IMMUNOHISTOCHEMICAL DETERMINATION OF BOVINE HAPTOGLOBIN IN EXPERIMENTAL MASTITIS

Hiss S[1], Petzl W[2], Zerbe H[3], Schuberth HJ[2], Seyfert HM[4], Sauerwein H[1]
[1]Institute of Physiologie, Biochemistry and Animal Hygiene, University Bonn, [2]Immunology Unit, School of Veterinary Medicine, Hannover, [3]Clinic for Ruminants, LMU München, [4]FBN, Dummerstorf, Germany

Introduction

The origin of milk Hp can be attributed to serum Hp passing the blood milk barrier during the acute phase reaction but also to local sources within the mammary gland (1). The objective of the present study was to evaluate the relationship between systemic Hp and immunohistochemically detectable Hp in mammary glands infected with two different pathogens.

Methods

Eight lactating Holstein Friesian cows were used for the experiment. Each cow was inoculated three times with either 500 colony-forming units (cfu) E. coli or 10,000 cfu Staph. aureus (4 cows each) through the teat canal. The right front quarter was inoculated first (24 h until slaughter), the right rear quarter was inoculated 12 h later, the left rear quarter was inoculated after 18 h (6 h until slaughter) and the left front quarter served as the control quarter. Blood samples were taken before, 12, 18 and 24 h after the first inoculation. All cows were slaughtered 24 h after the first inoculation. Milk and tissue samples were collected from each quarter. Serum and milk Hp concentrations were determined using an ELISA (1). Tissue samples were fixed in Bouin`s solution and embedded in paraffin. Tissues were cut into serial sections of 5 µm. Immunhistochemistry (IHC) was performed using polyclonal rabbit antibodies against bovine haptoglobin (1). For negative controls non-immune rabbit serum was used. Slides were counterstained in Mayer´s Haemalaun. IHC was quantified by counting cells positive for Hp with a reference grid in the microscope ocular. Due to the restricted number of animals, the presentation of results is limited to descriptive statistics.

Results

Tab.1: Hp concentrations in milk and serum (medians)

	Hp in quarter milk samples (µg/ml) hours after inoculation of individual quarter				serum Hp (µg/ml) hours after first inoculation			
	0	6	12	24	0	12	18	24
E. coli	2.0	3.8	80.7	537.5	15.1	33.4	70.4	1195.0
Staph. aureus	2.9	5.1	2.8	2.0	22.9	30.0	30.1	15.0

An intense immunostaining was observed in the lumina of alveoli, blood vessels and in the cytoplasm of leukocytes. Few epithelial cells were positive for Hp. Hp staining showed no obvious differences neither between control quarters and infected quarters nor between the two pathogens used.

Discussion: The inoculation of E. coli into the mammary gland lead to increased somatic cell counts (SSC, not shown) and increased levels of milk and serum Hp whereas the response towards Staph. aureus (SSC and Hp) was negligible. However the immunohistochemical results do not reflect these differences. The response towards Staph. aureus inoculation has to be confirmed in further studies.

Reference

1) Hiss, S.; Mielenz, M.; Bruckmaier, RM; Sauerwein, H., 2004: J Dairy Science 87 (11), 3778-3784