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## Distribution of bovine immunodeficiency virus in the organs of experimentally infected cows

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### Summary

The distribution of bovine immunodeficiency virus (BIV) in the organs of experimentally infected cows was investigated by use of nested polymerase chain reaction (PCR). Two cows (Nos. 1 and 2) experimentally infected with BIV were alive without any clinical symptoms of BIV infection for 28 months. Viral and proviral genomes of BIV were continuously detected from peripheral blood leukocytes in those cows by nested PCR. Proviral genomes of BIV were also detected in liver, lung, and spleen cells in the two cows, and in the brain in cow No. 1. Viral genomes were detected in liver, lung and spleen cells in cow No. 1, and detected only in spleen cells in cow No. 2. These results suggest that BIV tended to be persistent in some organs, especially in the spleen.

Key words: BIV, bovine immunodeficiency virus, distribution, provirus

### Introduction

The clinical signs of cattle infected with bovine immunodeficiency virus (BIV) have been reported as lymphadenopathy, lymphocytosis, neuropathy, and wasting<sup>16)</sup>. Only three different isolates were detected from naturally infected cattle<sup>13,16)</sup>. In cattle experimentally infected with BIV, significant clinical symptoms have not developed. The pathogenicity of BIV in cattle, therefore, is still unclear.

BIV is a member of family *Retroviridae* genus *Lentivirus*, most cases of lentivirus infections have a latent period of several years for the development of clinical manifestations of the disease<sup>4)</sup>. Although a long-term detection of BIV from peripheral blood leukocytes (PBL) has been reported<sup>14)</sup>, it is as yet unknown whether BIV integrates only in PBL or not. A provirus of BIV has been detected from monocytes in the peripheral blood of infected cattle<sup>10,11)</sup>, and the gene of BIV was detected from milk-derived

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leukocytes and stud bull semen<sup>8,9)</sup>. However, the levels of viral RNA in peripheral blood mononuclear cells were less than 1/10 the levels observed in persistently infected cells *in vitro*<sup>1)</sup>. In order to estimate the pathogenesis of BIV infection, it is necessary to investigate the distribution of BIV within the body of infected cattle.

In this paper, the distribution of BIV within the body of experimentally infected cows was investigated using nested polymerase chain reaction (PCR). Experimentally infected cattle with BIV were observed for 28 months, and they did not show any clinical symptoms during the experimental period. After euthanasia, some organs were collected, then DNA and RNA were extracted for detection of BIV genomes by PCR method.

#### Materials and Methods

*Experimental animals*: Four 2-month-old Holstein calves were used in this study. Two were intravenously inoculated with BIV (R29 strain which was isolated from naturally infected cattle by VANDERMAATEN et al.<sup>16)</sup>) infected bovine embryonic spleen cells. The other 2 calves were inoculated with physiological saline as a control. Each of the calves was kept for 28 months in an isolation pen under the same conditions. Clinical conditions of all calves were monitored by physical examination including measurement of body temperature and palpation of body surface lymphonodi, every week, and complete blood counts examination was performed every 2 weeks. The two BIV-inoculated calves were confirmed to be infected with BIV by syncytia assay<sup>10)</sup> and PCR method described below. After the observation period, all of the calves were killed, and the cervicales superficial and mesenteric lymphonodi, spleen, liver, lung, kidney, and brain were collected. RNA and DNA were extracted from these organs as soon as possible after the collection.

*Samples for the detection of BIV by PCR*: Five ml of peripheral blood was collected from the jugular vein using EDTA-2Na as an anticoagulant and centrifuged for 15 minutes at  $800\times g$ . Ten  $\mu\text{l}$  of the buffy coat were aspirated and used as peripheral blood leukocytes (PBL). Extraction of DNA and RNA from PBL, and cDNA synthesis using random primers were described previously<sup>7,15)</sup>.

Few pieces of each organ were washed thoroughly with phosphate buffered saline (PBS, pH 7.4), and cut up with scissors in PBS. The minced tissue suspension was filtered with gauze for exclusion of cell clots. The filtrate was centrifuged, and the pellet was washed three times with PBS. An aliquot of the cell suspension was stained with Giemsa's solution and no contamination of PBL was recognized by the microscopic observation of the size and shape of the cells. Then DNA and RNA were extracted from these cells ( $0.5\text{--}1.0\times 10^6$  cells) according to the same method for PBL, and used as templates for PCR.

*Polymerase chain reaction*: The genome of BIV was detected by the nested PCR. Four primers which recognize the *pol* coding region of BIV gene<sup>5)</sup> were synthesized with an Applied Biosystems DNA Synthesizer Model 380B. The nucleotide sequences of the primers are: OF, 5'-AGTAGATACGCCTATGAGGG-3' (location and direction: 3492-3511>), OR, 5'-GCTGTAGCTCTGAATCTACC-3' (<3818-3837), IF, 5'-TGAAGGCCATATGCATGGCT-3' (3731-3755>), IR, 5'-ATGTCTGGTGAGGT-ATGCCA-3' (<3943-3964). The contents of reaction mixture and the conditions for PCR were according to our previous report<sup>15)</sup> except for the annealing temperature of 60°C in both steps of the nested PCR. The first-step primers were OF and OR, and the second-step primers were IF and IR. In the second-step PCR, 1.5  $\mu\text{l}$  of first-step PCR products was used as a template. Aliquots of 5  $\mu\text{l}$  of the amplified PCR products

were analyzed by 1.5% agarose-gel electrophoresis.

### Results

*Detection of the genomes of BIV R29*: In order to estimate the specificity of the primers, DNA and RNA were extracted from BIV-infected bovine embryonic spleen (BIV-BESP) cells and used for PCR. A 346bp amplified DNA was detected in the first-step PCR using DNA and cDNA from BIV-BESP cells as a template, respectively. A 234bp amplified DNA was detected using second-step primers in both DNA and cDNA from BIV-BESP cells and first-step PCR products as a template, respectively. All these amplified DNAs had the expected nucleotide sizes in primer design. As negative controls, BIV-uninfected BESP cells and bovine leukemia virus (BLV)-infected leukocytes were used for PCR. No amplified DNA was detected in the negative controls. By the use of this PCR method, both viral and proviral genomes of BIV could be detected from the BIV-infected cells.

*Detection of BIV genomes from PBL*: All experimental animals were tested by nested PCR at 22, 25, 27 and 28 months after BIV inoculation. In two BIV-infected cows, amplified DNAs were detected from both DNA and cDNA of PBL in every experimental time. This result indicated that both viral and proviral genomes were present in PBL of the infected cows. In cow No. 1, amplified DNA was detected by only first-step PCR using DNA as a template. The significant clinical symptoms such as depression, lymphadenopathy, lymphocytosis and wasting were not observed in either BIV-infected cow during the experimental period. In the control animal, amplified DNA by nested PCR was not detected.

*Distributions of BIV genomes in infected animals*: As shown in Table 1, proviral genomes of BIV were detected in the liver, lung, and spleen of both cows infected with BIV. Proviral genome was also detected in the brain of one

Table 1. Summary of the detection of BIV viral or proviral genomes\* from organs

Organ	Template	Cattle No. **			
		1	2	3	4
PBL	DNA	++	+	-	-
	cDNA	+	+	-	-
Liver	DNA	+	+	-	-
	cDNA	+	-	-	-
Lung	DNA	+	+	-	-
	cDNA	+	-	-	-
Brain	DNA	+	-	-	-
	cDNA	-	-	-	-
Spleen	DNA	++	+	-	-
	cDNA	+	+	-	-
Lymphonodi	DNA	-	-	-	-
	cDNA	-	-	-	-
Kidney	DNA	-	-	-	-
	cDNA	-	-	-	-

\*: viral or proviral genomes are detected by nested PCR using cDNA or DNA as template, respectively

\*\* : Nos.1 and 2 are BIV-infected, 3 and 4 are mock-infected cattle.

-: absence of BIV genome,

+: detect BIV genome by nested PCR,

++: detect BIV genome only by first-step PCR

BIV-infected cow (No. 1). In this cow, the amplified product from the spleen was detected by only first-step PCR. The viral genomes were detected in the liver, lung, and spleen of cow No. 1. In the cow No. 2, the viral genome was detected only in the spleen. No amplified products by nested PCR was detected in the kidney or lymphonodi of the BIV-infected cows, or in any organs examined in the control cows.

### Discussion

Although there are many reports concerning the experimental infection of cattle with BIV, only three cases of natural infection of BIV have been reported<sup>13,16</sup>. The pathogenicity of BIV has not been clarified. In general, a long latency period occurs before the outbreak of this lentivirus infection disease. Feline immunodeficiency virus (FIV) and human immunodeficiency virus

(HIV), a member of genus *Lentivirus*, replicate within blood mononuclear cells, which result in the progressive dysfunction of the immune system of the host during the long-term infection. These viruses can also infect the cells of the central nervous system such as microglia, astrocytes and endothelial cells<sup>4</sup>). The infection of BIV in bovine peripheral monocytes and the subsequent dysfunction of the cells have been observed in calves experimentally infected with BIV<sup>10,11</sup>). In these experimental calves, no clinical symptom was recognized. Since monocytes/macrophage lineage is distributed in many organs, BIV may persist in those cells for a long period.

For the estimation of the distribution of virus within the body, *in situ* hybridization method is available using specific probe for viral genome. It has been reported that the levels of BIV RNA in virus-infected cells *in vivo* were at least 10-fold lower than RNA levels detected in *in vitro* cell culture<sup>1</sup>). This suggested that it might be difficult to detect BIV genome in the organs by *in situ* hybridization method. In the present study, therefore, nested PCR was utilized for the detection of BIV in the organs. As shown in Table 1, BIV genomes were detected in the lung, liver, spleen and brain, but not in the kidney and lymphonodi of calves infected with BIV. In order to avoid the contamination of PBL in the organ samples, each organ was washed carefully before DNA and RNA were extracted from the cells. Microscopic observation revealed no PBL in the samples for extraction. Therefore, there was little possibility of detecting BIV genomes derived from PBL. Although the target cells of BIV infection could not be confirmed in this study, these results indicate that BIV might preferentially replicate in phagocytic cells of some organs, such as alveolar macrophages, kupffer cells, reticular cells and microglia.

However, BIV genome was not detected in the kidney and lymphonodi, in which many

phagocytic cells were present. One possible explanation for this result was a localization of BIV in each organ. The organ samples for detection of BIV genomes were obtained as several pieces of small masses from different portions of organs. Thus, we thought that the results of nested PCR for detection of BIV genomes in organs did not depend on the localization of BIV within organs. The results also indicate that our preparation method of organ samples might prevent the contamination of PBL.

There is a report that nested PCR could detect the BIV genomes after examination by virus isolation and a protein immunoblot assay became negative in experimentally infected cattle<sup>14</sup>). As shown by the results of the present study, it was impossible to detect viral genome only by first-step PCR from any organ. This indicates that the amount of viral production was very small in the organs. In contrast, proviral genomes were detected only by first-step PCR from the PBL and spleen of one BIV-infected cow (No. 1). In the other BIV-infected cow, viral genomes were detected only from the PBL and spleen. Although the number of permissible cells such as macrophages or phagocytes per organ tested was not quantified, these results suggest that BIV might be likely to integrate in PBL and spleen DNA, and to replicate more viruses in PBL and spleen cells than in the other organs.

In viral infectious diseases, clinical symptoms or lesions are closely correlated with the distributions of virus in the host. In the present study, BIV genome was detected in the brain, which may explain the cause of neuropathy observed in cattle naturally infected with BIV. In this study, however, no direct correlation between clinical symptoms and the distribution of BIV was recognized. Mixinfection of FIV and feline leukemia virus in the cat often results in the development of severe clinical symptoms<sup>12</sup>). In the cases of mixinfection of BIV and BLV,

however, clinical symptoms have not been observed<sup>2,3</sup>). In the present study, BIV was not detected in the lymphonodi of the experimental animals. However, BLV is known to be latent in lymphonodi for a long time. This difference of virus distribution in the host body might be one possible explanation for the lack of outbreak of clinical disease in mixinfection with BIV and BLV.

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