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Lectin Histochemistry of Tracheo-Esophageal Region in Chick Embryos

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Abstract

Background Glycosylation is an important modification involved during embryonic development. Lectins are specific carbohydrate-binding proteins; they can be employed as specific probes to localize defined monosaccharide and oligosaccharides on cell surface and on cytoplasmic structures, and in extracellular matrix.

Objectives The lectins (SBA, PNA, WGA, SWGA, UEA-I) binding were used as a sensitive, stable, and easy tool that can provide an extraordinarily sensitive detection for changes glycosylation and carbohydrate expression that may occur during embryogenesis and development of trachea-esophageal region.

Methods Fertilized chick eggs were incubated at 38 °C, embryos were fixed with Bouin's solution. Sections were treated with fluoresce ineisothiocyanate (FITC) labeledlectins.

Results The histochemical study during the 2nd and 3rd days of development revealed variable tempo-spatial variability of lectin bindings to the mesenchymal tissues and other embryonic structures at the trachea-esophageal region.

Conclusions The lectin bindings could be an indicator for the glycoconjucate changes that play an essential role in developmental phenomenon of trachea-esophageal morphogenesis by marking cellular differentiation, cellular migration, and cellular interactions.

Key words Trachea, esophagus, chick, embryo, lectin, histochemistry.

Introduction

The digestive and respiratory systems have different physiological functions and are generally considered to be and studied as two independent systems. Although at birth they are separated, they were derived from a common and transiently developed structure, the foregut, which is the anterior part of the gastrointestinal (GI) tract. The development of the foregut is not well documented in comparison to that of other parts of the digestive system [(1)].

Glycosylation is an important post-translational modification of proteins involved in cell-cell interaction during embryonic development. Specific carbohydrates moieties of the oligosaccharide side chains of the glycoconjugates are among the factors involved in these interactions and the developmental morphogenetic processes are correlated with changes in the sugar content of glycoconjugates located on cell surfaces or in extracellular matrix [(2)]. Moreover glycocojugates that are present in the extracellular matrix are involved in

regulatory cellular migration and play critical roles during early embryonic development [3].

Lectins are specific carbohydrate-binding proteins of non-immune origin, which agglutinates cells and/or precipitates polysaccharides or glycoconjugates", and does not have an enzymatic function. Lectins selectively and specifically bind non-covalently to carbohydrate residues without modifying them. An important property of a lectin is the ability to bind to carbohydrates [4,5]. It is for this reason that they can be employed as specific probes to localize defined monosaccharides and oligosaccharides on cell surface, on cytoplasmic and nuclear structures, and in extracellular matrix in cells and tissue from throughout the animal and plant kingdom, down to bacteria and viruses. Lectins have more than one binding site and therefore they are able to cross-link cells through interactions with carbohydrates in the cell membrane. They are sensitive, stable, and easy-to-use tools. Lectin histochemistry and cytochemistry can provide an extraordinarily sensitive detection system for changes in glycosylation and carbohydrate expression that may occur during embryogenesis, growth, and disease [6,7]. In the present study we used 5 lectins (Glycinmaximus (soya bean) SBA, Arachis hypogeal (peanut agglutinin) PNA, Triticum vulgaris (wheat germ agglutinin) WGA, Triticum vulgaris (succinylated WGA SWGA, Ulexeuropaeus UEA-I) in the study of trachea-esophageal region development.

Methods

Chick embryos: Fertilized chick eggs were obtained from a local hatchery and incubated at 38°C. Fifty chick embryos were removed out and were staged according to the criteria of Hamburger and Hamilton [8]. About 2-3 embryos for each of the different stages (stage 14 to 19) were used.

The tissues were fixed in Bouins solution for 8 hours at room temperature and were processed for paraffin sectioning [9]. All lectins used were obtained from Sigma. They were fluoresceine isothiocyanate (FITC) labeled. The procedure for Fluorescein Isothiocyanate Labelled Lectins was done according to Allison [10].

Results

Lectin Histochemical Bindings:

The binding pattern of the different lectins used in this study showed variable tempro-spacial criteria. These binding patterns were summarized in tables 1 and 2.

Table 1. The lectins binding pattern with epithelial and mesenchymal tissue of 2 day embryo

Lectin regions in 2 days	head mesenchyme	ateral to cardinal ventral vein	Perinotochord mesenchyme	Pharyngeal wall	Pharyngeal arch	Dorsomedial to cardinal vein	Traingular pharynx
SBA	O	*O	O	O	Oo	O	Oo
UEA I	*O	*O	O	*O	*O	*O	*O
PNA	O	*O	O	O	*	*	*
WGA	O	O	O	O	*	*O	*
SWGA	*O	*O	O	*O	*	O	*

O = Cell surface binding, *= Intracellular binding, Oo = extracellular, *O = Mixed intracellular and cell surface binding.

Table 2. The lectins binding pattern with epithelial and mesenchymal tissue of 3 day embryo

Lectin Regions in 3 days	Ventrolateral to cardinal vein	Head mesenchyme	Perinotochord mesenchyme	Slit-like pharynx	Pharyngeal Arch	Dorsomedial to cardinal vein	Tracheal bud	Mesenchyme around esophagus & bronchial buds
SBA	O	O	O	*O	*	Oo	*O	*O
UEA-I	*	*	O	*	*	*	O	O
PNA	*O	*O	O	*	*	*	*O	*O
WGA	*	*O	O	*	*	*O	*O	*O
SWGA	*O	*O	O	*	*	*O	*O	*O

O = Cell surface binding,* = Intracellular binding, Oo = extracellular, *O = Mixed intracellular and cell surface binding

The Lectin Binding During the 2nd Day of Incubation:

The binding f SBA during the 2nd day of development is shown in (Figures 1A, 1B, and 1C). The binding of UEA-I during the 2nd day was shown in (Figures 1D, 1E, and 1F). The PNA binding pattern in the same developmental period was shown in (Figures 1G, 1H, and 1I). That of WGA shown in (Figures 1J, 1K, and 1L). The binding of SWGA was shown in (Figures 1M, 1N, and O).

The Lectin Binding During The3rd Day of Incubation:

The lectin binding during the 3rd day of incubation was shown in (Figures 2A, 2B, 2C, and 2D) for SBA. The binding of UEA-I is shown in (Figures 2E, 2F, 2G, and 2H). The binding of PNA is shown in (Figures 2I, 2J, 2K, and 2L). WGA binding was shown in (Figures 2M, 2N, 2O and 2P). The SWGA binding pattern was shown in (Figures 2Q, 2R, 2S and 2T).

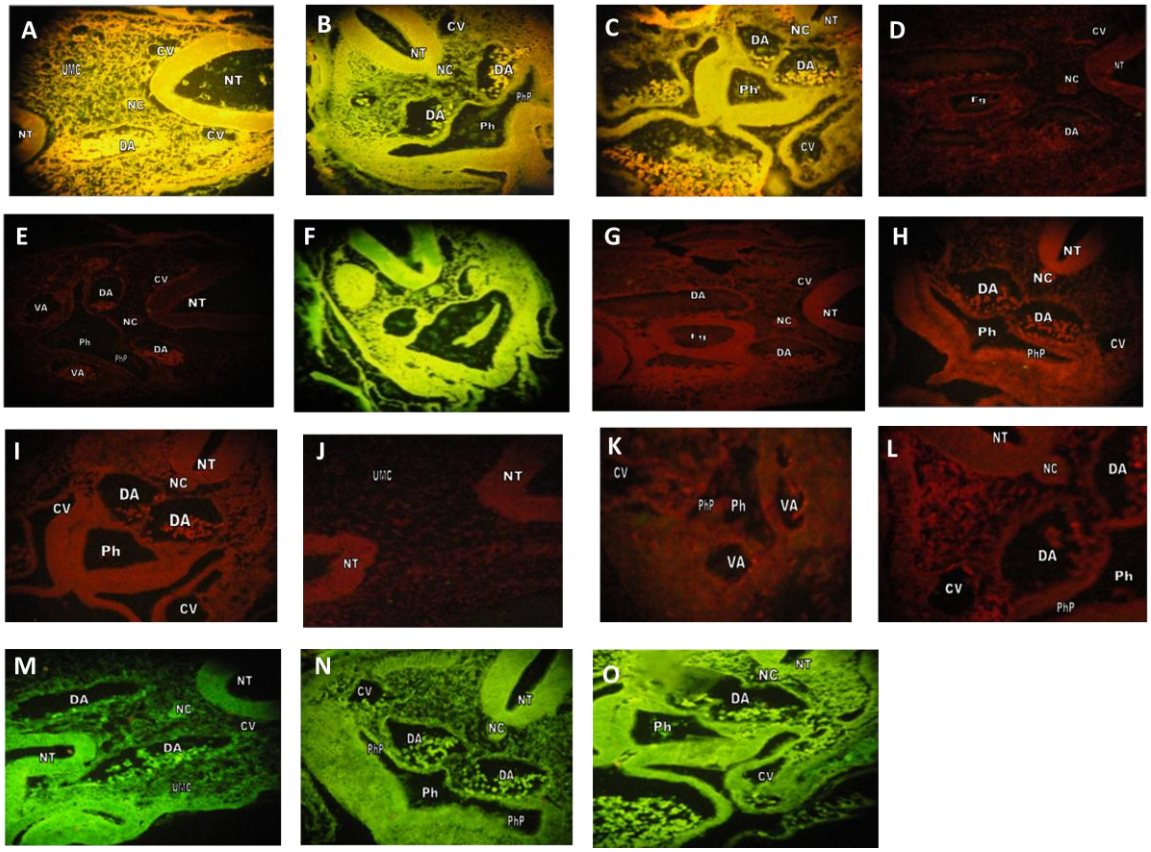

Figure 1. Transverse section of 2 day embryo, A-C incubated with SBA. 4X, D-F incubated

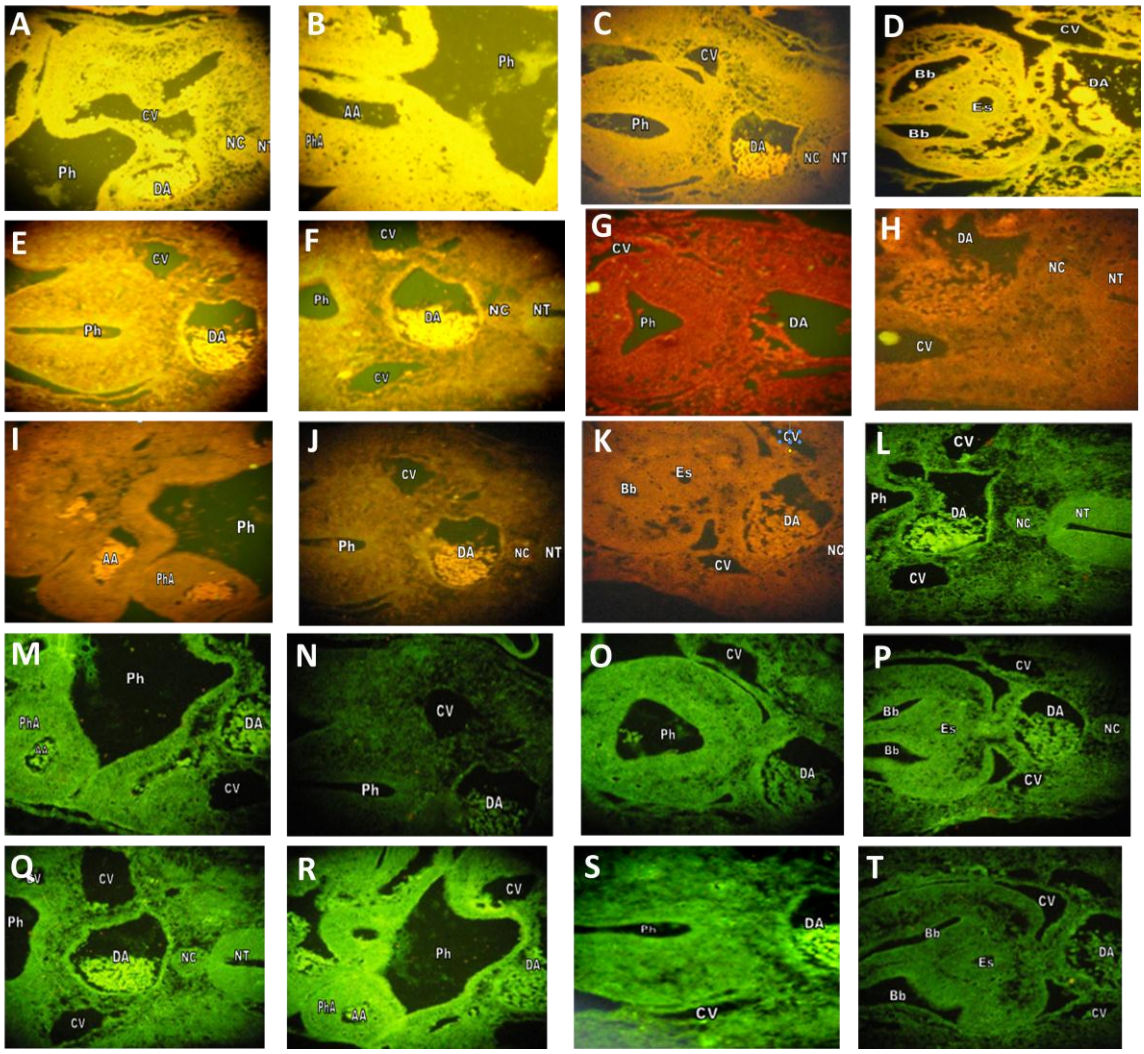

Figure 2. Transverse section of 3 day embryo, A-D incubated with SBA. 10X, E-H incubated with UEA-I. 10X, I-L incubated with PNA. 10X, M-P incubated with WGA. 10X, Q-T incubated with SWGA. 10X

Discussion

The Lectin Histochemical Reactions:

Thorpe *et al*. reported striking temporal and spatial patterning of specific carbohydrate sequences in the developing chick embryo [11]. The variable lectin bindings found in this study supported this concept and also supported the conclusion of Adiet *et al*. that Ulexeuropeus I (UEA-I), Glycine maximus (SBA), Arachishypogaea (PNA) and Triticumvulgare (WGA) binding showed a time-related variability of staining intensity and binding sites that depends upon the stage of differentiation and maturation [12].

The lectin binding to the mesenchymal tissue seen in this study supported the mesenchymal lectin binding reported by Gheri *et al*. [13]. The lectin binding pattern found in this study supported the view of Mohammad *et al*. reported that cell surface and extracellular glycoconjugate played an essential role in many developmental phenomenon as cell differentiation, cell migration, and cellular interactions [14].

The color of the fluorescence was reported to be variable, the fluorescence reactivity had been shown to be in different colors, green/ yellow and TRITIC or Texas Red (which fluoresces red) [7,15]. This conclusion was also found in the

fluorescence color of the lectin binding examined in this study, specially the fluorescence of WGA binding.

The lectin bindings should be logically considered at the mesenchymal tissues enclosing the embryonic structures around the developing pharyngeal and tracheo-esophageal regions.

Interpretation of the MesenchymalLectin Binding:

Around the Developing Cranial Part of the Foregut and Ventro-Lateral to the Cardinal Veins:

The Binding of PNA, SWGA, and UEA-I:

It seems that the mixed cell surface and intracellular binding of PNA and SWGA is a criterion of mesencymal tissues propagation ventrolateral to the deviated cardinal veins during the second and third days, the same criterion is seen at the mesenchyme propagation around the cranial part of the foregut at the third day.

The UEA-I binding varies at these mesenchymal regions showing temprospatial changes from mixed cell surface and intracellular binding at the second day to intracellular binding at the third day.

This conclusion goes with the concept of Zalik *et al*. reported that galactose specific lectin is expressed in spreading cells [(16)]. The PNA pattern binding (which is a galactose specific lectin could be related to spread of the mesenchymal tissues lateral to the deviated cardinal veins and around the developing cranial part of the foregut [(17)].

Also, according to this, conclusion the SWGA binding specific for N-acetylgalactosamine may be considered for the same interpretation as the binding pattern of SWGA simulate that of PNA [(18)]. The UEA-I binding specific for fucose may be related to cellular differentiation, this interpretation is based on the role of lectin binding in determination of the cellular differentiation [(19,20)].

Catt *et al*. stated that it is likely that in most tissues the high concentrations of lectin are particularly active in connective tissue during the extensive tissue reorganization [(21)]. The results of this study agree with that hypothesis.

The Binding of SBA and WGA:

SBA and WGA bindings showed variable tempro-spatial pattern that did not follow a conclusive chronological pattern in the mesenchyme around the cranial part of the pharynx and ventrolateral to the cardinal veins.

The variable SBA and WGA bindings at this mesenchyme are probably related to the masking effect described by Takahashi in the chick embryo [(22)].

Sinning *et al*. shown that SBA binding could be related to active proteins associated with mesenchyme formation that are localized to a particulate form of extracellular matrix, specific for matrix particulates in areas of the embryo that undergo an epithelial- mesenchymal interaction [(23)].

The results of this study reached a conclusion similar to that reported by Zschabitz *et al*. stated that WGA (Triticumvulgare) displayed a universal distribution of binding sites with differences in binding between focal areas of developing mesenchyme. These authors concluded that terminal sialic acid molecules, glucose, galactose-(β 1,4)-N-acetylglucosamine as well as galactose-(β 1,3)-N-acetylgalactosamine are diffusely distributed in mesenchymal tissue [(24)].

Dorsomedial to the Deviated Cardinal Veins:

The mesenchyme dorsomedial to the deviated cardinal veins lies on the sides of the neural tube represented the sclerotomal mesenchyme. It was described that the cells of the ventral and medial walls of the somites lose their compact organization, become polymorphous, and shift their position. These cells collectively known as the sclerotome that form a loosely woven embryonic connective tissue. This tissue will surround the spinal cord and the notochord to form the vertebral column [(25-29)].

In summary, during the 2nd day of development, the mesenchyme dorsomedial to the deviating cardinal veins showed cell surface reactivity with SWGA, and mixed reaction with UEA-I, and only intracellular reactivity with PNA. During the 3rd day of development, this mesenchyme showed

mixed reaction with SWGA, while PNA and UEA-I showed intracellular reaction. The binding at this mesenchyme showed cell surface SBA binding pattern during the second and third day of development. The WGA binding was of mixed cell surface and intracelluar pattern at the same period.

The PNA Binding:

This sclerotomal mesenchyme was shown in this study to be marked by intracellular PNA during the second and third day of development. This finding is in agreement with the suggestions of Gotz *et al*. whom reported conclusions supporting the results of this study; these authors reported that PNA and WGA were found in the developing sclerotomes [30].

Aulthouse and Solursh stated that PNA appears to be a marker for early precartilage cellular aggregates in chick embryo [31], also Zschabitz *et al*. suggested that in early stages of development, PNA selectively labeled the prevertebralblastema (the sclerotomes) [24].

Stringa *et al*. found specific binding to the lectin (PNA) in embryonic precartilage tissues and the developing somite. The hypothesis was that PNA-binding may be a characteristic of chondro progenitor cells in chick embryos [32]. Also, Mohammad *et al*. stated that PNA binding was found in the vertebral primordial at early stages [14].

The SWGA Binding:

The results of this study could support the hypothesis made by Griffith et al. that the undifferentiated mesenchymal transformation is accompanied by changes in the cell surface oligosaccharide complement of the differentiating cells. These authors stated that the pluripotential nature of the mesenchyme represents a developing system which is readily amenable to experimentation and should provide insights into the general mechanisms of cell differentiation and transformation [33].

The UEA-I Binding:

The binding pattern is probably a criterion of the mesenchymal tissue of the chick species during the third day of incubation, and thus this pattern could not be considered as a marker specific for the sclerotomal mesenchyme. This conclusion supported the finding of Zschabitz *et al*. that UEA-I failed to bind the vertebral primordial in rat embryo [24].

The SBA and WGA Binding:

The sclerotomal mesenchyme was shown in this study to be marked by cell surface and mixed cell surface and intracellular reactivity respectively during both the second and third days of development.

This pattern may be considered as a criterion of the sclerotomal cells of the chick embryo at this developmental period. This conclusion supported the finding of Gotz *et al*. that SBA did not react at all in the paraxial mesenchyme of the human embryos in comparison to chick embryos [30].

The Pharyngeal Arches:

The WGA binding maintained intracellular localization during the second and third days of development. The SBA pattern has been changed from extracellular binding at the second day to intracellular binding at the third day.

The PNA, SWGA and WGA Binding:

These lectins showed intracellular reaction with the mesenchyme of the pharyngeal arches during the second and third day of development, this pattern could be considered as a marker of this mesenchyme. This finding is in agreement with the report of Rojo *et al*., these authors stated that WGA reacted at every site of the bronchial region thus showing the presence of N-acetyl-D-glucosamine. These authors reported that other lectins, such as PNA and UEA-I, reacted also for a short time at some sites [34].

The same marker pattern of these lectins was found in the mesenchyme around the triangular pharynx at the second day and around the slit-like pharynx during the third day. This may indicate that this caudal pharyngeal lumen is surrounded by the mesenchyme of the pharyngeal arches. This interpretation depends on the consideration of Brooks and Leathem that the pattern of lectin binding could indicate the specific tissue type [6].

The UEA-I Binding:

The binding pattern of this lectin changes from a mixed cell surface and intracellular pattern at the second day to intracellular binding at the third day. Also this pattern was seen in the mesenchyme around the triangular and slit-like pharynx, and that could support the interpretation described with PNA, SWGA, and WGA binding.

The SBA Binding:

The pharyngeal arches mesenchyme changes the pattern of SBA from extracellular pattern at the second day to intracellular binding at the third day. Also the extracellular to intracellular changing pattern was seen in the mesenchyme around the triangular shaped pharynx at the 2nd day of developmentanl around the slit-like pharynx during the 3rd day, and that could supported the interpretation described with PNA, SWGA, and WGA binding.

Around The Respiratory Diverticulum, the Lung Buds, and The Esophagus:

The lectin binding pattern at the third day in these mesenchymal regions almost showed cell surface binding of the lectins with or without intracellular localization of pattern of the lectins used in this study. Such pattern could probably being considered to be specific to the type of the mesenchymal tissues at these regions.

These finding goes with the descriptions of Gheri *et al*. that lectin UEA-I was concomitant with the beginning of respiratory development [35]. The patterns of lectin binding appreciated in this study are the criteria that were applied as an indication of the topographic criteria or the type of the mesenchymal tissue separating the respiratory primordial from the esophagus.

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