

Differential Properties of Mucous Glycoproteins Produced by Allergic Inflammation and Lipopolysaccharide Stimulation in Rat Nasal Epithelium

Takeshi Shimizu · Shino Shimizu

Department of Otorhinolaryngology, Shiga University of Medical Science, Otsu, Shiga, Japan

Abstract

To examine the differential properties of mucous glycoproteins, we produced hyperplastic and metaplastic changes in goblet cells of rat nasal epithelium by intranasal instillation of ovalbumin (OVA) in OVA-sensitized rats, and by intranasal lipopolysaccharides (LPS) instillation. The epithelial mucosubstance was quantitatively examined by AB-PAS and lectin histochemistry. The newly produced mucin after OVA challenge or LPS instillation contained a high amount of sulfomucin and a low amount of neutral glycoprotein: LPS-induced mucin contained more sulfomucin (70.1% of total) and less neutral glycoprotein (8.6%) than OVA-induced mucin (sulfomucin 33.6%, neutral glycoprotein 41.8%). Four of the lectins, PNA, MAL II, SNA, UEA I, stained some of the mucosubstance, indicating the presence of galactose-N-acetylgalactosamine, α 2,3-linked and α 2,6-linked sialic acid-galactose, and fucose residues. After LPS instillation, the reactivity was higher for galactose-N-acetylgalactosamine (64.8% of total) and α 2,3-linked sialic acid-galactose (75.8%) than after saline instillation (3.5 and 19.1%, respectively) or OVA challenge (5.8 and 32.3%). A 2-fold increase in mucin mRNA (rat Muc5ac) expression was induced after LPS instillation or OVA challenge. These results indicate

that mucin mRNA expression increases similarly after LPS instillation or OVA challenge, however, carbohydrate compositions of newly produced mucin are different between the two groups.

Copyright © 2011 S. Karger AG, Basel

Mucous glycoproteins (mucin) are large heterogeneous macromolecules, containing oligosaccharide chains attached to peptide backbones encoded by several MUC genes. Hypersecretion of mucus is a common characteristic of airway inflammation. Mucin differs in the sugar groups and the extent of sialylation and sulfation of the oligosaccharide structure in different hypersecretory conditions. The alteration of these oligosaccharide structures or peptide backbones may affect the physicochemical properties of mucus in disease. However, little is known about the disease-related changes of mucin character and mucin gene expression in airway inflammation and biological roles of these changes are still unclear. We hypothesized that the extent of sialylation and sulfation of the terminal sugar residues, and mucin gene expression, are different between

mucins produced by allergic inflammation and by lipopolysaccharide (LPS) stimulation.

In the present study, we evaluated (1) the amount of sialomucin and sulfomucin by alcian blue-periodic acid-Schiff (AB-PAS) histochemistry, (2) the terminal sugar residues by lectin histochemistry, and (3) mRNA expression of the rat *Muc5ac* gene in rat nasal epithelium, produced by allergic inflammation or LPS stimulation.

Materials and Methods

We produced allergic inflammation and LPS-induced inflammation in rat nasal epithelium. Hypertrophic and metaplastic changes of goblet cells were induced by intranasal instillation of ovalbumin (OVA) in OVA-sensitized rats, and by intranasal LPS instillation. Rats were killed at 24 h after the last intranasal instillation. The nasal cavity was transversely sectioned at the level of incisive papilla, and histopathological changes of nasal septal epithelium were examined. The percent area of intraepithelial mucosubstance was quantitatively measured by the image analyzer.

Tissue sections were stained with AB-PAS and lectin histochemistry. Some of the mucosubstance in rat nasal epithelium reacted with four of the lectins: *Arachis hypogaea* (PNA), *Maackia amurensis* II (MAL-II), *Sambucus nigra* (SNA), and *Ulex europaeus* I (UEA-I). The expression of rat *Muc5ac* mRNA in rat nasal epithelium is also examined. The instillation methods, tissue preparations, AB-PAS and lectin staining, and methods for RT-PCR were described previously [1–3].

Results and Conclusions

Rat Muc5ac mRNA Expression

LPS stimulation and OVA challenge induces goblet cell metaplasia and mucus production in nasal epithelium of sensitized rats, whereas only a few goblet cells were observed in control rats. Quantitative examination revealed significant increase of epithelial mucosubstance after LPS stimulation and OVA challenge in OVA-sensitized rats. Both LPS stimulation and OVA challenge significantly increased rat *Muc5ac*

mRNA expression compared with animals treated with saline instillation. However, there was no difference between LPS stimulation and OVA challenge.

AB-PAS Histochemistry

Four types of mucosubstance were characterized: neutral glycoproteins, sialidase-sensitive sialomucin, sialidase-resistant sialomucin, and sulfomucin. In saline-instilled controls, most of the mucosubstance (75.7% of total) was neutral glycoprotein, and sulfomucin occupied only 13.2% of total. The newly produced mucosubstance after LPS instillation or OVA challenge contained higher amounts of sulfomucin and lower amounts of neutral glycoprotein ($p < 0.01$). LPS-induced mucosubstance contained more sulfomucin (70.1%) and less neutral glycoprotein (8.6%) than OVA-induced mucosubstance (sulfomucin 33.6%, neutral glycoprotein 41.8%, $p < 0.01$).

Increased mucus sulfation may be important for airway inflammation because of the increase in its negative charge. Mucin, the most plentiful high-molecular-weight polyanion on the respiratory surface, has been shown to react with cationic inflammatory proteins such as leukocyte elastase and lysozyme. The negatively charged sulfated carbohydrates are responsible for the inhibitory effects of mucin against these enzymes. Newly produced sulfomucin may play a role in protecting the mucosa against proteolysis by these cationic enzymes or by bacterial enzymes.

Lectin Histochemistry

We found that LPS-induced mucin contained more PNA-reactive and MAL-II-reactive mucin, compared with control and antigen-induced mucin. The amount of PNA-reactive mucosubstance increased significantly after LPS stimulation (64.8%), compared with saline instillation (3.5%) and OVA challenge (5.8%, $p < 0.01$). The amount of MAL-II-reactive mucosubstance also increased after LPS stimulation (75.8%), compared with

saline instillation (19.1%) and OVA challenge (32.3%, $p < 0.05$). PNA reacts with galactose-N-acetyl galactosamine, and MAL-II reacts with $\alpha 2,3$ -linked sialic acid-galactose. We found that these carbohydrate residues increased specifically in LPS-induced mucin.

These residues were reported to be potential sites for the attachment of many kinds of bacteria and viruses, promoting their trapping and removal by mucociliary clearance. The altered mucus may play an important role in host defense against bacterial infections, and it is interesting

that these alterations did not occur in allergic inflammation in our study.

Conclusions

Respiratory mucus is heterogeneous in its carbohydrate structure. The alteration of carbohydrate chains of mucin in airway inflammation is an important event for host defense, causing entrapment of micro-organisms and neutralization of proteolytic enzymes.

References

- 1 Shimizu T, Hirano H, Shimizu S, et al: Differential properties of mucous glycoprotein in rat nasal epithelium. *Am J Respir Crit Care Med* 2001;164:366–371.
- 2 Shimizu T, Takahashi Y, Kawaguchi S, et al: Hypertrophic and metaplastic changes of goblet cells in rat nasal epithelium induced by endotoxin. *Am J Respir Crit Care Med* 1996;153:1412–1418.
- 3 Shimizu T, Hirano H, Majima Y, et al: A mechanism of antigen-induced mucus production in nasal epithelium of sensitized rats. *Am J Respir Crit Care Med* 2000;161:1648–1654.

Takeshi Shimizu, MD
Department of Otorhinolaryngology, Shiga University of Medical Science
Seta-tsukinowa
Otsu, Shiga 520-2192 (Japan)
Tel. +81 77 548 2261, E-Mail shimizu@belle.shiga-med.ac.jp