CELLULAR & MOLECULAR BIOLOGY LETTERS
Volume 7, (2002) pp 1131 – 1136
http://www.cmbl.org.pl

Received 15 October 2002
Accepted 3 December 2002

Short Communication

THE ADJUVANT ACTIVITY OF LACTOFERRIN IN THE GENERATION OF DTH TO OVALBUMIN CAN BE INHIBITED BY BOVINE SERUM ALBUMIN BEARING α-D-MANNOPYRANOSYL RESIDUES

MAJA KOCIĘBA[1], MICHAŁ ZIMECKI[1], MARIAN KRUZEL[2] and JEFFREY ACTOR[2]
[1]Department of Experimental Therapy, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wrocław, Poland, [2]University of Texas, Houston Health Science Center at Huston, MSB 4.506, 6431 Fannin Street, Houston, TX 77030, USA

Abstract: Lactoferrin (LF) is an iron-binding glycoprotein present in the cytoplasmic granules of neutrophils and in external secretions of mammals. Although the biological role of human and bovine lactoferrin has been extensively studied, there is still uncertainty as to the nature and function of lactoferrin receptors. We recently determined that methyl-α-D-mannopyranoside given intraperitoneally (i.p.) could suppress the adjuvant activity of LF in the generation of delayed-type hypersensitivity (DTH) to ovalbumin (OVA). We concluded that the lactoferrin effects in DTH are mediated by carbohydrate-recognizing receptors like the mannose receptor (MR). This study indicates that subcutaneous (s.c.) administration of very small doses of the Man-bovine serum albumin (Man-BSA) complex, together with a sensitizing dose of the antigen, gives the same effects as i.p. administration of methyl-α-D-mannopyranoside. The latter is also a blocker of MR, although of a much lower affinity to the receptor than Man-BSA. The blocking of the adjuvant effect of LF by the Man-BSA complex (when given together with the sensitising dose of antigen) suggests that the function of antigen-presenting cells in the skin (presumably immature dendritic cells expressing MR) is inhibited. The results of our study indicate that a receptor with an affinity for mannose is essential for the mediation of adjuvant lactoferrin function in the generation of DTH.

Key Words: Delayed Type Hypersensitivity (DTH), Lactoferrin

INTRODUCTION

Lactoferrin (LF), an iron-binding protein, has the ability to stimulate the immune response. Our results indicate that lactoferrin has the potential to act as effectively as the bacterial component in the Freund's complete adjuvant for the generation of cell-mediated responses [1]. We previously compared the adjuvant property of bovine lactoferrin with human lactoferrin and demonstrated that bovine lactoferrin, administered as a component of incomplete Freund's adjuvant (iFa), augmented the cell-mediated DTH response to OVA more strongly than human lactoferrin. The question remained as to why bovine and human lactoferrin exhibit differential stimulatory activities. Since the protein sequence of this glycoprotein is highly conservative among different species, the differences in adjuvant activities may be due to structural differences in the lactoferrin glycan chains. Human and bovine lactoferrin contain glycans of the N-acetyllactosaminic type. However, bovine lactoferrin contains additional glycans of the oligomannosidic type. By contrast, human LF is characterized by N-acetyllactosaminic glycans possessing α-1,3-linked Fuc residues [2]. The presence of an additional fucose residue could be responsible for certain biological activities of human LF. Moreover, one hypothesis suggests that LF receptors directly recognize the glycan structure of lactoferrin [3]. In order to clarify the immunological function of lactoferrin glycans, we tested the effects of methyl-α-D-mannopyranoside on the adjuvant property of lactoferrin in the generation of DTH to OVA. We established that in mice, the adjuvant effects of both lactoferrins were blocked by an intraperitoneal (i.p.) pretreatment with methyl-α-D-mannopyranoside [4]. Since the immunological activities of lactoferrin were inhibited by methyl-α-D-mannopyranoside, administered by the distant route (i.p.) than the site of immunization (base tail) we decided to test the effect of Man-bovine serum albumin (Man-BSA) (another blocker of the mannose recognizing receptor), administered together with lactoferrin. To test the role of lactoferrin glycans, the adjuvant effects of human or bovine LF in the generation of DTH to OVA were measured in the presence or the absence of Man-BSA (high affinity), and in the presence or the absence of a control complex Gal-BSA (little affinity to the mannose-recognizing receptor).

MATERIALS AND METHODS

Animals

CBA mice (males and females, 10-12 weeks old) were delivered by the Animal Facility of the Institute of Immunology and Experimental Therapy, Wrocław, Poland. The mice were fed a commercial, pelleted food and water ad libitum. The animal ethics committee at the Institute of Immunology and Experimental Therapy approved the study.

Antigens, adjuvants and reagents

Low endotoxin bovine milk lactoferrin (< 44 E.U./mg, < 25% iron saturated), human milk lactoferrin. (< 10E.U./mg, < 20% iron saturated), ovalbumin lot 43H7010, complete Freund's adjuvant (cFa) lot F 5506, and incomplete Freund's adjuvant (iFa) were purchased from the Sigma Chemical Company, MO, USA. α-D-Man-bovine serum albumin (Man-BSA) was purchased from SIGMA, Germany. Gal-bovine serum albumin (Gal-BSA) was obtained from EY LABORATORIES, INC., USA. All the chemicals were of analytical grade.

Generation of the cellular immune response to ovalbumin

The mice were immunized s.c. into the base of their tails with 5 μg OVA in cFa or iFa. After 4 days, the DTH reaction was elicited by the s.c. injection of 50 μg OVA in iFa into their hind feet. 24 h later, the DTH reaction was measured using a very precise calliper with an accuracy of 0.05 mm. The specific DTH reaction was calculated by subtracting the foot pad thickness of naive mice given an eliciting dose of the antigen from the DTH reaction of sensitized mice.

Administration of LF and Man-BSA (Gal-BSA)

To achieve the adjuvant effect in the generation of DTH, LF was emulsified with iFa and antigen, and given s.c. into the base of the tail at a concentration of 200 μg. For inhibition of the effector phase of DTH, the Man-BSA (Gal-BSA) complex together with the sensitizing doses of the antigen was delivered subcutaneously.

Statistics

All the data are expressed in DTH units (1 unit = 0.1 mm) as mean values from 10 determinations ± SE. The differences between the experimental groups were analysed using the Student unpaired t test (t-test) (two groups).

RESULTS

The results of this study are consistent with the data from previous experiments using methyl-α-D-mannopyranoside given intraperitoneally. The presented results showed that subcutaneous administration of very small doses of Man-BSA complex, together with a sensitizing dose of the antigen, significantly ($p<0.001$) reduced the specific DTH reaction to OVA emulsified in iFa + BLF (Fig. 1). The effect of Man-BSA on the adjuvant property of bovine lactoferrin was related to dose, with levels as low as 2 μg Man-BSA able to inhibit BLF adjuvant activity (Fig. 1). The inhibitory action of Man-BSA on the adjuvant effect of human lactoferrin was evident, but not as strong as the inhibition evident when Man-BSA was admixed with BLF (Figs. 2 and 3). The specificity for the high affinity mannose receptor was also demonstrated.

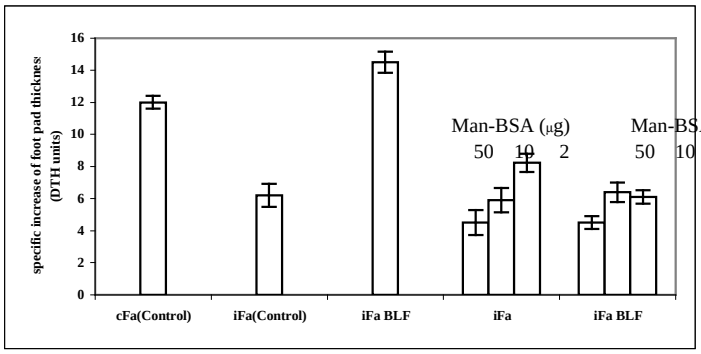

Fig. 1. The adjuvant effect of bovine lactoferrin in the generation of DTH to OVA is blocked by the subcutaneous administration of the Man-BSA complex together with sensitizing doses of the antigen. The results are presented as the mean values from 10 determinations (5 mice) ± SE: cFa/iFa ($p<0.001$); cFa/iFa + BLF ($p<0.01$); iFa/iFa + 50 μg Man-BSA (NS); iFa/iFa + 10 μg Man-BSA (NS); iFa/iFa + 2 μg Man-BSA ($p<0.05$); iFa/iFa + 50 μg Man-BSA + BLF ($p<0.05$); iFa/iFa + 10 μg Man-BSA + BLF (NS); iFa/iFa + 2 μg Man-BSA + BLF (NS); iFa + BLF/iFa + 50 μg Man-BSA + BLF ($p<0.001$); iFa + BLF/iFa + 10 μg Man-BSA + BLF ($p<0.001$); iFa + BLF/iFa + 2 μg Man-BSA + BLF ($p<0.001$).

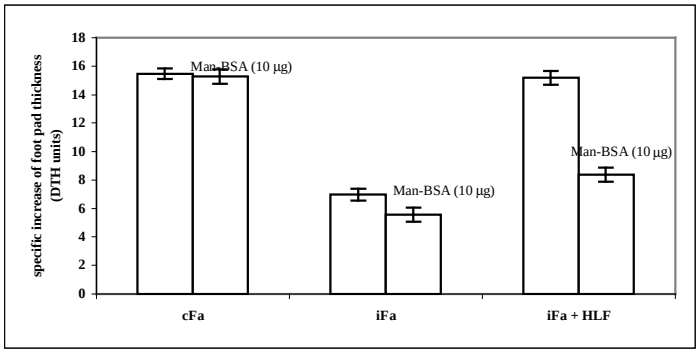

Fig. 2. The adjuvant effect of human lactoferrin in the generation of DTH to OVA is blocked by subcutaneous administration of the Man-BSA complex together with sensitizing doses of the antigen. The results are presented as mean values from 10 determinations (5 mice) ± SE: cFa/iFa ($p<0.001$); cFa/iFa + HLF (NS); iFa/iFa + 10 μg Man-BSA ($p<0.05$); iFa/iFa + 10 μg Man-BSA + HLF ($p<0.05$); iFa + HLF/iFa + 10 μg Man-BSA + HLF ($p<0.001$); cFa/cFa + 10 μg Man-BSA (NS).

The Gal-BSA complex inhibited the adjuvant action of BLF by only 20%, compared to an 80% inhibition by Man-BSA. Both Man-BSA and Gal-BSA emulsified with iFa + OVA or with cFa + OVA revealed no general inhibition of the DTH response in the absence of lactoferrin (Fig. 3). The adjuvant effects of HLF in the generation of DTH to OVA were not blocked by Gal-BSA (Fig. 3).

On the other hand, subcutaneous administration of Gal-BSA exhibited a modest but significant ($p<0.001$) suppression of the adjuvant property of bovine lactoferrin (Fig. 3).

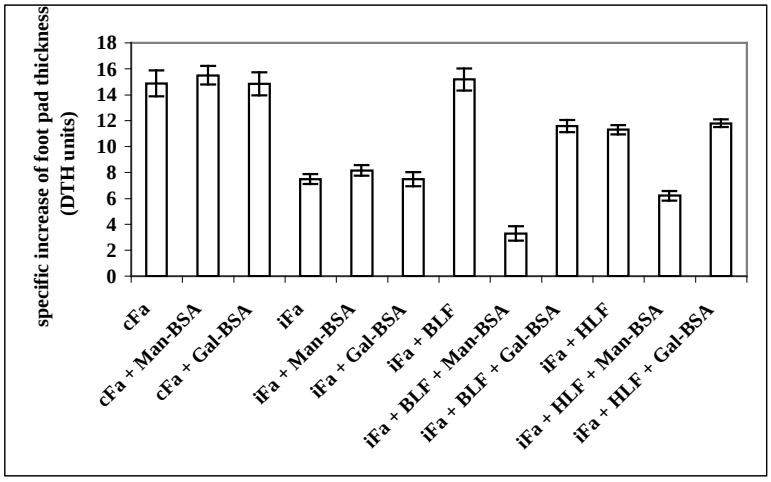

Fig. 3. Comparative studies between bovine and human lactoferrin. Differential effects of Man-BSA and Gal-BSA on the adjuvant property of lactoferrin. The results are presented as mean values from 10 determinations (5 mice) ± SE: cFa/iFa ($p<0.001$); cFa/iFa + BLF (NS); cFa/iFa + HLF ($p<0.01$); iFa + BLF/iFa + BLF + Man-BSA ($p<0.001$); iFa + BLF/iFa + BLF + Gal-BSA ($p<0.001$); iFa/iFa + BLF + Man-BSA ($p<0.001$); iFa + HLF/iFa + HLF + Man-BSA ($p<0.001$); iFa + HLF/iFa + HLF + Gal-BSA (NS); iFa/iFa + HLF + Man-BSA ($p<0.05$).

DISCUSSION

It has been previously shown that intraperitoneal pretreatment of mice with methyl-α-D-mannopyranoside blocked the adjuvant effect of the two lactoferrins. Our results suggested that the recognition of human or bovine lactoferrin is mediated in part by the carbohydrate-recognizing receptor, and that mannose or fucose residues play a most important role in the ligand-receptor association. Thus, the initiation and generation of DTH by lactoferrin as an adjuvant may have a functional basis, in part through binding to the macrophage mannose receptor. A recent report demonstrated that efficient T cell responses to cryptococcal mannoprotein require the recognition of terminal mannose groups by macrophage mannose receptors, and that T cell stimulation is functionally inhibited by a competitive blockade of the mannose receptor [5]. However, these data did not explain the difference between the adjuvant activity of bovine and human lactoferrin in the generation of DTH to OVA. In this study, the differential effects on the adjuvant property of the two lactoferrins of subcutaneous administration of Man-BSA or Gal-BSA were observed. Our data supported the concept of the mechanism of glycan-associated adjuvant activity

of lactoferrin, with possible direct effects on macrophage activation via a high affinity mannose-recognizing receptor. Such a possibility was further supported by the finding that the inhibition of the adjuvant action of BLF by the Gal-BSA complex was much smaller, which was probably associated with the low affinity of galactose to the MR [6]. Moreover, the possibility that Man-BSA may directly interact with BLF, thus preventing its adjuvant effect, is unlikely, because even 2 μg of the Man-BSA complex significantly inhibited the action of BLF; this represents about a 1:100 molecular weight ratio between Man-BSA and BLF (used at a concentration of 200 μg). Thus, almost all of the BLF would be not affected by Man-BSA binding. The affinity of the Man-BSA complex to the mannose receptor is exceptionally high [6]. It should be also noted that skin is rich in immature dendritic cells, which express the mannose receptor and are regarded as major APC in the generation of the immune response. Thus, our results led us to conclude that the inhibitory effects of Man-BSA were directed to the MR on dendritic cells, and the differential stimulatory activities of the two lactoferrins could be explained, at least in part, by the differences in glycan structure.

Acknowledgements. This work was partially supported by the Committee for Scientific Research (KBN), grant no. 6 P05A 103 21.

REFERENCES

1. Zimecki, M. and Kruzel, M.L. Systemic or local co-administration of lactoferrin with sensitizing dose of antigen enhances delayed type hypersensitivity in mice. **Immunol. Lett.** 74 (2000) 183-188.
2. Spik, G., Coddeville, B., Mazurier, J., Bourne, Y., Cambillaut, C. and Montreuil, J. Primary and three-dimensional structure of lactotransferrin (lactoferrin) glycans. **Adv. Exp. Med. Biol.** 357 (1994) 21-32
3. Rich, I.N. and Sawatzki, G. Lactoferrin, the signal for colony stimulating factor production? Negative-feedback regulation versus supply-and-demand regulation of myelopoiesis. **Blood Cells** 15 (1989) 400-406.
4. Zimecki, M., Kocięba M. and Kruzel, M. Immunoregulatory activities of lactoferrin in the delayed type hypersensitivity in mice are mediated by a receptor with affinity to mannose. **Immunobiol.** 205 (2002) 120-131.
5. Mansour, M.K., Schlesinger, L.S. and Levitz, S.M. Optimal T cell responses to Cryptococcus neoformans mannoprotein are dependent on recognition of conjugated carbohydrates by mannose receptors. **J. Immunol.** 168 (2002) 2872-2879.
6. Kery, V., Krepinsky, J.J, Warren, C.D., Capek, P. and Stahl, P.D. Ligand recognition by purified human mannose receptor. **Arch. Biochem. Biophys.** 298 (1992) 49-55.