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Effect of Transportation Stress on Bovine Lymphocyte and Neutrophil Functions

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Abstract
Two sets of experiments consisting of 1 hr- and 4 hr-transportation were undertaken to examine effects of road transportation of calves on their cellular immunity. From the results obtained, it is concluded that the calves transported by road fall into disorders of lymphoreticular cell functions, and that sera from the calves after transportation impair activities of normal lymphocytes and alveolar macrophages obtained from healthy donors. The cell and serum disorders may play an important role in the pathogenesis of respiratory tract disease complex, knows as shipping fever.

Discipline: Animal health
Additional key words: calf, cell immunity, shipping fever

Introduction

In the veterinary context, the term of "stress" is defined as "an abnormal or extreme adjustment in the physiology of an animal to cope with adverse effects of its environment and management[2]". The adverse effects are called stressors. Among those stressors on calves, transportation has been recognized as important because of its association with the outbreak of respiratory tract disease complex, known as shipping fever[1,14]. It is also reported that the transportation may change the susceptibility of animals to diseases by altering their metabolic and immune systems[4].

A series of experiments pertaining to the effects of road transportation of calves on their cellular immunity have been undertaken by evaluating lymphoreticular cell functions as indicators. The results obtained from those experiments[5-11] are summarized in this paper.

Lymphocyte and neutrophil functions in bovine affected by road transportation

The following two sets of experiments were undertaken separately by the authors' group, comprising 1 hr- and 4 hr-transportation treatments. The procedures employed and the results obtained are briefly reviewed as follows.

1) 1 hr-transportation[6]

Six castrated Holstein calves, all 6 months of age, were transported for 1 hr by a truck with a hood on a flat road at an average speed of 40 km/hr. Heparinized blood was collected immediately before the departure, 6 hr and 24 hr after transportation. The control group (n = 4) was kept in a single pen, one animal each, and left rested during the experiment. It was bled in the same manner with the transported group. Total leukocyte count, mitogen-induced lymphocyte blastogenesis and nitroblue tetrazolium (NBT) reduction activity (a bactericidal indicator) of neutrophils were measured as follows: the leukocyte count was determined with an electronic particle counter. The lymphocytes isolated by density gradient centrifugation were cultured in the mitogen-free medium or concanavalin A (Con A, a T-lymphocyte mitogen)-containing medium at 37°C in a humidified air containing 5% CO_2 for 3 days. The cell culture was then post-incubated with ^{3}H-thymidine and harvested onto a filter paper. The

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Table 1. Changes of total leukocyte number, lymphocyte blastogenesis and NBT reduction activity of neutrophils of calves under 1 hr-transportation and control conditions

Group (Number)	Item evaluated (Unit)	Stage of sampling		
		Before transportaion	After transportation	
			6 hr	24 hr
Transported (6)	Leukocyte number ($10^9/l$)	8.5 ± 0.7[a)]	11.9** ± 1.2[b)]	9.2 ± 1.2
	Blastogenesis (10^3dpm): Background	0.7 ± 0.2	0.5 ± 0.2	0.7 ± 0.5
	Con A-stimulated	35.9 ±14.5	47.0* ±16.4	34.8 ±10.2
	NBT reduction (OD at 565 nm)	0.21± 0.03	0.35**± 0.03	0.20± 0.04
Control (4)	Leukocyte number ($10^9/l$)	8.7 ± 1.0	9.3 ± 0.9	9.2 ± 1.8
	Blastogenesis (10^3dpm): Background	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.1
	Con A-stimulated	30.6 ± 4.3	32.9 ± 4.0	29.9 ± 3.6
	NBT reduction (OD at 565 nm)	0.26± 0.04	0.30 ± 0.04	0.30± 0.05

a): Values represent mean ± standard deviation.
b): The mean value is different (* $p<0.05$, ** $p<0.01$) from the initial value before transportation.

Table 2. Changes in leukocyte numbers, lymphocyte subpopulations, lymphocyte blastogenesis and NBT reduction activity of neutrophils of calves under a 4 hr-transportation condition

Item evaluated (Unit)	Stage of sampling				
	Before transportation	After transportation			
		0 hr	4 hr	20 hr	4 days
Leukocyte number ($10^9/l$)					
Total	11.6 ±0.5[a)]	13.6 ± 1.9	17.1* ± 3.6[b)]	13.1 ±1.9	11.2 ±1.9
Neutrophil	3.2 ±1.3	7.9* ± 2.0	9.7* ± 2.0	5.4 ±1.8	4.0 ±1.8
Lymphocyte	7.3 ±1.2	5.5 ± 1.2	6.8 ± 1.6	6.5 ±1.5	6.5 ±1.2
B-lymphocyte	1.7 ±0.2	1.4 ± 0.5	1.2 ± 0.5	1.7 ±0.3	1.4 ±0.4
T-lymphocyte	2.8 ±0.5	0.3* ± 0.2	0.4* ± 0.2	0.9*±0.8	2.1 ±0.7
Blastogenesis (10^3dpm)					
Background	6.2 ±3.0	2.0* ± 0.9	4.8 ± 3.2	4.8 ±3.5	6.4 ±4.1
Mitogen[c)]-stimulated	42.0 ±2.7	31.8 ±18.8	37.3 ±10.5	38.5 ±6.8	41.5 ±3.3
NBT reduction (OD at 565 nm)	0.28±0.05	0.35*± 0.07	0.38*± 0.07	0.30±0.08	0.32±0.07

a): Values represent mean ± standard deviation (n=4).
b): The mean is different (* $p<0.05$) from the initial value before transportation.
c): Phytohaemagglutinin-P.

radioactivity of the papers were counted in a liquid scintillation counter. The blastogenesis was evaluated by ^{3}H-thymidine incorporation of mitogen-free or mitogen-stimulated lymphocytes. Neutrophil isolation and NBT reduction test were undertaken with a modified method of the procedures developed earlier by the authors[5]. The reduction activity was expressed as the optical density of the cell precipitates determined at 565 nm with a spectrophotometer.

Table 1 shows the changes of total number of leukocytes and lymphocyte and neutrophil functions in the transported and control calves. The transported calves showed stimulated leukocytosis and enhancement of the cell functions caused by the transportation. The altered levels were recovered in 24 hr after transportation.

2) 4 hr-transportation[7]

The purpose of this experiment was to investigate the effects of a longer (4 hr) road transportation on lymphocyte subpopulations in addition to the previous indicators. Four castrated Holstein calves, 4 to 6 months old, were loaded on a truck and transported for 4 hr on a steep mountain road at an average speed of 20 km/hr. Heparinized blood was collected immediately before and at 0 hr, 4 hr,

Table 3. Immunosuppressive effect of sera just after 4 hr-transportation and their ultrafiltration fractions on Con A-stimulated lymphocyte blastogenesis[a)]

Sera before transportation	Sera just after transportation	Ultrafiltration fraction of sera just after transportation with molecular weight range		
		>50 Kdal	50–10 Kdal	<10 Kdal
106.3±9.8[b)ABCD]	84.5±6.1[c)AEF]	80.6±4.4[BGF]	71.6±4.0[CEG]	66.1±6.6[DFG]

a): Blastogenesis is expressed as ^{3}H-thymidine incorporation of lymphocytes (dpm).
b): Values represent mean ± standard deviation (n=4).
c): Figures having the same superscripts differ significantly ($p<0.05$).

Table 4. Immunosuppressive effect of sera after 2 day-transportation on lymphocyte blastogenesis and cytochrome C reduction activities of neutrophils and alveolar macrophages

Item examined (Unit)	Mean value (±SD) of cells preincubated with sera	
	Before transportation	After transportation
Blastogenesis (10^3dpm)		
Background	2.0±0.9	1.2**±0.3[a)]
Con A-stimulated	91.6±4.8	85.6* ±6.0
Cyt C reduction (nmole O_2^-)		
of neutrophils	2.2±0.2	2.3 ±0.3
of macrophages	2.0±0.3	1.7* ±0.2

a): The mean value (n = 12) is significantly different (* $p<0.05$, ** $p<0.01$) from the value before transportation.

20 hr and 4 days after transportation. Lymphocyte subpopulations, i.e., T- and B-lymphocytes, were identified as the cells forming rosettes with sheep erythrocytes and those bearing surface immunoglobulin, respectively. Leukocyte counts, lymphocyte blastogenesis and neutrophil function were evaluated in the same manner as was employed in the 1 hr-transportation experiment.

Table 2 shows the changes of the cell indicators in 4 hr-transported calves. The calves developed leukocytosis in association with neutrophilia, T-lymphocyte depletion, impairment of blastogenesis and neutrophil NBT reduction activity just after transportation. Similar impairment was observed in the other 4 hr-transportation experiment, in which a whole blood culture assay was adopted[9)].

Lymphoreticular cell functions suppressed by sera from road-transported calves

Some investigations were made to confirm whether sera from transported calves could affect the cell functions[8,11)]. The experimental procedures and results obtained are summarized as follows:

Sera were collected from two different groups of the calves transported for 4 hr and 2 days. The sera were preincubated with normal lymphocytes, neutrophils and alveolar macrophages from healthy donor calves for 1 hr at 37°C. After the preincubation, the effect of the sera on cells was examined with lymphocyte blastogenesis and cytochrome C (Cyt C) reduction activity of neutrophils and macrophages as indicators.

Tables 3 and 4 show the suppression of the cells by sera just after transportation. As for 4 hr-transportation, the lymphocyte suppressive activity was observed in the sera and their ultrafiltrated fractions (Table 3). The sera from 2 day-transported calves indicated that they impaired not only lymphocytes but also macrophages as well (Table 4).

Conclusions

Based on the results of a series of the above-mentioned experiments, it is concluded that (1) the calves transported by road fall into disorders of lymphoreticular cell functions in a greater or less degree, and (2) sera from the calves after transportation impair activities of normal lymphocytes and alveolar macrophages obtained from healthy donors. Among the cell disorders, somewhat specific is the response of blastogenesis, which is enhanced after 1 hr-transportation, whereas it is impaired in 4 hr-transported calves. The serum immunosuppression is likely to take place in accordance with the cell disorders caused by unfavorable metabolic byproducts during the transportation.

It is a widely-accepted logical sequence that hypothalamic-pituitary-adrenal axis responses to a

stressor can modulate lymphoreticular cell functions[3,13]. Plasma glucocorticoid levels, when artificially increased, are known to affect the cell functions[10,12]. In the authors' experiments, an alteration of plasma cortisol concentrations was observed in the calves. Therefore, the cell and serum disorders in the calves may be triggered by their endocrine responses to road transportation. Those disorders may play an important role in the pathogenesis of shipping fever of calves.

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