Effect of Bovine Lactoferrin on Functions of Activated Feline Peripheral Blood Mononuclear Cells During Chronic Feline Immunodeficiency Virus Infection

Saori KOBAYASHI1), Reeko SATO1)*, Takako AOKI1), Katsuhiko OMOE1), Osamu INANAMI2), Careen HANKANGA1), Yuichi YAMADA1)-3), Nobuyuki TOMIZAWA1), Jun YASUDA1) and Juso SASAKI1)

1)Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, 3–18–8 Ueda, Morioka 020–8550, 2)Laboratory of Radiation Biology, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18 Nishi 9, Sapporo 060–0818 and 3)Department of Veterinary Clinical Science, The United Graduate School of Veterinary Sciences, Gifu University, 1–1 Yanagido, Gifu 501–1193, Japan

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ABSTRACT. Feline immunodeficiency virus (FIV) infection is characterized by chronic overactivation of immune and inflammatory system, resulting in anergic state and dysfunction of immune cells. Lactoferrin (LF), a glycoprotein present in exocrine secretions and neutrophils, plays an important role in host defense system. Our previous study showed that oral administration of bovine LF (bLF) suppressed oral inflammation, improved the clinical symptoms and decreased serum γ-globulin as a marker of inflammation in FIV-infected cats with intractable stomatitis. The anti-inflammatory effect was partly involved in regulation of neutrophil function by bLF. In this study, to clarify the relationship between anti-inflammatory effects of bLF and peripheral blood mononuclear cells (PBMC), we examined the effect of bLF on proliferation, cell cycle progression and cytokine expression in PBMC of FIV-infected cats with the asymptomatic carrier and AIDS-related complex (ARC) phase. Real-time RT-PCR showed that bLF increased interferon-gamma and interleukin-2 in cells of the ARC group regardless of the time of its addition to the medium. Results suggest the hypothesis that therapy with bLF may have the potential to improve and protect functions of overactivated lymphocytes by modulating the cell proliferation, cell cycle and cytokines expression in cats in terminal stage of FIV infection.

Key words: anti-inflammatory activity, bovine lactoferrin, FIV-infected cat, persistent immune activation.

Feline immunodeficiency virus (FIV) is the feline equivalent of HIV in human, and the infection leads to an AIDS-like syndrome in domestic cats [24]. Its distribution is worldwide, and it is one of the important concerns in companion animal health care. Lymphocytes and macrophages targeted by FIV induce a progressive breakdown of immune system. FIV-infected cats go through four clinical stages [13]: the acute phase, the asymptomatic carrier (AC) phase in which cats seem clinically healthy but CD4+ cell numbers continuously decrease, the AIDS-related syndrome (ARC) phase, characterized by episodes of chronic inflammation and infection, the AIDS phase, characterized by severe hematological dysfunctions. Immune and inflammatory systems in FIV-infected cats are activated consistently to AIDS phase by the persistent existence of FIV. Lymphocyte activation following FIV infection is shown by increased germinal center activity in lymph nodes and high expression of T and B cell activation markers [26, 31]. We also observed that adenosine deaminase in T cell and plasma, which is released during T cell activation, was significantly elevated in FIV-infected cats in the ARC phase [7]. Persistent immune activation in HIV and FIV infection results in progression to AIDS [6, 8, 23]. Recent studies reported that FIV targets activated CD4+ cells using its primary receptor upregulated by stimuli and cytokines, indicating the infection is propagated in activated and proliferating T cells preferentially [4, 14, 22]. Additionally, lasting activation of immune cells leads to an anergic state of the cells and a lack of sensitivity to immune response including failure of cytokine production, antigen recognition and transmission of signaling pathways [12, 16]. Therefore, control of the persistent overactivated state of immune cells in FIV infection is key to maintaining their protective functions.

Lactoferrin (LF), an 80 kDa iron-binding glycoprotein present in milk, various exocrine secretions and neutrophils, contributes to the primary host defense. Its specific receptors are found on the surface of neutrophils, monocytes and activated lymphocytes [20]. LF has the regulatory activities of immune and inflammatory responses [15]. Several studies showed that bovine or human LF modulated proliferation in human lymphocytes and monoblast cells [2, 19, 25, 37], and significantly decrease interferon (IFN)-γ, tumor necrosis factor (TNF)-α or interleukin (IL)-1 production in human, feline and ovine peripheral blood mononuclear cells (PBMC) [2, 15, 35]. Inflammatory models have shown that bLF administration has therapeutic effects on intractable stomatitis in FIV-infected cats [27] and on chemically induced colitis or muscle inflammation in rodents through a decrease in inflammatory TNF-α and IL-6 [36] or a increase of anti-inflammatory IL-4 and IL-10 [33]. Thus, it is

*CORRESPONDENCE TO: SATO, R., Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, 3–18–8 Ueda, Morioka 020–8550, Japan.

e-mail: reekos@iwate-u.ac.jp
inferred that the anti-inflammatory and immunomodulatory activities of bLF are due to regulation of release of inflammatory cytokines, proliferation and differentiation of immune cells.

In this study, we focused on anti-inflammatory activities of bLF against persistent immune activation status in FIV infection and examined whether bLF modulated cell proliferation and expression of pro-inflammatory IFN-γ, IL-2, IL-1β, TNF-α and IL-12 in mitogen-stimulated PBMC from FIV-infected cats. Furthermore, the relationship between the inhibitory effects of bLF and cell cycle progression was also examined.

MATERIALS AND METHODS

**Animals:** Peripheral blood samples were obtained from eight FIV-negative healthy mixed breed cats (four males and four females, 2–9 years old) and eleven FIV-positive cats (AC phase: two males and two females, 8–11 years old, ARC phase: three males and four females, 8–12 years old). FIV-positive cats in the ARC phase have some inflammatory symptoms such as intractable stomatitis and chronic conjunctivitis. We confirmed that FIV-negative cats were clinically healthy and negative for feline leukemia virus antigen and feline immunodeficiency virus antibody before experiments.

**Reagents:** Bovine LF was the naturally occurring type isolated from bovine milk and was kindly provided by the Morinaga Milk Industry Co., Ltd., Japan.

**Isolation of feline PBMC:** Heparinized whole blood was collected from the jugular vein, and was layered on Ficoll-Conray solution (density, 1.077) and centrifuged at 1,500 rpm for 30 min at 20°C. PBMC were harvested, then cells were suspended in RPMI 1640 medium (Invitrogen Co., Carlsbad, CA, U.S.A.) supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. The viability of isolated PBMC was determined by 0.2% trypan blue staining (>95%).

**Proliferation assay:** The PBMC (1.8 × 10^5 cells/well) were plated in 96-well flat-bottomed plates in triplicates. Cells were stimulated with 10 µg/ml concanavalin A (ConA) and treated with or without bLF (10 and 50 µg/ml) for 48 hr at 37°C in 5% CO₂. The cell proliferation was measured by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. The stimulation index (SI) was expressed as the optical density (OD) in ConA-stimulated cells with bLF (0, 10 and 50 µg/ml)/OD in unstimulated cells × 100.

Preliminary examination indicated that the treatment with bLF at a concentration higher than 50 µg/ml also inhibited the cell proliferation, however, the inhibitory efficiency was similar to that seen at 50 µg/ml. Meanwhile, cells were treated with bLF at 500 µg/ml showed more efficiency in other assays than that seen at 50 µg/ml.

**Measurement of DNA content:** PBMC (1 × 10^6) were treated with or without bLF (500 µg/ml) 30 min before ConA stimulation (10 µg/ml) and cultured for 24 and 48 hr. Harvested cells were fixed and permeabilized with cold 70% ethanol for 2 hr. After treatment with RNase A, cells were stained with propidium iodide (PI) and analyzed by flow cytometry. Data analysis was performed with CELLQuest pro software (BD Biosciences).

**RNA extraction and cDNA synthesis:** Bovine LF (500 µg/ml) was added to the culture medium in PBMC (5 × 10^6 cells) 30 min before or 40 min after addition of 10 µg/ml ConA. The PBMC were incubated at 37°C in 5% CO₂ for 4 hr. The method of RNA extraction was described previously [15]. The cDNA was synthesized from total RNA (0.1 µg) using avian myoblastosis virus (AMV) reverse transcriptase and an RNA PCR Kit (AMV) Ver. 2.1 (Takara Biomedicals, Shiga, Japan). The cDNA was stored at –80°C until use.

**Primers:** The nucleotide sequences of all feline PCR primers are as follows: IFN-γ, forward: AGACTTTTGAAGACTGAAAGAG, reverse: TTCATTATTGGCTTGCGCT, IL-2, forward: GCACCTGTCTCAAGCTCTAC, reverse: GATGCCTTGACAAAAAGGTAAATC, IL-1β, forward: TCCACCTCAAATGGACAATA, reverse: TTTCATCTTCTTCTTGGGTNF-α, forward: TCTACTCCAGTTCTCTTCAC, reverse: AAAGTTAGACCTGCCGGAC, IL-12 p40, forward: CCTGAAGAAGATGACATCACC, reverse: TCAGTG- GACAAAATTCCT, β-actin as an internal control, forward: AACCCTGAGAAGATGACATCACC, reverse: GTAGGACAGCTCTCCTTGATGT.

Reverse transcriptase polymerase chain reaction (RT-PCR): The amplification of feline cytokines was performed using an RNA PCR Kit (AMV) Ver. 2.1 (Takara-Biomedicals). The PCR profile used was 2 min at 94°C for the first cycle, and 30 s at 94°C, 30 s at melting temperature and 30 s at 72°C for 40 cycles. The PCR products were electrophoresed on 2% agarose gel. The density of each band of cytokine and β-actin was quantified by NIH Image Ver. 1.62, and was calculated by the ratio between the cytokine and the β-actin gene products for each sample.

**Real-time RT-PCR for measurement of IFN-γ and IL-2:** Real-time PCR was performed using the 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, U.S.A.) with SYBR® Green I as the detection format. We used method of absolute quantification of gene expression of IFN-γ and β-actin to compare with data of FIV-negative cats in previous study [15]. The expression of IL-2 was measured by relative quantification analysis, and the standard curve was generated by these standards, which were serial dilutions of a ConA-stimulated PBMC sample of FIV-negative cat, where concentration is expressed in relative units. The reaction was performed by 60 cycles of 15 s at 95°C, 15 s at 62°C and 40 s at 72°C. After measurement, the concentration of an unknown sample was calculated from its standard values. The results were expressed as the ratio of cytokine to β-actin.

**Statistical analysis:** Statistical differences were evaluated by the Tukey-Kramer method, and p value less than 0.05 were considered significant.
RESULTS

Effect of bLF on ConA-induced proliferation of feline PBMC: We examined the effect of bLF at 10 and 50 µg/ml on ConA-induced cell proliferation in FIV-negative and FIV-positive cats. SI of ConA-induced PBMC proliferation were 29.6 ± 4.5, 56.0 ± 24.0 and 43.8 ± 19.0 in the FIV-negative, AC and ARC group, respectively. As shown in Fig. 1, addition of bLF decreased ConA-induced proliferation in PBMC. The significant inhibitory effect was shown at 50 µg/ml of bLF in all groups (p<0.05) compared to ConA-induced proliferation without bLF, and SI were 11.8 ± 4.5, 8.0 ± 7.4 and 5.2 ± 6.3 in the FIV-negative, AC and ARC group, respectively.

Effect of bLF on ConA-induced cell cycle progression: Because bLF inhibited the ConA-induced proliferation, we examined the effect of bLF on cell cycle progression in cells from FIV-negative cats. After the 24 and 48 hr cultures, there was no significant difference between the ConA-unstimulated cells cultured with or without bLF (data not shown). We found that ConA induced progression of apoptosis in feline PBMC. The change was especially obvious in 48 hr culture, and the addition of ConA to the cells induced a 27% increase of the apoptosis cells with a 28% decrease of the G0/G1 phase cells compared to the ConA-unstimulated control (Fig. 2). Addition of bLF to ConA-stimulated cells inhibited the progression of ConA-induced apoptosis, and there was a 25% decrease in the number of the apoptotic cells and a 26% increase in the number of G0/G1 phase cells in comparison with ConA-stimulated cells at 48 hr. The percentage of each phase in ConA-stimulated cells treated with bLF was almost similar to the ConA-unstimulated control.

Effects of ConA and bLF on expression of cytokine mRNA in feline PBMC: When ConA-stimulated PBMC in the ARC group were treated with 500 µg/ml of bLF, bLF significantly inhibited ConA-induced expression of IFN-γ and IL-2 (Fig. 3). On the contrary, there were variations in response to bLF in the expression of other cytokines among individuals. Similar results were observed in the other three animals in the ARC group, and in PBMC treated with 50 µg/ml of bLF (data not shown). To confirm these data obtained by RT-PCR, we quantified the level of IFN-γ and IL-2 by real-time RT-PCR. The Rn of IFN-γ in ConA-stimulated PBMC without bLF was increased to about 30 cycles, whereas those in PBMC exposed to bLF 30 min before or 40 min after ConA stimulation started the increase at about 38 cycles. And these curves of Rn against PCR cycles were close to that of untreated control but not completely (Fig. 4). The basal level of IFN-γ in the ARC group ranged from 7.8
× 10^{-6} to 3.4 \times 10^{-5} (Table 1A), while those of FIV-negative group were from 2.7 \times 10^{-5} to 3.5 \times 10^{-4} in our previous study [15]. Two of four cats in the ARC phase had similar levels of spontaneous IFN-\gamma, and the rest had a slight decrease of the level when compared with FIV-negative cats. However, three of four cats in the ARC phase had lower expression of ConA-induced IFN-\gamma than that of FIV-negative cats (Table 1A, cats #2 to 4: 1.2 \times 10^{-4}–5.8 \times 10^{-4}). According to our previous paper [13], the values of ConA-induced IFN-\gamma of expression of FIV-negative cats were from 1.6 \times 10^{-3} to 1.1 \times 10^{-1}. Regardless of this response, bLF modulated ConA-induced expression of IFN-\gamma to the approximate unstimulated level in both groups. The Rn of IL-2 in ConA-stimulated PBMC of the ARC group without bLF was increased at about 47 cycles, whereas those of PBMC with bLF 30 min before or 40 min after ConA stimulation began to the increase at about 47 cycles (Fig. 4). The addition of bLF reduced ConA-induced expression of IL-2 to spontaneous level in both groups (Table 1B). Moreover, bLF prevented ConA-induced expression of not only IFN-\gamma but also IL-2 to that of unstimulated cells regardless of the time of addition of LF to the cells in the ARC phase as well as FIV-negative cats.

**DISCUSSION**

The present study showed that the addition of bLF suppressed the ConA-induced proliferation of PBMC from FIV-negative and the AC and ARC groups (Fig. 1). Similar *in vitro* studies have reported that there was a dose-dependent inhibition of bovine or human LF by phytohemagglutinin-induced cell proliferation in human PBMC [2, 37]. Typical anti-inflammatory drugs cyclosporine A and hydrocortisone also were reported to completely suppress induced T cell proliferation in humans, and show their strong anti-inflammatory activities [21]. Therefore, our data suggest that bLF may result in immunomodulatory and anti-inflammatory effects on chronically activated PBMC in FIV infection.

Several recent *in vitro* studies have indicated that LF modified cell cycle progression in various cell types. The addition of human LF inhibited cell growth by suppression of G1 cyclin-dependent kinases in human breast cancer [3] or cyclin D1 in human and murine squamous cell carcinoma [34]. We next examined the relationship between the suppressive effect of bLF on cell proliferation and the cell cycle progression in feline PBMC. After 24 and 48 hr culture, ConA-induced apoptosis was completely prevented by addition of bLF to level of ConA-unstimulated cells (Fig. 2). This finding concurs with results from several studies that bLF reduced apoptosis in human activated lymphocytes and fibroblasts [18]. Thus, our results suggest that bLF has a protective effect on activated cells by inhibition of apoptosis.

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**Fig. 3.** The effect of bLF on ConA-induced expression of cytokine mRNA in feline PBMC. Cells (5 × 10^6 cells/ml) were cultured for 4 hr after ConA stimulation (10 µg/ml). Bovine LF (bLF; 500 µg/ml) were added to the culture medium 30 min before the addition of ConA. PCR products were run on a 2% agarose gel. \(\beta\)-actin was used as a positive transcription control.

**Fig. 4.** The effect of bLF on ConA-induced expression of IFN-\gamma and IL-2 mRNA in feline PBMC. PCR amplification plot for IFN-\gamma, IL-2 and \(\beta\)-actin by real-time RT-PCR. After the cells were incubated with medium only ( ), medium containing 500 µg/ml bLF ( ), 10 µg/ml ConA ( ), or 500 µg/ml bLF plus 10 µg/ml ConA ( ) for 4 hr. Rn (normalized reporter fluorescence intensity) was plotted against cycle number. Bovine LF was added to the culture medium 30 min before ( ) or 40 min after ( ) the addition of ConA. A representative experiment out of four is shown.
status. While there were variations in response to bLF in the
ment with other studies [16, 30]. Thus, these findings sup-
IFN-γ of PBMC from FIV-positive cats [11, 32]. However,
was in agreement with the results in symptomatic HIV-
reduced by bLF in murine macrophage cell line (unpub-
perform PBMC functions properly despite their activated
and modulation of proliferation and cell cycle progression.
Since ConA is known to induce production of cell
growth-related or cellular immunomodulatory cytokines,
followed by cell proliferation, we examined the effect of
bLF on expression of IFN-γ, IL-2, IL-1β, TNF-α and IL-12
p40 in PBMC of the ARC group by RT-PCR (Fig. 3) or real-
time RT-PCR (Fig.4 and Table 1). We observed that addi-
tion of bLF inhibited ConA-induced increase of IFN-γ
and IL-2 expression (Table 1B), which was in agree-
ment of IFN-γ and IL-2 among individu-
als, bLF suppressed the expression of ConA-induced IFN-γ
and IL-2 to those of the ConA-unstimulated cells, not only
30 min before and but also 40 min after ConA stimulation in
FIV-positive cats. The results suggest that bLF seems to
modulate cytokine expression in activated cells rather than
resting cells. In fact, Mincheva-Nilsson et al. [20] reported
that LF receptors were expressed on only activated human
lymphocytes not on unstimulated cells, indicating a role for
LF as an intrinsic regulatory factor in modulation of acti-
vated lymphocyte response. Mainly two hypotheses about
the intrinsic modulation of LF have been proposed as fol-
loows: (1) LF released from neutrophil binds its receptor on
activated lymphocyte, then leads to stimulate several intrac-
tellar signaling pathways. For examples, human LF sup-
pressed antibody production of lymphocytes and induced
maturation of T and B cells in human including the expres-
sion of CD4 surface marker [2, 5]. (2) Internalization and
binding of LF to the nucleic acid leads to some transcrip-
tional activations including enhancement of the cytoxic
functions of lymphokine-activated killer cell and natural
killer cell in the inflammatory process [9, 28]. Feline LF has
not been identified, however, judging from distribution of
LF in the body, mammary animals seem to have LF because
of LF and its receptors

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Time of bLF addition to the culture medium is given in parentheses. bLF was added to the medium 30 min before ConA stimulation. +40 min, bLF was added to the medium 40 min after ConA stimulation.

Table 1. Effects of bLF on ConA-induced mRNA expression of cytokines in feline PBMC
A. The expression of IFN-γ of PBMC from FIV-positive cats (ARC phase)

B. The expression of IL-2 of PBMC from FIV-negative and positive cats (ARC phase)
on immune cells will probably exist in cats and may act as an immunomodulator in feline immune system. In present study, the binding of bLF to ConA could partly explain its inhibitory effect on the ConA-induced cytokine expressions [29]. However, our results showed that bLF inhibited expression of ConA-induced IFN-γ and IL-2 regardless of the time of addition of bLF to the cells. Moreover, our previous study suggests that bLF is able to abrogate of protein tyrosine kinase and extracellular signal-regulated kinase (PTK/ERK)-independent intracellular signaling activated after interaction between ConA and its receptor [15]. These results suggest that bLF inhibits ConA-induced responses of cells not only by its scavenger effect, but also by other mechanisms such as inhibition of intracellular signaling.

Taken together, it is conceivable that bLF has anti-inflammatory effects on feline activated PBMC, which can be explained by the observation that it mediates the suppression of stimulated proliferation, expression of IFN-γ and IL-2 and modulation of cell cycle progression on activated PBMC. A recent study reported that hydrocortisone showed anti-inflammatory activities through attenuation of human T cell response including downregulation of IFN-γ, IL-2 and IL-5 [21]. The inhibitory effects of bLF were observed not only in PBMC from FIV-negative group but also in those from the ARC group in vitro, supporting the notion that treatment of bLF may have potential in vivo to improve the hyperactivated state of PBMC in FIV-positive cats. Our previous in vivo report showed that topical application of bLF to oral mucous membrane had anti-inflammatory effect on intractable stomatitis and ameliorated oral inflammation in FIV-positive cats [27]. Concurrently, serum γ-globulin levels, a marker of chronic inflammation, were also obviously reduced. The amelioratory effect of bLF on mucosal inflammation may be partly associated with its anti-inflammatory effects on activated PBMC as well as neutrophils in inflammatory sites. Additionally, bLF shows inhibitory effects as well as upregulatory effects on immune cells. Oral administration of bLF increased neutrophil phagocytic activity in FIV-positive cats [27], stimulated renewal of lymphocytes in drug-induced immunocompromised mice [1] and increased the percentage of CD4+ cell count significantly in HIV-infected children [38], whereas glucocorticoids completely impaired both T cell responses and neutrophil functions [10]. These studies suggest that clinical application of LF, which has immunomodulatory properties on immune system to maintain the homeostatic function of immune cells, may be advantageous to immunosuppressed FIV-positive cats. Therefore, therapy with bLF may have potential via protective effects on functions of immune cells by improving the persistent activation of local or general immune and inflammatory systems in FIV-infected cats.

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REFERENCES

15. Kobayashi, S., Sato, R., Inanami, O., Yamamori, T., Yamato,


