ABSTRACT

LINDEL KRIGE
Coagulation Factors Involved in the Pathology of Placental Malaria
(Under the Direction of JULIE M. MOORE)

Pregnant women and children are most vulnerable to malarial infection. Malaria in pregnancy leads to intrauterine growth restriction and preterm deliveries, resulting in low birth weight babies. The underlying mechanisms that lead to these poor birth outcomes are not well understood, but it is known that severe inflammation in the placenta together with excessive fibrin deposition are common features of placental malaria, and correlate with low birth weight. These inflammation and coagulation processes in the placenta occur as an immunological response to the malaria infection. This study addresses the hypothesis that local malaria-induced inflammatory responses induce placental coagulopathy, which is turn leads to significant compromise in placental function, and therefore, fetal distress. This study will mainly focus on the role of coagulation and immunological factors in the disease response process of mice. Murine placental malaria has proven to be an effective model for P. falciparum infection in humans. Mouse RNA has been isolated, and primers for each coagulation factor have been developed. Using these primers and isolated RNA, the next step will be to conduct real time PCR to determine possible upregulation of the coagulation factors. Further research possibilities include determining whether fetal or maternal cells initiate this immunological response leading to inflammation and coagulation in the placenta. Identifying the role of coagulation factors involved in the immunological response to placental malaria will provide further understanding on malarial pathogenesis and ways to prevent fetal growth restriction.

INDEX WORDS: Placental Malaria, Coagulation, Mouse Model, Tissue Factor, Tissue Factor Pathway Inhibitor, Plasminogen Activator Inhibitor, Thrombomodulin, Protease Activated Receptors

COAGULATION FACTORS INVOLVED IN THE PATHOLOGY OF PLACENTAL MALARIA

by

LINDEL KRIGE

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LINDEL KRIGE

Approved:

Dr. Julie M. Moore 04/29/10
Faculty Research Mentor

Approved:

Dr. David S. Peterson 04/29/10
Reader

Approved:

Dr. David S. Williams 05/07/10
Director, Honors Program, Foundation Fellows and
Center for Undergraduate Research Opportunities

Approved:

Dr. Pamela B. Kleiber 05/07/10
Associate Director, Honors Program and
Center for Undergraduate Research Opportunities

DEDICATION

To my family, friends, and mentors who have supported me throughout my studies. I am so grateful for your continued encouragement in the pursuit of my goals and enthusiasm in my accomplishments.

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CHAPTER 1
INTRODUCTION

Every year, over 250 million malaria cases are reported around the world and over 1 million deaths occur.[1] Most of these cases are due to *Plasmodium falciparum* and occur in pregnant women and children. Previous infection provides no immunity to primigravid women, but with multiple pregnancies, immunity to malaria develops. Pregnant women infected by malaria exhibit intrauterine growth restriction (IUGR) and give birth to low birth weight (LBW) babies. McGregor et al. examined fetal: placenta ratios and found that placental weight was unaffected by malarial infection. Thus, low birth weight was due to placental insufficiency rather than limited placental growth.[30] Pre-term delivery has been associated with high levels of fibrin deposition in the placenta.[28] Although not all patients show fibrin deposition, the majority of patients have elevated d-dimers in their blood; d-dimers are the products of fibrinolysis. The presence of d-dimers in the blood serves as markers for presence of fibrin[2]. The Moore research group has hypothesized this excessive fibrin deposition leads to placental insufficiency and thus, low birth weight babies.

Immunologic Response to Placental Infiltration by the Parasite

P. falciparum infects red blood cells (iRBCs) and expresses the surface protein, *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), on the red blood cell membrane. PfEMP1 attaches to host molecules such as Intercellular Adhesion Molecule (I-CAM) in the brain microvasculature and Chondroitin Sulfate A (CSA) among other molecules in the placenta.[31] This leads to sequestration of iRBCs in the intervillous space (IVS) in the

placenta. The intervillous space is the interface of maternal blood and fetal structures that house the fetal blood circulation. The parasites digest hemoglobin in the red blood cell for nutrients. One of the byproducts of this digestion is heme, toxic to parasites, so the parasite converts heme into hemozoin.[32] Hemozoin laden monocytes and hemozoin trapped in fibrin are common features of placental malaria.[53] In response to HETE-15, a lipid component of hemozoin, macrophages secrete increased levels of the pro inflammatory cytokines, IL-1B and TNFα which leads to the production of metalloproteinase-9 (MMP-9). MMP-9 sheds TNFα from its precursor creating a positive feedback loop between TNFα and MMP-9.[52] The release of hemozoin also causes the syncytiotrophoblast (ST), the fetal cell, to mount an immunologic response against the parasite (D. Sarr et al, unpublished data).[29] The syncytiotrophoblast is able to phagocytize iRBCs (early in pregnancy but not at term) and release cytokines and pro inflammatory chemokines like interleukin-8 (IL-8), macrophage inflammatory protein 1 alpha and beta (MIP-1α & β), and macrophage chemoattractant protein 1 (MCP1), increasing macrophage recruitment to the placenta.[43,49-51,58] Menendez et al. have found a correlation between the mononuclear infiltration of the placenta and low birth weight babies.[28] This immunological infiltrate in the placenta decreases maternal blood flow and produces cytokines such as interferon-gamma (IFN-γ), interleukin-2 (IL-2), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), which may negatively affect maternal-fetal nutrient exchange.[28,33]

There are various types of immunological pathways. The placenta, an anti-inflammatory organ, usually follows a TH2 pathway which targets the adhesion of parasites and favors the production of anti-inflammatory cytokines like interleukin-10 (IL-10). However, malaria infection in primigravid women disrupts the balance between the pathways and shifts the immune pathway away from TH2 and closer to TH1, which is associated with spontaneous

abortions. The TH1 pathway focuses specifically on parasite clearance or suppression and leads to the release of cytotoxic cytokines such as IFN-γ, stimulating macrophages to produce TNFα, which leads to phagocytosis of the parasite and further cytokine production.[34-36] iRBCs lead macrophages to express TF through the stimulation from these inflammatory cytokines like IFN-γ and TNFα.[39-40] Connecting inflammation and coagulation, Imamura et al. determined macrophages in the placenta express tissue factor and are surrounded by fibrin and other coagulation factors.[41] Tissue Factor (TF) expression activates the extrinsic coagulation cascade.[2-17] When thrombin is activated in the coagulation cascade, it converts fibrinogen to fibrin and activates Protease Activator Receptors (PARs) 1,2, and 4. PAR 1 leads to further inflammation by upregulation of surface binding proteins and cytokines like TNFα and IL-1β (Figure 1.5).[2,18] TNFα and IL-1β work together to cause pathogenesis.[45] TNFα and apoptosis of iRBCs cause an increased amount of microparticles which express TF on their surface and propagate coagulation.[19-22] Patients with *P. falciparum* also show decreased levels of antithrombin III and increased levels of the thrombin-antithrombin III complex. [37-38] Thrombin is inactivated through binding to antithrombin and forming the thrombin-antithrombin complex. The mass infiltration of immune cells along with coagulation activation leading to fibrin deposition causes narrowing of the intervillous space in the placenta and most likely disrupts blood flow to the fetus resulting in fetal growth restriction.

Murine Malaria as a Model of Infection

It is hypothesized coagulation as well as a robust immunological response drives the pathogenesis seen in placental malaria, but the mechanism is not well understood. In order to study this mechanism, the Moore research group has developed a mouse model for placental malaria. Two types of mice are being studied, A/J and C57BL/6 (B6). The murine species of

malaria used, *Plasmodium chabaudi chabaudi AS*, causes similar effects to those produced by the human species, *P. falciparum*. Similar to humans, pregnancy increases vulnerability to infection. Non pregnant B6 mice survived infection, while pregnant mice were unable to maintain pregnancy past the mid gestational period.[42] Infected, non-pregnant (INP) mice exhibit a TH1 immune response, producing high levels of inflammatory cytokines like IL-12, TNFα, and IFN-γ to clear infection. IFNγ and TNFα suppress fetal development, and induce apoptosis in trophoblast cells. IL-10 production can counteract these effects and reverse fetal resorptions.[46-47] Previous lab studies have investigated the response to infection in pregnant mice. Fetal loss is associated with increased IFN, TNF, IL-1B with high levels of soluble TNF receptors. Regardless of pregnancy status, infected B6 mice show a steady production of IFN-γ in plasma with peak production at day 9 of infection.[42] B6 mice exhibit no difference in TNF production between infection groups except for day 10 where production is higher in infected pregnant (IP) mice, and proinflammatory IL-1β levels peaked at d10 of infection as well. Interestingly, soluble TNF Receptor II (sTNFRII) plasma levels increased in infected pregnant (IP) mice in comparison to uninfected pregnant (UP) and infected not pregnant (INP) mice. Counter regulation by IL-10 is also observed with a dramatic increase in production at d10.

Previous studies in the Moore research group have also examined local response to infection. Cultured fetal-placental units from infected mice produced higher levels of IFNγ than uninfected mice, but there were no comparable difference in TNFα production between the two groups. sTNFRII was produced at higher levels in infected mice. IL-10 levels were undetectable at the local, placental level.[44]

Another study examined infected IFNγ -/- mice in comparison to infected B6 mice. Parasitemia peaked earlier (d10 vs. d11) in mutant knockout mice. Both mice eventually

experienced pregnancy failure, but the knockout mice retained viable fetuses longer. In TNFα ablation studies, TNFα ablated mice retained placental structure in comparison to normal mice which exhibited hemorrhaging, thinning of the labyrinth, and thrombosis.[42] Looking at protein expression in tissue sections, infected mice show increased amounts of tissue factor compared to uninfected mice. Furthermore, TNFα ablated mice tissue sections show decreased amounts of Tissue Factor.[42]

The two types of mice, A/J and B6 exhibit very different immunological responses to infection. B6 mice, regardless of pregnancy status, are able to clear infection with a robust cytokine response.[44] There is increased interferon gamma (IFNγ) and IL-10 in B6 mice and increased IL-4 and TNFα in A/J mice. The high levels of IFNγ indicate a TH1 response in B6, which protects these mice against peak parasitemia and death. A/J mice produce high levels of IL-4, indicating a TH2 response which does not protect against high parasitemia and mortality.[23] Histologically, B6 mice show hemorrhaging, necrosis, and thrombosis, whereas A/J mice show inflammatory infiltrate into the labyrinth (Figure 1.1)

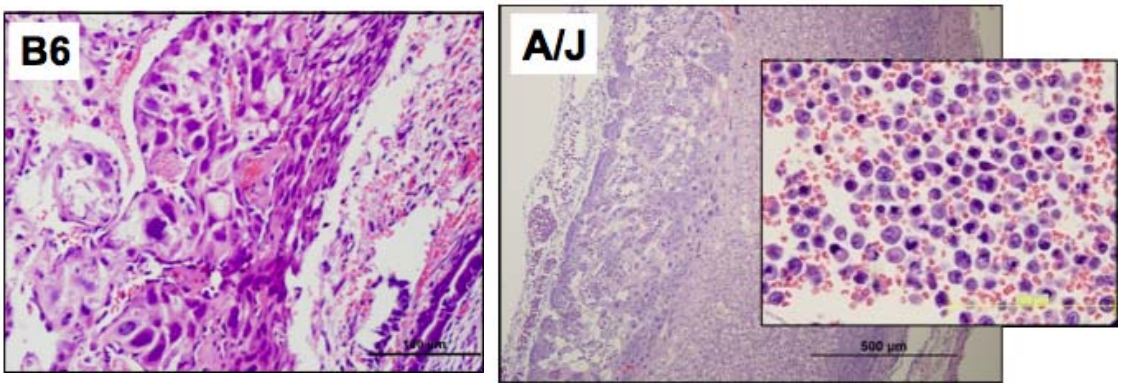

Figure 1.1: Comparison of B6 and A/J d11 Infected Mice Placental Tissue. B6 mice show hemorrhage, necrosis, and thrombosis. A/J mice show labyrinthine inflammatory infiltrate. Credit: J. Poovassery, D.

Examining Coagulation

The purpose of this experiment is to examine the B6 and A/J gene regulation of coagulation factors present in the placenta in relation to the presence of disease pathogenesis. The coagulation factors examined include Protease Activated Receptors (PAR) 1-4, Plasminogen Activator Inhibitor (PAI) 1 & 2, Tissue Factor Pathway Inhibitor (TFPI) 1 & 2, Tissue Factor (TF), and Thrombomodulin (TM), and their roles are discussed in Table 1.1.

The role of coagulation on malaria pathology has been studied in cerebral malaria. iRBC sequestration and related events such as hypoxia, cytokine and GPI production lead to the activation of Factor VIIa and TF, which serves as the initiation of the coagulation cascade. Factor VIIa/TF activate Factor X, which works along with Factor V, platelets, and iRBCs to convert prothrombin to thrombin leading to clotting and platelet aggregation during the propagation and amplification phase of coagulation. Thrombin along with Factor Xa and VIIa/TF lead to PAR activation by cleaving the surface protein on PAR. This leads to increased inflammation through cellular activation, expression of adhesion molecules, and inflammatory cytokines, which in turn initiate further coagulation through TF, propagating the inflammation-coagulation cycle.[54]

Table 1.1: Coagulation Factors Important to This Study

Coagulation Factor	**Effect**	**Overall Potential Effect (relation to fibrin)**
Tissue Factor (TF)	Binds & activates Factor VII which activates factor IX and factor X which activates thrombin	Increases fibrin deposition
Thrombomodulin (TM)	Activates protein C which inactivates thrombin	Decreases fibrin deposition
Plasminogen Activator Inhibitor (PAI)	Inhibits activation of plasminogen to form plasmin	Prevents fibrin breakdown
Tissue Factor Pathway Inhibitor (TFPI)	Binds to factor Xa inactivates TF	Decreases fibrin deposition
Protease Activated Receptors (PARs)	1) adhesion molecules (CSA) 2) cytokine release: Inflammation – TNFα and IL-1β 3) cellular activation --monocyte recruitment	Increases fibrin deposition
Thrombin	Fibrinogen → fibrin; platelet aggregation; Factor XI activation	Increases fibrin deposition

CHAPTER 2
MATERIALS AND METHODS

Experimental Design

A/J and B6 mice were used according to the model described in previous literature.[48] This study focuses specifically on expression of coagulation factors in mice at day 10 of infection and gestation in the midst of peak parasitemia. These mice start aborting at day 10, and by day 11, the embryos are no longer viable.[42]

Upon sacrifice, mouse uteri were examined for signs of active abortions, resorptions, and hemorrhaging. Uteri were collected from mice at time of sacrifice and placed in RNA later and frozen.

For this specific experiment, only day 10 mouse tissue was used. Day 10 mice were used for reasons cited in the introduction, specifically inflammation and coagulation seems to be at its peak. For comparison, four groups were examined: IP A/J, UP A/J, IP B6, and UP B6.

- 2 UP A/J: all factors
- 5 UP B6: PAR 1, PAR 2, TM, TF
- 4 UP B6: PAI 1, TFPI 1
- 4 IP A/J: TF, PAR 1, PAR 2, TFPI 1, and TM
- 3 IP A/J: PAI 1
- 5 IP B6: TF, PAR 1, PAI 1
- 6 IP B6: PAR 2, TFPI 1, TM

RNA Isolation

RNeasy mini-kit was used for RNA isolation. First, uterine tissue was thawed and placed in 600µL RLT buffer which lyses the cells and denatures the proteins. Tissue was then homogenized with the Tissue Miser and transferred to an Eppendorf tube. To precipitate RNA, 500 µL of 70% ethanol was added to the homogenized tissue. Sample was centrifuged for 15 seconds at 10,000rpm and flow through was discarded. 700 µL of RW1 buffer was added to wash and was centrifuged in the same manner. Then, 500µL of RPE buffer for washing was added and the sample was centrifuged again. 500 µL of RPE was added again but centrifuged for 2 minutes at 10,000rpm to dry the column. Lastly, sample was centrifuged with RNase free water for 1 minute and repeated with elute from the previous centrifugation to maximize concentration. Concentration of RNA was determined by measuring the optical density of the sample using a Nanodrop spectrophotometer.

DNA Removal

The Applied Biosystems DNA-free™ Kit was used to remove contaminating DNA from the RNA samples. 10x DNase I Buffer and rDNase were added to the sample and mixed gently to remove DNA. Sample tubes were then covered with parafilm and incubated in a hot water bath at 37ºC for 20 minutes. The DNases in the sample are then deactivated with DNase Inactivation Reagent to prevent the DNases from destroying cDNA when it was generated. The sample was incubated for 2 minutes and room temperature and centrifuged at 10,000rpm for 1.5 minutes.

cDNA Generation

The Applied Biosystems High Capacity cDNA Reverse Transcription Kit was used to generate cDNA from the treated RNA samples. A 2x master mix was prepared containing 10x RT Buffer, 25x dNTP mix, 10x Random RT Primers, Multiscribe™ Reverse Transcriptase, and nuclease free water. Equal volumes of master mix and RNA were added to a PCR reaction tube and centrifuged to eliminate air bubbles. These samples were loaded in the thermocycler and were run according to the program specified in the Applied Biosystems protocol. For controls in quantitative PCR, RT negative controls were prepared in the same manner except water was substituted for Multiscribe™ Reverse Transcriptase. Thus, any amplification seen in the RT negative control would be due to DNA contamination.

Primer Design

Primers for each target DNA sequence were designed using the Primer Express software system. Primers were designed to 1) have a similar length to the target DNA sequence 2) avoid forming secondary structures such as dimers 3) have similar melting points in the forward and reverse primers and 4) have a melting temperature suitable to the length and composition of the target DNA sequence.

Primers Used:

TFPI1for: GGATGTGAAGGGAACGAGAAC

TFPI1rev: TGGGGAAGGGGAGTGGA

TMfor: TAGGGAAGACACCAAGGAAGAG

TMrev: GAGAGAGAGAGGAGAGGAGAGG

PAR1for: TACATAACACCCCTTCGGCTAT

PAR1rev: AACACACCTTTCTCCTCTCGTC

PAI1for: GGCACAACACTTTCATTCAGC

PAI2rev: CGACTTTTCTTACACCCTTTCC

PAR2for: CACCTGGCAAGAAGGCTAAG

PAR2rev: CCCAGGGTTACTGACGCTAA

TFfor3: CCACCATCTTTATCATCCTCCT

TFrev3: AGCCTTTCCTCTATGCCAAGC

Primer Testing – Classic PCR

For each target, multiple primers were designed. Standard PCR using all available primers for each target was performed to optimize the primer selected for qPCR. The Roche Taq DNA Polymerase (1 U/μL), dNTPack was used for all standard PCR experiments. A master mix was prepared in PCR reaction tube containing PCR grade water, PCR grade nucleotide mix, forward primer, reverse primer, template DNA, 10x PCR reaction buffer containing $MgCl_2$, and Taq DNA Polymerase. Samples were loaded into the thermocycler and the program specified in the protocol was run adjusting for the melting temperature of the specific targets.

Amplification of targets was examined by running the amplified product through a 1.2% agarose gel containing ethidium bromide to visualize DNA under UV light. Samples were loaded with a loading buffer containing a dye and run with a 1000bp DNA ladder for size comparison. Using GeneSnap from Syngene software, gel was visualized and recorded on film. Size of target, absence of secondary structure formation, and amount of amplification (brightness) were used to select the best primers for use in qPCR.

Quantitative PCR

First, 96-well PCR plates were designed to run targets with similar annealing temperatures on the same plate. For each target, a mixture was prepared containing the forward and reverse primers, nuclease free water, and a SYBR green master mix. SYBR green is a fluorescent dye used to measure amplification during qPCR. cDNA for each mouse sample was added to the wells containing the target mixture. The plate was then covered and centrifuged at 2900rpm for 5 minutes to eliminate any air bubbles. The plate was placed in qPCR thermocycler and amplification was recorded in the Applied Biosystems software system. RT negative as well as nontemplate controls were used, and 18s was used as the normalizing gene.

Applied Biosystems qPCR thermocycler protocol:

Step 1: 50ºC for 2 minutes (1 cycle)

Step 2: 95ºC for 10 minutes (1 cycle)

Step 3: 95ºC for 15 seconds (40 cycles)

60ºC for 1 minute (40 cycles)

Statistical Analysis

For each target, A/J and B6 mice were separated and their relative quantification (RQ) values were calculated using the following equation:

$$RQ = \frac{2^{Target\ UP\ Average\ CT - Target\ IP\ Average\ CT}}{2^{18s\ UP\ Average\ CT - 18s\ IP\ Average\ CT}}$$

This gave an expression value normalized again 18s showing a fold increase relative to UP mice.

Standard Error of the Mean (SEM) was calculated using standard deviation (SD) according to the following equation:

$$S = \frac{2^{Target\ UP\ SD - Target\ IP\ SD}}{2^{18s\ UP\ SD - 18s\ IP\ SD}}$$

PAI-1 data were log transformed for best graphical appearance due to the large amplification difference between A/J and B6 mice.

CHAPTER 3
RESULTS

To examine the role of coagulation in placental pathology in A/J and B6 mice, coagulation factor gene upregulation in each mouse strain was determined through quantitative PCR. It was hypothesized B6 mice would show upregulation of coagulation factors in comparison to A/J mice based on previous lab data examining cytokine responses and histology in the two different mouse strains. In order to compare the two groups, each mouse strain was normalized against the housekeeping gene, 18s and calibrated against uninfected pregnant mice of their respective strains.

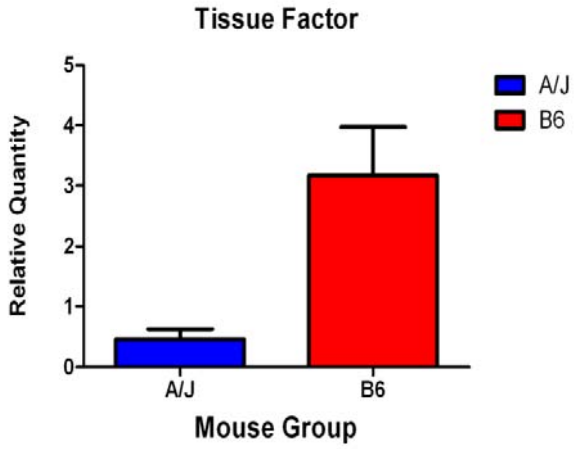

Figure 3.1: Tissue Factor (TF). *Gene expression in d10 A/J(n=4) and B6(n=5) mice compared to their respective UP groups.. A/J mice show downregulation. B6 mice show upregulation*

Procoagulants: TF, PAR1, PAR2

Tissue factor plays a significant role in coagulation as the initiator of the extrinsic coagulation cascade. As anticipated, the A/J mice show down regulation of Tissue Factor expression while the B6 mice show approximately three times upregulation in infected mice compared to uninfected mice.

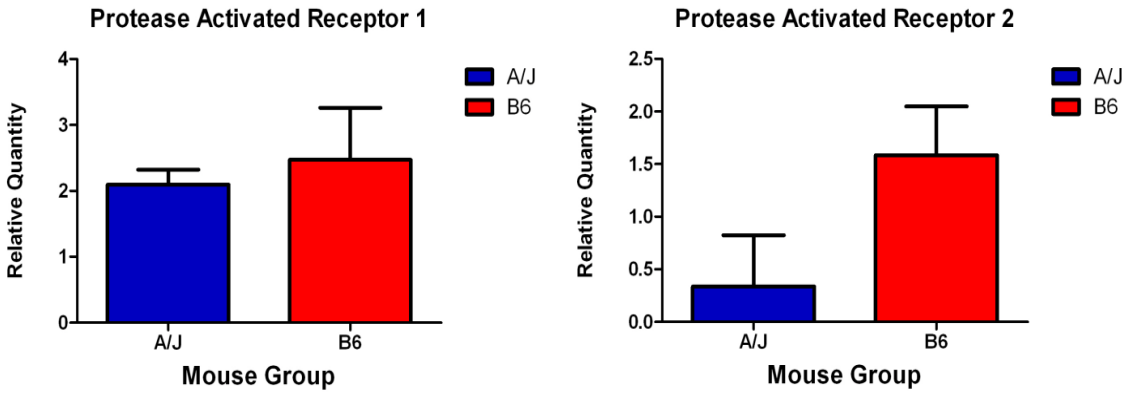

Figure 3.2: Protease Activated Receptors 1 & 2 (PAR 1 & 2). *Both d10 A/J (n=4) and B6 (n=5) infected mice show upregulation of PAR1 gene expression. Day 10 A/J (n=4) infected mice show downregulation of PAR2 gene expression, while d10 infected B6(n=6) mice show upregulation of PAR2 gene expression*

The Protease Activated Receptor (PAR) family consists of receptor g-coupled proteins that are activated by thrombin. The activation of these receptors lead to cellular activation ultimately resulting in release of inflammatory cytokines, upregulation of cellular adhesion molecules, and recruitment of other immunological cells. PAR 1 gene expression is upregulated two fold in infected A/J mice compared to uninfected A/J mice. B6 infected mice also show more than two fold PAR 1 gene upregulation compared to B6 uninfected mice. PAR 2 gene expression is down regulated in infected A/J mice, but B6 infected mice show about a 1.5 fold upregulation of PAR 2 gene expression.

Anti-fibrinolytic Factors: PAI-1

Plasminogen Activator Inhibitor 1 (PAI-1) prevents the degradation of fibrin by inhibiting the conversion of plasminogen to plasmin. Plasmin directly acts on the fibrin clot to break it down. A/J uninfected mice showed extremely little, basically no, PAI-1 expression, thus the infected A/J mice showed tremendous upregulation of PAI-1 in comparison to these uninfected A/J mice. Infected B6 mice also show upregulation of PAI-1 compared to uninfected B6 mice as was expected from the hypothesis.

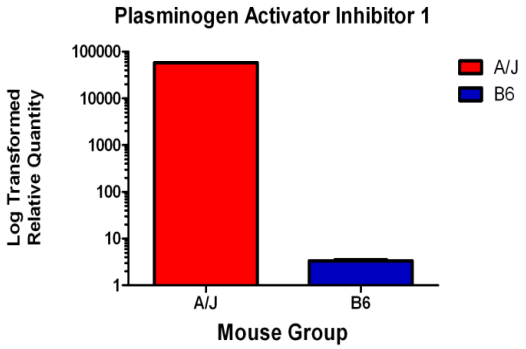

Figure 3.3: Plasminogen Activator Inhibitor 1 (PAI-1). *D10 infected A/J(n=3) and B6(n=5) mice show upregulation of PAI-1expression compared to uninfected A/J mice.*

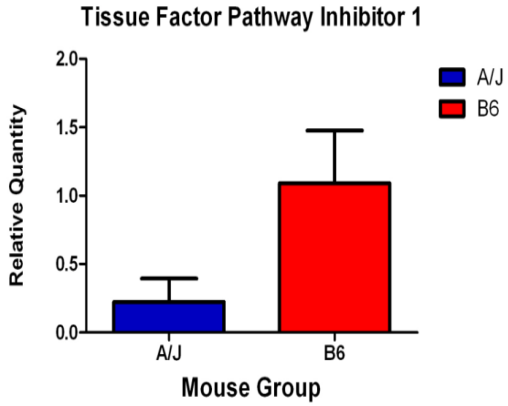

Figure 3.4: Tissue Factor Pathway Inhibitor 1 (TFPI-1). *D10 A/J(n=4) infected mice show downregulation of TFPI-1 gene expression. D10 infected B6(n=6) mice show slight upregulation of TFPI-1 gene expression.*

Anti-coagulants: TFPI-1 and TM

Tissue Factor Pathway Inhibitor 1 (TFPI-1) prevents Tissue Factor from initiating the extrinsic coagulation cascade. Infected day 10 A/J mice show downregulation of TFPI-1 gene expression compared to uninfected d10 A/J mice. Infected day 10 B6 mice show slight upregulation of TFPI-1 compared to d10 uninfected B6 mice.

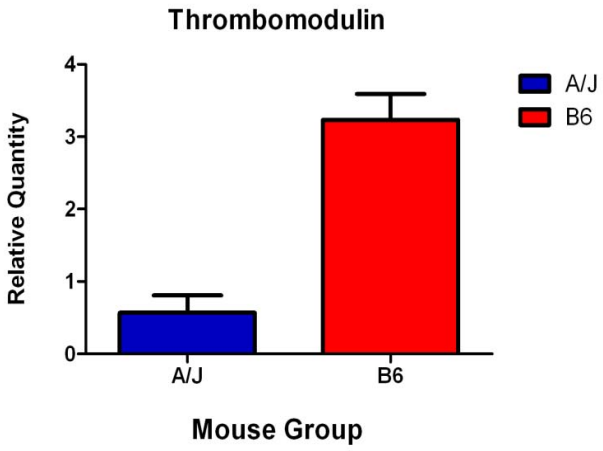

Figure 3.5: Thrombomodulin (TM). *D10 infected A/J (n=4) mice show downregulation of TM gene expression. D10 infected B6(n=6) mice show upregulation of TM gene expression.*

Thrombomodulin works with several other factors to inactivate thrombin and prevent fibrin formation. d10 infected A/J mice show downregulation of TM gene expression in comparison to uninfected d10 A/J mice. D10 B6 mice show approximately three fold upregulation of TM gene expression in comparison to d10 uninfected B6 mice.

CHAPTER 4
DISCUSSION

Fibrin deposition is associated with placental malaria and pre-term delivery.[28] Activation of the extrinsic coagulation cascade leads to fibrin deposition through the activation or inhibition of several coagulation factors. In the murine model for placental malaria, B6 mice have shown thrombosis and hemorrhaging while A/J mice have shown increased inflammatory infiltrate (Poovassery). The two different pathologies, present in these two genetically different mice, are both present in human malaria infection, establishing the relevance of this model for human placental malaria. Understanding the mechanisms of coagulation and inflammation in these mice provides further information and possibilities for research involving infection in humans.

In this study, B6 mice show increased gene expression of procoagulant activity compared to A/J mice. The upregulation of TF in infected B6 and downregulation of TF in infected A/J mice provide support for the proposition of increased coagulation in B6 mice but not in A/J mice since TF activates the extrinsic coagulation cascade leading to fibrin deposition. B6 mice also show upregulation in both PAR 1 and PAR 2. Since PARs are involved in producing proinflammatory cytokines such as TNFα which in turns increases TF expression, PARs propagate coagulation. The B6 upregulation of PARs provides additional support that B6 mice exhibit coagulation in placental malaria pathology. However, PARs are very complex g protein receptors with multiple effects. Thus, the upregulation of PAR 1 by infected A/J mice is not definitive as a sign of coagulation in A/J mice since PARs propagate coagulation indirectly though the release of proinflammatory cytokines, and A/J infected mice do show inflammatory infiltrate in the labyrinth of the placenta. Furthermore, previous studies in coagulation show that PAR 1 is activated by thrombin whereas PAR 2 is activated by Factor VIIa and Factor Xa, but

not thrombin.[55-57] This difference in activation of the two PARs might be why A/J mice show upregulation of PAR 1 but not PAR 2 and should be further investigated. Both A/J and B6 infected mice show upregulation of PAI-1. Since PAI-1 prevents the breakdown of fibrin, the upregulation by B6 is expected. However, the A/J mice show this upregulation because they are being compared to other uninfected A/J mice which show extremely low levels of PAI-1, so the upregulation in infected mice might not necessarily be an indicator of high levels of PAI-1 in the placenta, but should be investigated further perhaps using a variety of PAI-1 primers and including more mouse samples to test the consistency of the upregulation. Both anti-coagulants were upregulated in infected B6 mice and down regulated in infected A/J mice. This is not surprising as the upregulation of TM and TFPI-1 in B6 mice most likely serve as a compensation mechanism for the excessive coagulation occurring in the placenta.

Although examining coagulation factor gene expression reveals more about the process of pathogenesis in the placenta, the precise mechanism for this pathology is not yet well understood and provides a further avenue for research. In order to gain a more complete mechanistic view of placental pathology, a variety of experimental days should be examined to more closely study the relationship between increasing inflammatory cytokines, coagulation, and pregnancy failure. Studying additional coagulation factors such as TFPI-2, PAI-2, PAR-3, PAR-4, VEGF, EPCR-1, and ANG-1 will also provide more insight into the process of coagulation in the placenta. Lastly, gene expression and biological protein levels do not always correlate directly, so protein content should also be examined in these tissues.

Previous research has shown an intersection of inflammation and coagulation in the pathology of cerebral malaria.[2] The Moore research group is currently investigating this relationship in the placenta to determine how the inflammatory response and coagulation

activation contribute to disease pathology. Understanding the inflammatory and coagulation mechanisms leading to disease pathology is critical in the prevention of intrauterine growth restriction and low birth weight babies.

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