ACTIN BINDING ACTIVITY OF A 32kDa SURFACE PROTEIN OF *THEILERIA SERGENTI* PIROPLASM

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Theileria sergenti is a tick-borne protozoa in cattle and causes anemia as an intraerythrocytic piroplasm. This protozoan disease is one of the most important diseases in grazing cattle in Japan, because of the economical losses it causes. A 32kDa surface protein (p32) of *T. sergenti* piroplasm is a major immunodominant protein. There are several reports on its immunogenicity but not on its functions. In this study, we constructed a recombinant baculovirus (rAcNPV) into which the p32 open reading frame deleted of the nucleotide sequence encoding signal peptides was inserted downstream from the baculovirus polyhedrin promoter and investigated whether recombinant p32 (NSp32) expressed in *Spodoptera frugiperda* (Sf9) cells bound to cytoskeletal proteins, especially actin.

Expression of the NSp32 in rAcNPV-infected Sf9 cells was confirmed by immunoblotting analysis with an anti-p32 MoAb. The molecular weight of NSp32 was 32kDa, which was the same as that of authentic p32. The NSp32 was associated with a Triton X-100-insoluble fraction of Sf9 cells, and the amount of NSp32 bound to this fraction was reduced by treatment with an inhibitor of actin-binding protein, deoxyribonuclease. Double fluorescent staining using tetramethylrhodamine isothiocyanate-conjugated phalloidin in combination with a fluorescein isothiocyanate-conjugated anti-mouse immunoglobulin antibody and the anti-p32 MoAb revealed localization of NSp32 along microfilaments. These results indicated the binding activity of NSp32 to actin of Sf9 cells. Furthermore, NSp32 showed binding activity against bovine erythrocyte membranes treated with Triton X-100, and against polymerized (F–) actin. Peptides synthesized according to the deduced p32 amino acid sequence which contained charged amino acid-rich stretch polymerized rabbit muscle actin. These results indicated that p32 interacted with the erythrocyte membrane skeleton, possibly via actin.

The results of this study suggested that the interaction of piroplasm surface molecule(s) with the inner face of erythrocytes via membrane skeleton proteins is essential for growth and development of the parasite.