REVIEW *Immunology*

Host Immune Responses in the Course of Bovine Leukemia Virus Infection

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ABSTRACT. Bovine leukemia virus (BLV) is a type C retrovirus infecting bovine B cells and causing enzootic bovine leukosis. Since it takes long periods to develop the disease, it is believed that BLV and host immune responses are closely related. In this review, the accumulated data showing close relationship between BLV and host immune responses are summarized in 4 sections. First, we discuss the role of cell-mediated immunity in protecting hosts from BLV infection. Second, several reports showing the relationship between the disease progression and the change of cytokine profiles are summarized. In the third section, we have focused on tumor necrosis factor α (TNFα) and its two types of receptors, and the possible involvement of TNF α in the BLV-induced leukemogenesis is discussed. The expression of TNF α has been shown to be regulated by major histocompatibility complex (MHC) haplotype. The resistance to BLV infection is supposed to be established by some innate factors, which are closely related to MHC haplotype. Finally, we propose that a breeding strategy based on the MHC haplotype could be a good approach to control BLV infection. This review includes some recent data from us and other groups.

KEY WORDS: bovine leukemia virus, cell-mediated immunity, cytokine profile, major histocompatibility complex, tumor necrosis factor α.

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Bovine leukemia virus (BLV), the causative agent of enzootic bovine leukosis, is an oncogenic B-lymphotropic retrovirus [27]. The disease is divided into three stages; serologically positive, but negative for lymphocytosis (SP); serologically positive and positive for persistent lymphocytosis (PL); and leukemia. Like other members of chronic retrovirus infection, BLV infection results in a prolonged asymptomatic period with a low viral load that persists for 1 to 8 years. Thirty percent of infected animals progress to PL, characterized by a polyclonal expansion of B cells. Only 0.1 to 10 percent develop malignant lymphosarcoma [53]. Usually a long duration is required between these disease stages suggesting that BLV modulate host immune systems [22, 23].

The purpose of this review is to assemble the results of studies on the host immune responses in the course of BLV infection in an effort to provide a picture towards the control of BLV infection.

CELL-MEDIATED IMMUNITY AND BLV INFECTION

Both humoral and cell-mediated immunity (CMI) are known to be induced in natural BLV-infection, and these play roles in protection of hosts from BLV infection [20, 29, 41, 42, 45]. However, their relative roles in protection remain to be elucidated. In this regard, a series of recent studies have postulated that particularly CMI against BLV antigens contributes to the suppression of BLV replication, this leading to the delay of disease progression [42, 59]. Orlik and Splitter have shown that CMI responses to BLV antigens were suppressed in correlation to disease progression [46]. The positive effect of indomethacin on the recovery of the suppressed CMI response suggests involvement of prostaglandin E_2 (PGE_2) as a negative regulatory factor. PGE_2 is an immunosuppressant, inhibiting interleukin (IL-) 12 production by macrophages [64], production of type 1 (IL-2 and IFN-γ) cytokines by $CD4^+$ T cells and suppressing mitogen-induced T cell proliferation [3, 47, 64]. Thus, production of PGE_2 and the change of macrophage functions contribute to the suppression of CMI responses in the disease-progressed animals. Ohishi *et al.* also supported the critical roles of CMI to eliminate BLV infected cells [41, 42, 59]. Recombinant vaccinia virus encoding BLV*env* gene (rVV*env*) was constructed and injected into sheep. These sheep were challenged with BLV and the specific antibody and delayed-type hypersensitivity (DTH) responses were examined. Sheep which were immunized with rVV*env* and protected from the infection showed no antibody responses but good DTH response, which is supposed to be mediated by helper T cell type 1 (Th1) [7]. In addition, statistical analysis demonstrated the relationship among BLV titers in peripheral blood mononuclear cell (PBMC) and lymphocyte proliferative responses against BLV antigens [42].

In order to prevent BLV infection or disease progression, a vaccine should be able to induce BLV-specific CMI responses. Recently, it has been reported that inactivated protein antigens with adjuvants can induce CMI including the activation of cytotoxic T lymphocyte (CTL) response [33, 51, 56, 61]. Noguchi *et al.* have reported that a liposome encapsulated with mannan on its surface was successful to induce CTL responses against the enclosed antigens [40]. The mechanisms on how the antigens enclosed in mannan coated liposome are processed, however, remain to be elucidated. Using this liposome, BLV Env-specific CMI (Th1) responses have been shown to be induced in epitope peptide immunized Balb/c mice (Fig. 1) [43, 44]. Further-

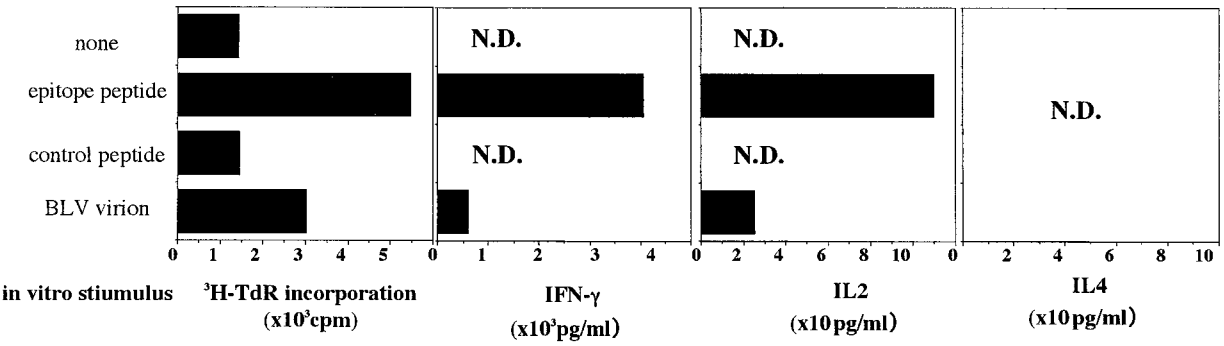

Fig. 1. Th1 / Th2 responses induced by the vaccination with the BLV Env epitope peptide encapsulated in mannan-coated liposome. Spleen cells were harvested from the immunized Balb/c mice and were incubated in the presence of the epitope peptide, or control peptide or BLV virion at a concentration of 100 μg/m*l*. The lymphocyte proliferative responses were examined by measuring the radioactivity into the DNA. The release of cytokines (IFN-γ, IL-2, Il-4) from the stimulated spleen cells were measured using a commercial ELISA kit. N.D. means not detected.

Table 1. Change of Th1 / 2 cytokine profiles in the course of BLV infection

Th1/2	cytokines	SP[a)]	PL[b)]	cells tested for cytokine production	References
Th1	IFN-γ	↑↑[c)]	N.T.[d)]	ConA-activated PBMC	68
		↑↑	↑	PBMC	49
		↑↑	→	PWM-activated PBMC	24
		↑↑	↑	LN[e)]	25
	IL-2	↑	N.T.	ConA-activated PBMC	68
		↑↑	↑	PBMC	49
		↑↑	↑	PWM-activated PBMC	24
		↑↑	↑	LN	25
	IL-12	↑	↑	PWM-activated PBMC	24
		↑↑	↓	monocytes / macrophage	50
Th2	IL-10	↑	N.T.	ConA-activated PBMC	68
		↑↑	↑↑	LN	25
		↑	↑↑	monocytes / macrophage	49

a) SP: Serologically positive for BLV infection but healthy.
b) PL: Persistent lymphocytosis.
c) By comparing uninfected control, ↑↑; much up-regulated, ↑; up-regulated, ↓; down-regulated, →; no change.
d) N.T.; not tested.
e) LN; lymph node.

more, sheep, which were immunized with Th epitope peptide encapsulated with mannan coated liposome, significantly eliminated BLV when experimentally challenged [21].

CHANGE OF CYTOKINE PROFILES ASSOCIATED WITH DISEASE PROGRESSION OF BLV INFECTION

Alternations in cytokine expression have been shown to be correlated with disease progression in chronic retroviral infections, suggesting that cytokine imbalances may contribute to disease progressions [8, 26, 36, 62]. In case of BLV infection, similar results were reported as summarized in Table 1. Pyeon *et al*. examined the cytokine profiles in cattle PBMC derived from each stage of the disease and demonstrated that the production of Th1 cytokine such as IL-2 was promoted in the PBMC from SP cattle than that of PL [49]. To the contrary, increased expression of Th2 cytokine such as IL-10 was shown in the macrophages of PL and cattle with leukemia [49]. Later, they have also reported that cattle in SP expressed much more amount of IL-12, which is a key cytokine that induce Th1 responses, though those were downregulated in cattle with progressed disease stages [50]. This finding was supported by the report by Yakobson *et al.*, showing that IL-12 expressions were increased at the early phase of infection, while those of IL-10 were induced in the PBMC of cattle, in which BLV replicated [69]. Thus, Th1 cytokines, which play important role to induce CMI was supposed to be critical to prevent disease progression of the BLV infection.

IL-2, however, is supposed to play a role to promote disease progression in case of BLV though IL-2 is one of Th1

Table 2. Association of TNF α gene polymorphism and susceptibility to, or severity of diseases

Disease	TNF α productivity	Effects of TNF α	References
Infectious disease			
Plasmodium falciparum	high	severe	37
Leishmania braziliensis	high	mucocutaneous leushmaniasis	5
Human T cell Leukemia Virus (HTLV)	high*	HTLV uveitis	54
Autoimmune disease			
Systemic lupus erythematosus	low	lupus nephritis	19
insulin-dependent diabetes mellitus	high	severe	48
rheumatoid arthritis	high	susceptible	38
celiac disease	high	susceptible	35

* Not confirmed.

cytokines. It is interesting that although IL-2 is originally known as a T cell growth factor, it can promote BLV-infected B cells to proliferation [63]. In addition, IL-2 increases the expressions of viral protein and IL-2 receptor in B cells from PL cow [18, 58, 63]. These results suggest that IL-2 contribute to the development of BLV-induced persistent lymphocytosis.

TUMOR NECROSIS FACTOR α AND BLV INFECTION

Tumor necrosis factor α (TNF α) is a pleiotropic cytokine, which is involved in diverse biological processes including immune and inflammatory reactions [2]. On one hand, TNF α may play an important role to eliminate some infectious agents, such as *Toxioplasma gondii* and Human immunodeficiency virus [4, 6, 9, 12, 15]. On the other hand, its function may promote disease progression [13, 16, 60]. The roles of TNF α in the course of BLV infection have not been fully established. To examine how TNF α contributes to the pathogenesis of BLV infection, mRNA expressions of TNF α were analyzed before- and after-BLV infection in the experimentally challenged sheep [21]. Interestingly, the expressions of TNF α were significantly upregulated after BLV infection in sheep, which eliminated BLV infected cells, though sheep in which BLV replicated showed down-regulation of the TNF α gene expression. This result suggests that at the early phase of infection, TNF α may be involved in the elimination of BLV.

It has been reported that the susceptibilities against infectious- and autoimmune- diseases depend on the individual differences in the production of TNF α as summarized in Table 2. In case of human and mice, it has shown that the gene for TNF α is located in major histocompatibility complex (MHC) class III region, and the regulation of the gene expression is associated with its polymorphism in the promoter sequence [52, 67]. However, the more production of TNF α does not always mean the more resistant to the disease. For example, several studies have shown that the individuals who produced more TNFα against malaria infection, showed a tendency to progress to severe symptoms [28, 37]. Furthermore, TNF α is thought to be one of the cytokines, which contributes to the control of B cell death [1, 30], the virus-induced B cell proliferation [10, 13, 34], and the leukemogenesis of B-1 cells [14, 65, 66]. Conflicting activities of TNF α, including induction and suppression of apoptosis have been introduced mainly through two distinct TNF-receptors; TNF RI and TNF RII [11, 15, 17]. These two distinct membrane receptors for TNF α have significant homologies in their extracellular domains with cystein-rich sequences. Contrary to this, there is no homology between their intracellular domains, suggesting that they utilize separate signaling pathways [55]. Furthermore, flow cytometric analysis revealed that though most resting human peripheral blood B cells expressed small amounts of TNF RII, it was markedly up-regulated upon stimulation with B cell mitogen. In contrast, the expression of TNF RI was low on resting as well as on activated B cells [11]. To examine the roles of TNF α and its receptors in the development of leukemogenesis induced by BLV infection, which has been known as B cell leukemia, we analyzed mRNA expressions of TNF α and its receptors in PBMC of BLV-challenged sheep. As summarized in Table 3, the mRNA expression of TNF RI was specifically downregulated in sheep showing PL. Furthermore, we found that no TNF RI mRNA were expressed in KU-1, which is a bovine B cell line transformed by BLV. It is important to examine what caused the downregulation of the level of TNF RI expressions, and whether this was specific to BLV-infected cells. The change of TNF RI/RII expression patterns has also been shown in other B cell leukemia cases including chronic lymphocyte leukemia and Burkitt lymphoma, and these consequence for the proliferative effects of TNF α [13, 65]. Then, we examined the proliferative responses of these sheep PBMC against TNF α stimulation. No proliferative responses were detected from the healthy sheep. However,

Table 3. Expression profiles of TNF-related genes and BLV-susceptibility

BLV-susceptibility	TNF α	TNF RI[a)]	TNF RII[b)]
resistant	↑[c)]	→	→
susceptible	↓	↓	→

a) TNF receptor type I.
b) TNF receptor type II.
c) Change of mRNA expressions after BLV infection; up-regulated: ↑, down-regulated: ↓, no change; →.

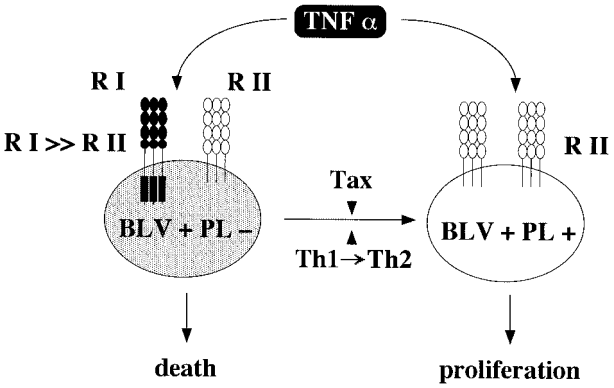

Fig. 2. The possible involvement of TNF α in the course of BLV infection.

PBMC from PL sheep showed proliferative responses against TNF α stimulation in a dose-dependent-manner. These results indicate that TNF α can induce lymphocyte proliferation in the BLV-infected PL sheep, in which expressions of TNF RI were downregulated. TNF α stimulation is supposed to contribute to leukemogenesis caused by BLV infection.

A hypothesis of the correlation between TNF α and pathogenesis of BLV infection is summarized in Fig. 2. At the early phase of infection, B cells infected with BLV express more amount of RI than RII. When the cells are stimulated by TNF α, apoptosis may be introduced and consequently BLV-infected cells will be cleared. Then, as a result of some changes, which may include the change of cytokine profiles [49, 50], some of the infected animals develop PL. In this stage, PBMC express much more amount of RII than RI and the stimulation of TNF α may induce lymphocyte proliferation and this cause leukemia development. Thus, TNF α and its receptors may play important roles in the pathogenesis of BLV infection.

BLV-RESISTANT ANIMAL

As mentioned in introduction, BLV-infected animals can be divided into 3 stages [53]. Recently, several reports showing the resistance to BLV infection is innately established by MHC have been accumulated [31, 32, 39, 57, 68]. Xu *et al.* demonstrated that there was significant difference between PL and healthy group in the amino acids sequence in the DRB3 region of bovine leukocyte antigen (BoLA) class II [68]. It has also shown that polymorphism in the MHC class II associated with disease resistance against experimental BLV infection. Nagaoka *et al.* analyzed statistically the sequence of ovine leukocyte antigen (OLA) class II region of the experimentally BLV-challenged sheep and found that the amino acids sequences at position 70 and 71 of OLA-DRB1 were statistically different between healthy and PL group sheep [39].

The disease causes significant economic problems in the dairy industry. For the purpose of classifying the bovine MHC allele, PCR-RFLP technique has been developed and this technique was applied to determine the disease resistance of BLV infection [68]. It is expected that the breeding strategy based on the MHC haplotype could be developed to produce BLV-resistant animals.

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