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Title	Immune responses and epitope analysis against bovine leukemia virus transactivator tax
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based on the gene of the piroplasm major surface protein, p32.

In this study, an expression system of I and C types of p32 in bacterial cells, and two hybridomas (4G10 and 5H9) producing monoclonal antibodies (MoAb) specific to the I type of p32 were established in order to compare immunogenicity between these two types. In an immunoblot assay to examine binding activity of polyclonal antibodies in infected calf sera and the monoclonal antibodies against each types of p32 demonstrated type-specific bindings of these antibodies to the corresponding p32 types, which indicated very poor immunological cross-reactivity between those two types. An indirect immunofluorescent antibody staining using unfixed piroplasms as antigens showed that MoAb 4G10 had binding activity to the cell surface of the parasite, but 5H9 did not. Serum from an infected calf was also positive in this assay. These results indicated a possibility that the serum antibodies might interfere biological activities of p32 and prevent the growth of the parasites.

In spite of host humoral immune responses against p32, cattle can not eliminate the parasites and several parasitemia peaks are observed in persistently infected animals. To investigate a possibility that antigenic variation or alternations of the piroplasm are involved in parasite evasion from host immune responses, blood containing the C and I types of the parasites were collected from two persistently infected calves at different parasitemia peaks, and diversities in nucleotide sequences of each type of p32 gene were analyzed. However, no nucleotide changes were detected, suggesting that antibodies are not enough to suppress the growth of the parasite. Since homologous molecules of p32 are also expressed at intraerythrocytic stages of other *Theileria* species, the molecule might have an important biological activity for parasite growth in the hosts. Possibly, antibodies directing to functional site(s) on the p32 might inhibit infection or growth of the protozoa. In order to develop an effective vaccine against bovine theileriosis, it is very important to study the function of this molecule and identify key motifs for its function.

#### Immune responses and epitope analysis against bovine leukemia virus transactivator tax

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Previous studies for the vaccine development against bovine leukemia virus (BLV) has focused only on the viral structural proteins, env and gag. Other studies, however, have revealed that the viral transactivator protein, tax can also induce the cell-mediated immunity against human T cell leukemia virus. In this study, the immune response against BLVtax was

investigated in mice and sheep immunized with a recombinant BLVtax protein derived from a baculovirus or *Escherichia coli* expression system. Furthermore, epitope mapping of BLVtax was performed in these animal species.

Spleen cells prepared from BALB/c mice immunized with BLV-producing fetal lamb kidney cells showed high lymphocyte proliferative reac-

tion when stimulated with the recombinant BLVtax protein. Furthermore, BALB/c and C57BL/6 mice, and sheep immunized with the recombinant BLVtax produced BLVtax-specific antibodies and showed proliferative responses against BLVtax.

Epitope mapping using 30 synthetic peptides of 20 mer covering the whole BLVtax polypeptide sequence was carried out. A lymphocyte proliferation assay revealed that, an epitope was located at positions of 111–130 and 131–150 for C57BL/6 and BALB/c mice respectively. In the case of sheep, there were different epitopes for the individual sheep. B cell epitope (position

51–70) and T cell epitope (position 181–200) were identified in one of the sheep which developed the strongest immune response against the whole BLVtax protein. In addition, the peptide at position 11–30 induced a non-specific proliferative response in both immunized and unimmunized sheep.

These results suggest that BLVtax is an immunogenic protein for mouse and sheep, and there are different epitopes for each experimental animal used in this study. The recombinant tax protein or peptides bearing B and/or T cell epitopes are good candidates for vaccine development against BLV infection.

#### Improvement of coproantigen detection methods for the diagnosis of the definitive hosts of *Echinococcus multilocularis*

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Improvement of coproantigen detection technique for diagnosis of *E. multilocularis* infection in definitive hosts were carried out. Initially, 5 different monoclonal antibodies (mAb) against *E. multilocularis* (EmA7, EmA8, EmA9, EmA10 and EmA11), were compared and their specificities were evaluated. Those mAb were tested for their cross-reactivities with somatic antigen extracts and coproantigens of *Taenia hydatigena*, *E. granulosus* and *E. multilocularis* in sandwich ELISA (sELISA). Out of 5 mAb, EmA8, EmA9 and EmA11 reacted strongly with *E. multilocularis* antigens, and used in an immunohistochemical study to observe the changes in antigen distributions during the development of *T. hydatigena* and *E. multilocularis*. Similar antigen distribution was observed in *T. hydatigena* as in *E. multilocularis* in all mAb.

However, the strongest reactions were recognized in the reproductive organs in *T. hydatigena*, while, in the tegment and parenchyma in *E. multilocularis*.

To improve the specificity and sensitivity of the coproantigen detection method, avidin-biotinylated peroxidase complex (ABC) method was applied to EmA9 based sELISA. sELISA's using either Rabbit anti-*E. multilocularis* excretory-secretory antibody or EmA9 as capture and biotinylated EmA9 as primary antibody (rAb/EmA9 or EmA9/EmA9, respectively) were determined to be the most appropriate for the detection of *E. multilocularis* coproantigens. The comparison between the OD values of ABC applied method and conventional method clearly showed an increase in reactivity in ABC sELISA's and, especially in rAb/EmA9, sELISA kept non-