Expression of a Major Piroplasm Surface Protein of *Theileria sergenti* in Sporozoite Stage

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**ABSTRACT.** A 32 kilodalton major piroplasm surface protein (MPSP) is expressed abundantly on the surface of intraerythrocytic piroplasms of *Theileria sergenti* and is considered to be a candidate antigen for vaccine development against piroplasmosis. In this study, transcripts of MPSP gene were detected in an expression cDNA library prepared from *T. sergenti*-infected tick salivary glands. Expression of MPSP in the sporozoite stage was also confirmed by immunoblot analysis. Its expression at the sporozoite and intraerythrocytic stages gives scope for possible induction of protective immunity being targeted at both stages by immunization with recombinant MPSP. —**KEY WORDS:** major piroplasm surface protein expression, sporozoite, *Theileria sergenti.*

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*Theileria sergenti* has a complex life cycle that involves two hosts, cattle and ticks, and is similar to other *Theileria* parasites. Common vectors of this parasite known in Asian countries are *Haemaphysalis* species (*H. longicornis* and *H. mageshimaensis*) [2]). Following sporozoites from tick salivary glands were injected into mammalian hosts, they differentiate into schizonts in nucleated cells, possibly non-lymphocytic origin, and thereafter into piroplasms in erythrocytes [15, 21]. Cattle infected with this parasite show elevated parasitemia, rapidly progressing anemia and mild hyperthermia [11].

The differentiation of protozoan parasites from one stage to another is accompanied by changes in the expression of proteins often recognized by host immunity [5]. These changes make development of immunological methods to control diseases difficult. In *T. parva* and *T. annulata*, antigenic proteins expressed in the sporozoite, schizont and piroplasm stages have been identified and characterized in detail [6, 18, 20]. These species induce transformation of infected bovine lymphocytes and schizont-infected cells can be cultured in vitro [14]. In *T. sergenti*, most studies on parasite antigens have been focused on antigens expressed at the piroplasm stage, because this is the stage mainly involved with the pathogenesis in cattle. In addition, difficulties of sample preparation have hindered analysis of molecules expressed in other stages of its life cycle. The fact that schizonts of *T. sergenti* are transiently detected in spleen, liver and lymph nodes at very low level [3], and that it lacks of capacity to transform infected host cells make analysis of schizont antigens difficult. Furthermore, preparation and purification of sporozoites are difficult due to very low infection rates in tick salivary glands. In piroplasm stage, the major piroplasm surface protein (MPSP) with a molecular mass of 32 kDa is highly immunogenic [7] and abundantly expressed on the cell surface as demonstrated by immunoelectron microscopy [17].

The MPSP homologues ranging from 30 to 34 kDa are conserved among *Theileria* species [16], which suggested its biological significance in parasite growth. This molecule is also a candidate antigen for vaccine development because passive immunization with a monoclonal antibody to this molecule produced partial protection against parasite challenge with reduced clinical symptoms [19]. MPSP-based synthetic peptide vaccine was effective in reducing the level of parasitemia and severe anaemia [13]. The effectiveness of an MPSP-based vaccine was also demonstrated in *T. annulata* [1]. A recombinant vaccine against sporozoite stage was effective against East Coast fever caused by *T. parva* [10]. The analysis of antigens expressed in the sporozoite stage of *T. sergenti* should be carried out. The aim of this study was to determine whether MPSP is expressed in sporozoite stage, the infective form for mammalian hosts.

*T. sergenti* Shintoku stock had been maintained in our laboratory by blood or tick passages in splenectomized calves. For the preparation of infected tick salivary glands and sporozoite materials, larvae of *H. mageshimaensis* provided by Dr. K. Takahashi, School of Veterinary Medicine, Rakuno Gakuen University, Japan, were fed on cattle showing parasitemia of approximately 10% and engorged larval ticks were kept in an incubator at 24°C until mouling was complete. Four months later, the nymphal ticks were fed on rabbits at room temperature for 3 days to induce the maturation of parasites to sporozoites within the salivary glands. The infected salivary glands were dissected out of the ticks, washed with cold phosphate-buffered saline (PBS), solubilized in sample buffer for sodium dodecyl sulfate-polyacrylamide gel electrophoresis [8], and analyzed by immunoblot analysis.

Figure 1 shows the result of immunoblot analysis using anti-MPSP monoclonal antibody (mAb), C9 [22]. This mAb recognized a protein with the a molecular mass of 32 kDa in sporozoite infected-salivary glands (Fig. 1A, lane 1). This was exactly the same size as MPSP expressed in

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piroplasms (Fig. 1 A, lane 2). The 32 kDa band was also recognized by a rabbit immune serum which had been raised against sporozoites by repeated infestations with *Theileria*-infected ticks (Fig. 1 B, lane 1 and 3) and also by a serum from *T. sergenti*-infected cattle (data not shown). The rabbit anti-sporozoite serum also reacted with a 60 kDa band in piroplasms and two bands > 100 kDa in sporozoite infected salivary glands. The former band was also detected in the sporozoite stage but reacted very weakly with the antiserum. No bands were detected in uninfected tick salivary glands with the rabbit anti-sporozoite serum (Fig. 1 B, lane 2), or with control rabbit serum (data not shown).

Transcription of the MPSP gene at the sporozoite stage was confirmed by isolation of cDNA clone from a sporozoite cDNA library. The cDNA library was prepared from infected tick salivary glands and immunoscreened with a serum from infected cattle which mainly contained antibodies against MPSP, essentially as described by Matsuba *et al.* [9]. The nucleotide sequences of two positive cDNA clones were determined. The sequence analysis revealed that one clone, SPZ-5, contained a 1.1 kbp-cDNA insert with the entire open reading frame for MPSP and its nucleotide sequence was identical to those of other MPSP previously reported [9] (data not shown) and another clone contained a 1.5 kbp-cDNA insert encoding a 60 kDa antigenic protein which was expressed in sporozoite and piroplasm stages (submitted for publication). The result in this study show that MPSP gene is transcribed in the sporozoite stage and that the mature polypeptide may be expressed after similar posttranslational processing in piroplasms, as indicated from their similar molecular sizes.

In *T. parva* and *T. annulata*, surface proteins of 67 kDa and 54 to 85 kDa [4, 12], respectively, are expressed, but we could not detect bands recognized strongly by anti-*T. sergenti* sporozoite serum at similar molecular mass range. Instead, our result indicated that MPSP is one of major antigens at the sporozoite stage of *T. sergenti*.

Immunization with MPSP-derived peptides partially protect animals from developing high parasitemia and severe anemia after sporozoite challenge. This indicates that immunity against this molecule interfere with parasite growth, but do not completely inhibit it [13]. A vaccine induced immunity against the piroplasm stage may play a major role through inhibition of interaction between parasites and host erythrocytes. In addition, expression of this molecule at sporozoite stage indicates that immunity against this molecule is also induced by vaccination with MPSP and affect parasites at steps of invasion into host cells and subsequent development into schizonts. A recent study indicated that *T. parva* expresses a MPSP-homologue at the piroplasme stage (Dr. Y. Yagi personal communication). Therefore, the inclusion of MPSP into a recombinant vaccine under development using a sporozoite surface molecule, p67 will be a valuable addition to a vaccine that is effective for bovine theileriosis.

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REFERENCES
