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Peanut lectin binding to the alveolar lining layer in hyaline membrane disease*

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Summary. The authors have studied the histochemical pattern of Peanut lectin (PNA) binding sites in lungs of seven newborns with hyaline membrane disease (HMD) and ten controls. The alveolar lining layer was positive in HMD and no changes in the PNA pattern was noted after neuraminidase digestion. Hyaline membranes were generally unstained but occasional reactivity was encountered in some parts. No reaction with PNA was observed in control lungs, but positivity was seen after neuraminidase pretreatment. Our histochemical data document the presence of accessible galactosyl residues with absence of terminal sialic acid in the alveolar lining layer of newborns with HMD. The authors suggest that PNA reactivity in HMD reflects an histochemical feature described in fetal lungs at the pseudoglandular and canalicular stages.

Key words: Peanut lectin – Hyaline membrane disease – Alveolar lining layer – Histochemistry

Introduction

Several studies have demonstrated a continuous layer lining the surface of the alveolar epithelial cells of the lung (Luke and Spicer 1965; Kuhn 1968; Scarpelli 1968; Bernstein et al. 1969); this alveolar lining material is composed of a phospholipid moiety belonging to surfactant and another part containing sialic acid and carbohydrate residues (Luke and Spicer 1965; Kun 1968; Bernstein et al. 1969; Adamson and Bowden 1970).

Surfactant deficiency provides an explanation for pulmonary neonatal atelectasis due to "Hyaline Membrane Disease" (HMD) (Reynolds et al. 1968; Adams et al. 1970; Boughton et al. 1970; Laweryns 1970; Farrell and Avery 1975). Consequently, this pathological condition may be suitable for an histochemical study of sialoglycoconjugates in the alveolar lining layer without the problems induced by the influence of the surfactant component. These glycoconjugates have been thought to play a key role in the regulation of protein conformation, ion transport across membranes, protection from proteolytic attack, cell-cell recognition and binding of hormones (Schulte and Spicer 1985). Structurally, the majority of sialic acid residues in N-glycosylproteins found at the cell surface are linked to penultimate β-Gal by an $\alpha 2 \rightarrow 6$ glycosidic bond (Montreuil 1980).

More recently lectins, proteins and glycoproteins found in plants and animals have been utilized for histochemical detection of specific terminal sugar and, in some instances, internal sugar complex carbohydrates (Goldstein and Hayes 1978; Spicer et al. 1981). In particular Peanut lectin (PNA) a protein with high affinity to the terminal disaccharide D-Gal $(1 \rightarrow 3)$-GalNAc, has been considered to be a specific cytochemical probe for detecting and localizing galactosyl residues in cell surfaces (Stoward et al. 1980). Therefore, in the present study, we have utilized PNA conjugated with fluorescein isothiocyanate (FITC) in combination with neuraminidase digestion in order to determine galactosyl residues and sialic acid sites in the alveolar lining layer of newborns dying of HMD and controls.

Materials and methods

Seven newborns, spontaneously delivered at 36–38 weeks of gestation (average weight ranging from 2000–2600 g), died

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within 48 h after birth with symptoms of respiratory distress syndrome. Their mothers were primiparas, nondiabetic and apparently healthy.

At autopsy, performed within 12 h after death, pulmonary atelectasis was observed; routinary histopathological methods revealed the typical picture of HMD. Fragments of lung obtained from all cases were fixed in 10% buffered formalin (pH 7.2, 300 mOsmol) at room temperature for 12–24 h and embedded in paraffin at 56° C. To detect PNA binding sites, 5 μ thick sections were incubated with FITC coupled Arachis Hypogaea Agglutinin (0.064 μg/m) (Sigma Chemical Co., St. Louis, MO, USA) (working dilution 1:50) for 30 min in a moist chamber, rinsed with 0.1 M phosphate buffered saline (PBS, pH 7.4) for 10 min, carefully blotted and mounted with PBS medium. Parallel sections were treated with neuraminidase from Vibrio Cholerae (Calbiochem Behring Corp.) 1 IU/ml at room temperature for 30 min or 100 IU/ml in 0.1 M acetate 0.04 M $CaCl_2$ buffer at pH 5.5 for 16 h at 37° C (Cooper 1982) and after they were incubated with FITC labelled PNA. A solution of the same lectin concentration containing sugar hapten (0.1 M lactose or 0.1 M galactose) to inhibit lectin binding was used on adjacent control sections (Stoward et al. 1980); sections were also incubated with FITC or PBS alone.

The pattern of fluorescence was examined with a ZEISS photomicroscope equipped with II FL epi-illuminator an filters for FITC (I excitation filter BP 550 to 590, dicroic mirror FT 510, and emission filter 520; II excitation filter BP 546/10, dicroic mirror FT 580, and emission filter 590), and Osram HBO 50 watt high pressure mercury lamp. Lung control sections, obtained from 10 age-matched newborns without HMD or pulmonary inflammatory processes, were submitted to all the histochemical procedures described above.

Results

In HMD a thin rim of FITC-PNA positivity, with occasional discontinuity, was observed in the alveolar lining layer (Fig. 1a). Hyaline membranes were generally unstained, although a slight focal positivity was noted in some parts of them. The pattern of FITC-PNA reactivity in lining layer and hyaline membranes was unmodified after neuraminidase pretreatment (Fig. 1b).

In control lung sections, no reaction was noted using FITC-PNA alone (Fig. 2a), but after neuraminidase digestion an evident reactivity of alveolar lining layer was observed (Fig. 2b).

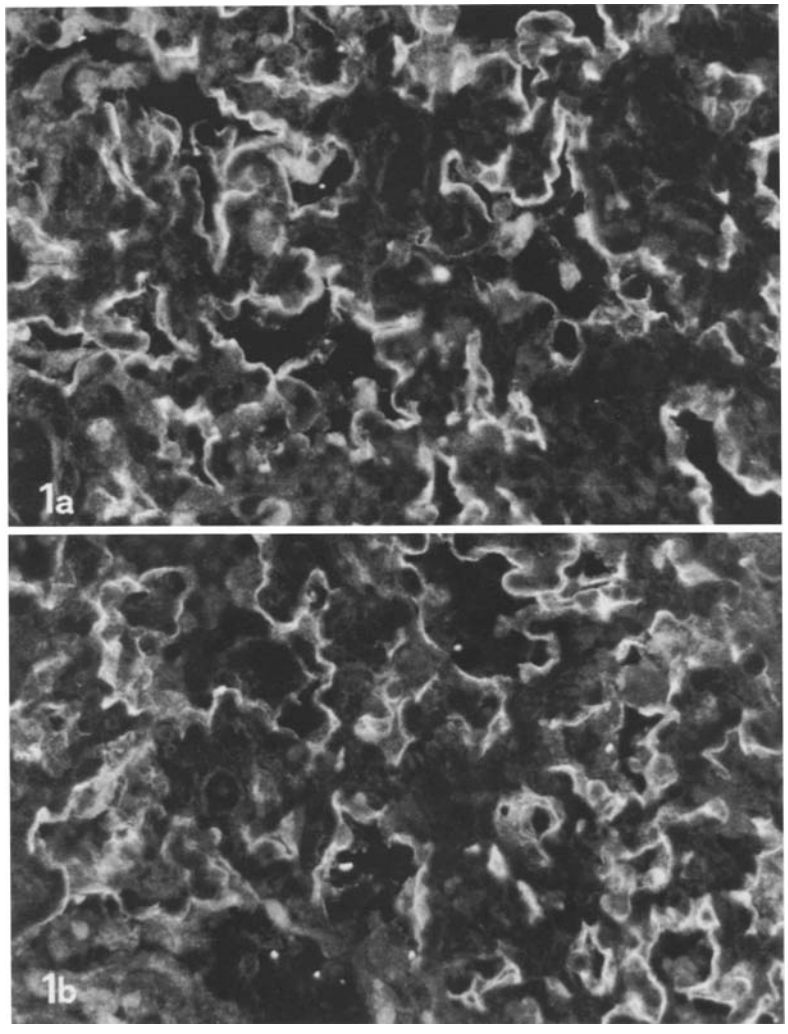

Fig. 1a, b. Lung with HMD. PNA-binding sites are well represented in the alveolar lining layer (**a**). The PNA staining pattern of the alveolar lining layer is unmodified after neuraminidase digestion (**b**). FITC-PNA (**a**) ×240; neuraminidase-FITC-PNA (**b**) ×220

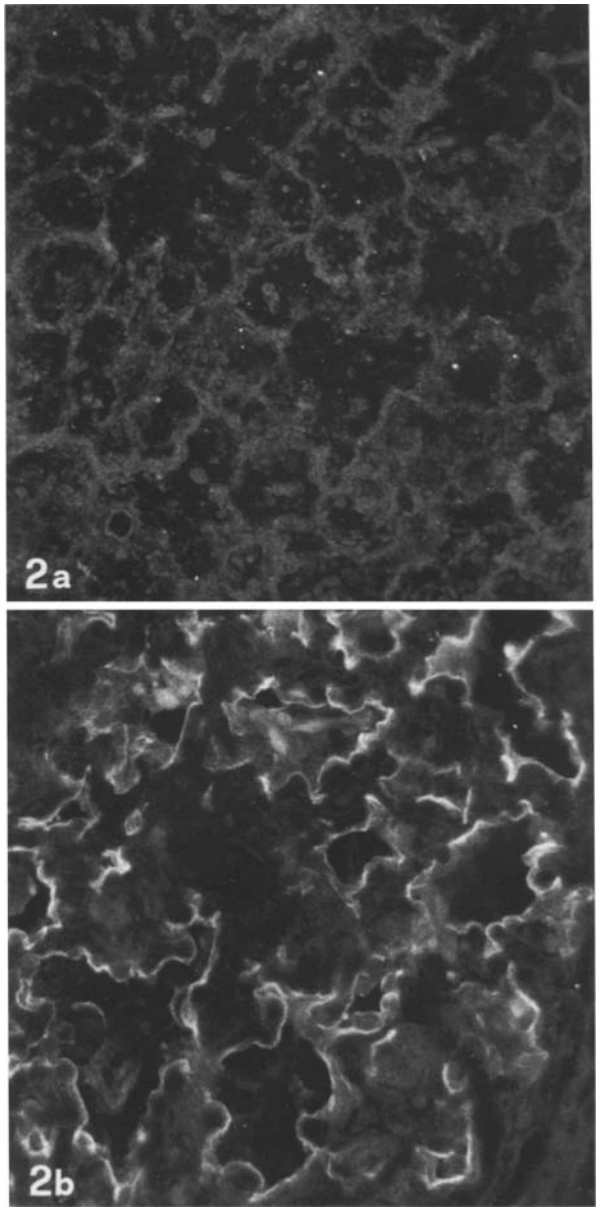

Fig. 2a, b. Control lung. The alveolar lining layer fails to show PNA binding (**a**); after neuraminidase digestion, an evident reaction is encountered at the alveolar surface (**b**). FITC-PNA (**a**) ×147; neuraminidase-FITC-PNA (**b**) ×258

When serial sections of HMD and control lungs were incubated with FITC-PNA supplemented with inhibitor, no staining of the alveolar lining layer was encountered; the same results were obtained after incubation with FITC or PBS alone.

In the bronchial mucosa of newborns with HMD and in controls, numerous goblet cells were intensely stained with FITC-PNA. Moreover, the apical part of bronchial epithelial cells was also stained; a slight increase of this positivity was obtained after neuraminidase digestion.

Discussion

We have documented the presence of accessible galactosyl residues in the alveolar lining layer of newborns with HMD. These results are consistent with the in vitro specificity of the PNA lectin for galactose and galactosamine (Stoward et al. 1980); this histochemical specificity is also indicated by the ability of 0.1 M galactose or lactose to prevent the lectin binding to the alveolar lining layer. Moreover, we have observed the abolition of staining in sections treated with FITC or PBS alone.

Commonly, the oligosaccharide side chains possess a terminal galactose-sialic acid dimer and neuraminidase digestion exposes lectin binding galactose residues, enhancing thus the reaction (Stoward et al. 1980; Spicer et al. 1981). In our cases of HMD, digestion of tissue glycoproteins with neuraminidase had the unexpected effect of failing to modify PNA staining in the alveolar lining, indicating the absence of terminal neuraminic acid which impedes the capacity to bind PNA lectin. However, in our control lungs a PNA-reactive layer was evident only after neuraminidase pretreatment, thus documenting the sialic acid presence. This latter finding has been also decribed in two full-term newborns without cardiopulmonary lesions (Faraggiana et al. 1986).

The biological significance of PNA binding to the alveolar lining layer in HMD remains unknown. Changes in lectin binding to the surface carbohydrates of rat lung alveolar epithelial cells have been reported in streptozotocin-induced diabetes (Dixon and Jersild 1983); these changes may include changes in molecular composition that involve biosynthetic pathways associated with glycosylation, changes in the rate of turnover of specific glycoconjugates or changes in membrane configurations that affect availability for lectin binding (Dixon and Jersild 1983). Moreover, recent observations in developing pulmonary vasculature of the pig lung suggest that as the cells mature the carbohydrate surface antigens to which the lectin binds may be masked or disappear (Mills and Haworth 1986). The PNA positive patterns observed in our cases with HMD, in comparison with the negative reaction of controls, would appear to involve exposure of sugar residues which are either new or inaccessible in controls; how the histochemical changes observed in the PNA binding to the alveolar lining layer relate to the changes in biochemical surface composition remains an intriguing problem. In HMD, the surfactant deficiency may make available galactosyl residues, which are not coupled with terminal sialic acid. The absence

of sialic acid has been demonstrated in fetal lungs at pseudoglandular and canalicular stages by PNA-neuraminidase pretreatment (Faraggiana et al. 1986); moreover, it has been described that sialic acid is added to fetal lungs as the alveoli mature (Faraggiana et al. 1986). In HMD, the PNA reactivity with the absence of sialic acid in the alveolar lining layer may reflect an histochemical feature present in the surface of fetal respiratory tree and, at the same time, suggests arrested maturation of the alveolar lining layer in this neonatal pathological condition.

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