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<th>Instructions for use</th>
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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 encounters 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 unin­
fected 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 serodiag­
nosis 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. multilocu­
laris* 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 re­
sponses 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 coproan­
tigen 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. multilocular­
is* infected dogs showed distinct band patterns in
IgG and IgA reactions.

Secondly, four beagle dogs were divided into
two groups, two dogs into reinfection group which
were infected with *E. multilocularis* and purged
at 21 DPI, reinfeated 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 coproan­
tigen excretion was also examined by sandwich
ELISA using monoclonal antibody EmA9. The
peak of the OD value in reinfection 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 reco­
vered in reinfection group at the autopsy were