duction of IFNα cDNA, were confirmed but the expression levels of IFNα were too low to demonstrate the biological activity as measured by anti-viral effects. In the case of LAP cDNA-introduced potatoes, no transformants were obtained because of mutations in the vector plasmid.

The low expression levels of IFNα in the transformants could be explained by that (1) the expression levels of foreign genes depend on the sites of their insertion because the foreign DNAs are randomly inserted into the plant chromosomal DNA, (2) the translation of the transcript in plant cells is inefficient because of the differences in the codon usages between plant and mammal genes. The results obtained in this study suggest that mammalian genes being introduced into the plant cells should be converted into suitable forms to achieve high expression in plant cells.

Molecular cloning and characterization of *Babesia caballi* antigen genes for development of serodiagnosis for equine babesiosis

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Babesia caballi and *Babesia equi* which are tick-transmitted intra-erythrocytic protozoan cause equine babesiosis. The complement fixation test (CFT) and indirect immunofluorescent antibody test (IFAT) which are currently used for detecting *B. caballi* infection are not sensitive and specific enough. Therefore, in this study, aiming at more simple, sensitive and specific serodiagnostic methods for equine babesiosis on enzyme-linked immunosorbent assay (ELISA) using recombinant antigens, molecular cloning and characterization of *B. caballi* antigen genes were performed.

B. caballi cDNA library was constructed and screened with a serum of an experimentally infected horse with *B. caballi*. Several clones obtained by immunoscreening, out of which two clones, i.e. B.cA1 and B.cA2 were further analyzed.

B.cA1 contained a 2307bp insert with an open reading frame of 1887bp, which encoded polypeptide of 629 amino acids. Homology search revealed that B.cA1 was a member of heat shock protein 70 (hsp70) gene family. Horse antibodies affinity-purified with B.cA1 products reacted with not only 69kDa protein of *B. caballi*, but also 67kDa protein of *B. equi* antigens. This suggests that cross-reactivity between *B. caballi* and *B. equi* which ELISA using merozoite lysates sometimes encouters is partly due to the presence of common epitopes between hsp70s.

B.cA2 containing a 729bp insert encoded a polypeptide of 212 amino acids which showed high homology to the C-terminal sequences of rhoptry associated protein-1(RAP-1) of several *Babesia* species. Southern blot analysis showed that *B. caballi* genome contained multiple copies of this gene as is the case in other Babesia species. Horse antibodies affinity-purified with B.cA2 products strongly reacted with 53 and 55kDa proteins of *B. caballi* but not with *B. equi* antigens. Moreover, on immunoblot analysis, B.cA2 recombinant proteins expressed in *E. coli*

specifically and strongly reacted with sera from *B. caballi*-, but not *B. equi*-infected and uninfected horses. Therefore, B.cA2 product is thought to be suitable for serodiagnosis of equine babesiosis caused by *B. caballi*.

Antigenic diversities among strains cause problems in specificity and sensitivity of serodiagnosis of many protozoan diseases. However, RAP-1 molecules of other *Babesia* species have been reported to be species-specific antigens. Therefore, B.cA2 is thought to contain species-specific epitopes among strains. ELISA based on B.cA2 recombinant product will be developed after fine analysis of antigenic homogeneity of this molecule among *B. caballi* stocks.

Specific antibody responses in dogs experimentally infected with *Echinococcus multilocularis*.

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Specific serum IgG, IgA and IgM, and copro IgA against somatic and excretory/secretory antigens of adult tapeworms and protoscolices of *Echinococcus multilocularis* were analyzed in dogs experimentally infected with *E. multilocularis* by enzyme-linked immunosorbent assay (ELISA).

First, two mongrel dogs were infected with *E. multilocularis* and their sera and fecal samples were collected until 33 days post infection (DPI). IgG and IgA responses against adult antigens showed a gradual increase from 12 DPI whereas IgM against adult antigens showed a transitory rise in one dog. Similar time course of responses against protoscolex antigens were observed, but the responses were weak. IgG against EmA9 (designated by Khono *et al.*, 1995)-antigen which specifically reacts with monoclonal antibody, EmA9, used in coproantigen detection assay also showed a gradual increase from 12 DPI, indicates host immune system recognized EmA9-antigen excreted in the host feces. Western blot analysis of adult and protoscolex antigens with sera of *E. multilocularis* infected dogs showed distinct band patterns in IgG and IgA reactions.

Secondly, four beagle dogs were divided into two groups, two dogs into reinfecion group which were infected with *E. multilocularis* and purged at 21 DPI, reinfected at 35 DPI, and the other two into control group which were infected with *E. multilocularis* at 35 DPI, the same time as reinfection in reinfection group. Sera and fecal samples were collected until the autopsy (80 days after reinfection). IgG and IgA against adult antigens and IgG against EmA9 antigen showed an evident increase from 14 or 15 days post primary infection in both groups. Higher titer was correlated with long-standing infections. Rapid and strong IgG responses after reinfection were not observed. Time course of coproantigen excretion was also examined by sandwich ELISA using monoclonal antibody EmA9. The peak of the OD value in reinfecion group was less than that in control group and the decrease of the OD value after the peak was more rapid in reinfection group. The number of worms recovered in reinfection group at the autopsy were