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Short communication

Expression of tumor necrosis factor-α in IgM$^+$ B-cells from bovine leukemia virus-infected lymphocytotic sheep

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Abstract

Tumor necrosis factor (TNF)-α is thought to be one of the cytokines that account for bovine leukemia virus (BLV)-induced B-cell lymphoproliferative disorder, however, information on TNF-α expression in B-cells is limited. In this study, the expression of TNF-α in IgM$^+$ B-cells from BLV-infected sheep with or without lymphocytosis was determined. Freshly isolated IgM$^+$ B-cells from three sheep with lymphocytosis constitutively transcribed TNF-α mRNA. Although TNF-α mRNA expression in IgM$^+$ B-cells was transiently up-regulated after cell culture, TNF-α mRNA expression was markedly higher in lymphocytotic sheep when compared to that of non-lymphocytotic sheep or uninfected sheep. Expression of membrane-bound TNF-α on IgM$^+$ B-cells was also augmented in lymphocytotic sheep. TNF-α expression in lymphocytotic sheep may support the proliferation of B-cells.

Keywords: BLV; TNF-α; B-cell

1. Introduction

Bovine leukemia virus (BLV) induces a B-cell lymphoproliferative disorder in cattle. Approximately 30% of naturally infected cattle shows persistent lymphocytosis (PL) that is a polyclonal, non-neoplastic B lymphocytosis. PL often precedes the development of B-cell leukemia observed in fewer than 5% of infected cattle (Schwartz and Levy, 1994). Although the pathogenesis of BLV infection clearly involves several host factors (Kabeya et al., 2001b), the exact mechanism of the disease progression is unknown. One hallmark of PL animals is spontaneous proliferation of peripheral blood mononuclear cells (PBMC) when cultured in the absence of exogenous antigens or mitogens (Stone et al., 2000; Konnai et al., 2006). Because there is a clear association between PL and spontaneous lymphocyte proliferation, it is suggested that this proliferation is an in vitro correlate of the disease process (Stone et al., 2000). Indeed, interleukin (IL)-2, a T-cell-derived stimulatory cytokine, is indispensable for spontaneous proliferation of

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PBMC and thus thought to be critical to induce PL in vivo (Trueblood et al., 1998). In a previous report, tumor necrosis factor (TNF)-α was also shown to be involved as one of growth factors in the spontaneous proliferation of PBMC derived from PL cattle (Konnai et al., 2006). TNF-α is a pleiotropic cytokine produced by several kinds of cells including B-cells and its activity is mediated by two functionally different cell surface receptors, TNF-R1 and TNF-R2. TNF-R1 contains an intracellular death domain required for signaling pathways associated with apoptosis, while TNF-R2 can induce gene transcriptions for cell survival, growth and differentiation (MacEwan, 2002). In human, activation of TNF-R2 in T-cells and thymocytes has been shown to be proliferative (Tartaglia et al., 1993; Grell et al., 1998; Kim and Teh, 2004) and cause human immunodeficiency virus (HIV)-induced local proliferation of T-cells (Agostini et al., 1995). Previously, we have also shown that the attenuated TNF-R1 mRNA expression in PBMC was closely associated with lymphocytosis in sheep experimentally infected with BLV (Kabeya et al., 2001a). These findings suggest the ascription of TNF-α signaling via TNF-R2 to the progression of the disease in BLV-infected animals.

The fact that B-cell subset expands during PL stage raises the intriguing possibility that expression of cellular genes in B-cells, the main target of BLV, may be altered by BLV infection. To date, little has been reported on the expression of TNF-α and its receptors in B-cells from BLV-infected animals. In this study, we conducted a brief investigation of the expression of TNF-α in B-cells derived from BLV-infected lymphocytotic or non-lymphocytotic sheep.

2. Materials and methods

2.1. Sheep and quantification of BLV-positive cells

Four 2- to 3-years old castrated *Suffolk* sheep (S2–S5), which had been experimentally infected with BLV for 2 years, and an uninfected 2 years old male sheep (S1) were used in this study (Table 1). All sheep were kept under controlled conditions according to the Guidelines of Hokkaido University. At 4–6 months of age, four sheep (S2–S5) were intravenously inoculated with PBMC (1×10^6) obtained from a BLV-infected sheep (Tajima et al., 2003). The inoculation dose of PBMC produced approximately 1000 syncytia in the indicator cells. Total and differential leukocyte counts were determined on whole blood, and the absolute numbers of IgM^+, $CD4^+$ or $CD8^+$ cells were estimated after flow cytometric analysis. Total cellular DNA was extracted from PBMC with the Wizard genomic DNA purification kit (Promega, Madison, WI, USA). The percentage of BLV-infected cells among PBMC was determined by applying real-time quantitative PCR system (LightCycler; Roche Diagnosis, Mannheim, Germany) as described previously (Tajima et al., 2003).

Table 1
Tested sheep in this study

Sheep no.	% of BLV^+ cells[a]	WBC (/μl)	PBMC (/μl)	No. (%) of lymphocytes (/μl)[b]			[^{3}H]-thymidine incorporation (cpm)[c]	
				IgM^+	$CD4^+$	$CD8^+$	Non-stimulated	ConA
S1	ND[d]	6700	3652	1282 (35.1)	1055 (28.9)	89 (2.4)	301 ± 74	54531 ± 1749
S2	0.7	7200	4284	1174 (27.4)	942 (22.0)	548 (12.8)	572 ± 255	77532 ± 3358
S3	19.6	9000	5535	2862 (51.7)	1024 (18.5)	609 (11.0)	15731 ± 1033	73664 ± 2112
S4	29.7	11700	7605	3932 (51.7)	1217 (16.0)	1202 (15.8)	7479 ± 1275	47442 ± 335
S5	30.6	26300	22750	19383 (85.2)	1294 (5.7)	1101 (4.8)	5045 ± 862	18984 ± 852

[a] Percentage of BLV-infected cells was calculated from genomic DNA derived from PBMC using real-time PCR as described previously (Tajima et al., 2003).

[b] Single-color flow cytometric analysis was performed to determine the percentage of IgM^+, $CD4^+$ or $CD8^+$ cells among PBMC. The absolute number of IgM^+, $CD4^+$ or $CD8^+$ cells were quantitated from total and differential leukocyte counts.

[c] The amount of incorporated [^{3}H]-thymidine into DNA was expressed as the mean cpm ± S.D. of triplicate wells.

[d] ND, not detected.