The Veterinary Journal 197 (2013) 291–296

Contents lists available at SciVerse ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Polymeric immunoglobulin receptor expression and local immunoglobulin A production in bovine sublingual, submandibular and parotid salivary glands

K. Sakaguchi [a], H. Yokota [b], T. Miyasho [b], N. Maeda [b,c], K. Nakamura [d], T. Onaga [e], M. Koiwa [f], K. Matsuda [a], M. Okamoto [a], K. Hirayama [a], H. Taniyama [a,*]

[a] Department of Veterinary Pathology, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan
[b] Department of Veterinary Biochemistry, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan
[c] Department of Technology, Association of Meat Science and Technology Institute, 1-5-6 Ebisu, Shibuya, Tokyo 150-0013, Japan
[d] Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan
[e] Department of Veterinary Physiology and Nutrition, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan
[f] Department of Large Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

ARTICLE INFO

Article history:
Accepted 21 December 2012

Keywords:
Polymeric immunoglobulin receptor
Immunoglobulin A (IgA)
Salivary gland
Cattle
Immunology

ABSTRACT

The submandibular and parotid glands are the main sources of immunoglobulins A (IgAs) in human and rat saliva. These glands express the polymeric immunoglobulin receptor (pIgR), which transports IgAs into saliva. The main source of IgAs in saliva and pIgR expression in salivary glands has not been well documented in cattle. Expressions of pIgR were determined in the major bovine salivary glands (sublingual, submandibular, and parotid) by RT-PCR for mRNA and by Western blot analysis and immunohistochemistry (IHC) using an anti-human pIgR antibody for protein. The protein detected with the antibody was identified by nano-liquid chromatography-quadrupole time of flight mass spectrometry. Additionally, the distribution of Ig-producing plasma cells was analyzed by IHC.

RT-PCR showed that pIgR was expressed in the sublingual and submandibular glands, but not in the parotid gland. Higher protein levels were observed in sublingual glands than in submandibular glands by Western blot. By IHC, pIgR was mainly located on the apical side of the cytoplasmic membrane in the sublingual gland, whereas it was observed only on the basal side in the submandibular gland. The highest density of plasma cells expressing IgAs was observed in the sublingual gland. These results suggest that the sublingual gland plays an important role in first-line defence of the oral cavity in cattle in contrast to humans and rats.

© 2013 Elsevier Ltd. All rights reserved.

Introduction

The mucosal immune system is the first line of defence against a variety of antigens. The main players in adaptive immunity in this system are the polymeric immunoglobulin As (IgAs) (Kaetzel, 2005; Brandtzaeg, 2009; Corthésy, 2010). In humans and rats, the salivary glands, especially the parotid and submandibular/sublingual glands, are the major source of IgAs in the mouth (Korsrud and Brandtzaeg, 1982; Carpenter et al., 2004; Seemann et al., 2004; Brandtzaeg, 2007). IgAs are produced by plasma cells and transported across epithelial cells by polymeric immunoglobulin receptors (pIgRs) (Brandtzaeg, 2007; Asano and Komiyama, 2011). IgAs are captured by pIgRs expressed on the basolateral surface of epithelial cells and transported to the apical surface. At the apical surface, the extracellular domain of pIgRs, known as the secretory component (SC), is cleaved and released in free form or as a component of secretory IgAs (Kaetzel, 2005; Asano and Komiyama, 2011).

In cattle, the daily volume of salivary secretion, which consists mostly of parotid saliva, may amount to as much as 100–190 L/day (Kay, 1960; Bailey, 1961; Bailey and Balch, 1961a,b). The detection of IgAs has been reported in ruminant whole saliva, but was not detectable from parotid saliva (Hurlimann and Darling, 1971; Mach and Pahud, 1971; Watson and Lascelles, 1971). Additionally, the role of pIgR expression in ruminant salivary glands remains unclear.

In mammals, the mechanism which regulates the secretion of salivary IgAs has not been well documented. To understand in greater detail the regulatory mechanism of IgA secretion, we con-

* Corresponding author. Tel.: +81 11 388 4763.
E-mail address: taniyama@rakuno.ac.jp (H. Taniyama).

1090-0233/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tvjl.2012.12.030

ducted a comparative investigation of pIgR expression in bovine sublingual, submandibular, and parotid glands.

Material and methods

Animals

Tissue samples from the sublingual, submandibular and parotid glands were collected during the course of post-mortem examinations of 21 Holstein Friesian cattle. In all cases, the cause of death or euthanasia was not related to salivary gland disease and the glands were macroscopically normal. Details of the samples examined, age, sex and disease background of the cattle are described in Table 1. For reverse transcription polymerase chain reaction (RT-PCR) and Western blot analysis, samples were immediately frozen in liquid nitrogen and stored at −30 °C.

Reverse transcription PCR

Total RNA was isolated from the three major salivary gland tissues in four cattle using the MagNA Pure LC mRNA Isolation kit for tissues (Roche Diagnostics). PCR was performed according to the manufacturer's protocol using self-designed primer pairs Bos pIgR-1 F (5′-TGAAGAGTCCCATCTTCGGT-3′) and Bos pIgR-4 R (5′-TTGAGGATGACGGTGTAGGT-3′), which were designed according to the nucleotide sequence of the *Bos taurus pIgR* gene, as previously reported (GenBank Accession number BC149032). The size of the product was predicted to be 1231 and 574 base pairs (bp), corresponding to the long and short forms of the pIgR, respectively (Kulseth et al., 1995).

The PCR conditions used were 94 °C for 2 min, 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 2 min, and a final extension at 72 °C for 7 min using a thermocycler (iCycler, Bio-Rad Laboratories). PCR products were separated by 1% agarose gel electrophoresis and stained with ethidium bromide.

Western blot analysis

For Western blot analysis, fractions of plasma membrane were prepared as follows: a salivary gland sample (200 μg) from six cattle was mixed with a 4-fold volume of phosphate buffered saline (PBS), homogenized at 4 °C, and centrifuged at 9000 *g* for 20 min at 4 °C. The supernatants were centrifuged at 50000 *g* for 60 min at 4 °C; then the sediments were homogenized with 100 μL of PBS and used as plasma membrane (Omura and Sato, 1964; Xiong et al., 2009). The supernatants were used as soluble fractions.

Equal amounts of the sample were separated on a 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The resultant gels were stained with Coomassie brilliant blue (CBB) or transferred to PVDF membranes (ATTO) for Western blot. The membranes were blocked overnight at 4 °C with 2% (weight in volume) non-fat dry milk in PBS, then probed overnight at 4 °C with a primary antibody (rabbit polyclonal anti-human pIgR, H-300, Santa Cruz), diluted 1: 200 in PBS. After washes with PBS-Tween (0.1%), the membranes were incubated with a horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody diluted 1:5000 in PBS for 60 min at room temperature. After washes with PBS-Tween, immunodetection was performed by a chemiluminescence-based detection kit (ECL, GE Healthcare) and scanned with Ez-capture (ATTO).

In-gel digestion

The protein band observed in preparative gels stained by CBB that corresponded to the band observed by Western blot analysis with the anti-human pIgR antibody was excised. The excised piece of gel was de-stained and digested according to the methods of Nomura et al. (2009), and then analyzed by nano-liquid chromatography-quadrupole time of flight mass spectrometry (nano-LC-Q-TOF-MS).

Nano-LC-Q-TOF-MS

The Easy-nLC (Bruker Daltonics) system, consisting of an autosampler nanoflow pump, was used. The analytical column was L-column micro (C_{18} 0.075 × 150 mm, 3-μm particle size), and the trapping column was 0.3 × 5 mm, 5-μm particle size, all from Chemicals Evaluation and Research Institute. The sample injection volume was 1 μL and separation was achieved on the analytical column at a flow rate of 300 nL/min. The Easy-nLC system was coupled to a micrOTOF-QII time-of-flight mass spectrometer (Bruker Daltonics) and a captive spray ionization (Bruker Daltonics) equipped with a nanospray source. Positive ion mode scanning of a gradient mobile phase consisting of (A) 0.1% formic acid solution, and (B) acetonitrile with 0.1% formic acid solution, was used. The gradient started at 92% (A) and decreased at 40% (A) in 60 min. The mobile composition decrease to 5% (A) was done in 5 min. The instrument was operated with positive ions using a range of 50–2500 m/z and an auto MS/MS mode. Capillary voltage of the ion source, flow rate of dry gas, and dry temperature were set to 1500 V, 3.0 L/min, and 160 °C, respectively.

Database searching was performed using MASCOT against Swiss-Prot. Taxonomy was restricted to other Mammalia. Carbamidomethylation was considered as a fixed modification, and various modifications were investigated. All results were stored and further analyzed using Data analysis 4.0 and biotools 3.2 software (Bruker Daltonics).

Immunohistochemistry

Seventeen samples from the salivary glands were fixed in 4% paraformaldehyde for 24 h, and then the tissues were dehydrated through a series of graded paraffin and sectioned at 4 μm. Sections were immunostained by the avidin–biotin-peroxi-

Table 1
Age, sex and disease background of the cattle.

Case	Age (years)	Sex	Cause of euthanasia and other health conditions	Tissues examined			
				PCR	WB	IHC[a]	IHC[b]
1	2	F	Peritonitis	–	–	+	+
2	2	F	Mastitis	+	+	–	–
3	3	F	Ventral fracture, pneumonia	–	–	+	+
4	3	F	Dislocation of the hip joint	–	+	+	+
5	4	F	Dysstasia, myorrhexis of the hind limb	+	–	–	–
6	4	F	Ventral fracture, endometritis	+	–	–	–
7	4	F	Ligament injury of the hip joint	–	–	+	+
8	4	F	Dislocation of the hip joint	–	–	+	+
9	4	F	Dysstasia, subcutaneous abscess of the hind limb	–	–	+	+
10	4	F	Endometritis	–	–	+	+
11	4	F	Mastitis, fracture of the rib	–	–	+	+
12	5	F	Ligament injury of the hip joint	–	+	+	+
13	5	F	Lung abscess	–	–	+	+
14	5	F	Dysstasia, myorrhexis of the hind limb	–	+	+	+
15	5	F	Dysstasia, myorrhexis of the hind limb	–	+	+	+
16	6	F	Dysstasia, myorrhexis of the hind limb	–	–	+	+
17	6	F	Peritonitis	–	–	+	+
18	7	F	Ligament injury of the hip joint	–	–	+	+
19	7	F	Bladder papilloma	–	–	+	+
20	10	M[A]	Cervical mass (mesenchymal tumour)	+	–	–	–
21	15	F	Peritonitis, mastitis, pancreatolithiasis	–	+	+	+

F, female; M, male; PCR, reverse transcription PCR; WB, western blot analysis.
+, samples examined.
[a] IHC, immunohistochemistry (IHC) using anti-human pIgR antibody.
[b] IHC using anti-bovine IgA, IgM, and IgG antibodies.
[A] Case 20 is a bull used for breeding, while all others are dairy cattle.

Download English Version:

https://daneshyari.com/en/article/5798238

Download Persian Version:

https://daneshyari.com/article/5798238

Daneshyari.com