Niger. J. Physiol. Sci. 32(December 2017) 171-177
www.njps.com.ng

Niger. J. Physiol. Sci.

Potential Link Between Complement 5a Receptor and Mood Disorders in Mouse Exposed to Experimental Malaria *In Utero*

***Okojie A.K.[1,3,4*], Okobi O. E.[2], Dar H.[3], Malik H.[3], Arif M.[3], Rauf K.[3], and Iyare E.E.[1]**

[1]Reproductive and Developmental Programming Research Group, Department of Physiology, University of Nigeria, Enugu Campus, Enugu, Nigeria. [2]Department of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria. [3]Neuropharmacology Unit, Department of Pharmacy, COMSATS Institute of Information Technology, Abbottabad, Pakistan. [4]Department of Physiology, Madonna University, Elele, Rivers State, Nigeria.

Summary: In Africa, a large number of pregnancies are exposed to *Plasmodium falciparum* infection. The in-utero environment extremely influences childhood neurodevelopment and behaviour. The complement 5a receptor (C5aR) is linked to several disease conditions. However, the influence of *Plasmodium berghei* during pregnancy on maternal complement 5a receptor and subsequently on fetal behaviour is unknown. Pregnant mice were intra-peritoneally inoculated on gestational day 13 with $1.02x10^5$ infected red blood cells (iRBCs). iRBCs used in this experiment were gotten by in vivo passage of *P. berghei* in mice when the level of iRBCs have gotten to about 10-20%. A section of pregnant mice (both test and control groups) were earmarked to give birth and their offspring monitored up to postnatal day 42 when depression-like behaviour was evaluated using tail suspension test model. The other pregnant mice were subjected to cardiac puncture on gestational day 19 for C5a receptor estimation using Elisa assay. Results showed that pregnant mice infected with *P. berghei* had elevated C5a receptor compared with uninfected pregnant females. It was also shown that *P. berghei*-exposed offspring presented a depressive-like behaviour compared to unexposed controls. It may be concluded from this study, that complement 5a receptor demonstrates a pathogenic role in signaling and its possible role in mediating depression linked to *Plasmodium berghei* exposure in utero.

Keywords*:* Complement 5a receptor, *Plasmodium berghei*, *In-utero* malaria, Depression

©Physiological Society of Nigeria

**Address for correspondence*: pintos4live@yahoo.com; +2348157512145

Manuscript Accepted: November, 2017

INTRODUCTION

It is estimated that 125 million pregnant women are exposed to malaria infection yearly. This exposure may present deleterious consequences for the offspring and mothers, mainly first and second-time mothers (Dellicour et al 2010). Malaria in pregnancy (MIP) is a leading public health problem in Africa due to its high maternal and fetal morbidity resulting in about 200,000 infants' death yearly (Hartman et al 2010). Pregnancy is known to compromise the immune system thereby increasing malaria susceptibility and worsen the clinical manifestation to both mothers and fetus when there is maternal lack of pre-immunization or is very low (Nosten et al 2004, Menendez 1995). Pregnancy associated malaria has obvious maternal and fetal health unpleasant effects as well as high probability of maternal anemia, preterm birth, stillbirth, fetal growth restriction (FGR) and low birth weight infants (LBW), (Guyatt and Snow 2001).

Malarial in pregnancy is marked by aggregation of parasites in the intervillous spaces of the placenta tissue, leading to adhesion of iRBCs to the endothelial lining. This sequestration or adhesion seen in placental malaria is brought about by a unique parasite-encoded variant surface antigen (VSA) present on cell membrane of iRBCs (Salanti et al 2003). VSA confines to chondroitin sulphate A on the syncytiotrophoblast covering the intervillous space. Adhesion leads to the libration of inflammatory leukocytes, causing death of the placental tissue. Usually, to some extent, multigravid in endemic areas are protected from placental malaria which could be as a result of maternal antibodies inhibiting cell-adhesion of the iRBCs to the placenta (Beeson et al 2005, Staalsoe 2004). However, the immunity is absent if a multigravida leaves an endemic area. Consequently, such individuals are at danger should they return.

The complement system is an important part of the innate immune system response to infections (Horiuchi and Tsukamoto 2016). The initiation of complement system resultss in the formation of membrane attack complex (MAC), causing the

production of pores in the membrane, resulting in the elimination of target cells. Complement fragment notably C5a trigger inflammation as anaphylatoxins and chemotactic factors, and continous initiation of the complement system results in different inflammatory conditions (Ricklin and Lambris 2013, Horiuchi and Tsukamoto 2006). C5a is a 74-amino acid peptide that is produced through breakdown of complement protein C5. C5a is a powerful chemoattractant molecule mediated through interaction with its G-protein-coupled receptor C5aR, which is present on most leukocytes and central nervous system (Humanyun et al 2009, Chenoweth et al., 1982).

Complement system is activated by malaria infection via different pathways (e.g. thrombin and serine proteases), and current reports suggest that C5a has a probable pathologic role in complicated malaria and malaria infection during pregnancy (McDonald et al 2013, Silver et al 2010, Conroy et al 2009, Conroy et al 2012, Patel et at 2008, Kim et al 2014). Poor pregnancy outcome has been attributed to activated complement system as a common pathway without any visible infection (Holers et al 2002, Salmon et al 2002). Increased C5a production has been suggested to be a facilitator of injury associated with the placental in experimental models of spontaneous miscarriage and FGR (Girardi and Salmon 2003). Recently, studies in human have shown a link between C3a, C5a and complications in pregnancy (Banadakoppa et al 2014).

The role of the complement 5a receptor in mood disorders of pups delivered by mouse infected with *P.berghei* is unknown. Using wild type (WT) mouse model of experimental malaria in pregnancy, we hypothesized that WT pregnant mice exposed to *P. berghei* would exhibit raised plasma levels of the C5a receptor than the pregnant non-exposed mice. Using tail suspension test (TST), we hypothesized that time of immobility and latency to first immobility would differ in pups of pregnant mice exposed to *P. berghei* compared with the non-exposed mice.

MATERIALS AND METHODS

Animals and parasites

All animal work was approved by the ethics committee of Department of Pharmacy, Comsats Institute of Information Technology (CIIT), Abbottabad campus and College of Medicine, University of Nigeria, Enugu campus. Non-pregnant mice (BALB/c) were purchased from National Institute of Health, Pakistan and maintained in conventional housing at CIIT. Pregnant dams were housed individually in their plastic cages approximately 35 cm × 50 cm × 35 cm. Animals were provided with normal rat food and water ad libitum. Adult female mice of 10-12 weeks of age were used for infection experiments. *P.berghei* was gotten from Nigerian Institute of Medical Research (NIMR) Lagos, Nigeria. Parasitized RBCs employed in this experiment were gotten from in vivo passaAge in male mice when percentage iRBCs is approximately 10-20%. Giemsa staining was done daily to monitor parasitemia using thick and thin blood.

Pregnancy timing/monitoring

Presence of vaginal plug in collaboration with body weight in kg were employed to detect period of pregnancy (Freyre et al. 2006). Female mice (n=2-3) were placed together with one male of proven fertility until the detection of vaginal plug which was done every 6 hours. The day of detecting vaginal plug was taken as gestation day one (GD1) and pregnancy progression was monitored every other day by weighing the pregnant mice. Positive fertilization was ascertained between GD10 and GD13 when the mice showed a body weight gain of 3–4g (Freyre et al. 2006). Therefore, gain in body weight was used as a reference point of pregnancy while, loss in weight was used as an index of pregnancy loss.

Innoculation of Pregnant Mice with P. berghei Parasite

Pregnant mice were intra-peritoneally (IP) infected on GD13 with $1.02x10^5$iRBCs in 0.5ml normal saline, and parasitemia was monitored daily. This model employed in this present study has been previously validated as a murine model of MIP as it replicates important pathogenic features of malaria in pregnancy and infection earlier than GD13 will result in LBW and stillbirth (Neres et al 2008). We used 10^5 iRBCs in our study to remove LBW and possibly boost the number of live births as malaria during pregnancy is associated with stillbirth. Non-infected pregnant mice used as controls were administered 0.5ml of normal saline IP. A set of the pregnant mice (whether infected or non-infected) were allowed to deliver and the offspring were monitored up to postnatal day (PND) 42. The other pregnant mice were subjected to cardiac puncture on GD19 for complement 5a receptor estimation. Foster mothers were used in newborn post-natal follow-up studies as *P. berghei* infection is lethal in mice. Thus, both offspring from infected mice and those from non-infected mice were all transferred to foster mothers to limit weight differences which could result from differential maternal nourishment. The offspring were weighed every day.

Estimation of Complement 5a receptor by Elisa

The withdrawn blood sample was put in a heparinized tube and centrifuge for 15 minutes at 1000rpm at -4^0C. The plasma was then stored at -80^0C until the time for analysis. Estimation of C5aR1 was carried out according to the procedure described in the Elisa kit manual (Elabscience, China; Catalog No: E-EL-M1328). The absorbances of the samples were immediately measured at 450 nm using Pro Reader-96 microplate reader.

Tail suspension test

This experiment employs hemodynamic stress to be hanging the mice by its tail (Thierry et al. 1986). In this protocol, mice were suspended about 100cm above the stand by an adhesive tape which was placed 1cm from the tip of the tail. The test was videotaped for 6 min and during this period, latency to the first immobility episode and time of immobility were evaluated.

Statistical Analysis

All results are presented as means ± standard error of mean (SEM). In this case, between group comparisons were analyzed with Student's unpaired-test. Differences were considered statistically significant when values P < 0.05. GraphPad Prism 5 statistical software (La Jolla, CA) was used for all statistical analysis.

RESULTS

It was observed that exposure to *P. beghei* during pregnancy from GD13 led to gradual increase in % infected red blood cells as shown in figure 1. Our result showed that exposure to malaria during pregnancy induced a significant increase in plasma complement 5a receptor concentration compared with controls (10.55 ± 0.78 vs 2.87 ± 0.32, Fig. 2).

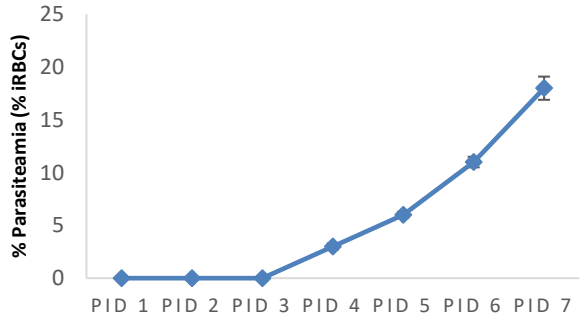

Fig.1: Maternal parasitaemia (post infection day (PID) one to seven, n=7) shown as percent of iRBCs per total red blood cells counted in mouse.

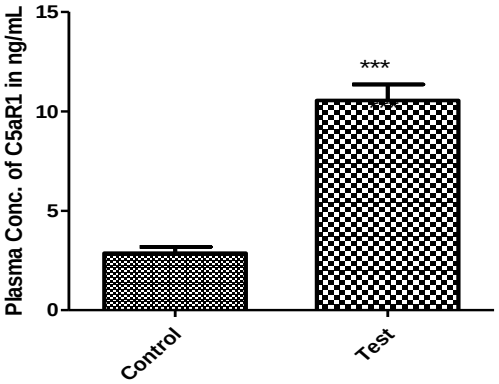

Fig. 2: Estimation of plasma complement 5a receptor concentration in pregnant female mouse on gestational day 19 infected with *P. berghei*. Each bar represents mean ± SEM of the data (n=5). ***P < 0.0001 compared to the control.

Our result showed that exposure to *P. berghei* during pregnancy did not significantly affect litter size compared with controls (6.33 ±1.0 vs 6.29 ± 0.6, Fig.3). It was observed that exposure to *P. berghei* infection during pregnancy on GD13 led to gradual increase in the body weight of pups which were significantly increased at PND 42 compared with controls (26.4±0.8 vs 20.8±1.0, p< 0.05 Fig.4). Our result showed that exposure to *P. berghei* during pregnancy did not significantly affect brain weight compared with controls (0.4566 ±0.03 vs 0.4570 ± 0.01, Fig.5).

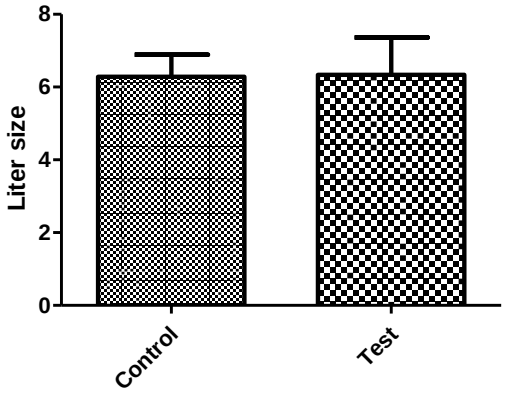

Fig. 3: Average litter size in pregnant female mouse exposed to *P. berghei* during pregnancy. Each bar represents mean ± SEM of the data (n=7).

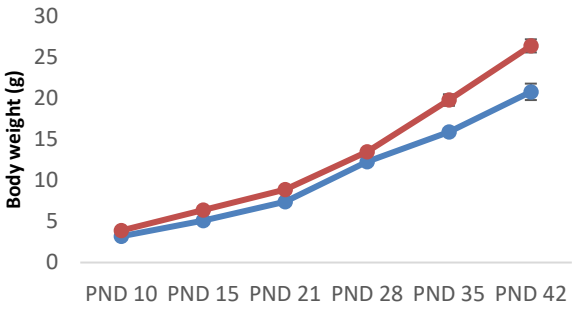

Fig. 4: Offspring weight from PND 10 to PND 42 of age in unexposed (n=10) and *P. berghei* exposed offspring (n=8).

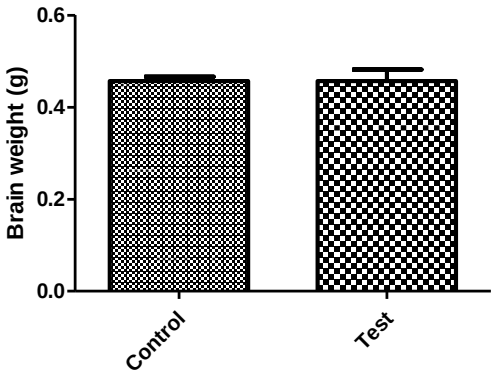

Fig. 5: Brain weight at PND 42 in pups of female mouse infected with *P. berghei* during pregnancy. Each bar represents mean ± SEM of the data (n=5).

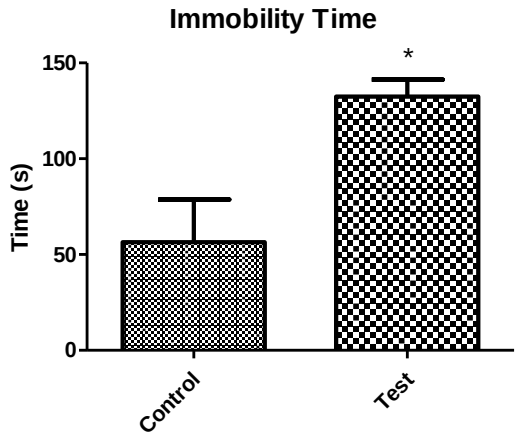

Fig. 6: Evaluation of depression-like behavior in tail suspension test in pups of female mouse infected with P. berghei during pregnancy. Each bar represents mean ± SEM of the data (n=5). *P < 0.0132 compared to the control.

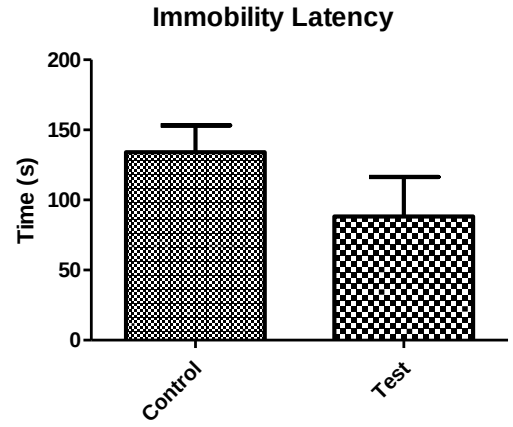

Fig. 7: Evaluation of depression-like behavior in tail suspension test in pups of female mouse infected with P. berghei during pregnancy. Each bar represents mean ± SEM of the data (n=5).

Exposure to *P. berghei* infection during pregnancy induced a significant increase in time of immobility compared with controls (132.4 ± 8.9 vs 56.4 ± 22.3, Fig. 6). Result showed that exposure to *P. berghei* infection during pregnancy induced a non-significant decrease in latency to first immobility compared with controls (88.2 ± 28.2 vs 134.0 ± 19.2, Fig.7).

DISCUSSION

Although malaria infection during pregnancy is very common in Sub-Sahara regions, there are very limited studies that demonstrate how maternal infection with malaria affects Complement 5a receptor and its subsequent role in depressive-like behaviour in the offspring. The only available study, used pregnant mice (C5aR-/-) evaluating memory and depression in the offspring (McDonald et al 2015). This study is likely the first to directly evaluate complement 5a receptor level in plasma of pregnant mice infected with *P. berghei*. This study has shown that maternal infection with *P. berghei* which led to significantly raised complement 5a receptor resulted in an interesting offspring's behaviour characterized by increased depressive-like behaviour as indicated by increased immobility time and decreased latency to first immobility. The offspring that were exposed to *P. berghei* were neither congenitally infected nor presented with any phenotypic adverse outcome associated with malaria in pregnancy.

The complement system makes up the first line of defense against foreign microbial pathogens. The complement cascade can be amplified by one or more of the three activation pathways: 1) the classical pathway, activated by the attraction of C1q to antigen-antibody complexes or directly to the surface of a microorganism; 2) the alternative pathway, activated by deposition of spontaneously activated complement components directly on microbial surfaces; or 3) the mannan binding lectin (MBL) pathway, activated by the binding of MBL to mannose-containing carbohydrates on microorganisms. These pathways converge in C3 protein with the subsequent cleavage of C5 that then leads to the generation of the membrane attack complex (MAC), which is important for complement function. C5 can also be directly cleave by both thrombin and serine protease. Malaria infection is associated with activation of coagulation cascade; increased production of thrombin and leukocytes bond with serine protease (McDonald et al 2013).

Studies have shown that immune infection during pregnancy is linked with obvious danger of neurodevelopmental conditions in offspring (Bilbo and Schwarz 2009). Malaria during pregnancy is marked with the accumulation of iRBCs including white blood cells within the intervillous space, leading to specific body defense system activation within the placenta (Umbers et al 2011). Raised peripheral and placental levels of C5a have been shown in females with malaria in pregnancy and are often linked to poor pregnancy outcomes (Conroy et al 2013). C5a is an active aggressor of pro-inflammatory and anti-angiogenic pathways (Silver et al 2010). These findings are in line with other possible mechanisms associated with dysfunctional neurodevelopment as well as dysregulated neuro-angiogenesis, dysregulated complement system-facilitated neurodevelopment, or neuroinflammatory processes. (McDonald et al 2013).

Our results showed a persistent but gradual increase in parasitized erythrocytes (PEs). Nevertheless, 10^5 inoculums resulted in low maternal parasiteamia (fig.1) when compared to previously reported inoculum of 10^6 PEs (Neres et al 2008). These findings indicate that, similarly to humans, pregnant mice showed great intensity of predisposition to malaria infection that could ultimately have a negative impact on their offspring or pregnancy as we observed a persistent but gradual increase in PEs.

In this study, though the body weight of the experimental group was significantly higher at six weeks of age than the control group; however, the whole brain weight of both *P. berghei* exposed offspring was same with that of the control group as shown in the figure (fig. 4 & 5). This is in agreement with Rijken et al [2012] that have previously shown maternal malaria in humans not to have any gross phenotypic effect on foetal cortical brain development.

Most studies use sucrose preference test (SPT), forced swim test (FST) or tail suspension test (TST) in laboratory animals to characterize depression-like behaviour (Babrie et al 2014, Fernandez et al 2014, Ge et al 2014). Immobility in FST and TST are thought to mirror behavioural despair present in humans which is our main behaviour of interest in this study. Though a single behavioural test is likely not sufficient as various divergence may exist in different depressive-like behaviour; however, TST may be a better reference point in this animal model (Zhou et al 2017, McDonald et al 2015). Offspring exposed to *P. berghei* displayed depressive behaviour which is in agreement with previous report (McDonald et al 2015). There is already evidence linking depression to proinflammatory cytokines (Postnal and Appenzeller 2015). Proinflammation can cause increase in indoleamine-2,3-dioxygenase (IDO), which is associated with inflammatory processes and serotonergic systems (Xu et al 2015); however, the concentration of proinflammatory cytokines were not measured in this study present study as Silver et al (2010) has shown that C5a is a strong initiator of proinflammation.

During pregnancy, inflammatory conditions are associated with poor neurodevelopmental outcomes (Leitner et al 2014, Elovitz et al 2011). Gallagher et al (2013) showed that maternal IL-6 cytokine surge leads to an elevated forebrain neural precursor pool through initiation of embryonic neural stem cell self-renewal pathway. It is our opinion that these changes in early neurogenesis as a result of inflammation could have high potential impact on neuro-behavioural function. Conroy et al (2009) have shown that C5a potentiate pro-inflammatory cytokine responses, as well as IL-6 to malaria iRBCs.

Interestingly, there is some evidence that suggests inflammatory pathways, particularly the complement 5a receptor is linked to several disease conditions like cardiac disease, amyotrophic lateral sclerosis (ALS), and adverse pregnancy outcome in malaria associated pregnancy (John et al 2017, Khor et al 2016, Conroy et al 2013). As shown in our study, offspring of pregnant mice exposed to *P. berghei* showed depressive-like behaviour. It would therefore appear possible that C5a receptor could be important in mediating adverse outcome associated with malaria in pregnancy. However, its uncertain if this relationship is direct or causal. The importance of the complement system, mainly the physiological relevance of C5a and its receptors has been well known; however, the role of the complement system in general, and C5a receptor in particular in normal and pregnancy with complications are still unresolved.

In conclusion, this study has shown that C5a receptor is elevated in experimental model of malaria in pregnancy which possibly indicates the pharmacological implications for the inhibition of the innate immune complement C5a receptor as an important pathway with possible link to neuroprotective effects since in utero exposure to malaria in mice is associated with neurobehavioural deficits in their offspring.

Acknowledgement

Comsats Institute of Information Technology and The World Academy of Science (CIIT-TWAS) are gratefully acknowledged for providing funding through CIIT-TWAS sandwich fellowship to the first author. We would also like to pay special thankfulness, warmth and appreciation to Dr. O.O. Aina, Malaria Research Group, Nigerian Institute of Medical Research, for gifting us the Plasmodium berghei used for this study.

REFERENCES

Babrie S, Doosti M.H., and Salari A.A (2014): Tumor necrosis factor-alpha during neonatal brain development affects anxiety and depression-related behaviors in adult male and female mice. *Behav.BrainRes.* 261, 305–314.

Banadakoppa M, Chauhan MS, Havemann D, Balakrishnan M, Dominic JS, et al. (2014): Spontaneous abortion is associated with elevated systemic C5a and reduced mRNA of complement inhibitory proteins in placenta. ClinExpImmunol 177: 743–749.

Beeson JG, Rogerson SJ, Elliott SR, Duffy MF (2005): Targets of protective antibodies to malaria during pregnancy. J Infect Dis;192:1647.

Bilbo SD, Schwarz JM (2009): Early-life programming of later-life brain and behavior: a critical role for the immune system. Front BehavNeurosci 3: 14.

Chenoweth DE, Goodman MG, Weigle WO (1982): Demonstration of a specific receptor for human C5a anaphylatoxin on murine macrophages. J Exp Med 156:68–78.

Conroy A, Serghides L, Finney C, Owino SO, Kumar S, et al. (2009): C5a enhances dysregulated inflammatory and angiogenic responses to malaria in vitro: potential implications for placental malaria. PLoS One 4: e4953.

Conroy AL, McDonald CR, Kain KC (2012): Malaria in pregnancy: diagnosing infection and identifying fetal risk. Expert Rev Anti Infect Ther 10: 1331–1342.

Conroy AL, Silver KL, Zhong K, Rennie M, Ward P, et al. (2013): Complement activation and the resulting placental vascular insufficiency drives fetal growth restriction associated with placental malaria. Cell Host Microbe 13: 215–226.

Dellicour, S. et al. (2010): Quantifying the number of pregnancies at risk of malaria in 2007: a demographic study. PLoS Med. 7, e1000221.

Elovitz MA, Brown AG, Breen K, Anton L, Maubert M, et al. (2011): Intrauterine inflammation, insufficient to induce parturition, still evokes fetal and neonatal brain injury. Int J Dev Neurosci 29: 663–671.

Fernandez J.W, Grizzell J.A, Philpot R.M, and Wecker L (2014): Postpartum depression in rats: differences in swim test immobility, sucrose preference and nurturing behaviors. *Behav.BrainRes.* 272, 75–82.

Freyre A, Falcon J, Mendez J, Rodriguez A, Correa L, et al. (2006) Refinement of the mouse model of congenital toxoplasmosis. ExpParasitol 113: 154–160.

Gallagher D, Norman AA, Woodard CL, Yang G, Gauthier-Fisher A, et al. (2013) Transient maternal IL-6 mediates long-lasting changes in neural stem cell pools by deregulating an endogenous self-renewal pathway. Cell Stem Cell 13: 564–576.

Ge J.F, Peng Y.Y, Qi C.C, Chen F.H and Zhou J.N (2014): Depression-like behavior in subclinical hypothyroidism rat induced by hemi-thyroidelectrocauterization. *Endocrine* 45, 430–438.

Girardi G, Salmon JB (2003): The role of complement in pregnancy and fetal loss. Autoimmunity 36:19–26.

Guyatt HL, Snow RW (2001): Malaria in pregnancy as an indirect cause of infant mortality in sub-Saharan Africa. Trans R Soc Trop Med Hyg 95: 569–576.

Hartman TK, Rogerson SJ, Fischer PR (2010). "The impact of maternal malaria on newborns". *Annals of Tropical Paediatrics*. **30** (4): 271–82.

Holers VM, Girardi G, Mo L, Guthridge JM, Molina H, et al. (2002): Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. J Exp Med 195: 211–220.

Humayun S, Gohar M, Volkening K, Moisse K, Leys traLantz C, Mepham J, McLean J, Strong MJ(200 9): The complement factor C5a receptor is upregulated in $NFL^{-/-}$ mouse motor neurons. J Neuroimmunol 210:52–62.

John D Lee, Vinod Kumar, Jenny NT Fung, Marc J Ruitenberg, Peter G Noakes, Trent M Woodruff (2017): Pharmacological inhibition of complement C5a-C5aR1 signaling ameliorates disease pathology in the $hSOD1^{G93A}$ mouse model of amyotrophic lateral sclerosis. British Journal of Pharmacology. DOI: 10.1111/bph.13730.

Khor KH, Moore TA, Shiels IA, Greer RM, Arumugam TV, Mills PC (2016): A Potential Link between the C5a Receptor 1 and the β1-Adrenoreceptor in the Mouse Heart. PLoS ONE 11 (1): e0146022.

Kim H, Erdman LK, Lu Z, Serghides L, Zhong K, et al. (2014) Functional roles for C5a and C5aR but not C5L2 in the pathogenesis of human and experimental cerebral malaria. Infect Immun 82: 371–379.

Leitner K, Al Shammary M, McLane M, Johnston MV, Elovitz MA, et al. (2014): IL-1 receptor blockade prevents fetal cortical brain injury but not preterm birth in a mouse model of inflammation-induced preterm birth and perinatal brain injury. Am J Reprod Immunol 71: 418–426.

McDonald CR, Lindsay S. Cahill, Keith T. Ho, Jimmy Yang, Hani Kim, Karlee L. Silver, Peter A. Ward, Howard T. Mount, W. Conrad Liles, John G. Sled, Kevin C. Kain (2015): Experimental Malaria in Pregnancy Induces Neurocognitive Injury in Uninfected Offspring via a C5a-C5a Receptor Dependent Pathway. PLoS Pathog;11(9): e1005140.

McDonald,C.R., Elphinstone,R.E. and Kain,K.C (2013): The impact of placental malaria on neurodevelopment of exposed infants: a role for the complement system? *Trends Parasitol.* 29, 213–219.

Menendez C (1995): Malaria during pregnancy: a priority area of malaria research and control. Parasitol Today 11: 178–183.

Neres R, Marinho CR, Goncalves LA, Catarino MB, Penha-Goncalves C (2008): Pregnancy outcome and placenta pathology in Plasmodium berghei ANKA infected mice reproduce the pathogenesis of severe malaria in pregnant women. PLoS One 3: e1608. doi: 10.1371/journal.pone.0001608 PMID:18270595.

Nosten F, Rogerson SJ, Beeson JG, McGready R, Mutabingwa TK, et al. (2004): Malaria in pregnancy and the endemicity spectrum: what can we learn? Trends Parasitol; 20: 425–432.

Patel SN, Berghout J, Lovegrove FE, Ayi K, Conroy A, et al. (2008) C5 deficiency and C5a or C5aR blockade protects against cerebral malaria. J Exp Med 205: 1133–1143.

Postnal M and Appenzeller S (2015): The importance of cytokines and autoantibodies in depression. *Autoimmun. Rev.* 14, 30–35.

Ricklin D, Lambris JD (2013): Complement in immune and inflammatory disorders: pathophysiological mechanisms. J Immunol; 190:3831–8.

Rijken M. J., Merel C., Eduard J.H.M, Suporn K., Noaeni K., Tamalar P., Gerard H.A.V., Rose McGready, François H Nosten and Lourens R Pistorius (2012): Effect of malaria in pregnancy on

foetal cortical brain development: a longitudinal observational study. Malaria Journal; 11:222.

Salanti A, Staalsoe T, Lavstsen T, Jensen AT, Sowa MP, Arnot DE, Hviid L, Theander TG (2003): Selective upregulation of a single distinctly structured var gene in chondroitin sulphate A-adhering Plasmodium falciparum involved in pregnancy-associated malaria. MolMicrobiol; 49:179–91.

Salmon JE, Girardi G, Holers VM (2002): Complement activation as a mediator of antiphospholipid antibody induced pregnancy loss and thrombosis. Ann Rheum Dis 61 Suppl 2: ii46–50.

Silver KL, Higgins SJ, McDonald CR, Kain KC (2010): Complement driven innate immune response to malaria: fuelling severe malarial diseases. Cell Microbiol 12: 1036–1045.

Staalsoe T, Shulman CE, Bulmer JN, Kawuondo K, Marsh K, Hviid L(2004): Variant surface antigen-specific IgG and protection against clinical consequences of pregnancy-associated Plasmodium falciparum malaria. Lancet; 363:283.

Takahiko Horiuchi and Hiroshi Tsukamoto (2016): Complement-targeted therapy: development of C5- and C5a-targeted inhibition. Inflammation and Regeneration; 36:11.

Thierry B, Steru L, Simon P, Porsolt RD (1986): The tail suspension test: ethical considerations. Psychopharmacology 90(2):284–285.

Tsukamoto H and Horiuchi T (2006): Clinical aspects of the complement system. RinshoByori; 54:757–62.

Umbers AJ, Aitken EH, Rogerson SJ (2011) Malaria in pregnancy: small babies, big problem. Trends Parasitol 27: 168–175.

Xu Y, Sheng H, Tang Z, Lu J, and N. X (2015): Inflammation and increased IDO in hippocampus contribute to depression-like behavior induced by estrogen deficiency. *Behav.BrainRes.* 288, 71–78.

Zhou Y, Wang X, Zhao Y, Liu A, Zhao T, Zhang Y, Shan Z and Teng W (2017): Elevated Thyroid Peroxidase Antibody Increases Risk of Post-partum Depression by Decreasing Prefrontal Cortex BDNF and 5-HT Levels in Mice. Front. Cell. Neurosci. 10:307.