Identifying the Protein Interactions between *Plasmodium* Sporozoites and Hepatocytes that Lead to Malaria

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Introduction

Claiming more than a million lives in 2008 alone, malaria is now the 5th leading cause of death in low-income countries worldwide (World Health Organization, 2009). Malaria is spread mainly by the bite of the *Anopheles* mosquito that injects the infectious sporozoites, one of many stages in the relatively complex life cycle of the parasite *Plasmodium*. These sporozoites invade the liver of the mammalian host, and within a membrane bound structure called the parasitophorous vacuole, they differentiate into exoerythrocytic forms (Silvie, 2003). Though the entry into the hepatocytes has remained largely unknown, some studies in both mice and human cells shed new light by demonstrating that the host protein CD81 is required for infection by sporozoites of *P. falciparum* and *P. yoelii* (Silvie, 2003).

CD81, a tetraspanin transmembrane protein found on the surface of hepatocytes, has been known to contribute to cell membrane remodeling events in various cells such as sperm-egg fusion and formation of syncytia in viruses (Silvie, 2003). It was also recently shown that the tetraspanin CD81 is responsible for the internalization of *Listeria monocytogenes*, the bacterial pathogen responsible for lysteriosis (Tham, 2010). Similarly, CD81 is believed to be involved in the fusion of a sporozoite membrane with a hepatocyte during entry, since rodent hepatocytes that lack CD81 are incapable of being infected by *P. yoelii* sporozoites (Silvie, 2003).

Other studies in mice hepatocytes show that there is an important interaction between CD81 and another transmembrane protein, CDP9-1. When knockout CD9P-1 hepatocytes from mice are exposed to *P. yoelii* sporozoites, the cells are more prone to infection (Charrin, 2009). Over-expression of CD9P-1, on the other hand, leads to decreased infectivity of host cells indicating that CDP9-1 is a negative regulator of CD81(Charrin, 2009). Given the role CD81 has on internalization and fusion of membranes in biological systems, and its regulation in mice hepatocytes by CD9P-1 contributing to malarial infectivity, it would be interesting to see if this role is conserved in the internalization of the sporozoite by the hepatocyte as well.

Hypothesis

This study will test the hypothesis that a membrane surface protein on *P. yoelii* sporozoites binds to the CD81 transmembrane protein on mice hepatocytes. As seen in other cells where CD81-protein interactions contribute to cell membrane rearrangement events, a sporozoite surface protein and CD81 hepatocyte protein interaction seems likely. Results from this study will bridge the gap in knowledge regarding the formation of the parasitophorous vacuole that allows the infection and differentiation of *Plasmodium* sporozoites in hepatocytes.

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Specific Aims

1. The goal of this study is to determine if there is a strong interaction between any membrane protein on *P. yoelii* sporozoites and the CD81 transmembrane protein on rodent hepatocytes using a “photoactivatable cross-linking reagent” in Label Transfer.
2. Gel electrophoresis and autoradiography will be used to separate and identify an approximate molecular weight of the unknown radiolabeled sporozoite protein.

Experimental Procedure

In an effort to identify the membrane protein on sperm that binds to a known protein on oocytes, scientists during the early 1990’s utilized a radioactive cross-linking reagent, known as the Denny-Jaffee reagent, that is bound to the known protein and is transferred onto a distal partner protein within a series of steps (Bleil, 1990). This method provides an innovative way in which unknown proteins hypothesized to interact with known proteins can be tagged and isolated, and will be used in determining the protein-protein interaction in this study. Thus, the materials and methods used here will be very similar to those used by Bleil (1990).

Modification of CD81 with Denny-Jaffee reagent (numbered illustration, Fig 1, given on p. 4.)

Purified CD81 rodent hepatocyte proteins and *Plasmodium yoelii* sporozoites will serve as good models since past studies leading up to this were conducted mainly on both of these models. (1)Treatment of isolated hepatocyte CD81 proteins with 125I-Denny-Jaffee reagent in a sodium bicarbonate solution allows the cleavable photoactivated cross-linker to remain deactivated yet covalently bound to the CD81 protein. (2)Separately cultured *P. yoelii* sporozoites will be combined with the radiolabeled CD81 ready for photoactivation that will link the CD81 and the interacting sporozoite protein. (Preparation of controls described differently below).

A potential pitfall of this study is that the binding of the Denny-Jaffee reagent could interfere with the interaction between the CD81 complex and a sporozoite membrane protein. If the reagent does not bind to the positive control, and the proteins did not bind with each other in the test condition due to interference from the reagent, then no conclusions can be made regarding the characteristics of the protein-protein interaction. However, the inclusion of a negative control would yield results of what is expected when the proteins are incapable of binding with each other due to interference of the reagent and this will help us identify the problem.

Photoactivated cross-linking of proteins and cleavage of cross-linkers.

Photoactivation is performed by exposure to a high pressure mercury lamp.(3)This photoactivation causes the cross-linker to bind to the protein that CD81 is bound to, thus tagging the protein of interest. (4)Finally, incubation in a solution of sodium dithionite reduces and cleaves the cross-linkers resulting in two individually labeled proteins.

Preparation of negative and positive controls.
Preparation of the negative control consists only of step (1) as mentioned above. This negative control is expected to demonstrate that the Denny-Jaffee reagent is functional with CD81 proteins. This will also serve as the protein molecular weight indicator of a CD81-125I-DJ complex during electrophoretic analysis. (5)Preparation of the positive control consists of CD81 rodent hepatocyte proteins exposed to a solution of centrifuged sporozoites. The positive control will then be (6)radiolabeled, (7) photoactivated, and (8)finally reduced to demonstrate functionality of the cross-linker. In the electrophoretic analysis described below, this sample should show the identical results as the test sample described above.

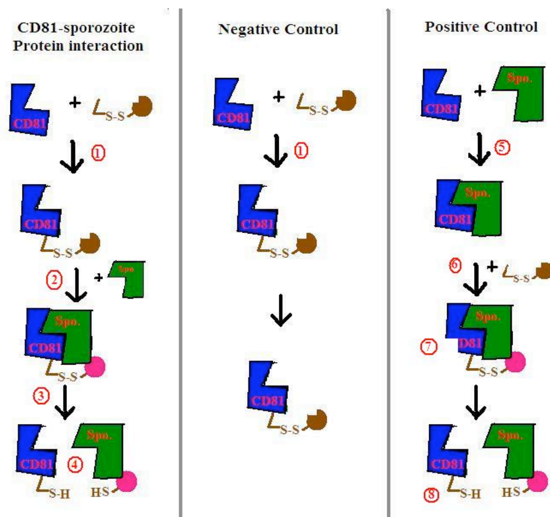

Figure 1: Attachment,photoactivation (pink), and reduction of Denny-Jaffee reagent (brown) to CD81 proteins (blue) and sporozoite protein (green).

Electrophoresis of products
When gel electrophoresis is run with a molecular weight marker on the final radiolabeled protein products and the gel is exposed to x-rays to distinguish proteins, the test condition and the positive control should yield identical results. The combined CD81-125I-DJ complexes and sporozoite protein-125I-DJ complexes should be seen at different marker lengths in the each of their lanes. The negative control should yield a band at a single molecular weight since only radiolabeled CD81-125I-DJ complexes were formed. Additionally, 125I-DJ alone, as well as CD81 alone will be run in two separate lanes to identify the molecular weights of those individual molecules that will aid as a reference. Using these results, we can arrive at an approximate molecular weight of the sporozoite protein of interest.

Another potential pitfall is that CD81 is being used without any of its partner proteins, such as CD9P-1, that contribute to the infectivity of the hepatocyte. The inclusion of these proteins in these experiments, however, is beyond the scope of this study and can be incorporated into a future study based on the results from this experiment. In the future, the proteins of interest can be excised from the gel and protein-NMR can be run to understand the structure and binding characteristics of the sporozoite protein.

Conclusion

The results of this study may allow us to draw parallels to membrane fusion events involving CD81 in other systems, giving us a better understanding of how a parasitic *Plasmodium sporozoite* is capable of invading hepatocytes, one of the primary and most crucial stages of malarial infection in mammals.

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