Serum Concentration of a Bovine Mannan-Binding Protein Reactive with a Ra Chemotype Strain of Salmonella Typhimurium: No Significant Changes Associated with Mastitis

Shunji SUGIH, Kazuo AKIYAMA1, and Yoshikazu HIROTA2

Department of Serology and Immunology, School of Medical Technology, Kitasato University, 1-15-1 Kitasato, Sagamihara, Kanagawa 228, 1Ashoro Branch, Hokkaido Tokachi Agricultural Mutual-Aid Association, 1-17-18 Khonan, Ashoro, Hokkaido 089-37, and 2Laboratory of Immune Cytology, National Institute of Animal Health, 3-1-1 Kannondai, Tsukuba, Ibaraki 305, Japan

(Rceived 14 December 1993/Accepted 18 February 1994)

ABSTRACT. The serum concentration of a bovine mannann-binding protein reactive with a Ra chemotype strain of Salmonella Typhimurium in sera from cows with or without mastitis were determined by sandwich enzyme-linked immunosorbent assay. From the results obtained for 10 healthy cows aged 2 to 7 years, mean ± SD serum concentrations of MBP in bovine sera were 77.5 ± 33.1 μg/ml. Concentrations in 7 healthy heifer calves aged 6 months were 52.2 ± 10.2 μg/ml, whereas those in 7 healthy bullocks 65.8 ± 21.8 μg/ml. Concentrations in 4 cows aged 4 to 7 years with mastitis were slightly lower (34 to 72 μg/ml). After recovery, the serum concentrations rose to the normal concentrations in healthy cows. These findings indicate that the serum concentrations of bovine MBP decrease during mastitis, suggesting that bovine MBP may not be an acute phase reactant.—KEY WORDS: bovine MBP, mastitis, serum concentration.


Bovine serum contains at least three Ca2+-dependent mannan-binding proteins such as conglutinin (Kg), serum lectin (CL-43), and mannan-binding protein (MBP) [3, 6, 8, 17]. The Kg serum concentration of cattle is well known to be lowered by the season, nutritional state, parturition, and infections [1, 5, 9-11, 14]. Human serum MBP reactive with a Ra strain of Salmonella Typhimurium (Ra-reactive factor, RaRF) [14] has been reported to slightly increase in infection and inflammation [7]. Although bovine serum MBP antigenically cross-reactive with bovine RaRF has been most recently identified and isolated [15], it is not yet known about changes(s) in the serum MBP concentration associated with microbial infection and/or inflammation. To study the relationship between changes in the serum MBP concentration and different infectious diseases, therefore, attempts were made to determine the MBP concentrations in sera from cows with or without mastitis by sandwich enzyme-linked immunosorbent assay (ELISA).

Bovine sera for ELISA were obtained from the blood samples collected from healthy and infected Holsteins in the areas of Ashoro Branch, Hokkaido, Tokachi Agricultural Mutual-Aid Association, Hokkaido, Japan as reported previously [1, 2].

Yeast mannan and CNBr-Sepharose 4B were purchased from Nacalai Tesque Inc., Kyoto and Pharmacia, Uppsala, Sweden, respectively. Mannobiose-Sepharose 4B and Protein A-Sepharose 4B were the products of Seikagaku Co. Ltd., Tokyo and Sigma Chemical Co., St. Louis, MO, U.S.A., respectively.

Bovine serum for isolation of MBP was purchased from Itoh Ham Foods Inc., Ibaraki, Japan. The MBP was isolated by affinity chromatography on mannan-Sepharose 4B followed by affinity chromatography on mannobiose-Sepharose 4B as described previously [15].

Antiserum against bovine MBP was produced in a rabbit by the methods described previously [16]. Rabbit IgG was isolated by affinity chromatography on Protein A-Sepharose 4B (E. Y. Laboratories, San Mateo, CA, U.S.A.). Antibodies to bovine IgM and IgG in the isolated IgG fraction were absorbed by affinity chromatography on bovine IgM and IgG-coupled Sepharose 4B as described previously [4]. One hundred μg of NHS-LC-Biotin (Pierce, IL., U.S.A.) was added to 1 ml of purified IgG (1 mg/ml) equilibrated with 0.1 M NaHCO3. The mixture was allowed to stand at room temperature for 4 hr. After that, the mixture was dialyzed at 4°C against 10 mM Tris-HCl, pH 7.4 containing 0.15 M NaCl and 1 mM Na2N3 (Buffer I) to remove unbound biotin.

Sandwich ELISA was carried out in triplicate with microtiter ELISA plates (Corning Inc., N. Y., U.S.A.). Each well of ELISA plates was coated at 4°C overnight with 100 μl of purified normal IgG or anti-MBP IgG (5 μg/ml) diluted in 0.1 M carbonate buffer, pH 9.5. After washing the plates 4 times with 0.05% Tween 20 in Buffer I (Buffer II), 200 μl of 0.1% gelatin in Buffer I (Buffer III) was added to each well. The plates were incubated at 37°C for 1 hr for blocking. After the plates were similarly washed, 100 μl of purified MBP or bovine sera diluted in Buffer III containing 5 mM EDTA was added to each well. The plates were incubated at 37°C for 1 hr. After similarly washing the plates, 100 μl of biotinylated anti-MBP antibody (diluted to 1:500) in Buffer III was added to each well. After incubation at 37°C for 1 hr, the plates were similarly washed. After washing the plates, 100 μl of ExtrAvidin-alkaline phosphatase (Sigma Chemical Co., St. Louis, U.S. A., diluted to 1:4,000) in Buffer III was added to each well. After incubation at 37°C for 1 hr, the plates were similarly washed. After washing the plates, 100 μl of ExtrAvidin-alkaline phosphatase (Sigma Chemical Co., St. Louis, U.S.A.) in 10% diethanolamine, pH 9.8, containing 0.5 mM MgCl2 and 1 mM Na2N3 was finally added to each well and allowed to develop color at room temperature for 45 min. To stop the reaction, 50 μl of 5 N NaOH was added to each well. The enzyme-substrate reaction was determined spectrophotometrically with Immuno Reader NJ-2000 (Japan InterMed Co., Ltd., Tokyo) at 405 nm. Absorbances with three determinations were averaged, from which the average was plotted against MBP concen-
Fig. 1. Serum concentration (μg/ml) of MBP in the sera obtained from healthy and infected cows. Healthy, healthy cows; Mastitis, cows suffered from mastitis; Recovered, clinically treated and recovered cows. Numbers show cow No. presented in Table 2 of reference 1. Concentrations (μg/ml) in sera from cows with mastitis shown in Fig. 1 were 34 (No. 2), 58 (No. 4), 61 (No. 5), and 71 (No. 6), respectively, whereas those in sera from the same cows after recovery 130 (No. 2), 118 (No. 4), 80 (No. 5), and 92 (No. 6), respectively.

Concentration (ng/ml).

Dose-response curve for coating antibody at concentration of 5 μg/ml over concentration range for purified bovine serum MBP of 20–1,000 ng/ml was determined. The assay system was considered to be accurate for determination of the serum MBP at the concentration as low as 20 ng/ml.

Mean ± SD concentrations (μg/ml) of MBP in bovine sera from healthy male and female Holsteins, which were described previously [2], were determined by use of the ELISA. Concentrations in sera from 10 healthy adult cows aged 2 to 7 years were 77.5 ± 33.1 (range 26–126 μg/ml), whereas those in 7 healthy heifer calves aged 6 months were 52.2 ± 10.2 (range 34–66 μg/ml). Concentrations in sera from 7 healthy bullocks aged 6 months were 65.8 ± 21.8 (range 45–105 μg/ml). These findings suggest that young and adult cattle may show a wide range of serum MBP concentrations as found for human serum MBP [13, 20]. Moreover, the MBP concentrations in sera from adult cattle were found to be slightly higher than those from young cattle. However, the Kg concentrations in sera from the same adult cattle have been reported to be much higher than those from the same young cattle [2].

The MBP concentrations in sera from 4 Holsteins aged 4 to 7 years with mastitis (No. 2, 4, 5 and 6) and in those from the same cows after recovery, which were presented in Table 2 of reference 1, were determined by sandwich ELISA. The results are presented as μg/ml in Fig. 1. As shown in Fig. 1, the MBP concentrations in sera from cows suffered from mastitis were found to be from 34 to 72 μg/ml. Following treatment, the MBP concentration in the sera from the same recovered cows were found to be from 92 to 140 μg/ml (Fig. 1). These findings indicate that the MBP serum concentrations in the sera from recovered cows trend to rise to the normal concentration in healthy cows aged 2 to 7 years after recovery. Although similar changes have been found in K concentrations associated with mastitis [1], changes in the K concentrations in sera from cows with mastitis have been reported to be much larger (about 30-fold) than those from healthy cows [1]. These suggest that bovine serum MBP may not be an candidate for one of acute phase reactants in contrast to human serum MBP [8]. Moreover, bovine serum mannan-binding proteins such as MBP, Kg, and CL-43 are suggested to be differently produced in response to mastitis and/or microbial infection. Thus, more detailed studies will be needed to elucidate their roles in bovine nonimmune system against microbial infection.

ACKNOWLEDGEMENTS. This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan and by the Special Coordination Fund for Promoting Science and Technology from the Science and Technology Agency of Japan.

REFERENCES