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Title	Molecular cloning of genes of Theileria sergenti and comparative genome structure analysis of Theileria parasites
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Citation	Japanese Journal of Veterinary Research, 47(1-2), 46-48
Issue Date	1999-08-31
Doc URL	http://hdl.handle.net/2115/2733
Type	bulletin (article)
File Information	KJ00003408065.pdf

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provided. Results from the present work and others have shown that anti-tick vaccines based on a single antigen may not be inclusively protective. In addition, immature and mature ticks tend to show different sensitivities to host anti-tick immune responses, and hence a cocktail vaccine that contains multiple antigens will be more protective. Further search for candidate antigens was conducted.

Despite the potential of proteolytic enzymes as possible target molecules in immunological control of ticks, studies on these molecules in ticks have been quite limited. In this study, a generic approach was used to clone and express *in vitro* 2 genes each of serine and cysteine proteinases. The generic approach proposes to use oligonucleotide primers designed from conserved motifs among proteinase families. In other studies, the polymerase chain reaction with these primers was carried out to amplify gene fragments which were used as probes to clone full length genes. In this study, PCR primers designed from the partial gene sequences were used in the 5' and 3' RACE protocols to clone the full length genes. While the primers used in this study have been used by others to clone cysteine proteinase and serine proteinase genes in other parasite species, the results in the present study were the first reports on molecular cloning and characterization of both cysteine and serine proteinase genes from a hard tick. Two serine proteinase genes, HLSG-1 and -2 encoded polypeptides with molecular masses of 37.7 and 31.2 kDa, respectively. Two cysteine proteinase genes, HLCG-A and -B encoded polypeptides with molecular masses of 33.7 and 37.0 kDa, respectively. Rabbit antisera raised against recombinant product of HLSG- I and -2 expressed in *E. coli* recognized authentic molecules expressed in ticks, which suggested that the genes were expressed in ticks.

In conclusion, this study has provided relevant information towards the development of a vaccine against ticks. Results from the vaccination trial with rp29 in rabbits were very promising and needs further studies to enhance the protective capacity of the vaccine. Data from this work on tick proteolytic enzymes provided fundamental information with respect to obtaining efficacious anti-tick vaccine antigens using generic approaches. Further work is needed for further characterization of the proteolytic enzymes reported in this study.

Molecular cloning of genes of *Theileria sergenti* and comparative genome structure analysis of *Theileria* parasites

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Theileria sergenti is a tick-borne protozoa of cattle and causes anaemia as an intraerythrocytic piroplasms, which is one of the most economically important disease of grazing cattle in Japan. Cattle which are persistently infected with this parasite, show elevated parasitaemia, rapid progress of anaemia and mild hyperthermia, which is occasionally fatal, under conditions of stress or coinfection with other pathogenic microorganisms. The methods of controlling this parasite

have not been established. Additionally, the dynamics of this parasite in hosts and anaemia mechanisms were not elucidated.

In this thesis, the author performed the molecular cloning of genes of *T. sergenti* to develop the effective preventive and therapeutic applications. The results obtained are summarized as follows.

1. The gene cloning of cysteine proteinase was carried out with the aim of chemotherapeutic exploitation. Sequencing of one cDNA clone of cysteine proteinase showed that it contained an open reading frame (ORF) which could encode a polypeptide of 402 amino acids with predicted molecular mass of 46.4 kDa. Southern blot hybridization and restriction fragment length polymorphism (RFLP) analyses of this gene between I- and C-type parasites showed the diversity of the gene.

2. Two sets of oligonucleotide primers were designed to specifically amplify either of the two allelic forms of p23 gene by polymerase chain reaction (PCR). The analysis by allele-specific PCR clarified that stocks maintained in laboratories and field isolates of *T. sergenti* consisted of various combinations of the two allelic forms similar to major piroplasm surface protein (MPSP) genes. By PCR, the ORF of *T. buffeli* (B)-type p23 was amplified from the genomic DNA and its nucleotide sequence was determined. Comparison of B-type sequence with that of C-type resulted in 93.5 % homology at amino acid level.

3. The genes cloning of antigenic proteins which were expressed in both sporozoite and piroplasm stages were performed and two positive clones were isolated. The sequence analysis revealed that one clone contained a 1.1 kbp-cDNA insert with the entire ORF for MPSP and another clone contained a 1.5 kbp-cDNA insert encoding a 60 kDa antigenic protein (Ag60). MPSP expression in sporozoite stage was confirmed by immunoblot analysis. In immunoelectronmicroscopy using anti-Ag60 sera, the labelling was located exclusively in the cytoplasm but not on the surface of piroplasm. Particularly, the signals were associated with the electron-dense organelles that were thought to be rhoptry organelles. By Southern blot and immunoblot analyses, genetic and antigenic polymorphisms of Ag60 between I- and C-type parasites were also demonstrated.

From the results of genes cloning, genetic and antigenic diversity in *T. sergenti* were observed and mixed-parasite populations existed in cattle. For controlling the parasite by immunological methods, the antigenic diversity was thought to be a key factor, which may be produced at the sexual stage in tick vector. The analysis of chromosome provided us the basic information to study the generation of genetic and antigenic polymorphism. In addition, by comparing the genome structure between parasites, this may help us to know the evolutional relationship and pathogenic differences. The results obtained are summarized as follows .

4. *T. sergenti* has four chromosomes similar to *T. parva*. The genome of *T. sergenti* has much higher C+C content or more G+C rich regions than that of *T. parva* but less than *T. mutans*. From the results of Southern blot analysis, MPSP gene was located on the chromosome I (Chr 1), p23, Hsp70 and cysteine proteinase genes were on Chr 2 and SSUrRNA gene was on Chr I and Chr 314.

5. Most of genes used for molecular karyotype showed a conserved synteny between *T. sergenti* and *T. parva*. However, in *T. sergenti*, cysteine proteinase and Hsp genes were located on the same chromosomes (Chr 2), whereas in *T. parva*, cysteine proteinase gene (Chr 3) was located on the different chromosome of Hsp genes (Chr 2). There was a possibility of interchromosome exchange between Chr 2 and Chr 3 during specification into *T. parva* and *T. sergenti*. This exchange is hypothesized to affect the expression

of the sporozoite surface molecule, p67, which plays an essential role in parasite invasion into host cells, and the biological properties of parasites.

Hereafter, we must examine the utilities of genes obtained in this study for controlling *T. sergenti* infection. And the information of genome structure and karyotype make possible to analyze the generation mechanism of genetic polymorphism via a sexual stage.

Interacting host immune responses affecting on the disease progression in animals experimentally infected with bovine leukemia virus

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Only a few animals infected with bovine leukemia virus (BLV) develop leukemia after long latent period for 7–8 years. This observation shows that BLV has some mechanisms to modulate host immune systems and that several interactions between BLV and hosts determine the course of disease progression. Several studies have been carried out to determine the factors which have effects on the disease progression of BLV infection, including major histocompatibility complex (MHC) haplotypes, cell-mediated immune responses (CMI), changes in cytokine profiles, and mutations in the p53 gene. In this study, to develop the therapy to prevent the disease progression of BLV infection, the following were examined ; 1) Development of a peptide vaccine which can induce CMI against BLV, 2) Relationship between the changes in cytokine responses during the early phase of infection and the susceptibility to BLV-infection, and 3) Mechanisms to modulate the host immune systems by BLV.

1) Development of a peptide vaccine which can induce CMI against BLV

Epitope peptides derived from the BLV envelope (BLVEnv) Induced specific CMI in some of immunized sheep, and these sheep were prevented from the viral expressions. Neutralizing antibodies to BLV were detected even in sheep in which virus replicated, suggesting that CMI rather than humoral immunity play an important role in the prevention of BLV replication. However, it was also shown that some sheep immunized with the peptide did not show immune responses against BLV, suggesting the restricted effectiveness of the peptide vaccine.

In addition, the mannan-coated liposome has also been developed as a candidate for the effective delivery system of the peptide antigen. BALB/c mice immunized with a peptide (BLVEnv 98–117) encapsulated in the mannan-coated liposome developed peptide-specific Th 1 immune-responses.

2. Relationship between the changes in cytokine responses during the early phase of infection and the susceptibility to BLV-infection

The mRNA expressions of the interferon-γ (IFNγ), interleukin- (IL-) 2,4,6 and 10 genes were suppressed in the peripheral blood mononuclear cells (PBMC) from all of the sheep at two weeks after the BLV-challenge. Tumor necrosis factor α (TNF α) mRNA expression was enhanced in all of the sheep which were prevented from the BLV replication, whereas reduced in the sheep which showed BLV expansion. Some of sheep with enhanced expression of TNF α