

The Leukocyte Population in the Peripheral Blood and the Colostrum of Cows Infected with Bovine Leukemia Virus is Skewed Towards Humoral Immunity

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ABSTRACT

Infection of cows with Bovine leukemia virus (BLV) occurs throughout the world, and is especially prevalent in dairy cows. Thus, the current study determined the immune status of cows infected with BLV by examining the leukocyte population and the cytokine mRNA expression of mononuclear cells in the peripheral blood and colostrum of clinically healthy BLV-positive cows. All samples were obtained on the day of calving within 12 hr after calving. Eighteen cows were seropositive for BLV infection (BLV+ group) and 31 cows tested negative (BLV-

group), as determined by the agar gel immunodiffusion assay. The number of major histocompatibility complex class II (MHC-II)+ cluster of differentiation (CD)14- B-cells was significantly higher in BLV seropositive cows in both the peripheral blood and the colostrum. Interleukin (IL)-4 mRNA expression levels in the peripheral blood and the colostrum of the BLV+ group were significantly higher than those of the BLV- group. These results suggest that the immune status of BLV infected cows was skewed toward humoral immunity shortly after calving.

INTRODUCTION

In 2007, a seroepidemiological survey of Japanese dairy cattle for Bovine leukemia

virus (BLV) infection was performed that revealed the prevalence of BLV infection was 34.7%, suggesting that BLV is widespread in Japan.¹⁴ Most cattle infected with BLV do not exhibit persistent lymphocytosis (PL) or enzootic bovine leukemia (EBL) with the development of tumors.^{12,20} However, lactating cows infected with BLV show abnormal immune cell populations in the peripheral blood, which suggests a greater susceptibility to other infections.⁸ Periparturient cows are susceptible to various infectious diseases due to decreased immune function.^{9,21} Older periparturient cows have even higher susceptibilities to infection, perhaps owing to the profound reduction in the number of T cells in the periphery on the day of calving that may also be associated with a higher incidence of mastitis.¹⁷

Previous studies have also shown that the shedding of virus and the number of virions in the peripheral blood increase around the time of calving in BLV-infected cows, both of which might increase the risk of transmission of BLV.^{6,13} BLV infection becomes clinically apparent around parturition, especially in cows with EBL, and the development of the signs of disease is caused by the decreased immune function in cows during the periparturient period.⁷ One of the main transmission routes of BLV from dams to neonatal calves is via the colostrums.^{6,11} Thus, it is hypothesized that BLV affects the immune cells both in the peripheral blood and in the colostrum due to the presence of a greater number of viruses. However, to date, the immune status in the periphery and the colostrum of BLV-infected cows on the day of calving has not been elucidated. Therefore, the present study examined the immune cell populations and cytokine expression levels in the peripheral blood and the colostrum on the day of calving to clarify the immune status of BLV-infected cows.

MATERIALS AND METHODS

Clinically healthy Holstein cows (n=49) were divided into two groups based on the results of the AGID assay using serum collected prior to calving. Eighteen positive

cows (BLV+ group) and 31 negative cows (BLV- group) were found in the current study population. The peripheral blood and the colostrum were obtained within 12 hr after calving. There was no significant difference between the ages of BLV+ cows (4.08 ± 0.37 ; mean \pm standard error of the mean [SEM]) and BLV- cows (4.63 ± 0.27 years old). Peripheral blood was obtained from either the tail vein or the jugular vein into vacutainer tubes containing either ethylenediaminetetraacetic acid (EDTA-2K) for white blood cell (WBC) counts or with heparin-Na for cell population and cytokine expression assays. The colostrum (100 mL per cow) was obtained from one healthy quarter.

Total WBC counts were determined using an automated blood cell counter (Celltac α MEK-6358; JASCO, Tokyo, Japan). Complete blood cell counts in the blood and the colostrum were analyzed by staining for cell surface markers, as previously reported.^{15,18} Monoclonal antibodies for the following cell surface markers were used: CD3 (MM1A; VMRD, Pullman, WA), CD4 (CACT183A; VMRD), CD8 (BAT82A; VMRD), MHC-Class II (CAT82A; VMRD) and CD14 (MY-4; Coulter Immunology, Hialeah, FL). The percentage of each cell type was determined by flow cytometry (FACScan; Becton Dickinson, Franklin Lakes, NJ).

For cytokine mRNA analysis, peripheral blood mononuclear cells (PBMCs) and mononuclear cells in the colostrum were isolated, quantified, and the cell number adjusted to 5×10^6 /mL. Cells were left unstimulated or were stimulated with 5 mg/mL phytohemagglutinin (PHA) (Sigma-Aldrich, St. Louis, MO) for 12 h at 37 °C, as previously reported.¹⁷ After incubation, RNA was extracted and quantified. First strand cDNA was synthesized by reverse transcription and real-time polymerase chain reaction (PCR) was performed, as previously described.^{16,17} The primers for interferon (IFN)- γ , IL-4, IL-10, IL-12 and β -actin were prepared as previously described.¹⁹ Data produced were

Table 1. Immune cell populations of the PBMC and colostrum mononuclear cells in cows.

	PBMC				Colostrum Cells			
	BLV+ (n=18)		BLV- (n=31)		BLV+ (n=18)		BLV- (n=31)	
CD3+ (%)	15.60 ± 1.99	20.16 ± 1.18	*	61.09 ± 4.54	68.45 ± 1.70			
CD4+ (%)	6.25 ± 0.91	9.81 ± 0.37	**	28.92 ± 2.79	39.24 ± 2.15	**		
CD8+ (%)	4.19 ± 0.42	4.40 ± 0.34		15.36 ± 1.92	22.25 ± 1.34	*		
MHC-II+CD14- (%)	59.86 ± 4.93	25.43 ± 1.61	**	30.76 ± 4.15	13.67 ± 1.19	**		
CD14+ (%)	19.05 ± 3.29	37.08 ± 1.88	**	19.73 ± 4.42	10.87 ± 0.92			
CD4+/CD8+ (ratio)	1.60 ± 0.21	2.58 ± 0.20	**	3.74 ± 1.40	2.03 ± 0.18			

Values are expressed as means ± SEM.

*Denotes significantly different between two groups ($P < 0.05$).

**Denotes significantly different between two groups ($P < 0.01$).

presented as ΔCt values, where ΔCt is the difference in the threshold cycles between the target and β -actin, which served as an internal control.⁹ The fold-changes in expression ($\Delta\Delta Ct$) were calculated relative to the mRNA levels in the same sample that was not stimulated. The Mann-Whitney U-test was used to determine the differences between the BLV+ and BLV- groups. A P-value < 0.05 was considered significant. The data were expressed as the mean ± SEM.

RESULTS

The total numbers of WBCs in the blood were $18,788.9 \pm 1,408.0$ cells/mL and

$13,274.2 \pm 854.7$ cells/mL for the BLV+ and BLV- groups, respectively. The numbers of PBMCs for the BLV+ and BLV- groups were $10,619.0 \pm 871.3$ cells/mL and $2,722.6 \pm 151.7$ cells/mL, respectively. Overall, the BLV+ group showed significantly higher ($P < 0.01$) observed changes in both WBC and PBMC counts. The percentage of MHC-II+CD14- B cells was significantly higher in the BLV+ group, whereas the percentages of CD3+ T cells, CD4+ T cells, and CD14+ monocytes were significantly lower in the BLV+ group. In addition, the percentages of CD4+/CD8+ ratio was significantly lower in the BLV+ group (Table 1).

Table 2. Cytokine mRNA expression levels in the PBMC and colostrum mononuclear cells.

		PBMC				Colostrum cells			
		BLV+ (n=18)		BLV- (n=31)		BLV+ (n=18)		BLV- (n=31)	
IL-4	PHA(-)	13.23 ± 0.41	8.29 ± 0.59	**	18.90 ± 0.27	11.02 ± 0.61	**		
	PHA(+)	17.65 ± 0.27	9.89 ± 0.42	**	18.88 ± 0.36	13.47 ± 0.56	**		
IL-10	PHA(-)	4.79 ± 0.37	4.92 ± 0.26		5.32 ± 0.31	4.33 ± 0.34			
	PHA(+)	5.13 ± 0.24	4.96 ± 0.27		4.75 ± 0.36	4.45 ± 0.36			
IL-12	PHA(-)	7.82 ± 0.62	6.02 ± 0.35		7.54 ± 0.39	8.51 ± 0.56			
	PHA(+)	8.95 ± 0.61	6.37 ± 0.38	*	7.42 ± 0.42	8.70 ± 0.59	*		
IFN-g	PHA(-)	4.43 ± 0.37	4.39 ± 0.40		7.70 ± 0.31	7.86 ± 0.53			
	PHA(+)	6.11 ± 0.33	4.69 ± 0.53	*	7.29 ± 0.45	8.24 ± 0.54	*		
IFN-g/IL-4	PHA(-)	0.33 ± 0.02	0.50 ± 0.05	**	0.41 ± 0.01	0.72 ± 0.04	**		
	PHA(+)	0.34 ± 0.02	0.46 ± 0.04	**	0.38 ± 0.02	0.62 ± 0.04	**		

Values are expressed as means ± SEM.

*Denotes significantly different between two groups ($P < 0.05$).

**Denotes significantly different between two groups ($P < 0.01$).

Overall, the percentage of MHC-II+CD14- B-cells was significantly higher in the colostrum of cows in the BLV+ group, whereas the percentages of CD4+ and CD8+ T cells were significantly lower in the BLV+ group (Table 1). In PBMCs and colostrum cells, significantly higher levels of IL-4 mRNA and significantly lower IFN- γ /IL-4 ratios with or without PHA stimulation were found in the BLV+ group compared with those in the BLV- group. The levels of IL-12 and IFN- γ in PBMCs from the BLV+ group were significantly higher, but expression levels of these cytokine in the colostrum mononuclear cells were lower than those in the BLV- group (Table 2).

DISCUSSION

The number of B cells in the peripheral blood increases in BLV infected cattle as the disease progresses.^{12,20} Since B cells and monocytes express MHC-II in the peripheral blood, CD14-negative MHC-II-positive cells are recognized as B cells.³ The percentage of B cells among peripheral MHC-II-positive cells from BLV+ cows has been shown to increase considerably more than uninfected animals.⁴ In the current study, this result was confirmed by the significant increase in the number of MHC-II+CD14- B cells both in the peripheral blood and in the colostrum, and an increased percentage of B cells was found both in the peripheral blood and the colostrum from BLV+ cows.

CD4 is expressed on T-helper cells that can be classified into type 1 (Th1) and type 2 (Th2) cells depending on the types of cytokines produced.¹ Th1 cells produce IL-2 and IFN- γ that stimulate cell-mediated immunity, whereas Th2 cells produce IL-4, IL-5, IL-6, and IL-10 that skews the immune response toward humoral immunity.^{1,5,10} During the periparturient period in dairy cows, there is a decrease in cell-mediated immunity, skewing immune responses toward humoral immunity.^{9,21}

In the present study, the CD4+/CD8+ and IFN- γ /IL-4 ratios following higher IL-4 expression were significantly lower in BLV+ cows, both in the peripheral blood and in the

colostrum. Consistent with the data in the present study, cows with decreased IFN- γ /IL-4 and CD4+/CD8+ ratios after calving have been reported to exhibited lower levels of cell-mediated immunity.²³ Similarly, humans infected with human immunodeficiency virus (HIV), that along with BLV belongs to the *Retroviridae*, also show lower CD4+/CD8+ ratios in the peripheral blood after parturition.¹⁸ These data suggest that the immune system in BLV+ cows at parturition was skewed drastically toward Th2-type responses, in which humoral immunity dominates along with a lower level of cell-mediated immunity that could lead to increased susceptibility to mastitis and other periparturient diseases.^{14,23} It is reported that BLV+ cattle were predisposed to the development of EBL and do not spontaneously recover from *Trichophyton verrucosum* infection.²² Taken together, these data indicate an increased susceptibility to infections in addition to EBL in BLV+ cows around the time of calving.

IFN- γ is a pivotal Th1-type cytokine that activates natural killer (NK) cells and macrophages. IL-12 is produced by B cells and macrophages to activate NK cells and induces the differentiation of T-helper cells into Th1 cells.² The Th1 type cytokines have been show to be important for preventing the advancement of diseases in BLV+ cattle.⁷ In the present study, the IFN- γ and IL-12 mRNA levels in PBMCs were higher in BLV+ cows and suggests these Th1 type cytokines produced by the increased number of B cells, might have certain inhibitory effects on disease advancement in BLV+ cows even through humoral immunity. However, IFN- γ and IL-12 expression levels were markedly lower in the colostrum mononuclear cells in the BLV+ group. The current study did not provide evidence to explain why the expression levels of these Th1 type cytokines were depressed only in the colostrum and further investigation is needed to elucidate this mechanism.

CONCLUSION

In conclusion, BLV+ cows had increased

numbers of B cells in both the peripheral blood and colostrum on the day of calving. The cytokine expression pattern was found to favor the activation of humoral immunity and thus there is likely to be an increased risk for the development of EBL and the contraction of other infectious diseases.

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