Effect of Administration of Serum Thymic Factor (FTS) in Calves and Rabbits Infected with Bovine Immunodeficiency-Like Virus

Nobuaki HIRAI, Hiroyuki FURUYAMA1, Akira AWAYA2, and Misao ONUMA**

Department of Epizootiology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido 060, 1School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069, and 2Institute of Biological Science, Mitsui Pharmaceuticals, Inc., Mobara, Chiba 297, Japan

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ABSTRACT. The effect of serum thymic factor (FTS) administration in bovine immunodeficiency-like virus (BIV)-infected calves and rabbits was examined. We previously found that some of the macrophage functions were depressed and humoral immune responses against foreign proteins were delayed in BIV-infected calves compared to uninfected calves. After FTS administration, however, no delay of antibody responses against antigenic proteins was observed in BIV-infected calves. Though the chemiluminescence (CL) responses of macrophages in BIV-infected calves were significantly depressed (p<0.05), FTS administration resulted in the recovery of the CL responses in the BIV-infected calves comparable to those in the control calves. Antibody responses against foreign proteins in BIV-infected rabbits were significantly depressed (p<0.025) as compared with those in uninfected rabbits, though the depression became no significant after FTS administration. -KEY WORDS: bovine immunodeficiency-like virus (BIV), immunodeficiency, lentivirus, serum thymic factor (FTS).


The lentivirinae subfamily of retroviruses is a group of exogenous, nononcogenic viruses that cause chronic, multisystemic disease in susceptible hosts [17]. Bovine immunodeficiency-like virus (BIV) is a lentiviral pathogen of cattle which is genetically, antigenically and structurally similar to human immunodeficiency virus (HIV) type 1 [6, 11, 24]. Calves inoculated with BIV developed a mild lymphocytosis and a moderate lymphoproliferative reaction in the small subcutaneous lymph nodes but did not develop severe clinical symptoms [7]. Some of the macrophage functions; superoxide anion release, phagocytic activity, and chemotactic responsiveness were depressed in BIV-infected calves, and a slight delay of antibody responses against mouse serum proteins were observed in BIV-infected calves [19]. Although cattle is the natural host of BIV, BIV can persistently infect to rabbits [12, 20, 23], and BIV-infected rabbits show depression of the humoral immune response against foreign proteins [12].

Serum thymic factor (FTS; zinc-free thymulin) is a nonapeptide hormone [3, 21] which has been demonstrated in thymic epithelial cells [10, 14, 22]. In vivo studies on biological activities of FTS have shown its various modulatory effects on immune system [4]. Therefore, it is interesting to know whether FTS administration causes immune modulation in BIV-infected animals which showed a delay in humoral immune responses against foreign proteins. In the present study, we investigated the effects of FTS administration on humoral immune responses against foreign proteins and chemiluminescence responses of the macrophages in BIV-infected animals.

MATERIALS AND METHODS

Experimental BIV infection to calves and rabbits: Three 2-month-old calves were inoculated intravenously with BIV materials as described previously [19]. The first calf (No. 10) was inoculated intravenously with 1 × 10⁶ of bovine embryo spleen (BESP) cells infected with BIV-R29 strain (BESP-BIV). When calf No. 10 was confirmed to be infected, the second calf (No. 39) was inoculated with 5 × 10⁶ of live peripheral blood mononuclear cells (PBMC) prepared from calf No. 10. The third calf (No. 30) was inoculated in the same way with 5 × 10⁶ of PBMC of calf 39. Five normal 2-month-old calves were used as controls. BIV infection was monitored by the detection of BIV antigen in cocultured PBMC with BESP cells, and by the detection of BIV antibody.

Three 3-month-old male Japanese albino rabbits (Nos. 4, 5 and 7) were inoculated intravenously with 1 × 10⁷ of BESP-BIV cells. Two rabbits (Nos. 11 and 12) received 1 × 10⁶ of uninfected BESP cells, and two nontreated rabbits (Nos. 10 and 13) were used as controls. To monitor BIV infection in rabbits, polymerase chain reaction and Western blotting were done to detect proviral DNA in PBMC and BIV antibody as described previously [12].

Effect of FTS administration on the humoral responses in BIV-infected calves: Three BIV-infected calves (Nos. 10, 30 and 39) which showed a delay in the antibody responses against mouse serum proteins (Fig. 1a) were injected intramuscularly with 0.25 ml of chicken serum plus 0.25 ml of Freund’s complete adjuvant at 57, 27 and 39 weeks after BIV infection, respectively. At the same time of the immunization, 0.5 mg of the synthetic FTS...
(zinc-free thymulin: <Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-
Asn-OH; Mitsui Pharmaceuticals, Inc., Tokyo, Japan) was inoculated subcutaneously to each calf, and the same
dose of FTS was inoculated weekly until 6 weeks after the
first immunization. Twenty days after the first immuniza-
tion, chicken serum was immunized as a booster. Serum
samples were collected from these calves every 3 days, and
antibody titers were determined by enzyme-linked im-
munosorbent assay (ELISA).

Chemiluminescence responses of the macrophages in
BIV-infected calves: Lumilond-dependent chemilumines-
cence (CL) responses of macrophages in the BIV-infected
(Nos. 10, 30 and 39) and control calves were measured
with a bioluminescence reader (Biolumat LB 9500) at
14–46 weeks post-inoculation (PI) as described previously
[1]. The CL responses of macrophages were monitored at
3, 6 and 9 weeks after the first FTS administration.

The effect of FTS administration on humoral responses in
BIV-infected rabbits: At 133 days after BIV inoculation,
three BIV-infected (Nos. 4, 5 and 7) and uninfected control
rabbits (Nos. 10, 11, 12 and 13) were inoculated with
1 × 10^9 of sheep red blood cells (SRBC). From 1 to 7
weeks after SRBC inoculation, the rabbits were injected
intravenously with 50 µg (Nos. 4, 5, 10 and 11) or 10 µg
(Nos. 7, 12 and 13) of FTS once per week. Two weeks
after SRBC inoculation, the same number of SRBC was
injected to the rabbits as a booster. Antibody titers against
SRBC membrane proteins were determined by ELISA as
described previously [12].

Statistical analysis: The statistical significance of the
data was evaluated by Student’s t-test.

RESULTS

The effect of FTS on humoral immune responses in
BIV-infected calves: A slight delay in the humoral immune
responses against mouse serum proteins was observed in
BIV-infected calves during 2–5 weeks after immunization
(Fig. 1a). Especially, antibody response was delayed in
BIV-infected calf No. 30. While FTS administration
resulted in no delay in antibody responses against chicken
serum protein in the same BIV-infected calves at 27–57
weeks after BIV inoculation (Fig. 1b). Though the
antibody titers of calf No. 30 were still lower than those of
uninfected control calves, recovery of humoral immune
responses was observed after FTS administration (Fig.
b).

The effect of FTS on CL responses in BIV-infected
calves: CL responses of macrophages were significantly
(p<0.05) suppressed in all three BIV-infected calves before FTS administration (Nos. 10, 30 and 39; Table 1).
The CL responses of BIV-infected calves were still lower than those of uninfected control calves at 3 and 6 weeks
after FTS inoculation. However, at 9 weeks after FTS administration, there were no significant differences in CL
responses between two BIV-infected calves (Nos. 30 and
39) and uninfected calves (Table 1).

The effect of FTS on humoral immune responses in

![Fig. 1. Effects of FTS administration on humoral immune responses in BIV-infected calves. (a) Antibody responses (top) against mouse serum protein in BIV-infected (●, No. 10; ▲,
No. 39, and ●, No. 30) and uninfected control (○) calves after inoculation with mouse serum (arrowhead) (adapted
from reference [19]). (b) Antibody responses against chicken serum protein in BIV-infected and uninfected calves
(the same symbol of (a)) after inoculation with chicken serum (arrowhead) and FTS (↑) as determined by ELISA.]

<table>
<thead>
<tr>
<th>Calf No.</th>
<th>Before FTS administration</th>
<th>Weeks after FTS administration</th>
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<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
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<td>30</td>
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<td>39</td>
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<td>734</td>
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<tr>
<td>Control calves^a</td>
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<td>3014</td>
</tr>
</tbody>
</table>

Table 1. Effects of FTS administration to luminol dependent chemiluminescence (CL) responses in BIV-infected calves

BIV-infected rabbits: Antibody responses against SRBC were significantly (p<0.025) suppressed in BIV-infected rabbits during 3–7 weeks after immunization (Fig. 2a). To
know the effect of FTS, BIV-infected rabbits were also administered with FTS, and the humoral immune
responses against SRBC were determined. The differences

^a Control is the mean counts of five uninfected calves.
of antibody titers between BIV-infected and control rabbits became less relevant after FTS administration (Fig. 2b) as compared to those of the rabbits before FTS administration (Fig. 2a). Significant difference between the antibody titers of BIV-infected and uninfected rabbits after FTS treatments was only observed at 5 weeks after immunization (p<0.05). There were no differences between the antibody titers of the rabbits received 50 μg and 10 μg of FTS per week (data not shown).

DISCUSSION

FTS is a nonapeptide hormone originally isolated from pig serum [2, 3, 21]. FTS has been shown to be involved in T-lymphocyte maturation and differentiation [3], restoring cell-mediated cytotoxicity [5] and contact delayed-type hypersensitivity [8] in thymectomized mice. Blood FTS levels are low in patients of Down's syndrome [9] and several immunodeficiency diseases [13], and FTS is known to induce differentiation of T-cells and enhances several functions of various T-cell subsets in normal or partially thymus-deficient human recipients [4]. Though T-cell stimulation has been considered to be one of the major effects of FTS, recent studies showed that this nonapeptide hormone elicits more wide range of immunological effects. FTS injection enhances hematopoiesis and reduces mortality of mice after whole-body X-irradiation [15]. Experimental allergic encephalomyelitis in guinea pigs [16], reovirus-induced diabetes in mice [18] and leptospira-induced nephritis in gerbils [25] were suppressed by FTS administration.

We previously showed that the major target cells of BIV in experimentally infected calves are monocytes/macrophages, and some of the monocyte functions; superoxide anion release, phagocytic activity, and chemotactic responsiveness were depressed in BIV-infected calves [19]. A slight delay in the humoral immune responses against foreign proteins was also observed in BIV-infected calves (Fig. 1a). In BIV-infected rabbits, depression of the humoral immune response against foreign proteins was also observed (Fig. 2a). BIV antigen-positive cells in spleen appeared to be macrophages in BIV-infected rabbits [20], and we also detected proviral DNA in adherent (macrophage) fractions of alveolar and peritoneal cells [12]. Thus, one of the target cells of BIV in rabbits is also macrophages and the infection of BIV in macrophages may cause immune dysfunction as suggested in BIV-infected calves.

After inoculation with FTS, no delay of antibody responses to chicken serum was observed (Fig. 1b), and recovery of CL responses of monocytes were also observed (Table 1) in BIV-infected calves. The antibody responses against foreign proteins were remarkably depressed in BIV-infected calf No. 30 (Fig. 1a) with a good correlation of depression of the phagocytic activity [19] and CL responses of macrophages (Table 1). Recovery of humoral immune response was observed in calf No. 30 after FTS administration (Fig. 1b). Previously we observed that macrophage function of mice was activated by FTS administration in vivo (unpublished data). Together with these results, it is presumable that FTS induced the recovery of macrophage function in BIV-infected calves. Recovery of CL responses was observed at 9 weeks (Nos. 30 and 39), but not at 3 and 6 weeks after FTS administration (Table 1), indicating that the effect of FTS may require repeating injections. Since blood concentrations of FTS in BIV-infected calves were not monitored in this study, the exact reason of this result is still unclear.

In addition to calves, recovery of humoral immune responses by FTS administration was also observed in BIV-infected rabbits (Fig. 2b). We couldn't detect any significant differences between the antibody titers of the rabbits received different doses of FTS in this study.

Fig. 2. Effects of FTS administration on humoral immune responses in BIV-infected rabbits. (a) Antibody responses against SRBC in BIV-infected (●; No. 4, ▲; No. 5, and ■; No. 7) and uninfected control (○) rabbits after inoculation with SRBC (arrowhead). Vertical bars represent standard errors of control rabbits (quoted from reference [12]). (b) Antibody responses against SRBC in BIV-infected (the same symbol of (a)) and uninfected control (○; average titers of four rabbits received 50 μg or 10 μg of FTS per week) rabbits after inoculation with SRBC (arrowhead) and FTS (†). Antibody titers were determined by ELISA.
However far more animals are needed to determine the dose dependent effects of FTS administration.

FTS is a nontoxic natural peptide hormone which is available in synthetic form, and clinical use of FTS to immunodeficiency diseases has been expected. However previous studies showed that FTS exerts suppressive effects on cell-mediated hypersensitivity in normal mice [8]. In the present experiment, the CL responses of uninfected control calves were decreased at 6 and 9 weeks after FTS administration. The sequential administration of FTS may cause suppressive effect on macrophage functions of normal calves. Thus, even we observed recovery of immune response in BIV-infected animals, immunosuppressive effects induced by FTS administration should also be considered. Furthermore, although restoring T-cell function in animals with immune dysfunction is considered to be one of the major effects of FTS, it is still unclear whether BIV infection affects lymphocyte function. More detail and long term observations are necessary to further define the effect of FTS on BIV-induced immune dysfunctions.

REFERENCES


