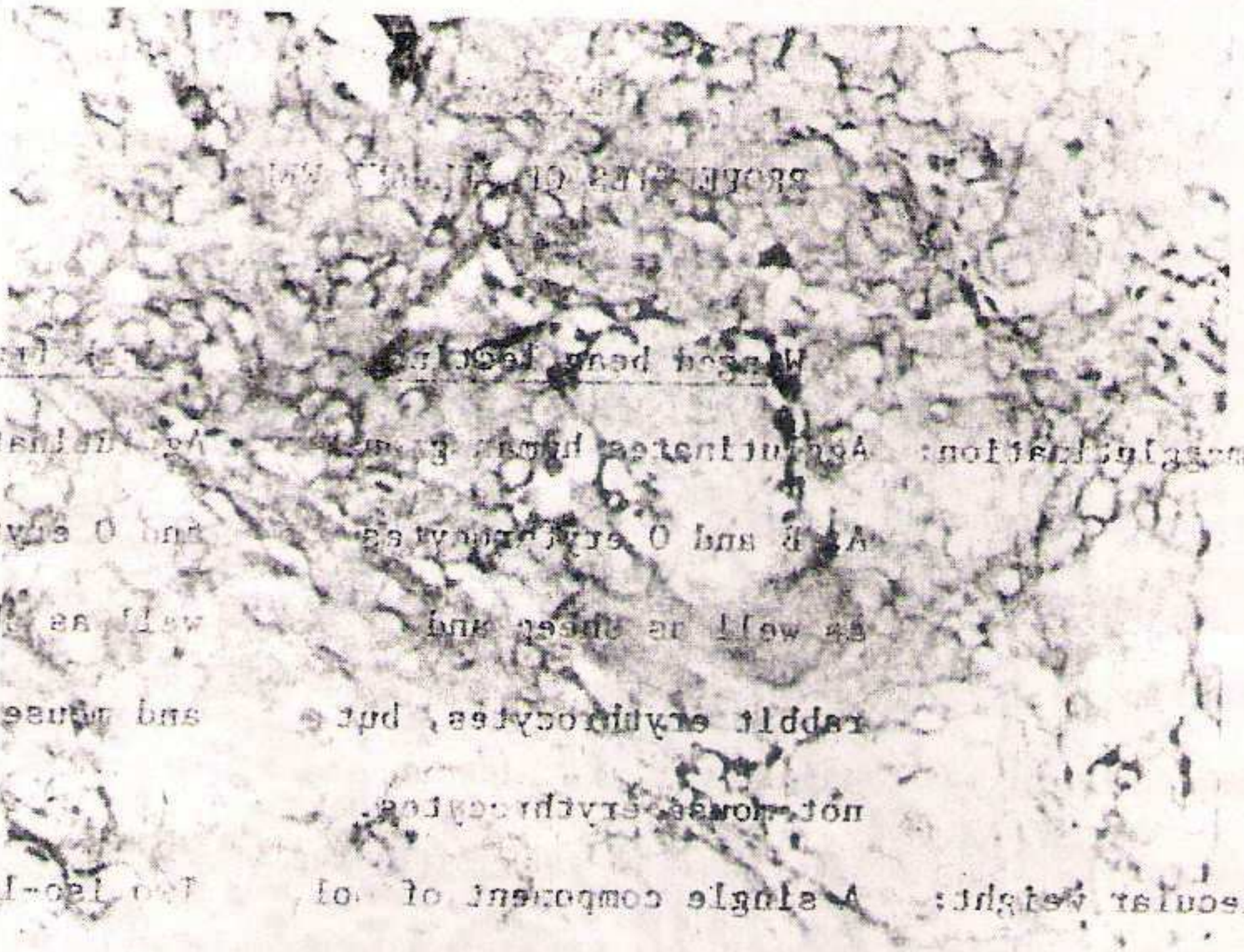

Figure 4 : Verrucous carcinoma tissues showing irregular cytoplasmic binding and membrane binding.

Figure 5 : Squamous cell carcinoma - infiltrating carcinoma cells show dense binding.

TABLE 1

PROPERTIES OF JFL AND WBL

	Winged bean lectins	Jack fruit lectins
1. Haemagglutination:	Agglutinates human group A, B and O erythrocytes as well as sheep and rabbit erythrocytes, but not mouse erythrocytes.	Agglutinates human A, B and O erythrocytes as well as sheep, rabbit and mouse erythrocytes.
2. Molecular weight:	A single component of mol. wt. 35,000; unaffected by reduction.	Two iso-lectins of mol. wts. 11,500 and 15,000; unaffected by reduction.
3. Isoelectric point:	A single band pI, 4.0.	A spread of components between pI 6.0 and 8.3.
4. Breast irhibitor:	N-acetyl-D-galactosamine.	N-acetyl-D-galactosamine
5. Yield of lectin:	6.0 mg/gm of defatted meal retaining more than 80% of the haemagglutinating activity.	2.0 mg/gm of defatted meal retaining more than 75% of the haemagglutinating activity.

6. Amino acid compositions:

Amino acid	Molar percent		Amino acid	Molar percent	
	WBL	JFL		WBL	JFL
Aspartic acid	9.06	9.24	Methionine	0.67	1.16
Threonine	5.57	7.02	Isoleucine	2.35	7.02
Serine	4.40	8.70	Leucine	10.94	6.36
Glutamic acid	13.76	7.08	Thyrosine	3.49	7.43
Proline	10.44	4.51	Phenyl alanine	7.10	6.85
Glycine	2.58	14.73	Histidine	3.05	0.72
Alanine	8.69	2.28	Lysine	10.00	6.41
Valine	6.17	9.13	Arginine	4.03	1.36

TABLE 2

JFL BINDING PATTERN IN PREMALIGNANT AND MALIGNANT LESIONS OF THE ORAL CAVITY

Tissue	Intracellular Cytoplasm	Area	Keratinised Area	Cells at Basement Membrane
Normal (n=10)	Uniform +(10/10)	Uniform ++(10/10)	Nil	Diffuse (10/10)
Oral Leukoplakia (n=10)	Uniform +(10/10)	Uniform +++(10/10)	Uniform ++(6/10) Irregular +++(4/10)	Uniform ++(6/10) Irregular ++(4/10)
Oral Submircous Fibrosis (n=10	Uniform ++(10/10)	Uniform +++(10/10)	Uniform ++(7/10) Irregular ++(3/10)	Uniform ++(10/10)
Verrucous Carcinoma (n=10)	Focal ++(10/10)	Irregular +++(10/10)	Irregular +++(8/10) ++(2/10)	Uniform +++(10/10)
Squamous Cell Carcinoma (n=10)	Irregular +++(10/10)	Irregular +++(10/10)	Irregular +++(7/10) ++(3/10)	Focal +++(10/10)

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