



Title	Analyses of antigenic diversity of a major surface protein of <i>Theileria sergenti</i> and host immune responses against the parasite
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Development of a nasal vaccine of infectious bovine rhinotracheitis: Protective immunogenicity of baculovirus-expressed glycoproteins of bovine herpesvirus 1

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Intramuscular immunization with attenuated infectious bovine rhinotracheitis (IBR) live virus vaccines induces serum antibodies against bovine herpesvirus 1 (BHV-1) in cattle and decreases the severity of the clinical signs induced by IBR. However it does not induce protective immunity on the mucosae, where the primary infection occurs in cattle, resulting in the following latent infection. The attenuated vaccine viruses may revert to virulent wild strains. In the present study, to develop more effective and safer IBR vaccines, intranasal immunization of cattle and rabbits with test subunit vaccines containing baculovirus-expressed gC and/or gD glycoproteins of BHV-1 was performed. Cholera toxin B subunit (CTB) was added to the vaccines as a mucosal adjuvant.

Cattle immunized intranasally with gC or gD subunit vaccines produced the neutralizing antibodies against BHV-1 in the nasal secretions. After challenge with $10^{7.8}$ PFU of BHV-1, all of

the cattle immunized showed less severity of clinical signs and less virus shedding. However, the complete protection of the animals from BHV-1 infection was not established.

Rabbits were primarily immunized with intramuscular injection of antigens together with Freund's complete adjuvant, followed by intranasal administration of antigens with CTB. Each vaccine containing gC, gD or inactivated BHV-1 virions, as well as gC + gD-combined one, induced anti-BHV-1 antibodies in the sera and nasal secretions of the animals. gD and gC + gD-combined vaccines induced neutralizing antibodies in the nasal secretions and conferred protection from challenge with $10^{6.0}$ PFU of BHV-1.

These findings indicate that intranasal vaccination with the baculovirus-expressed glycoproteins is a promising measure to confer protective immunity to animals against BHV-1 infection.

Analyses of antigenic diversity of a major surface protein of *Theileria sergenti* and host immune responses against the parasite

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Theileria sergenti, a causal agent of bovine piroplasmiasis in Japan, is a tick-borne protozoan

parasite. The parasites distributed in Japan are divided into two major genotypes, I and C types,

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-