Histochemical and Lectinhistochemical Studies on Nasal Mucosa of Pigs with or without Respiratory Diseases

Carlos J. PERFUMO, Nelson MORES[1)], Armando D. ARMOCIDA, Itamar A. PIFFER[1)], Adriana R. MASSONE and Shin-ichi ITAGAKI[2)]

Institute of Pathology, Faculty of Veterinary Sciences, La Plata National University, CC 296, 1900 La Plata, Argentina, [1)]EMBRAPA, Concordia SC, Brazil, and [2)]Department of Biomedical Science, Faculty of Agriculture, The University of Tokyo, Bunkyo, Tokyo 113–8657, Japan

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ABSTRACT. Histochemical and lectinhistochemical examinations were carried out on nasal mucosa of pigs with or without respiratory diseases. As the results, both acid and neutral mucins coexisted in nasal mucosa of normal pigs while acid sialomucins were mainly observed in nasal mucosa of pigs infected with *Bordetella bronchiseptica* and/or *Pasteurella multocida.* Lectinhistochemistry revealed that the nasal epithelial cells of normal pigs were rich in N-acetylgalactosamine, fucose and N-acetyl-glucosamine residues which showed a tendency to disappear in porcine cytomegalovirus infection and to increase in atrophic rhinitis, respectively. — KEY WORDS: lectinhistochemistry, nasal mucosa, swine.

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Mucous epithelia which are in contact with environments are covered with mucus which acts as a selective physicochemical barrier. Mucus is composed of about 95% water and 5% mucins. Mucins are glycoproteins mainly characterized by high level of *O*-linked oligosaccharides which are produced by goblet cells and mucous cells of the submucosal glands at the level of the upper respiratory tract [4]. The properties of the mucus, e.g. viscosity and elasticity, are associated with intact mucins. Therefore, under diseased condition affecting the upper respiratory tract, the inflammatory response may also induce some differences in chemical compositions of mucins which in turn may lead to change in their physical properties [2]. The application of classical histochemical methods in association with lectinhistochemistry to normal and diseased nasal mucous membrane of pigs enables us to investigate glycoconjugates stored in cells and secretory mucins "*in situ*" as well as their reactive and functional changes during the host response against virus and bacteria.

The animals were divided into 4 groups; group A: 2 respiratory disease-free pigs (70 days old), group B: 2 pigs (55 and 70 days old) naturally infected with porcine cytomegalovirus, group C: 2 pigs (70 days old) inoculated twice with 10^7 colony-forming units (CFU) of *Bordetella bronchiseptica*, and killed at 35 days postinoculation, and group D: 3 pigs (70 days old) inoculated first with 10^7 CFU of *Bordetella bronchiseptica* and then with 10^7 CFU of toxigenic *Pasteurella multocida* one week later. Nasal samples were taken at the level of the first upper premolar teeth, fixed in 10% neutral buffered formalin, and then decalcified by formic acid-sodium citrate. Paraffin sections (4 μm) were stained with hematoxylin and eosin (H.E.) for histological examination. In addition, for histochemical examinations, alcian blue (pH 2.5)-periodic acid-Schiff (AB-PAS) method was used for differentiating blue-stained acid from red-stained neutral mucosubstances, and high iron diamine alcian blue (HID-AB) procedure for distinguishing grey-black-stained acid sulfated glycoconjugates from blue-stained nonsulfated sialomucins, respectively [3]. For lectinhistochemical examinations, seven biotinylated lectins (Table 1) were used. In brief, the staining procedure was as follows: after blocking endogenous peroxidase activities in H_2O_2 in methanol, non specific background staining was reduced by covering the deparaffinized tissue sections with bovine serum albumin. Then sections were reacted with biotin-labeled lectins in humid chamber at 5°C overnight and treated with ABC reagent solution (Vectastain ABC Kit, Vector Laboratories, Burlingame CA, U.S.A.). Reaction products were visualized by diaminobenzidine-H_2O_2. Counterstain was done with hematoxylin. A graded score system used was : 0=negative, 1=weak, 2=moderate and 3=strong reaction.

Control animals showed no histopathological changes. The most characteristic changes in group B were prominent mucoprulent exudates adherent to the nasal mucous membrane, metaplasia or desquamation of epithelial cells and degenerative to necrotic changes in the epithelial cells of submucosal glands with basophilic intranuclear inclusion bodies. Some areas of nasal mucous epithelial cell hyperplasia with partial loss of cilia and thinner bone trabeculae were found in group C. In group D, there were marked infiltration of mononuclear cells, lymphocytes and plasma cells, almost disappearance of bone trabeculae with increased number of active osteoclasts, degeneration of osteoblasts, and proliferation of fibrous tissue.

The results of PAS-AB and HID-AB stainings on the normal and diseased nasal mucosa (Table 2) showed that goblet cells of normal pigs usually secreted acid sialomucins and some cells in group B lost their stainability. In groups C and D, hyperplastic changes in epithelia led to the formation of acid sialomucin-filled cysts. In the superficial tubuloacinar glands of normal pigs, both acid and neutral mucosubstances coexisted. On the other hand, in group B, neutral mucosubstances were hardly seen, indicating changes

Table 1. Lectins used in this studies

Lectin	Abbreviation	Major carbohydrate specificity (a)	Concentration ($\mu g/ml$)
Canavalia ensiformis	Con A	α-D-Glc, α-D-Man	30
Triticum vulgaris	WGA	β-D-GluNAC, NeuNAc	10
Dolichos biflorus	DBA	α-D-GalNAc	30
Glycine max	SBA	α-D-GalNAc, α-D-Gal	30
Archis hypogaea	PNA	β-D-Gal-(1–3)-GalNAc	30
Ricinus communis	RCA-1	β-D-Gal	30
Ulex europaeus	UEA-1	α-L-Fuc	30

(a) Fuc=fucose; Gal=galactose; GalNAc=N-Acetylgalactosamine; Glc=glucose;GluNAc=N-acetylglucosamine; Man=mannose; NeuNAc=N-acetyl-neuraminic acid.

Table 2. Summary of histochemical findings of swine nasal mucosa

Group	A	B	C/D
Goblet cells	as	a	as
Superficial gland epithelial cells	as*/n	as	as*/n*
Deeper gland epithelial cells	as	neg.	as

a=acid; as=acid sialomucin (non sulfated); n=neutral; neg.=negative. *=apical part. A=Respiratory disease-free pigs; B=Pigs naturally infected with porcine cytomegalovirus; C=Pigs experimentally infected with *Bordetella bronchiseptica*; D=Pigs experimentally infected with *Bordetella bronchiseptica* and *Pasteurella multocida*.

in glycosilation processes associated with degenerative and necrotic changes in the glandular epithelial cells.

As shown in Table 3, such lectins as DBA, SBA, PNA and RCA-1 that bound selectively to N-acetylgalactosamine showed moderate to strong positive reactivities to mucous epithelial cells and gland epithelial cells in all groups. Binding reactivity of respiratory epithelia to WGA specific for N-acetylglucosamine was negative or slightly positive in control samples while it was strongly positive in hyperplastic and cystic epithelia of atrophic rhinitis material (Fig. 1). However WGA also exhibited strong affinity to sialic acid in terminal position. Its binding pattern therefore indicates the localization of this carbohydrate in the epithelial cells of bacteria-infected samples.

In pigs, normal respiratory epithelial cells gave a positive reaction with UEA-1 specific for fucose, and hyperplastic epithelia of atrophic rhinitis samples showed also a marked reactivity. In this regard, previous work [1] reported that bronchiolar epithelia were negative for UEA-1. This difference might reflect both differences in mucus composition secreted at the different level of airway and the histological structures involved, i.e. pseudostratified ciliated epithelia in the nose and simple cuboidal epithelia in the bronchiole. The results that ConA did not bind to respiratory epithelia and showed only slight binding reactivity to gland epithelia indicate that mucins contain scarce N-linked complex type oligosaccharides under both normal and diseased conditions [4].

With reference to submucosal glands, except for group B, in which degenerative to necrotic changes led to loss of lectins affinities, the more interesting finding was a difference in lectin affinity pattern between superficial and deeper epithelia indicating a difference in terminal sugar or aminosugar involved than variation among groups.

In conclusion, results obtained in the present study on

Table 3. Summary of lectinhistochemical findings of swine nasal mucosa

Lectins		Con A	WGA	DBA	SBA	PNA	UEA-1	RCA-1
	Group							
Respiratory	A	0	0–1	3	3	2–3	3	3
epithelial	B	0	1	0–3	ns	0	1	0
cells	C/D	0	3	3	3	2–3	3	3
Superficial	A	1–2	2	3	3	0	3	0
gland epithelial	B	1	1	1	3	0	0	0
cells	C/D	1–2	2–3	3	3	0–1	ns	0
Deeper	A	0	1	3	2	2	3	0
gland epithelial	B	0	1–3	0–3	2	0	0	3
cells	C/D	0	1–2	3	2	1–3	3	0

0=negative, 1=weak, 2=moderate, 3=strong reaction, ns=non specific reaction. See the footnote of Table 2.

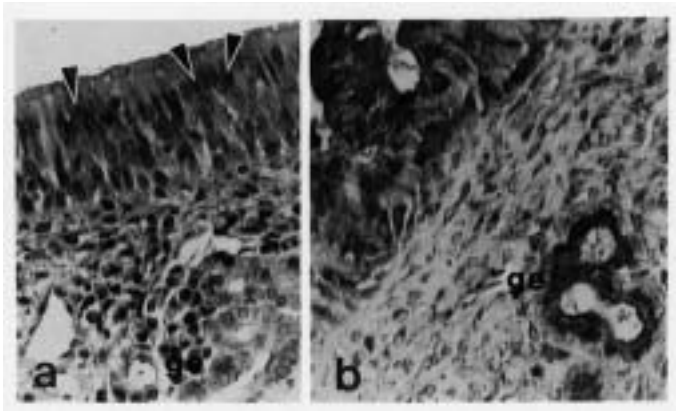

Fig. 1. Nasal mucosa of a pig free from respiratory disease (a) and a pig experimentally infected with *Bordetella bronchiseptica* and *Pasteurella multocida* (b). Cillia and supranuclear cytoplasm (arrowheads) of superficial cells are positive for WGA, but glandular epithelia (ge) are negative (a). WGA strongly stains both hyperplastic respiratory epithelial cells and glandular epithelial cells (b). Lectinstaining, × 400.

nasal mucosa showed that both acid and neutral mucins coexist in normal pigs, while acid sialomucins were mainly observed in bacteria-infected animals. In porcine cytomegalovirus-infected pigs, the lack in sialomucins was mainly due to the severe necrosis of glandular epithelial cells in which virus replication occurred. Lectinhistochemistry revealed that the epithelial cells were rich in Gal-NAc, Fuc and Glu-NAc residues which showed a tendency to disappear in porcine cytomegalovirus infection and to increase in atrophic rhinitis.

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