Interactions Between Natural Killer Cells, Cortisol and Prolactin in Malaria During Pregnancy

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Natural killer cells derived from pluripotent hematopoietic stem cells are important cells of the immune system that have two main functions: a cytolytic activity and a cytokine-producing capacity. These functions are tightly regulated by numerous activating and inhibitory receptors, including newly discovered receptors that selectively trigger the cytolytic activity in a major histocompatibility complex independent manner. Based on their defining function of spontaneous cytotoxicity without prior immunization, natural killer (NK) cells have been thought to play a critical role in immune surveillance and cancer therapy. New insights into NK cell biology have suggested their major roles in the control of infections, particularly in *Plasmodium falciparum* infection and in fetal implantation. *P. falciparum* is the main protozoan parasite responsible for malaria causing 200-300 million clinical cases and killing over 3 million people each year. This review provides an update on NK cell function, ontogeny and biology in order to better understand the role of NK cells in pregnancy in regions where malaria is endemic. Understanding mechanisms of NK cell functions may lead to novel therapeutic strategies for the treatment of human disease, in general, and particularly in the fight against malaria.

Keywords: Natural killer cells, *Plasmodium falciparum*, malaria, pregnancy

In areas of intense *Plasmodium falciparum* transmission, pregnancies are associated with substantial malaria-related morbidity and mortality. The mechanisms of protection against malaria are not fully understood. Protective immunity is acquired during childhood. Until recently, it has been poorly understood why the clinical protection against malaria is rendered inefficient when young women become pregnant. Over the last century, the epidemiology of pregnancy associated *P. falciparum* malaria has been the subject of numerous research articles with excellent reviews of this literature published.¹⁻⁴ However, most of these reviews focus on the mounting evidence that protective immunity to pregnancy-associated malaria depend on acquisition of antibodies directed against parasite-encoded variant antigens on the surface of the infected red blood cells and only one gives attention on the "cortisol hypothesis."² This review will illustrate the cortisol hypothesis, and because natural killer (NK) cells play a crucial role in this hypothesis, an update on NK cell function and biology is provided in order to better understand the role of NK cells in the susceptibility of pregnant women to malaria.

Characterization of NK Cells

NK cells are large granular lymphocytes that comprise approximately 5% to 15% of peripheral blood mononuclear cells (PBMC) and 5% to 10% of lymph nodes. NK cells were first identified by their ability to lyse tumor cells without prior immunization. Actually, NK cells are defined as lymphocytes lacking the T cell receptor (TCR) complex and are CD3 negative. The best surface antigen to identify human NK cells is the neural cell adhesion molecule (CD56) first identified in brain tissue.⁵ Although CD56 may have adhesion function in neural tissue based on homotypic interactions,⁶ its function in NK cells remains unknown. Monoclonal antibodies to several other antigens can be used to identify important subsets of NK cells. CD16 (FcγRIII) is the Fc receptor expressed on the surface of CD56^{dim} NK cells that identifies the most mature cell population belonging to this lineage. Well-characterized unique NK cell subsets circulating in peripheral blood are CD56^{bright} NK cells⁷ that are minimally cytotoxic⁸ and highly proliferative.^{8,9} CD56^{bright} NK cells constitutively express interleukin (IL)-2 receptor and lack FcγRIII.¹⁰ They also express c-kit¹¹ and the receptor for T cell factor-1

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Table 1. Characteristics of NK cell receptors.

Receptor	Family	Genes	Species	Function	Known ligands
KIR	Ig	Multigene	H, Hd, Ho	Activating or inhibitory	Broad MHC I specificity
LIR/ILT	Ig	Multigene	H	Activating or inhibitory	Broad MHC I specificity
NKp30	Ig	Single	H, Hd, Ro	Activating	?
NKp44	Ig	Single	H	Activating	Hemagglutinins
NKp46	Ig	Single	H, Hd, Ro	Activating	Hemagglutinins
NKp80	Ig	Single	H	Activating	?
Siglec-7	Ig	Single	H	Inhibitory	Sialic acid
LAIR	Ig	Single	H	Inhibitory	Ep-CAM
2B4	Ig	Single	H, Ro	Activating	CD48
NKG2D	CLD	Single	H, Hd, Ro	Activating	MICA/B, ULBP
Ly49	CLD	Multigene	H, Hd, Ro	Activating or inhibitory	HLA I, M157, Hm1-C4
CD94/NKG	CLD	Multigene	H, Hd, Ro	Activating or inhibitory	HLA-E, Qa-1b
KLRB1	CLD	Multigene	H, Hd, Ro	Activating or inhibitory	C-type lectin related molecule
TLR	Toll like	Multigene	A, V	Activating	Pathogen-associated molecules

A, arthropods; CLD, C-type lectin domain family; H, human; Ho, humanoids; HLA, human leukocyte antigen; Ig, immunoglobulin superfamily; ILT, immunoglobulin-like transcript receptors; KIR, killer immunoglobulin-like receptors; LIR, leukocyte immunoglobulin-like receptors; MHC, major histocompatibility complex; NK, natural killer; Ro, rodents; TLR, toll-like receptors; V, vertebrates.

(TCF-1).¹² The CD56^{dim} NK cells can be divided into subpopulations based upon CD2 expression. CD2 is the ligand for lymphocyte function-associated antigen-3 (LFA-3, or CD58). All NK cells express the lymphocyte marker CD7.¹³ CD8, also present on the cytotoxic population of T-cells, is expressed by approximately 35% of CD56^{dim} NK cells.⁹ In contrast to CD8⁺ T cells, where CD8 is composed of both α and β chains, CD8 expressed on the surface of NK cells is composed of predominantly CD8- α chains.¹⁴

Identification of Target Cells of NK Cells

Before their phenotypic characterization, NK cells were functionally defined as cells freshly isolated from blood with the ability to lyse tumor cell targets. Activation of NK cells with IL-2 induces cytolytic machinery and the capacity to lyse a broad range of tumor targets not lysed by resting NK cells. Although there has been much progress in understanding the signaling machinery of NK cells, the recognition events may

be more complex. It is well known that $\beta 2$ integrins expressed on the surface of NK cells recognize intracellular adhesion molecule-1 (ICAM-1) on target cells.^{15,16} However, this may be one of several receptor ligand pairs that are involved in the recognition process. Another adhesion ligand pair, CD2 on NK cells, recognizes LFA-3 on target cells.¹⁷ Antibodies against $\beta 2$ integrins and CD2 variability interrupt cytotoxicity in a target-specific manner.¹⁸ Activating and inhibitory NK cell receptors (table 1) have now been shown to recognize a wide range of self and non-self antigens.

Regulation of NK Cell Function by Major Histocompatibility Complex (MHC) Class I Restricted Receptors

In contrast to T cells, NK cells kill a broad array of targets in a human leukocyte antigen (HLA)-unrestricted manner. However, recent observations counter the notion that NK killing is totally MHC-unrestricted. While antigen recognition

NK cell receptor function may be activating (+) or inhibitory (-)

Figure 1. NK cells function through a diverse repertoire of activating and inhibitory receptors. Activating receptor ligation triggers the cytotoxic mechanism, while inhibitory receptors protect from NK cell lysis. Since multiple receptors are involved between NK cells and their targets, the decision of whether or not to kill a target is the net sum of all receptor interactions, and inhibitory receptor signaling is dominant. Both classical and nonclassical class I MHC (left side) and non-MHC ligands (right side) are known for many NK cell receptors.

presented by MHC class I molecules activates T cells, class I antigen presentation can render targets resistant to lysis by NK cells. Thus, NK cell receptor function may be activating and/or inhibitory (figure 1). As originally proposed by Ljunggren and Kärre¹⁹ in the “missing self” hypothesis, it is now well recognized that class I downregulation by transformation or infection may make cells susceptible to NK cell lysis.^{20,21} Class I-recognizing NK receptors were identified first in the mouse as the Ly49 proteins, which are type II lectins capable of binding sugar moieties on class I molecules.²² Although a truncated nonfunctional Ly49L has been found in man, humans do not use Ly49 receptors.²³ In contrast, human class I-recognizing NK cell receptors are type I membrane glycoproteins and members of the immunoglobulin superfamily that are not present in mice.^{24,25} Although thought initially to represent a paradox, it is now known that human NK cells express both immunoglobulin and lectin-type receptors.²⁶⁻²⁸

Chromosome 19 is a hot spot for NK cell receptors defined by immunoglobulin structural moieties. Hence, they are termed killer immunoglobulin-like receptors (KIR). The individual receptor names are assigned by the number of corresponding immunoglobulin domains (e.g., KIR2D, KIR3D) and by the length of their associated cytoplasmic tail, either long (L) or short (S) (e.g., KIR2DL, KIR2DS1).²⁹⁻³¹ A KIR can signal inhibition or activation when class I MHC is recognized based on the length of its cytoplasmic tail. Long cytoplasmic tails contain immunoreceptor tyrosine-based

inhibitory motifs that signal inhibition when the KIR is appropriately ligated. At least three of these inhibitory receptors (KIR2DL1, KIR2DL2, KIR3DL1) have class I ligands identified (cw4, cw3, bw4). KIR with short cytoplasmic tails non-covalently associates with cytoplasmic DAP12 molecules that signal activation when the KIR is ligated. DAP12 is a disulfide-bonded homodimer containing an immunoreceptor tyrosine-based activation motif and its gene is also found on chromosome 19.^{32,33} The function of inhibitory KIR is summarized in figure 2.

The Role of NK Cells During Infection

There are two mechanisms by which NK cells may affect disease. As already discussed above, NK cells do not kill targets that express “self” class I HLA molecules. Viral infection, like some cancers, may downregulate class I MHC, allowing for killing of virally infected cells by direct cytotoxic mechanisms. A second major role of NK cells is their production of cytokines, which may be critically important to eliminate infections. Understanding of the pathophysiology of viral infection and immune recognition by NK cells has been advanced by the discovery of NK cell-inhibitory receptors that recognize MHC molecules. For instance, NK cell clones were isolated from peripheral blood to study the lysis of HeLa cells before and after infection with herpes simplex virus or cytomegalovirus (CMV). Both viral infected targets downregulate MHC class I molecules, making them undetectable by T cells, but more susceptible to NK cell killing than uninfected control cells.³⁴ However, the factors

Function of inhibitory KIR

Figure 2. Specificity of NK cell function. In contrast to T cells, which require class I MHC to kill their targets, inhibitory KIRs have exactly the opposite effect.

that influence MHC class I expression are complex and may underlie the mechanisms of escape from NK cell-mediated lysis of virus-infected targets.

Several studies have evaluated the mechanisms of CMV infection and specificity of MHC downregulation. Unlike many viral proteins that downregulate class I molecules, CMV US2 and CMV US11 are specific to HLA-A and -B alleles, but do not affect HLA-C molecules showing allelic specificity.³⁵ CMV UL40 has been interesting because of the homology between the signal sequence (amino acids 3-11) of most HLA-C alleles and the UL40 peptide (amino acids 15-23). Two independent laboratories have recently found UL40 sequence homology to HLA-E binding peptides.^{36,37} The first study used fibroblasts that constitutively express low levels of HLA-E to test UL40 constructs. UL40 bound HLA-E and increased its expression, without affecting expression of HLA-C, and protected against NK cell attack through CD94 lectin inhibitory receptors.³⁶ The second study co-transfected HLA-E and UL40 into K562 cells, which became resistant to killing by NKG2A⁺ NK cell clones.³⁷ Blocking antibodies to CD94 or HLA-E restored lysis, showing the specificity of this interaction. Therefore, some CMV proteins (US2 and US11) promote NK cell attack by downregulation of MHC, but UL40 has an opposing effect by stabilizing and upregulating HLA-E. Other viral proteins may have similar allelic specificity, but not all downregulation of MHC is necessarily associated with increased susceptibility to NK killing. Kaposi's sarcoma-associated herpes virus (KSHV) proteins K3 and K5 dramatically downregulate class I molecules. K3 downregulates HLA-A, -B, -C and -E, while K5 downregulates HLA-A and -B. Surprisingly, KSHV K5 transduced targets, which do not downregulate Cw4, were resistant to an NK cell line that lacked a Cw4-specific KIR.³⁸ Thus, K3 induces target sensitivity by MHC downregulation, and K5 induces resistance independent of MHC expression. The mechanism of this K5-induced resistance was found to be

by downregulation of target cell intracellular adhesion molecule-1 needed for NK recognition and downregulation of B7-2 needed for effector cell activation. This illustrates a novel mechanism by which KSHV downregulates class I molecules, which should make it more susceptible to NK lysis but, by other mechanisms, escapes this immune control.

Interferon (IFN)- γ is probably the most important cytokine produced by NK cells with anti-infection activity. Mice that lack IFN- γ receptors have an increased susceptibility to murine CMV, listeria monocytogenes and the protozoan parasite, *Toxoplasma gondii*.³⁹ Detailed studies of murine CMV infection show that IFN- γ and tumor necrosis factor (TNF)- α levels are upregulated in mice infected with murine CMV.⁴⁰ By selective depletion of NK cells, IFN- γ production is eliminated, suggesting that NK cells are the primary producers of this cytokine. The role of IFN- γ in the pathogenesis of murine CMV is suggested by studies using monoclonal antibodies to neutralize IFN- γ , resulting in an increase in viral replication and subsequent severity of viral hepatitis. Interestingly, neutralizing IFN- γ does not alter direct cytotoxicity, suggesting that direct killing of infected cells is not the mechanism of NK cell control of murine CMV. Further studies showed that IL-12 stimulates IFN- γ in NK cells to mediate a protective effect against murine CMV. This may be virus-specific since lymphocyte choriomeningitis virus does not induce IFN- γ and was not affected by IL-12 neutralization.⁴¹

NK Cells and Malaria

In *P. falciparum* infection, disease occurs only as a result of the asexual blood stage after the parasite leaves the liver and begins to invade and grow inside red blood cells. Important insight into the role of NK cells in immunity to malaria was provided on IL-12-related mechanisms in early stages of the disease in the model.⁴² Indeed, infection of mice can be ameliorated by administration of IL-12 early in the course of

the infection. Untreated mice died 12 days after infection, while IL-12 treated mice survived. Administration of IL-12 early in the course of the disease improves the outcome of the infection in normal mice, leading to the development of IFN- γ , TNF- α and nitric oxide-dependent immunity.⁴³ The role of nitric oxide was investigated by the administration of aminoguanidine, a selective inhibitor of cytokine inducible nitric oxide synthetase. Significantly increased mortality was observed following treatment twice daily with 9 mg of aminoguanidine, but there was no effect on parasitemia. Furthermore, *in vivo* depletion of NK cells after anti-GM1 antibody treatment results in a severely increased pathology, and IL-12-treated but NK cell-depleted A/J mice fully fail to control the infection.⁴⁴ More recently, the role of NK cells in control of human malaria has begun to be investigated. Artavanis-Tsakonas and Riley⁴⁶ have revealed that, in non-immune donors, NK cells are among the first cells in peripheral blood to produce IFN- γ in response to *P. falciparum*-infected red blood cells. In this setting, the authors observed that NK cells are activated during the first 18 hours of exposure to parasitized erythrocytes. This activation was dependent on IL-12 and also to a lesser extent on IL-18. Interestingly, cells from semi-immune African adults produce less IFN- γ , which might reflect haplotypic variation in KIR genotype among individuals and/or extensive functional allelic polymorphisms in KIR and toll like receptors.⁴⁶⁻⁴⁸ These results were obtained with whole blood cells. Using purified cells, we have recently shown that NK cells from nonimmune individuals can kill plasmodia-infected erythrocytes in the absence of opsonizing antibodies directly through cell-cell interaction and that this activity was inhibited by anti-Fas antibody and granzyme B inhibitor.⁴⁹ We also provide evidence that purified NK cells can inhibit growth of Plasmodia-infected erythrocytes opsonized with antibody from semi-immune African adults by around 25%, and that pre-treatment of NK cells with anti-Fas and granzyme B inhibitor prevents this growth inhibition.⁴⁹

The Role of NK Cells in the Pregnant Uterus

Any discussion of the normal physiology of NK cells would be lacking without mention of their putative role in pregnancy. It has long been recognized that large granular lymphocytes of NK cell origin are the major lymphocyte populations lining the pregnant uterus.⁵⁰ Uterine NK cells achieve peak numbers during the first trimester, when they represent 70% of all lymphocytes, and are not found at term.⁵¹ Uterine NK cells are different than mature circulating NK cells, yet phenotypically resemble the smaller unique blood NK cell subset, CD56⁺bright/CD16⁻/CD3⁻,⁵² and has low direct cytotoxicity. Since IL-2 is not produced in the placenta, proliferation of uterine NK cells is through production of IL-15 by placental macrophages and the IL-15 receptor on CD56⁺bright uterine NK cells.⁵³ Significant progress has been made in understanding the role of NK cells by evaluating mice that lack NK cells. These mice are able to bear offspring but with 64% fetal loss.⁵⁴ In addition, they

have no endometrial glands, decreased placental size, decidual edema and abnormalities involving the large maternal arterioles supplying the placenta.^{50,55} The causal relationship between this phenotype and the role of NK cells was established by showing that T cell-deficient mice do not exhibit these abnormalities and transplantation of stem cells from severe combined immune deficient mice (which have normal NK cells but no T cells) into the NK cell-deficient mice reverses the phenotype.⁵⁶ These studies show that CD56⁺bright NK cells occupying the mesometrial side of the pregnant uterus contribute to fetal implantation. Recent progress has been made in understanding this mechanism.

Since transgenic ϵ 26 mice have a 10-fold lower concentration of uterine IFN- γ ,⁵⁶ Ashkar and colleagues⁵⁷ investigated mice lacking IFN- γ or IFN- γ receptor α -receptors. These latter two knockouts had an excessive number of uterine NK cells that were small and had limited numbers of cytoplasmic granules. The decidual arteries did not undergo normal gestation-induced remodeling. In contrast to the hypocellularity of the decidua in the NK cell knockout mice, decidua in the IFN- γ deficient mice progressed to overt necrosis during the second half of gestation. Further experiments used the RAG-2/common γ chain knockout mice, which also lack NK cells. As expected, these mice had very low concentrations of IFN- γ in the uterus. Engraftment of these NK-deficient mice with IFN- γ knockout marrow resulted in normal numbers of NK cells in the uterus, but an inability to initiate pregnancy-induced remodeling of spiral arteries.⁵⁸ Interestingly, treatment with murine IFN- γ (100-1000 units per day x 6 days) reversed this defect and resulted in normal decidual artery remodeling. Taken together, these studies suggest that IFN- γ modulates the NK cell pool resident in the uterus, that uterine NK cell-derived IFN- γ is not necessary for initiation of decidualization, but appears essential for decidual maintenance in the second trimester.

It is intriguing that trophoblasts lack class I and class II HLA molecules but highly express nonclassical HLA-G, which is restricted to the placenta.^{59,60} Since NK cells have receptors for nonclassical class I molecules, some have hypothesized that HLA-G protects fetal cells from lysis by maternal NK cells through this mechanism. Using a migration assay, NK cell trafficking through the HLA-G-expressing endothelial cells was reduced.⁶¹ Recent studies show that HLA-G expression may interact with HLA-E molecules. Trophoblast cells were purified by flow cytometry and found to express HLA-E by reverse transcriptase polymerase chain reaction and immunoprecipitation.⁶² Staining with HLA-E tetrameric complexes refolded with the leader peptide derived from HLA-G identified 93% of decidual NK cells compared to 55% from the peripheral blood of non-pregnant women, suggesting the importance of NK cell lectin receptors in pregnancy. KIR may also be important, since uterine NK cells in the first trimester of pregnancy and essentially on all NK cells obtained from the placenta at term were found to express the p49 receptor, now termed KIR2DL4. KIR2DL4 was

shown to directly bind HLA-G and may protect trophoblast cells from lysis by NK cells.⁶⁰ Lanier⁶² has pointed out that it is still not clear whether KIR2DL4/HLA-G interactions are physiologic. These concerns are based on two possibly contradictory findings: 1) a woman found to have an HLA-G^{null} mutation had normal fetal development and 2) fetal mice with β -2 microglobulin genes knocked out are not rejected by their heterozygous mother. Further studies into the role of NK cells in pregnancy will define these interactions and their physiologic relevance. Abnormal functions of NK cells in women with recurrent spontaneous abortions have been postulated. There seems to be a correlation with recurrent spontaneous abortion and increased NK cell cytotoxicity and an abnormally high decidual CD56⁺/CD16⁻ ratio compared to normal pregnancies.⁶³ A better understanding of NK cells in pregnancy may lead to important new therapies to prevent fetal loss.

Malaria During Pregnancy

Malaria is endemic in more than 100 countries. Over two billion people living in these endemic countries are exposed to *P. falciparum* infection. Children less than 6 years old and pregnant women are at increased risk of *P. falciparum* malaria and its associated complications. Approximately 50 million women become pregnant every year in malaria endemic regions.⁶⁴ Pregnancy-associated malaria is estimated to be responsible for a third of preventable low birth weight babies in sub-Saharan Africa and to cost the lives of approximately 200,000 infants annually.⁶⁵ Women are more susceptible to infection when pregnant, and both the frequency and the severity of the disease are higher in pregnant than non-pregnant women.¹ In areas where malaria is highly endemic, a protective semi-immunity against *P. falciparum* is acquired during the first 10-15 years of life with the majority of malaria-related morbidity and mortality happening in young children.⁶⁶ However, in contrast with low malaria prevalence in adults, pregnant women in endemic areas are highly susceptible to malaria.¹

In pregnancy, there is a transient depression of cell-mediated immunity that allows fetal allograft retention but also interferes with resistance to various infectious diseases.⁶⁷ Cellular immune responses to *P. falciparum* antigens are depressed in pregnant women in comparison with non-pregnant control women.^{65,68}

Anti-adhesion antibodies against chondroitin sulfate A-binding parasite are associated with protection from maternal malaria, but these antibodies develop only over successive pregnancies, accounting for the susceptibility of primigravidae to infection.⁶⁹ Indeed, women in first and second pregnancies are the most affected, with both gravidity and premunity influencing susceptibility to malarial infection.⁷⁰

Cortisol, Prolactin and Susceptibility of Pregnant Women to Malaria

The immune system destroys infected cells and rebuilds tissue.⁷¹ Similar processes are driven by hormones. In women, during the 9 days preceding ovulation (the proliferative phase) the endometrium becomes built up, highly vascularized and infiltrated with small NK cells.^{72,73} Ovulation defines the beginning of the 14-day secretory phase during which NK cells proliferate and differentiate. In the secretory phase, an embryo can implant or die off, in the presence or absence of NK cells, respectively. Upon implantation of an embryo, the menstrual cycle is broken and pregnancy starts.

Whereas NK cells represent approximately 10% of cells in peripheral blood, uterine NK cells constitute 60% to 90% of the leukocytes in the decidua, a tissue in which B lymphocytes and T lymphocytes are rare. Two types of NK cells are distinguished, those expressing low levels of CD56 and specialized in cytolysis, and those expressing high levels of CD56 and involved in cytokine secretion.⁷⁴ Uterine NK cells express the highest amount of CD56.⁷⁵

Cortisol, an adrenocortical hormone in humans suppresses the immune system and directly inhibits NK cell activity.⁷⁶⁻⁷⁸ Conversely, prolactin, a 24 kDa single chain hormone secreted by the anterior pituitary gland, is an immunostimulatory "cytokine"^{79,80} that directly affects NK cell function.⁸¹

NK cell cytolytic activity decreases significantly during pregnancy and NK cytotoxic activity defect has been reported in pregnant women.^{82,83} We measured NK cell cytotoxicity in peripheral venous blood samples obtained from pregnant Gabonese women at the time of delivery. The NK cell-mediated cytotoxicity against *P. falciparum*-infected erythrocytes *in vitro* was lower in samples obtained from primiparous women than in multiparous ones.⁸⁴ However, these findings were discussed and contested by Pearson.⁸⁵ He supported the "prolactin hypothesis" and pointed out that labor was not an appropriate time to assay for prolactin levels because they naturally fall 24 hours preceding the onset of delivery. Nevertheless, we and others have demonstrated that the surface expression and function of the triggering receptors responsible for NK-mediated recognition and killing of tumor cells is regulated by hormones.^{85,86} Prolactin selectively upregulates the surface expression of NKp46 and NKp30. In contrast, cortisol downregulates the expression of NKp30.⁸⁶ Our data are interesting because NKp30 acts together with NKp46 to induce cytotoxic activity against a variety of target cells. These results have important implications for the understanding of the involvement of NK cells in the susceptibility of pregnant women to *P. falciparum* malaria. Pregnant women living in endemic areas are most susceptible to malaria between the second trimester and the early postpartum period.⁸⁷

We recently reported that cortisol and prolactin concentrations increase during pregnancy, regardless of parity. We found that cortisol concentrations were higher in primigravidae than in multigravidae, and also higher in *P. falciparum*-infected primigravidae than in uninfected primigravidae throughout pregnancy and at the time of delivery.⁸⁸ We showed that prolactin concentration did not differ according to *P. falciparum* status. The low NK cell cytotoxic activity we found was correlated with high cortisol concentrations. Some studies have found that exogenous cortisol suppresses NK cell cytotoxicity⁸⁹ and that subphysiological concentration of cortisol results in direct functional inactivation.^{74,75} Moreover, the surface density of natural cytotoxicity receptors is directly correlated with the magnitude of NK cell cytolytic activity against several target cell types.⁹⁰ We, therefore, hypothesize that the difference in NK cell cytotoxic activity against *P. falciparum*-infected erythrocytes observed between primiparous and multiparous women can be partially explained by the difference in cortisol concentrations found in these two groups of pregnant women.

Conclusion

NK cells are important cells of the immune system derived from stem cells in the marrow. Their function in infectious diseases and pregnancy is controlled through a complex system of cell receptors to control cell proliferation, cytotoxicity and cytokines. The production of hormones and other pregnancy regulatory factors in primigravidae may alter cell function, thereby conferring an advantage for malaria infection. A causal relationship between high cortisol levels and depressed NK cell cytotoxicity against *P. falciparum*-parasitized erythrocytes and susceptibility to malaria has been demonstrated. Parasitized erythrocytes become sensitive to NK cytotoxicity and prolactin, and cortisol serum levels were related with NK cells cytolytic activity. Despite limited success in currently reported therapeutic trials, understanding the biology of NK cells and their homing mechanisms *in vivo* may lead to novel therapeutic strategies. However, much research remains to be done.

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