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Author(s)	SHIKI, Masako
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Epitope Mapping of *E. coli* 54K Protein Using Monoclonal Antibodies

Akiko Yagi

Laboratory of Microbiology,
Department of Disease Control,
School of Veterinary Medicine,
Hokkaido University, Sapporo 060–0818, Japan

Herpesvirus establishes a latent infection in a convalescent host. It is difficult to control herpesvirus infection due to reactivation of latent virus from an apparently healthy animal. During latnt infection with bovine herpesvirus 1 (BHV1), viral transcripts are detected in the trigeminal ganglia and translated to 41 K proteins. The latency related (LR) protein binds to cyclin A, which regulates host cell cycle, to play a role in maintenance of latency.

To provide the information about structure of LR proein, the author tried to make a panel of monoclonal antibodies using recombinant LR protein fused to histidine-tag generated in *E. coli* as antigens. Western blot analysis demonstrated that most monoclonal antibodies recognized a polypeptide with molecular mass of 54 K, which was expected for the recombinant LR protein. It is, however, revealed that the 54 K protein was a component of *E. coli.* The auther described here antigenic mapping of the 54 K protein of *E. coli.* At least 4 antigenic regions were obserbed on the molecule by competition binding assay. The results indicate that the panel of monoclonal antibodies would be useful for providing information about function of the 54 K protein of *E. coli.*

Vaccination trial against theileriosis using a recombinant vaccinia virus

Masako Shiki

Laboratory of Infectious Diseases,
Department of Disease Control,
School of Veterinary Medicine,
Hokkaido University; Sapporo, 060–0818, Japan

Bovine theileriosis is a tick-borne disease of cattle caused by the protozoan parasite *Theileria sergenti* which causes anemia as intraerythrocytic piroplasma and occasionally death in severe cases. Due to economic losses, new control methods are urgently needed. An immuno dominant major piroplasm surface protein (MPSP) is expressed on the surface of the merozoites. In this study, a recombinant vaccinia virus expressing the MPSP was used as live antigen delivery system to immunize cattle against *T. sergenti.*

The expression of MPSP in recombinant vaccinia virus-infected RK13 cells was confirmed by immunoblot analysis. Expression was detected 3 hrs after infection, and MPSP with molecular mass larger than the predicted size was detected which is believed to be due to N-linked glycosylation.

Immunogenicity of the recombinant MPSP was examined by viral inoculation to mice and calves. In both animals, antibodies to MPSP were produced after the second inoculation. In the calf vaccination trial, low level peripheral

blood mononuclear cells (PBMC) proliferation against the recombinant MPSP was detected afier the third inoculation. These results indicate that recombinant vaccinia virus can induce both humoral and cellular immunities. Following challenge with *T. sergenti*-infected erythrocytes, higher level of antibody responses as well as PBMC proliferations were detected in immunized calves compared to controls. However, immunized and control calves showed the same levels of parasitemia and packed cell volume (PCV). Therefore, immunization of calves with recombinant vaccinia virus did not confer protective anti-merozoite immunity. These present findings are based on observations done over on short period of time. The course of disease progression in the later stages of infection was not evaluated. Therefore, further experiments will be required to examine the potential of the vaccinia virus expression system as a control method for bovine theileriosis.

Appearance of apoptosis induced by Marek's disease virus infection

Hiroyasu Takahashi

Laboratory of Infectious Diseases,
Department of Disease Control,
School of Veterinary Medicine,
Hokkaido University, Sapporo 060–0818, Japan

Marek's disease virus (MDV), a causative agent of Marek's disease (MD) characterized by T cell lymphoma formation and peripheral nerve enlargement, causes immunosuppression in infected chickens. The immunosuppression can be divided into two phases ; the primary phase characterized by humoral immunosuppression, and the secondary one characterized by T cell unresponsiveness to mitogen stimulation. Previously, it was shown that $CD4^+$ $CD8^+$ T cells in the thymus and $CD4^+$ T cells in the spleen and peripheral blood underwent apoptosis at 1 and 3 weeks after MDV infection, respectively. However, the relationship between apoptosis and immunosuppression after MDV infection remains unclear.

In this study, to demonstrate the involvement of apoptosis in immunosuppression induced by MDV, lymphoid tissues, such as spleen, bursa of Fabricius, thymus and cecal tonsils, obtained from chickens experimentally infected with MDV were examined histologically. The immunohistochemical staining to detect MDV-specific phosphorylated protein, pp38, and TUNEL (Terminal deoxynucleotidyl transferase mediated dUTP-biotin nick end labeling) method were used in order to identify MDV-infected cells and apoptotic cells, respectively. TUNEL-positive cells were detected in bursa of Fabricius obtained from MDV-infected chickens at 1 to 3 weeks after infection. Throughout the experimental period, pp38-positive and TUNEL-positive cells were observed not only in primary lymphoid tissues such as the thymus and bursa of Fabricius, but also in secondary lymphoid tissues such as the spleen and cecal tonsils. These results suggest that MDV could induce apoptosis in several lymphoid tissues. In addition, the number of TUNEL-positive cells was higher than that of pp38-positive cells in those tissues. Since chickens were depressed and their thymus and bursa of Fabricius were severely atrophied at that time, apoptosis of uninfected cells may be one of the causes of immunosuppressions induced by