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Apicomplexan parasites in the genera *Babesia* and *Plasmodium* infect mature erythrocytes and cause disease in animals and humans. A principal vaccine strategy is to direct immune responses against merozoite surface proteins and block entry into the host erythrocyte. Previous work in both *B. bovis* and *P. falciparum* has identified potential vaccine candidates that may induce immune protection during natural infection and are thought to be involved in the erythrocyte invasion process. Although many of these proteins have been characterized and their sequence derived, their function and role in invasion are not yet fully understood. Merozoite surface proteins are thought to be involved in initial binding of the merozoite to the erythrocyte surface. In malaria, there is compelling evidence that merozoite surface protein –1 (MSP-1) of *P. falciparum* is involved in erythrocyte binding and invasion. In babesial parasites, the ability of antibodies against *B. bovis* merozoite surface antigen – 1 (MSA-1) to prevent merozoite attachment to the erythrocyte and inhibit erythrocyte invasion in vitro, supports a role for MSA-1 in mediating merozoite attachment to the erythrocyte. Since breakthrough isolates in vaccinated cattle express allelic variants of MSA-1, there may be selection of an MSA-1 variant population under immune pressure. Therefore, I am interested in better understanding the role of MSA-1 in invasion with the goal of defining conserved molecular targets for immune control.

I will test the **hypothesis that merozoite surface antigen – 1 (MSA-1) of *Babesia bovis* mediates erythrocyte invasion through binding to the erythrocyte surface.** To more definitively determine the role of MSA-1 in erythrocyte invasion, two questions will be addressed. First, does MSA-1 mediate binding of the merozoite to the erythrocyte surface? To address this question, in **Specific Aim 1**, I will determine if normal erythrocytes bind to COS7 cells expressing recombinant *B. bovis* MSA-1 on the surface. Second, is MSA-1 function necessary for erythrocyte invasion? This question will be addressed in specific aims 2 and 3. In **Specific Aim 2**, I will determine if inhibition of MSA-1 expression by antisense RNA disrupts erythrocyte binding and invasion. Merozoites will be transfected with plasmids containing *msa-1* in the antisense orientation. Reduction in MSA-1 expression will be detected in immunoblots using a monoclonal antibody to MSA-1. The effects of the reduced MSA-1 expression will be detected by binding assays and monitoring the growth cycle and percentage parasitized erythrocytes (PPE) in culture. Finally, in **Specific Aim 3**, I will determine if disruption of *msa-1* inhibits erythrocyte binding and invasion. Merozoites will be transfected with plasmid DNA containing a selectable marker and a 469 base pair internal region of *msa-1* for gene targeting. The effects of MSA-1 disruption will be similarly detected by monitoring the in vitro growth cycle and percentage parasitized erythrocytes (PPE). The two alternative, but complementary approaches that will be used, inhibition (specific aim 2), and deletion (specific aim 3), are each essential for adequate interpretation of the potential outcomes, particularly if *msa-1* deletion is a lethal event.

The primary purposes of this research are to increase our understanding of the mechanisms of erythrocyte invasion used by babesial parasites, and to develop a system for investigating the function of other babesial molecules using genetic manipulation. The information obtained will expand our knowledge of comparative invasion mechanisms used by apicomplexan hemoparasites, including *Theileria* and *Plasmodium* spp. Characterizing the proteins involved in erythrocyte invasion is ultimately necessary to optimize the selection of vaccine components. The results of this project, as well as the transfection techniques that will be developed, will allow myself and others to begin targeting regions of functional significance for further study.