A lectin histochemical study of the thoracic respiratory air sacs of the fowl

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ABSTRACT

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The lectin-binding characteristics of the epithelial lining of the thoracic air sacs of the chicken were determined. Con A, LCA and PSA bound to the apical membrane as well as to the cytoplasm distal to the nucleus of the surface epithelium, indicated the presence of α-linked mannose as well as N-acetylchitobiose-linked α-fucose residues in the glycoproteins. GSL I bound to the apical membrane and cytoplasm distal to the nucleus, but not to the cilia of the epithelium, where-as MPL, DBA and RCA120 bound to the apical membrane, cilia and cytoplasm, indicated the presence of α-linked N-acetylgalactosamine residues. However, neither SJA or SBA showed any binding, indicating the absence of β anomers of galactosyl (β1.3)N-acetylgalactosamine and β-linked N-acetylgalactosamine residues. UEA I bound to the apical membrane and cilia, as well as to the cytoplasm of a few cells, indicated the presence of α-linked fucose residues. PNA bound to the apical membrane of some, but not all, surface epithelium cells, indicated the presence of galactosyl (β1.3)N-acetylgalactosamine residues. WGA bound to the apical membrane and cilia, as well as to the cytoplasm of a few cells, indicated the presence of neuraminic acid residues.

Keywords: Fowl, lectin binding, thoracic air sacs

INTRODUCTION

The gross, light and electron microscopic structure of the respiratory air sacs of the fowl are well documented (Carlson & Beggs 1973). A simple epithelium resting on a basal lamina supported by a layer of fibro-elastic connective tissue lines the respiratory surface of the air sacs. The epithelium varies from simple squamous to a ciliated columnar epithelium with goblet cells. Air sacs of birds are prone to infection by various microorganisms, including fungi, bacteria and viruses. Some microorganisms or their toxins need to attach to glycoproteins or glycolipids on cell surfaces in order to cause disease, e.g. the S glycoproteins of the infectious bronchitis virus bind to neuraminic acid-containing glycans (Schultze, Cavanagh & Herrler 1992). The relative expression of glycoconjugates is important because of the significant role membrane glycoconjugates appear to play in respiratory host defenses (Lasky 1992; Abdi, Kobzik, Li & Mentzer 1995).

The diversity of glycoconjugates and the application of lectin histochemistry is extensively reviewed by Spicer & Schulte (1992). Most lectins are multimeric proteins that share the common property of binding to defined sugar structures (Spicer & Schulte 1992). The assumed monosaccharide specificity of a lectin can be very different from the actual complex oligosaccharide(s) it recognizes in histochemical preparations. Because of the specificity that

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each lectin has toward a particular carbohydrate structure, even oligosaccharides with identical sugar compositions can be distinguished. Some lectins will bind only to structures with mannose or glucose residues, while others may recognize only galactose residues. Some lectins require that the particular sugar be in a terminal non-reducing position in the oligosaccharide, while others can bind to sugars within the oligosaccharide chain. Some lectins do not distinguish between α or β anomers while others require not only the correct anomeric structure, but also a specific sequence of sugars for binding. Some glycoproteins have terminal sialic acid residues that can block lectin binding and require desialylation before binding.

No data could be found in the existing literature on the lectin-binding glycoproteins or glycolipids of the epithelium lining the respiratory surface of air sacs in chickens. The present study was undertaken to determine the lectin-binding characteristics of the thoracic air sacs of the chicken using lectin histochemical techniques.

MATERIALS AND METHODS

Source and type of birds

For this study a total of 25 specific pathogen free (SPF) White Leghorn chickens were obtained from the Poultry Research Farm, Cornell University, Ithaca, New York. The birds were clinically healthy, of both sexes, 42–84 days old and weighed 140–900 g.

Collection of the air sac membranes

The birds were euthanased with CO_2 in a chamber designed for this purpose. After euthanasia they were placed in dorsal recumbency, the skin over the ventral abdomen and thorax was removed and the hips dislocated to provide stability in dorsal recumbency. The ventral abdominal wall was incised just caudal to the sternum to open the ventral hepatic peritoneal cavities. From here the incision was extended cranially along the lateral borders of the pectoral muscles, transecting the sternal ribs close to their junctions with the sternum. Both clavicles and coracoids were cut and the sternum reflected cranially. The ventral abdominal wall was removed and the gizzard freed from its attachments to facilitate access to the thoracic air sacs. The medial wall of the thoracic air sacs was visualized by cutting the left and right hepatic ligaments and reflecting the liver and gut medially. The septum separating the cranial and caudal thoracic air sacs was located and a small transverse incision was made over the septum along their ventral borders. The septum was then incised along its entire length to create one cavity consisting of both the cranial and caudal thoracic air sacs. A thin (2 mm thick) stainless steel ring was passed through the incision and placed so as to include the medial wall of both cranial and caudal air sacs. A thick (3 mm) stainless steel ring was placed on the peritoneal surface of the air sac and aligned with the thin ring. The two rings were clamped together with a modified hemostat and the membrane was removed by cutting the tissue along the outer circumference of the rings. The rings were then clamped in a small binder clamp and the hemostat removed. The entire specimen (clamp, rings and membrane) was placed in either 10 % phosphate-buffered formalin or Bouin's fixative. This ensured that the membranes did not shrink during fixing and processing.

Processing of specimens for light microscopy

For paraffin wax embedding the tissues were dehydrated through a graded series of ethanol, cleared in Propar (Anatech Ltd., Lake Rd., Battle Creek, MI 49015) and infiltrated with paraffin wax. After infiltration the membrane was cut from the rings, embedded in paraffin wax and sectioned at 4 µm on a rotary microtome. Paraffin wax-embedded sections were mounted on slides coated with Poly-L-lysine (Vector Laboratories, Burlingame, California).

Lectins used

Thirteen biotinylated lectins (Kit I and Kit II, Vector Laboratories) were screened. These lectins were obtained from the plants *Maclura pomifera* (MPL), *Dolichos biflorus* (DBA), *Glycine maxi* (SBA), *Arachis hypogaea* (PNA), *Ricinus communis* (RCA), *Triticum vulgaris* (WGA), *Ulex europaeas* (UEA), Concanavalin A (CON A), *Griffonia simplicifolia* (GSL), *Pisum sativum* (PSA), *Lens culinaris* (LCA) and *Sophora japonica* agglutinin (SJA), and wheat germ agglutinin (WGA). Optimal dilution concentration for each lectin was determined and varied from 5–50 µg/mℓ.

Lectin staining

Tissue sections were de-paraffinated in xylene and re-hydrated through a graded series of ethanol. Endogenous peroxidase activity was blocked by incubation of sections for 10 min at room temperature in 0.5 % (v/v) hydrogen peroxide in methanol. After rinsing the sections in 0.01 mol phosphate buffer

solution (PBS), the specific biotinylated lectin at the appropriate dilution in PBS was added to cover the tissue sections, and then incubated at 37 °C in a humid chamber for 1 h. Negative controls for each tissue were incubated with buffer only, and for binding specificity the lectins were blocked with their inhibitory sugars. The sections were washed with PBS and covered with pre-diluted streptavidin-peroxidase conjugate (Vector Laboratories) for 15 min at room temperature in a humid chamber. The sections were then washed six times with PBS and incubated with aminoethyl carbazole (AEC) substrate (Zymed Laboratories Inc., San Francisco, California) for 2–20 min at room temperature. Colour development was monitored under the microscope. Sections were washed in distilled water to stop further colour development, and then counterstained with haematoxylin. All sections were examined under an Olympus BH-2 light microscope and relevant areas photographed. Lectin staining was recorded as positive or negative.

RESULTS AND DISCUSSION

The results of staining with various lectins are summarized in Table I.

Processing paraffin wax embedded tissues for light microscopy requires removing the wax from the tissues prior to staining. This process also removes most of the glycolipids (Brooks, Leathem & Schumacher 1997) that are therefore excluded in the results of this study.

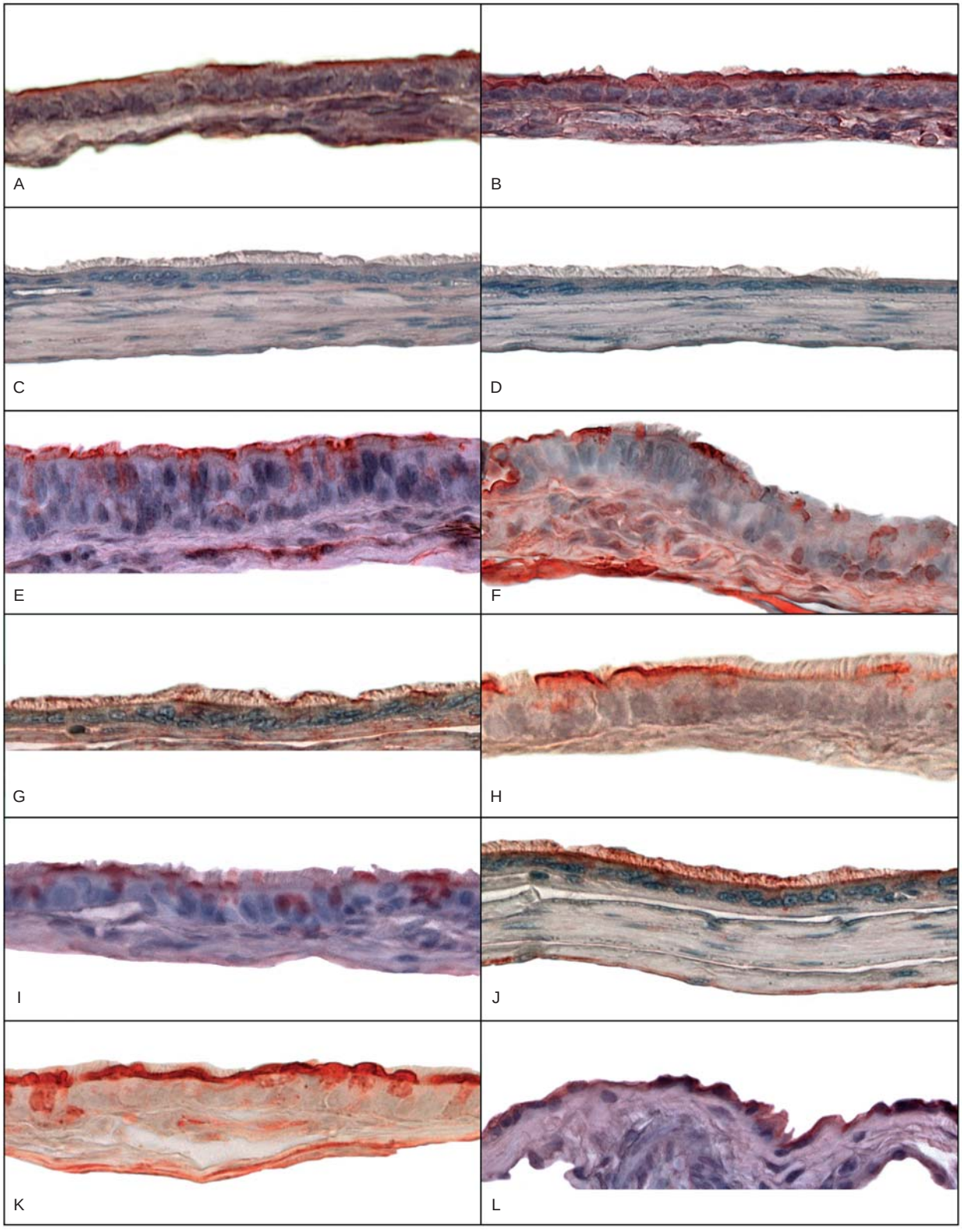
It is generally agreed that a squamous epithelium lines the air sacs, and that many of the cells contain surfactant in the form of myeloid inclusions. Well-defined areas of ciliated cuboidal to columnar cells, as well as goblet cells are randomly distributed over the surface of the membrane (Lucas 1970; Smith, Meier, Lamke, Neill & Box 1986). The results of the present study showed that areas of squamous epithelium reacted evenly for all the lectins, except for SJA and SBA that did not show any positive binding, irrespective of the concentration of the lectin that was used. Due to the very attenuated nature of the squamous epithelium it was not possible to distinguish between staining of the apical membrane and staining of the cytoplasm distal to the nucleus. Contrary to the squamous epithelium, areas of columnar and ciliated epithelium did not react uniformly with all the lectins that showed positive binding. Some lectins bound to the apical membrane of ciliated and non-ciliated cells, while others only bound to the apical membrane of non-ciliated cells. Binding to the cytoplasm also varied between the different lectins.

The lectins Con A, LCA and PSA bind to a wide variety of membrane glycoproteins that have a core structure that includes a-linked mannose. LCA rec-

TABLE 1 Results of lectin binding

Lectin	Binding	Sugar specificity	Blocking sugar
MPL 6.25 µg/mℓ	Positive	N-acetyl galactosamine	D+ galactose
UEA 50 µg/mℓ	Positive	Fucose	Fucose
PNA 50 µg/mℓ	Positive	Galactose	D+ galactose
RCA 50 µg/mℓ	Positive	Galactose and N-acetylgalactosamine	D+ galactose
Con A 50 µg/ℓ	Positive	Glucose and mannose	Methyl mannoside and methyl glucoside
DBA 25 µg/mℓ	Positive	N-acetyl galactosamine	N-acetyl galactosamine
GSL I 25 µg/mℓ	Positive	Galactose and N-acetylgalactosamine	Galactose and/or N-acetyl galactosamine did not block
WGA 12.5 µg/mℓ	Positive	N-acetylglucosamine	Chitobiose
PSA 12.5 µg/mℓ	Positive	Glucose and mannose	N-methyl mannoside and methyl glucoside
LCA 12.5 µg/mℓ	Positive	Glucose and mannose	N-methyl mannoside and methyl glucoside
SBA up to 100 µg/mℓ	Negative	N-acetyl galactosamine	
SJA up to 100 µg/mℓ	Negative	N-acetyl galactosamine	

ognizes additional sugars as part of the receptor structure and is therefore more specific than Con A. PSA is almost identical to LCA and binds to α-linked mannose with an N-acetylchitobiose-linked α-fucose residue included in the receptor sequence (Spicer & Schulte 1992; Brooks *et al.* 1997). All three lectins



require Ca and Mg cations for binding. Con A (Fig. 1L), LCA (Fig. 1A) and PSA (Fig. 1B) showed strong binding to the apical membrane, and to a lesser degree to the cytoplasm distal to the nucleus of the surface epithelium of the thoracic air sacs. This would indicate the presence of a-linked mannose, as well as α-linked mannose with an N-acetylchitobiose-linked α-fucose residue in the glycoproteins of the apical membrane and in the cytoplasm of the epithelium. The cytoplasm of the surface epithelium contains large amounts of glycogen (Carlson & Beggs 1973). Therefore the affinity of the cytoplasm for Con A is probably due to the presence of glucose residues in the glycogen (Pedini, Ceccarelli & Gargiulo 1994; Nadel 2003). N-methyl mannoside and methyl glucoside effectively blocked binding of the three lectins.

The lectins GSL I, MPL, RCA^{120} SJA, SBA and DBA preferentially bind to α-linked N-acetylgalactosamine residues of glycoproteins (Spicer & Schulte 1992). GSL I (Fig. 1H) showed binding to the apical membrane and to the cytoplasm distal to the nucleus of a few cells, but did not bind to the cilia of the epithelium. MPL (Fig. 1K), DBA (Fig. 1G) and RCA^{120} (Fig. 1E) showed binding to the apical membrane and cilia, as well as to the cytoplasm proximal to the nucleus of some cells. Neither SJA (Fig. 1C) nor SBA (Fig. 1D) showed any binding to the epithelium. This may be due to the fact that SJA preferentially binds to β anomers of galactosyl (β1.3)N-acetylgalactosamine and that SBA preferentially binds to oligosaccharide structures with terminal α or β-linked N-acetylgalactosamine residues (Spicer & Schulte 1992). This would indicate that a-linked N-acetylgalactosamine residues are present on the glycoproteins of the epithelium, but that it is neither present as β anomers, nor as terminal α or β residues. Binding of GSL I, MPL, DBA and RCA^{120} could effectively be blocked with galactose.

UEA I binds to glycoproteins and glycolipids containing α-linked fucose residues at a branch or terminal position on the glycoprotein (Spicer & Schulte 1992). The lectin showed positive binding to the apical membrane and cilia of the epithelium (Fig. 1I). A few cells also showed positive binding to the cytoplasm distal to the nucleus. This would indicate that the glycoproteins of the apical membrane and the cytoplasm of some cells contain α-linked fucose residues. Binding could effectively be blocked with fucose.

PNA binds specifically to galactosyl (β1.3)N-acetylgalactosamine (T-antigen) present in many glycoconjugates in soluble and membrane associated glycoproteins (Lotan, Skutelsky, Danon & Sharon 1975). The lectin showed positive binding to the apical membrane and cytoplasm of some cells of the epithelium, but not to all of them (Fig. 1F). This would indicate that the specific receptor is not present on all surface-lining cells. Binding could effectively be blocked with galactose.

WGA preferentially binds to oligosaccharides containing terminal dimers and trimers of N-acetylglucosamine or chitobiose, and can also interact with some glycoproteins via sialic (neuraminic) acid residues (Spicer & Schulte 1992). The lectin showed binding to the apical membrane and cilia, as well as to the cytoplasm of a few cells (Fig. 1J). This would

FIG. 1 Results of lectin binding

- A LCA bound to the apical membrane and cytoplasm distal to the nucleus of the surface epithelium
- B PSA bound to the apical membrane and cytoplasm distal to the nucleus of the surface epithelium
- C SJA showed no affinity to any part of the surface epithelium
- D SBA showed no affinity to any part of the surface epithelium
- E RCA 120 bound to the apical membrane, cilia and cytoplasm distal to the nucleus of the surface epithelium
- F PNA bound to the apical membrane and cytoplasm of some, but not all, cells
- G DBA bound to the apical membrane and cilia, and to the cytoplasm of some of the surface epithelium
- H GSL bound to the apical membrane, but not to the cilia of the surface epithelium
- I UEA I bound to the apical membrane and cilia, as well as to the cytoplasm distal to the nucleus of some cells of the surface epithelium
- J WGA bound to the apical membrane and cilia of the surface epithelium
- K MPL bound to the apical membrane and cilia, as well as to the cytoplasm distal to the nucleus of some surface epithelial cells
- L Con A bound to the apical membrane of the surface epithelium

indicate that the glycoproteins of the apical membrane and cytoplasm of some cells contain dimers and trimers of N-acetylglucosamine or chitobiose, or sialic acid residues as part of their structure. Binding was effectively blocked with chitin hydrolysate.

In conclusion, most cells of the epithelium lining the respiratory surface of the thoracic air sacs of the fowl showed an affinity to a wide variety of lectins. This indicated that the apical cell membrane and the cytoplasm distal to the nucleus contained α-linked mannose, α-linked mannose with an N-acetylchitobiose-linked α-fucose, α-linked N-acetylgalactosamine, α-linked fucose, galactosyl (β1.3)N-acetylgalactosamine and dimers and trimers of N-acetylglucosamine or chitobiose, or sialic acid residues as part of their glycoconjugate structure. This information will be useful in further studies of air sacculitis in poultry.

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