# Variations in Killer-cell Immunoglobulin-like Receptor and Human Leukocyte Antigen Genes and Immunity to Malaria

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## ABSTRACT

Malaria is one of the deadliest infectious diseases in the world. Immune responses to *Plasmodium falciparum* malaria vary among individuals and between populations. Human genetic variation in immune system genes is likely to play a role in this heterogeneity. Natural killer (NK) cells produce inflammatory cytokines in response to malaria infection, kill intraerythrocytic *Plasmodium falciparum* parasites by cytolysis, and participate in the initiation and development of adaptive immune responses to plasmodial infection. These functions are modulated by interactions between killer-cell immunoglobulin-like receptors (KIR) and human leukocyte antigens (HLA). Therefore, variations in *KIR* and *HLA* genes can have a direct impact on NK cell functions. Understanding the role of KIR and HLA in immunity to malaria can help to better characterize antimalarial immune responses. In this review, we summarize the different KIR and HLA so far associated with immunity to malaria.

**Key words:** Genetic variation, Human Leukocyte Antigen, Innate immunity, Killer-cell immunoglobulin-like receptor, malaria, natural killer cells

## Introduction

Malaria is one of the most serious infectious disease problems in the world (1). Malaria burden is greatly affected by human immunity (2, 3), especially in populations with moderate to high transmission intensity (4). Partial immunity to malaria is developed over years of exposure (5). Although this partial immunity does not provide complete protection, it reduces risks of parasitemia progressing to clinical illness and to severe disease (6). This explains why most malaria deaths in high transmission areas, such as much of sub-Saharan Africa, occur in children (7). Several studies have consistently shown a number of human genetic variants that are associated with protection from uncomplicated and severe *P. falciparum* malaria. Haemoglobin S heterozygous (HbAS) individuals are protected from uncomplicated and severe malaria (8, 9). Haemoglobin C (HbAC) heterozygotes (10, 11), and α–thalassemia heterozygotes (_α/αα) and homozygotes (_α/_α) are protected from severe malaria (8). However, these well-characterized polymorphisms only partially explain genetic variation in responses to malaria (12). It is important to identify additional human genetic variants that are associated with susceptibility or protection.

Genetic variants of human killer-cell immunoglobulin-like receptors (KIR) and human leukocyte antigens (HLA) are strongly associated with risks of infectious diseases (13), autoimmune disorders (13-15), success in cell transplantation for the treatment of haematopoietic malignancies (16), certain cancers (17), and pregnancy outcomes (18). *KIR* and *HLA* genes segregate independently on chromosomes 19 and 6, respectively, and are both highly diverse, with extensive allelic polymorphism (19). *KIR* and *HLA* genes are reported to be more polymorphic in African populations than in other populations (19). Evolutionary pressure from malaria pathogens may have in part driven the high *KIR* and *HLA* genetic diversity in Africa (20, 21). Data regarding associations between *KIR* and *HLA* variants and malaria risk have been inconsistent, but since interactions between the genetically diverse KIR and HLA molecules modulate the functionality of natural killer (NK) cell response to malaria infections, these genes remain good candidates to understand the role of immune cells in malaria.

Despite recent reports indicating improvement in the control of malaria in some populations, and the potential for elimination of malaria from many regions of the world, *P. falciparum* malaria still causes extensive morbidity and mortality, particularly in sub-Saharan Africa (22). In response to the persistent malaria burden, there have been increased efforts in both vector control, using insecticides and malaria treatment, and chemoprevention using antimalarial drugs (23). However, these approaches have faced challenges of both insecticide and drug resistance (24). Drug discovery for new antimalarials is challenging and costly (24), and parasite resistance develops readily (25). Given the limitations of insecticides and antimalarial drugs, a highly effective malaria vaccine would significantly contribute to malaria control (26). Among major challenges to the development of vaccines against malaria are failure to induce strong innate immune responses and lack of potentiation and maintenance of adaptive immune responses (27).

There have been efforts to develop malaria vaccines since the 1940s (28). Despite several promising candidates, an effective vaccine that provides long-lived protection from malaria has not been developed (29). One vaccine candidate, RTS,S/AS01, has recently been approved for pilot implementation trials in sub-Saharan Africa (30). However, RTS,S/AS01 offers only modest and short lived protection (31, 32), and the efficacy of this vaccine varies with malaria transmission intensity (27). Other approaches are under study, but none has yet yielded a highly efficacious vaccine (32). A better understanding of the role of human genetic variation in heterogeneous immune responses to malaria infection may facilitate vaccine approaches. In this review, we provide a concise overview of evidence for associations between *KIR* and *HLA* genetic variants and susceptibility to or protection against malaria.

**Killer-cell Immunoglobulin-like Receptors**

Killer-cell immunoglobulin-like receptors (KIRs) are a family of highly polymorphic type 1 transmembrane glycoproteins expressed on the surface of NK cells and some T cells (33), that bind HLA class I molecules (34) and regulate NK cell functions (35). KIRs are encoded by a set of highly polymorphic genes located within the leucocyte receptor complex on human chromosome 19q13.4 (36). *KIRs* are the second most genetically diverse family in the mammalian genome after *HLA*, and they differ between individuals mainly at three levels, copy number variation, allelic diversity and variation in the binding specificity of individual *KIRs* to *HLA* class I ligands (37).

Sixteen *KIR* genes have been described to date, including genes that encode inhibitory (*KIR3DL1-3, KIR2DL1-3* and *KIR2DL5*) and activating (*KIR3DS1* and *KIR2DS1-5*) receptors (38). *KIR2DL4* is unique because it can trigger both activation and inhibition (39). *KIR2DP1*

and *KIR3DP1* are pseudogenes that do not encode cell surface receptors (40). The nomenclature of *KIR* genes is based on structural and functional characteristics (41). Depending on whether *KIR* have two or three extracellular immunoglobulin domains (D), they are named *KIR2D* or *KIR3D* (42). Functionally, *KIR* with short (S) intracytoplasmic tails activate NK cells by pairing with the immunoreceptor tyrosine-based activation motif (ITAM) containing adaptor protein DAP12 and those with long (L) intracytoplasmic tails inhibit NK cell functions, because they contain the immunoreceptor tyrosine-based inhibitory motif (ITIM) that recruits phosphatase SHP-1 (43). Inhibitory *KIRs* however can also prime NK cells for functional competence if they bind to self *HLA* class I molecules, a process known as NK cell education (44). *KIR* genes with two or three extracellular immunoglobulin domains and a short intracytoplasmic tails are named *KIR2DS* or *KIR3DS*, with specific genes identified by a suffix, such as *KIR2DS2*, *KIR2SD4 or KIR3DS1* (45). *KIR* genes with two or three extracellular immunoglobulin domains and long intracytoplasmic tail are named *KIR2DL* or *KIR3DL,* with specific genes identified by a suffix, for example *KIR2DL1*, *KIR2DL2*, *KIR3DL1* and *KIR3DL2* (45). The human *KIR* genes are grouped into *KIR A* and *KIR B* haplotypes (**Fig. 1**). Haplotype *A* comprises a fixed number of 7 *KIR* genes, including 3 'framework' genes present in all haplotypes (*KIR3DL3*, *KIR2DL4* and *KIR3DL2*) and *KIR2DL1*, *KIR2DL3*, *KIR3DL1* and *KIR2DS4. KIR2DS4* is the only activating KIR in this haplotype, and because it often carries a 22bp deletion, haplotype *A* is thought to be mostly inhibitory. About half of the individuals in any population studied to date will have haplotype *A* (46). Most diversity in haplotype *A* is conferred by allelic polymorphism (46). By definition, all other combinations of 4-16 *KIR* genes are classified as haplotype *B*, including many activating *KIR* such as *KIR2DS1, KIR2DS2, KIR2DS5* and *KIR3DS1,* in addition to *KIR2DL5* (46). Diversity of haplotype *B* is conferred by gene content (46). However, within the centromeric (*Cen*) and telomeric (*Tel*) ends of the *KIR* locus, there are recombination hot spots, so that crossover can occur and generate hybrid haplotypes such as *CenA-TelB* or *CenB-TelA*, which by definition belong to haplotype *B* (47). *KIR* genes exhibit strong linkage disequilibrium (LD), and are inherited together (36). Because of the tight linkage disequilibrium, many disease association studies with *KIR* genes actually analyse the association with haplotypes because it is difficult to isolate the role of single *KIR* genes (48).

A well supported hypothesis is that *KIR A* is specialized in fighting infectious pathogens (49), while *KIR B* is important in influencing successful reproduction (50). How inhibitory receptors in haplotype *A* may protect against infection diseases is unclear, however there are at least two possibilities. The first relates to the degree of inhibition, in that weaker inhibition may be beneficial for successful immune responses (51). The second relates to NK cell education, a process that primes NK cell function through the binding of inhibitory receptors to self MHC molecules (52). The strength of *KIR* and *HLA* binding varies depending on the specific receptor-ligand pair, as well as the affinity of the pairs (53) and, in turn, it impacts on regulation of NK cell activity (54). Heterogeneity in *KIR* gene content combined with allelic polymorphisms may lead to extensive haplotypic diversity and highly diverse NK cell populations within an individual (55). *KIR* diversity may in turn contribute to heterogeneous NK cell responses to *P. falciparum* malaria infection (36).

**Human Leucocyte Antigens**

The HLA complex encodes the most polymorphic human genes, and it is usually thought that their diversity is driven by resistance to pathogens (56). The HLA complex is composed of genes on chromosome 6 that code for molecules that mediate antigen recognition and presentation, as well as immunity against infectious pathogens including *P. falciparum* (57).

There are two classes of polymorphic HLA molecules based on their structure and function, HLA class I (HLA-A, -B and -C) and HLA class II (HLA-DR, -DQ, and -DP). HLA class I molecules are expressed on the surface of most nucleated cells. HLA class I and II molecules generally present antigenic peptides from infectious pathogens to CD8+ and CD4+ T cells, respectively (58). But HLA class I molecules are also major ligands for *KIRs* (59), and as such they play a role in regulation of NK cell activity (59). KIR binding to HLA class I molecules involves 4 epitopes named A3/A11, Bw4, C1 and C2 found on some HLA-A, some HLA-B and all HLA-C allotypes. Non-classical HLA class I molecules HLA-G (60), is expressed only on placental cells and may play roles in reproduction (61), binding to *KIR2DL4* and leukocyte Ig-like receptor B (LILRB) while the other non-classical class I HLA-E (60) presents peptides derived from other HLA class I molecules to the inhibitory CD94/NKG2A receptor on NK cells (62) and some T cells. HLA class II molecules are confined to immunocompetent cells such as B lymphocytes, dendritic cells, macrophages, endothelial cells, and activated T-lymphocytes, but their expression may be induced on other cell types (63).

**Immunity to malaria**

Acquired immunity to *P. falciparum* malaria infection is complex, requiring a balance of control of both parasite growth and inflammation, as overproduction of inflammatory cytokines results in adverse pathological consequences (64). Innate and adaptive immunity are both essential for limiting *P. falciparum* parasite growth and the severity of malaria infection (3, 65). Repeated exposure to *P. falciparum* results in attenuation of inflammation (66), and generation of antimalarial antibodies that are essential in the control of blood stage malaria infection (67, 68). Antimalarial antibodies play an essential role in immunity to malaria in areas with high *P. falciparum* malaria transmission intensity (69). Individuals living in malaria endemic regions acquire immunity against severe malaria quickly, but immunity against symptomatic malaria and control of parasitemia requires years of repeated exposure to malaria parasites, and remains incomplete (70-72).

It has been observed that humans are protected from malaria by antibodies through two mechanisms. First, antibodies bind to circulating free malaria parasites and prevent them from invading red blood cells (73, 74), and second, antibodies from malaria-immune individuals bind to *P. falciparum* proteins on the surface of infected red blood cells. This induces antibody dependent cellular cytotoxicity (ADCC) by NK cells (75), phagocytosis by monocytes, and engagement of effector cells via Fc-mediated interactions to mediate clearance of opsonized red blood cells (73)

**NK cells and innate immunity to *P. falciparum* malaria**

In humans, NK cells are usually defined as $CD3^{-}CD56^{+}$ cells (76), and can be further subdivided based on CD56 expression (77). Typically, $CD56^{dim}$ NK cells constitute the majority (90%) of peripheral blood NK cells (78), whereas $CD56^{bright}$ NK cells are more abundant in secondary lymphoid tissues (79). $CD56^{dim}$ NK cells express high levels of the low-affinity Fc receptor CD16, display variegated expression of inhibitory KIR for HLA class I, and express high levels of perforin (80). In contrast, $CD56^{bright}$ NK cells express no or low levels of CD16, exclusively express the inhibitory receptor CD94/NKG2A but no KIR and have 10-fold lower perforin expression than $CD56^{dim}$ NK cells (81). Therefore, $CD56^{dim}$ and $CD56^{bright}$ NK-cell subsets are considered to perform different functional roles (Fig. 2 A). Another subset of NK cells, referred to as adaptive or memory NK cells develop with age in people with certain infections. For example, in $HCMV^{+}$ individuals, adaptive NK cells can be found in blood that are identified by the expression of the CD94/NKG2C receptor and the CD57 marker (82).

Adaptive NK cells are differentiated from the rest of NK cells by absence of transcription factor promyelocytic leukemia zinc finger (PLZF) and of signalling Fc receptor γ-chain (FcRγ) that are lost through epigenetic modifications (83).

NK cells play a vital role in the innate immune response to falciparum malaria infections by production of IFN-γ, inhibition of parasite growth, and cytotoxic killing of intraerythrocytic parasites (75). NK cells are the first cells in peripheral blood to produce IFN- γ in response to *P. falciparum* infection (84) and also participate in the initiation and development of adaptive immune responses (85). It has been shown that terminally differentiated $CD56^{neg}$ NK cells expand in children after chronic malaria exposure and in those diagnosed with endemic Burkitt lymphoma (86). These NK cell activities can be triggered by three different but complementary pathways: cytokine activation (75), antibody-dependent cell-mediated cytotoxicity (ADCC) (75), and loss of inhibitory signalling due to downregulation of HLA class I ligands, also known as missing-self recognition (75). It was recently shown that $CD56^{dim}$ NK cells inhibit *P. falciparum* growth and kill *P. falciparum* within red blood cells flagged with IgG antibodies from individuals who live in malaria endemic areas through ADCC (**Fig. 2 A**). This is important during blood stage *P. falciparum* malaria infection (75). Liver NK cells also express KIR which, through interactions with HLA class I molecules expressed on the surface of hepatocytes (**Fig. 2B**), may modulate NK cell response to liver stage malaria infection (87).

In a recent study in Mali, multi-parameter flow cytometry showed an increase in the proportion of adaptive NK cells which were associated with lower parasitemia and protection from malaria infection (88). Indeed these cells enhanced ADCC response to *P. falciparum*–infected red blood cells (iRBCs) in the presence of naturally acquired antibodies from malaria-resistant individuals (88). Malaria susceptible individuals with a higher proportion of PLZF and FcRγ negative NK cells during the transmission season had improved odds of getting protected from malaria infection during the subsequent season (88). Although the most prominent cytokines produced by NK cells are IFN-γ and tumour necrosis factor-α (TNF-α), they also secrete other cytokines, including IL-10, the growth factor GM-CSF, and chemokines MIP-1α, MIP-1β, IL-8, and RANTES. It has been demonstrated that, production of the regulatory cytokine IL-10 by NK cells prevents overt pathology and death from cerebral malaria in mouse models (89).

Some studies have demonstrated that individuals may vary in their ability to elicit an innate immune response to malaria infection with clear implications for disease manifestations (4). This variation possibly arises as a result of several factors, including the strength of the cytokine and costimulatory signals provided to NK cells by accessory cells and differential NK cell maturation, depending on age and infection history. For example human cytomegalovirus (HCMV) modulates NK cell activity through engaging inhibitory receptors without triggering the activating surface receptors (90). HCMV also leads to suppression of NK cell receptor recognition by their HLA class ligands (91). Heterogeneity of NK cell responses to plasmodial infection may be attributed to interactions between NK cell receptors and their ligands, HLA class I molecules (84).

**NK cells and placental malaria**

Placental malaria results from sequestration of *P. falciparum* infected erythrocytes within the intervillous spaces of the placenta and may cause serious pregnancy complications such as abortion, stillbirth, and intrauterine growth restriction (92). Cytolysis of *P. falciparum* infected red blood cells by peripheral NK cells may be important in defence against placental malaria (93, 94). Previous studies have indeed shown that an increase in IFN-γ expressing

NK cells in the intervillous blood of the placenta at term is associated with a reduced risk of placental malaria (95)

A distinct population of NK cells is the most abundant leukocyte population in the decidua, where early in pregnancy these cells contribute to placentation and pregnancy outcomes by regulating trophoblast invasion and uterine vascular remodelling (96). Decidual NK cells may also provide first-line innate immune defence against infectious diseases including placental malaria (97). Decidual NK cells mainly express inhibitory *KIR2DL1*, *KIR2DL2*, and *KIR2DL3*, activating *KIR2DS1*, as well as *KIR2DL4*, which may perform inhibitory or activating functions (97). *KIR* diversity may be driven by balanced selection between pregnancy success and defence against pathogens (98)**.** Certain specific combinations of *KIR* and *HLA* genes affect the susceptibility to certain pregnancy complications (96). Other combinations may be associated with susceptibility to or protection from placental malaria (99). In a study of 688 placental malaria and HIV-coinfection in Kenya, *KIR BB* homozygosity was associated with protection from placental malaria in HIV negative pregnant women, but with susceptibility in HIV positive pregnant women (97). This reverse association between *KIR* genes and placental malaria remained only in women with high CD4 cell counts but not in those with low CD4 cell counts.

**KIR and immunity to *P. falciparum* malaria**

The activity of NK cells in immunity against *P. falciparum* infection depends on a fine balance between the strengths of the activating and inhibitory signals induced by KIR molecules (100). It has been clearly shown that KIR molecules regulate NK cell mediated production of IFN-γ in response to *P. falciparum* infection, and consequently influence the pathogenesis and severity of malaria (101). For example, NK cells from individuals with *KIR AB* haplotypes produce greater IFN-γ responses *in vitro* in response to *P. falciparum* infection compared to responses in either *KIR AA* or *KIR BB* homozygous individuals (102).

A study of 477 malaria cases in Thailand showed association of the *KIR2DL3* gene that marks haplotype *A* and of its ligand *HLA-C1* with cerebral malaria. Moreover, this receptor-ligand pair was found at lower frequency in malaria high-endemic populations, suggesting evidence of selection due to unhelpful NK cell responses which may increase susceptibility to cerebral malaria (103). However, comparing *KIR* genotypes of 321 children with severe and uncomplicated malaria with those of 314 control children, the *Cen-AB* haplotype was found to be more frequent in malaria cases in the Gambia, with more activating KIR genes (*KIR2DS2* and *KIR2DS5*) in very ill children, suggesting that exaggerated NK cell activation may contribute to the pathogenesis of severe malaria (100). Furthermore, parasitemia was higher in *KIR AA* homozygote individuals, and more frequent in controls, suggesting that more inhibitory *A* haplotype may not favour parasite clearance but may protect from severe malaria (100).

In Southwest Nigeria, *KIR2DL5, KIR2DS3* and *KIR2DS5* genes were overrepresented in individuals with asymptomatic *P. falciparum* parasitemia compared to those with either uncomplicated or severe malaria (104). Furthermore, the frequency of *KIR2DS3* and *KIR2DS5* was higher in individuals with uncomplicated malaria compared to those with severe malaria (104). These results suggest a protective role for inhibitory *KIR2DL5* and activating *KIR2DS3* and *KIR2DS5* genes from severe malaria. Heterozygosity at the *KIR* centromeric region, *Cen-AB2*, has also been shown to be more frequent in asymptomatic controls compared to individuals with severe malaria (104). This implies that, KIR heterozygous *AB* haplotypes may be associated with protection from severe malaria, and it has been suggested that this may be due to the greater proportion of educated NK cells in *KIR AB* heterozygous individuals. In Africans the centromeric region of the *KIR* locus, which encodes *HLA-C* receptors, is highly

diverse, whereas the telomeric region encoding Bw4-specific receptors (*KIR3DL1*) lacks diversity (105). These features of *KIR* are consistent with ongoing selection imposed by malaria pathogens in Africa (105).

In northern India, individuals possessing *KIR2DS2, KIR2DL1* and *KIR2DL3* genes were susceptible to severe malaria (106). In this same study, a combination of *KIR2DS2-HLAC1, KIR2DL1-HLAC2* and *KIR2DL3-HLAC1* was found to be associated with increased risk of getting severe malaria (106). In a study of 77 Melanesian individuals with history or symptoms of malaria in the malaria high-endemic Solomon Islands, *KIR AB* heterozygosity showed higher frequency among 37 Plasmodium-positive individuals compared to 40 negative individuals (107). The non-deleted allele of *KIR2DS4* (*001) was also more frequent in malaria positive individuals (107). Other studies have focused on functional responses of lymphocytes from healthy donors to *P. falciparum*-infected red blood cells (iRBC) and found heterogeneity in donor's NK-cell IFN-γ production, which was significantly associated with *KIR* genotypes (108), showing that KIR *AB* heterozygosity was associated with high responses of CD56dim cells (109). It is difficult to draw a conclusive picture from the available evidence. While *KIR AB* heterozygosity may favour a larger number of 'educated $CD56^{dim}$ NK cells that can respond more effectively to the infection, individuals with numerous activating KIR may mount too vigorous responses that may be deleterious in infected individuals. Individual *KIR* genes as well as *KIR* and their *HLA* class I ligands shown to be associated with susceptibility to or protection from severe malaria are summarised in Table 1.

## Table 1: KIR genetic variants and their HLA ligand combinations linked to susceptibility and protection from *P. falciparum* malaria

| Reference | Study | Findings |
|---|---|---|
| (108) | NK cell responses of healthy donors to *P. falciparum* iRBCs (n =27) | Individuals with *Tel-AA KIR* had a high NK cell response to *P. falciparum* in donors of European, Asian, and African descent |
| (107) | Plasmodium positive and negative malaria patients (n =77) | *KIR-AB* heterozygotes were more frequent in Plasmodium-positive Melanesians |
| (109) | NK cell responses of healthy donors to infected RBCs (n =81) | *KIR-AB* heterozygosity favoured IFN-γ production by $CD56^{dim}$ cells in response to *P. falciparum* infected RBCs |
| (103) | Severe malaria (n=133), uncomplicated malaria (n=188) and control (n=314) children | In the Gambia, parasitemia was higher in children with *KIR-AA*, however *Cen-AA* was more frequent in controls. *Cen-AB* more frequent in malaria cases with *KIR-BB* and *HLA-C1* more frequent in very ill children |
| (102) | Cerebral and non-complicated malaria cases (n = 477) | In Thailand, significant association of *KIR2DL3* and its ligand *HLA-C1* with cerebral malaria. Also, *KIR2DL3* and *HLA-C1* less frequent in malaria high-endemic populations |
| (96) | Placental malaria in 479 HIV positive and 209 HIV negative pregnant women | In Kenya, *KIR-BB* homozygosity was associated with protection from placental malaria in HIV negative pregnancies but susceptibility in HIV positive pregnancies |
| (104) | Severe malaria (n=201), uncomplicated malaria (n=153) and asymptomatic malaria (n=200) | In South West Nigeria, *Cen-AB* less frequent in severe malaria, with *KIR-BB* more frequent in severe and uncomplicated malaria compared to asymptomatic |
| (106) | Cerebral malaria (n=213) Uncomplicated (n=87) | North India, cerebral malaria associated with *KIR2DS2* (*B*) *KIR2DL1* (*A)* and *KIR2DL3* (*A*). *KIR-BB* associated with cerebral malaria. *AB* associated with uncomplicated malaria |

**HLA genetic variants and immunity to *P. falciparum* malaria**

The unique variability of the genes that encode HLA molecules and their haplotypic composition, especially in native Africans, is proposed to have resulted from needs to fight multiple and frequent deadly infectious pathogens (110). HLA class I molecules are major ligands for *KIRs*, and as such, they play a role in regulation of NK cell activity during malaria infection. HLA class I molecules also present malaria antigens to T cells and are therefore important in adaptive immunity to malaria, which is critical during liver stage *P. falciparum* malaria infection. HLA class II molecules mediate the clearance of red blood cells infected with *P. falciparum* parasites through stimulation of T helper cells (111). Some studies have clearly shown that HLA molecules influence antibody titres to malaria antigens, including glutamate rich protein and merozoite surface antigens (112).

Several studies have shown some *HLA* genetic variants to be associated with susceptibility or protection from severe malaria. However, results have been inconsistent (Table 2 A and 2B). The first study on the role of *HLA* in malaria described protection from cerebral malaria and severe malaria anaemia conferred by the *HLA* class I allele, *HLA-Bw53*, and the *HLA* class II haplotype, *HLA-DRBI*1302-DQAI*0102-DQBI*0501*. Molecular analysis revealed that liver stage antigen-I (LSA-1), a liver-stage specific antigen of *P. falciparum,* was recognized by *HLA-Bw53*-positive individuals. However, these findings have not been confirmed in other malaria endemic populations, and significant associations of malaria antigens other than LSA-1 with HLA molecules have not been discovered.

In studies of interactions between variable HLA molecules and polymorphic parasite factors, it has been demonstrated that the *P. falciparum* circumsporozoite protein binds to HLA-DR and HLA-DQ molecules in vitro as well as in animal models. Two HLA class II alleles, *DRB1*04* and *DPB1*1701*, have been observed to be more frequent in severe, compared to uncomplicated malaria. *HLA-B49, -A1, -B27* and *HLA-DRB1*0809* was shown to be strongly associated with severe malaria in a study conducted in Mumbai in India (113). *HLA-A19* and *HLA-DQB1 *0203* were associated with protection from severe malaria in the same population (113). In a study conducted in Thailand in patients with severe cerebral malaria, *HLA-B46* was significantly associated with risk of cerebral malaria, while *HLA-B56* and *HLADR1*1001* were associated with protection from cerebral malaria (114). In Senegal, *HLA-DR3* and *HLA-DR10* were strongly associated with cerebral malaria (115). In the Gambia, *HLA-B53* and *HLA-DRB1*1302* alleles were found to be strongly associated with protection from severe malaria (116).

**Table 2A: HLA class I genetic variants associated with susceptibility or protection against *P. falciparum* malaria**

| Reference | Country | HLA class I associated with susceptibility to severe malaria | HLA class I associated with protection from severe malaria |
|---|---|---|---|
| (117) | The Gambia | - | *B*53* |
| (118) | Malaysia | - | *B*1513* |
| (113) | Mumbai, India | *HLA-A1, HLA-B27* and *HLA-B49* | *HLA-A19* |
| (114) | Thailand | *HLA-B46* | *HLA-B56* |
| (119) | Sardinia, Italy | - | *B*35* |
| (120) | New Delhi, India | - | *A*0211* |
| (100) | The Gambia | - | *HLA-Cw*16:01* frequency (a C group 1 allele) |

**Table 2B: HLA class II genetic variants associated with susceptibility or resistance to *P. falciparum* malaria**

| Reference | Country | HLAs associated with susceptibility to malaria | HLAs associated with protection against malaria |
|---|---|---|---|
| (115) | Senegal | *DR*3, DR*10* (cerebral malaria) | - |
| (121) | The Gambia | - | *DRB1*1302-DQB1 *0501* |
| (122) | Vietnam | - | *HLA-DQ1*0502* |
| (113) | Mumbai, india | *HLA-DRB1*0809* | *HLA-DQB1*0203* |
| (114) | Thailand | - | *DRB1*1001* |

**Concluding remarks**

It is clear that interactions between KIR and HLA molecules modulate the activity of NK cells. The role of NK cells in responses to malaria has become established, and therefore it is important to decipher how *KIR* and *HLA* immunogenetics regulate susceptibility to or protection from malaria, in order to understand the heterogeneous responses of populations to malaria infection. This in turn may help understand malaria transmission and severity in specific populations and inform specific malaria control intervention.

Several studies have suggested that certain *KIR* and *HLA* variants are associated with malaria but there are challenges at different levels to draw a comprehensive picture of how *KIR* and *HLA* variants contribute to susceptibility to or protection against malaria. Some challenges are related to population genetics, some to parasite genetics and others to the biology of *KIR* and *HLA* immunogenetics. First of all, some of these studies are conducted with small sample sizes, emphasizing the need to study larger populations in Africa and other malaria endemic populations, particularly to accommodate for allelic diversity. Different populations may have selected different *KIR* and *HLA* variants. Other infectious pathogens prevalent in certain malaria endemic populations may also exert selective pressure on immune responses, thus shaping the diversity of *KIR* and *HLA* in those populations. Therefore, the role of other co-infections should be considered in studies involving *KIR* and malaria especially in populations with many infectious pathogens. Different species of parasites may infect different populations and unique antigenic drifts may occur in parasites in different populations. How the binding between certain KIR and HLA molecules regulate NK cell function in the context of any given disease is still very challenging to determine. In the case of malaria, knowledge of the rich *KIR* allelic diversity in African populations and their binding affinities to equally diverse *HLA* ligands is still being sought. KIR can either inhibit or enhance NK cell effector functions. Combinations of *KIR* and *HLA* determine both NK cell education – that is the priming of effector function – and effector function themselves. However, because inhibitory KIR and other inhibitory receptors (e.g. CD94/NKG2A) contribute to education and effector function in opposite ways (i.e. they promote education but suppress function), it may be difficult to recreate how *KIR* and *HLA* immunogenetics determine the biology of NK cells during malaria infection. For example, inhibitory interactions may be necessary to educate NK cell to mediate ADCC required to eliminate iRBC, but not required or even counterproductive to activate IFN-$\gamma$ production by NK cells upon interaction with HLA class I-expressing infected cells.

Finally, evolutionary pressure from *P. falciparum* malaria pathogens might have selected for certain *KIR* and *HLA* genetic variants that protect against severe malaria but increase the risk of other diseases, as it has been well documented for sickle cell disease and other hemoglobinopathies. For example, the high frequency in sub-Saharan Africa of certain *KIR* and *HLA* variants associated with pregnancy complications (123), may be the result of the trade-off for the selection of genetic variants that protect against malaria.

## Conflict of interest

Olympe Chazara started a role as an employee of AstraZeneca, UK at the time of manuscript preparation. Other authors have declared that no competing conflict of interests exist.


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## References


1. Ashley EA, Pyae Phyo A, Woodrow CJ. Malaria. Lancet (London, England). 2018;391(10130):1608-21.
2. Gomes PS, Bhardwaj J, Rivera-Correa J, Freire-De-Lima CG, Morrot A. Immune Escape Strategies of Malaria Parasites. Frontiers in microbiology. 2016;7(1617).
3. Gowda DC, Wu X. Parasite Recognition and Signaling Mechanisms in Innate Immune Responses to Malaria. Frontiers in immunology. 2018;9(3006).
4. Doolan DL, Dobaño C, Baird JK. Acquired Immunity to Malaria. Clinical Microbiology Reviews. 2009;22(1):13.
5. Nhabomba AJ, Guinovart C, Jiménez A, Manaca MN, Quintó L, Cisteró P, et al. Impact of age of first exposure to Plasmodium falciparum on antibody responses to malaria in children: a randomized, controlled trial in Mozambique. Malaria Journal. 2014;13(1):121.
6. Varo R, Crowley VM, Sitoe A, Madrid L, Serghides L, Kain KC, et al. Adjunctive therapy for severe malaria: a review and critical appraisal. Malaria Journal. 2018;17(1):47.
7. Maitland K. Severe Malaria in African Children - The Need for Continuing Investment. The New England journal of medicine. 2016;375(25):2416-7.
8. Mpimbaza A, Walakira A, Ndeezi G, Katahoire A, Karamagi C, Nsobya SL, et al. Associations between erythrocyte polymorphisms and risks of uncomplicated and severe malaria in Ugandan children: A case control study. PLoS One. 2018;13(9):e0203229-e.
9. Goheen MM, Campino S, Cerami C. The role of the red blood cell in host defence against falciparum malaria: an expanding repertoire of evolutionary alterations. British Journal of Haematology. 2017;179(4):543-56.
10. Agarwal A, Guindo A, Cissoko Y, Taylor JG, Coulibaly D, Koné A, et al. Hemoglobin C associated with protection from severe malaria in the Dogon of Mali, a West African population with a low prevalence of hemoglobin S. Blood. 2000;96(7):2358-63.
11. Mockenhaupt FP, Ehrhardt S, Cramer JP, Otchwemah RN, Anemana SD, Goltz K, et al. Hemoglobin C and Resistance to Severe Malaria in Ghanaian Children. The Journal of infectious diseases. 2004;190(5):1006-9.

12. Kwiatkowski DP. How Malaria Has Affected the Human Genome and What Human Genetics Can Teach Us about Malaria. The American Journal of Human Genetics. 2005;77(2):171-92.
13. Norman PJ, Hollenbach JA, Nemat-Gorgani N, Marin WM, Norberg SJ, Ashouri E, et al. Defining KIR and HLA Class I Genotypes at Highest Resolution via High-Throughput Sequencing. American journal of human genetics. 2016;99(2):375-91.
14. Matzaraki V, Kumar V, Wijmenga C, Zhernakova A. The MHC locus and genetic susceptibility to autoimmune and infectious diseases. Genome Biology. 2017;18(1):76.
15. Bodis G, Toth V, Schwarting A. Role of Human Leukocyte Antigens (HLA) in Autoimmune Diseases. Rheumatology and Therapy. 2018;5(1):5-20.
16. Cooley S, Parham P, Miller JS. Strategies to activate NK cells to prevent relapse and induce remission following hematopoietic stem cell transplantation. Blood. 2018;131(10):1053.
17. Torres AR, Sweeten TL, Johnson RC, Odell D, Westover JB, Bray-Ward P, et al. Common Genetic Variants Found in HLA and KIR Immune Genes in Autism Spectrum Disorder. Frontiers in Neuroscience. 2016;10(463).
18. Colucci F. The role of KIR and HLA interactions in pregnancy complications. Immunogenetics. 2017;69(8):557-65.
19. Jiang W, Johnson C, Simecek N, López-Álvarez MR, Di D, Trowsdale J, et al. qKAT: a high-throughput qPCR method for KIR gene copy number and haplotype determination. Genome Medicine. 2016;8(1):99.
20. Meyer D, C. Aguiar VR, Bitarello BD, C. Brandt DY, Nunes K. A genomic perspective on HLA evolution. Immunogenetics. 2018;70(1):5-27.
21. Quintana-Murci L. Understanding rare and common diseases in the context of human evolution. Genome Biology. 2016;17(1):225.
22. Dhiman S. Are malaria elimination efforts on right track? An analysis of gains achieved and challenges ahead. Infect Dis Poverty. 2019;8(1):14.
23. Burrows JN, Duparc S, Gutteridge WE, Hooft van Huijsduijnen R, Kaszubska W, Macintyre F, et al. New developments in anti-malarial target candidate and product profiles. Malaria Journal. 2017;16(1):26.
24. Ashley EA, Phyo AP. Drugs in Development for Malaria. Drugs. 2018;78(9):861-79.
25. Sinha S, Medhi B, Sehgal R. Challenges of drug-resistant malaria. Parasite. 2014;21:61.
26. Birkholtz L-M, Bornman R, Focke W, Mutero C, de Jager C. Sustainable malaria control: transdisciplinary approaches for translational applications. Malaria Journal. 2012;11(1):431.
27. Ssemaganda A, Giddam AK, Zaman M, Skwarczynski M, Toth I, Stanisic DI, et al. Induction of Plasmodium-Specific Immune Responses Using Liposome-Based Vaccines. Frontiers in immunology. 2019;10:135-.
28. Coelho CH, Doritchamou JYA, Zaidi I, Duffy PE. Advances in malaria vaccine development: report from the 2017 malaria vaccine symposium. npj Vaccines. 2017;2(1):34.
29. Ouattara A, Laurens MB. Vaccines Against Malaria. Clinical Infectious Diseases. 2014;60(6):930-6.
30. van den Berg M, Ogutu B, Sewankambo NK, Biller-Andorno N, Tanner M. RTS,S malaria vaccine pilot studies: addressing the human realities in large-scale clinical trials. Trials. 2019;20(1):316.
31. Gosling R, von Seidlein L. The Future of the RTS,S/AS01 Malaria Vaccine: An Alternative Development Plan. PLOS Medicine. 2016;13(4):e1001994.
32. Beeson JG, Kurtovic L, Dobaño C, Opi DH, Chan J-A, Feng G, et al. Challenges and strategies for developing efficacious and long-lasting malaria vaccines. Science Translational Medicine. 2019;11(474):eaau1458.
33. Pallmer K, Oxenius A. Recognition and Regulation of T Cells by NK Cells. Frontiers in immunology. 2016;7(251).
34. Wroblewski EE, Parham P, Guethlein LA. Two to Tango: Co-evolution of Hominid Natural Killer Cell Receptors and MHC. Frontiers in immunology. 2019;10(177).

35. Pende D, Falco M, Vitale M, Cantoni C, Vitale C, Munari E, et al. Killer Ig-Like Receptors (KIRs): Their Role in NK Cell Modulation and Developments Leading to Their Clinical Exploitation. Frontiers in immunology. 2019;10:1179-.
36. Middleton D, Gonzelez F. The extensive polymorphism of KIR genes. Immunology. 2010;129(1):8-19.
37. Parham P. The genetic and evolutionary balances in human NK cell receptor diversity. Semin Immunol. 2008;20(6):311-6.
38. Béziat V, Hilton H, Norman P, Traherne J. Deciphering the killer-cell immunoglobulin-like receptor system at super-resolution for natural killer and T-cell biology. 2016.
39. Faure M, Long EO. KIR2DL4 (CD158d), an NK Cell-Activating Receptor with Inhibitory Potential. The Journal of Immunology. 2002;168(12):6208.
40. Moffett A, Chazara O, Colucci F, Johnson MH. Variation of maternal KIR and fetal HLA-C genes in reproductive failure: too early for clinical intervention. Reproductive BioMedicine Online. 2016;33(6):763-9.
41. Campbell KS, Purdy AK. Structure/function of human killer cell immunoglobulin-like receptors: lessons from polymorphisms, evolution, crystal structures and mutations. Immunology. 2011;132(3):315-25.
42. Moesta AK, Parham P. Diverse functionality among human NK cell receptors for the C1 epitope of HLA-C: KIR2DS2, KIR2DL2, and KIR2DL3. Frontiers in immunology. 2012;3:336-.
43. Cheent K, Khakoo SI. Natural killer cells: integrating diversity with function. Immunology. 2009;126(4):449-57.
44. Orr MT, Lanier LL. Natural killer cell education and tolerance. Cell. 2010;142(6):847-56.
45. Moesta A, Parham P. Diverse functionality among human NK cell receptors for the C1 epitope of HLA-C: KIR2DS2, KIR2DL2, and KIR2DL3. Frontiers in immunology. 2012;3(336).
46. Colucci F, Traherne J. Killer-cell immunoglobulin-like receptors on the cusp of modern immunogenetics. Immunology. 2017;152(4):556-61.
47. Jiang W, Johnson C, Jayaraman J, Simecek N, Noble J, Moffatt MF, et al. Copy number variation leads to considerable diversity for B but not A haplotypes of the human KIR genes encoding NK cell receptors. Genome Res. 2012;22(10):1845-54.
48. Single RM, Martin MP, Meyer D, Gao X, Carrington M. Methods for assessing gene content diversity of KIR with examples from a global set of populations. Immunogenetics. 2008;60(12):711-25.
49. Penman BS, Moffett A, Chazara O, Gupta S, Parham P. Reproduction, infection and killer-cell immunoglobulin-like receptor haplotype evolution. Immunogenetics. 2016;68(10):755-64.
50. Parham P, Moffett A. Variable NK cell receptors and their MHC class I ligands in immunity, reproduction and human evolution. Nature reviews Immunology. 2013;13(2):133-44.
51. Khakoo SI, Thio CL, Martin MP, Brooks CR, Gao X, Astemborski J, et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. Science (New York, NY). 2004;305(5685):872-4.
52. Martin MP, Qi Y, Gao X, Yamada E, Martin JN, Pereyra F, et al. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. Nat Genet. 2007;39(6):733-40.
53. Guethlein LA, Norman PJ, Hilton HG, Parham P. Co-evolution of MHC class I and variable NK cell receptors in placental mammals. Immunological reviews. 2015;267(1):259-82.
54. Boudreau JE, Hsu KC. Natural Killer Cell Education and the Response to Infection and Cancer Therapy: Stay Tuned. Trends Immunol. 2018;39(3):222-39.
55. Korbel DS, Newman KC, Almeida CR, Davis DM, Riley EM. Heterogeneous Human NK Cell Responses to <em>Plasmodium falciparum</em>-Infected Erythrocytes. The Journal of Immunology. 2005;175(11):7466.
56. Choo SY. The HLA system: genetics, immunology, clinical testing, and clinical implications. Yonsei Med J. 2007;48(1):11-23.

57. Mosaad YM. Clinical Role of Human Leukocyte Antigen in Health and Disease. Scand J Immunol. 2015;82(4):283-306.
58. Rock KL, Reits E, Neefjes J. Present Yourself! By MHC Class I and MHC Class II Molecules. Trends Immunol. 2016;37(11):724-37.
59. Hollenbach JA, Pando MJ, Caillier SJ, Gourraud PA, Oksenberg JR. The killer immunoglobulin-like receptor KIR3DL1 in combination with HLA-Bw4 is protective against multiple sclerosis in African Americans. Genes and immunity. 2016;17(3):199-202.
60. Carlini F, Ferreira V, Buhler S, Tous A, Eliaou J-F, René C, et al. Association of HLA-A and Non-Classical HLA Class I Alleles. PLoS One. 2016;11(10):e0163570.
61. Durmanova V, Homolova M, Drobny J, Shawkatová I, Buc M. Role of HLA-G and other immune mechanisms in pregnancy. Central European Journal of Biology. 2013;8.
62. Rölle A, Jäger D, Momburg F. HLA-E Peptide Repertoire and Dimorphism—Centerpieces in the Adaptive NK Cell Puzzle? Frontiers in immunology. 2018;9(2410).
63. Greenbaum J, Sidney J, Chung J, Brander C, Peters B, Sette A. Functional classification of class II human leukocyte antigen (HLA) molecules reveal seven different supertypes and a surprising degree of repertoire sharing across supertypes. Immunogenetics. 2011;63:325-35.
64. Kumar R, Ng S, Engwerda C. The Role of IL-10 in Malaria: A Double Edged Sword. Frontiers in immunology. 2019;10(229).
65. Liehl P, Meireles P, Albuquerque IS, Pinkevych M, Baptista F, Mota MM, et al. Innate Immunity Induced by <span class="named-content genus-species" id="named-content-1">Plasmodium</span> Liver Infection Inhibits Malaria Reinfections. Infection and Immunity. 2015;83(3):1172.
66. Portugal S, Moebius J, Skinner J, Doumbo S, Doumtabe D, Kone Y, et al. Exposure-dependent control of malaria-induced inflammation in children. PLoS Pathog. 2014;10(4):e1004079-e.
67. Stanisic DI, Fowkes FJI, Koinari M, Javati S, Lin E, Kiniboro B, et al. Acquisition of Antibodies against <span class="named-content genus-species" id="named-content-1">Plasmodium falciparum</span> Merozoites and Malaria Immunity in Young Children and the Influence of Age, Force of Infection, and Magnitude of Response. Infection and Immunity. 2015;83(2):646.
68. Cockburn IA, Seder RA. Malaria prevention: from immunological concepts to effective vaccines and protective antibodies. Nature Immunology. 2018;19(11):1199-211.
69. Kusi KA, Manu EA, Manful Gwira T, Kyei-Baafour E, Dickson EK, Amponsah JA, et al. Variations in the quality of malaria-specific antibodies with transmission intensity in a seasonal malaria transmission area of Northern Ghana. PLoS One. 2017;12(9):e0185303.
70. Ly A, Hansen DS. Development of B Cell Memory in Malaria. Frontiers in immunology. 2019;10(559).
71. Farrington L, Vance H, Rek J, Prahl M, Jagannathan P, Katureebe A, et al. Both inflammatory and regulatory cytokine responses to malaria are blunted with increasing age in highly exposed children. Malaria Journal. 2017;16(1):499.
72. Valletta JJ, Recker M. Identification of immune signatures predictive of clinical protection from malaria. PLoS computational biology. 2017;13(10):e1005812.
73. Arora G, Hart GT, Manzella-Lapeira J, Doritchamou JYA, Narum DL, Thomas LM, et al. NK cells inhibit Plasmodium falciparum growth in red blood cells via antibody-dependent cellular cytotoxicity. eLife. 2018;7:e36806.
74. Paul AS, Egan ES, Duraisingh MT. Host-parasite interactions that guide red blood cell invasion by malaria parasites. Curr Opin Hematol. 2015;22(3):220-6.
75. Arora G, Hart GT, Manzella-Lapeira J, Doritchamou JY, Narum DL, Thomas LM, et al. NK cells inhibit Plasmodium falciparum growth in red blood cells via antibody-dependent cellular cytotoxicity. eLife. 2018;7:e36806.

76. Gharehbaghian A, Donaldson C, Newman J, Bannister G, Bradley BA. The correlation between the percent of CD3- CD56+ cells and NK precursor function. Iran J Allergy Asthma Immunol. 2006;5(4):167-75.
77. Rebuli ME, Pawlak EA, Walsh D, Martin EM, Jaspers I. Distinguishing Human Peripheral Blood NK Cells from CD56dimCD16dimCD69+CD103+ Resident Nasal Mucosal Lavage Fluid Cells. Sci Rep. 2018;8(1):3394.
78. Amand M, Iserentant G, Poli A, Sleiman M, Fievez V, Sanchez IP, et al. Human CD56(dim)CD16(dim) Cells As an Individualized Natural Killer Cell Subset. Frontiers in immunology. 2017;8:699-.
79. Michel T, Poli A, Cuapio A, Briquemont B, Iserentant G, Ollert M, et al. Human CD56<sup>bright</sup> NK Cells: An Update. The Journal of Immunology. 2016;196(7):2923.
80. Fauriat C, Long EO, Ljunggren H-G, Bryceson YT. Regulation of human NK-cell cytokine and chemokine production by target cell recognition. Blood. 2010;115(11):2167-76.
81. Jacobs R, Hintzen G, Kemper A, Beul K, Kempf S, Behrens G, et al. CD56bright cells differ in their KIR repertoire and cytotoxic features from CD56dim NK cells. European journal of immunology. 2001;31:3121-6.
82. Hammer Q, Rückert T, Dunst J, Romagnani C. Adaptive Natural Killer Cells Integrate Interleukin-18 during Target-Cell Encounter. Frontiers in immunology. 2018;8:1976-.
83. Schlums H, Cichocki F, Tesi B, Theorell J, Beziat V, Holmes TD, et al. Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function. Immunity. 2015;42(3):443-56.
84. Wolf A-S, Sherratt S, Riley EM. NK Cells: Uncertain Allies against Malaria. Frontiers in immunology. 2017;8:212-.
85. Obaid A, Naz A, Ikram A, Awan FM, Raza A, Ahmad J, et al. Model of the adaptive immune response system against HCV infection reveals potential immunomodulatory agents for combination therapy. Sci Rep. 2018;8(1):8874.
86. Forconi CS, Cosgrove CP, Saikumar-Lakshmi P, Nixon CE, Foley J, Ong'echa JM, et al. Poorly cytotoxic terminally differentiated CD56negCD16pos NK cells accumulate in Kenyan children with Burkitt lymphomas. Blood Adv. 2018;2(10):1101-14.
87. Liu P, Chen L, Zhang H. Natural Killer Cells in Liver Disease and Hepatocellular Carcinoma and the NK Cell-Based Immunotherapy. J Immunol Res. 2018;2018:1206737-.
88. Hart GT, Tran TM, Theorell J, Schlums H, Arora G, Rajagopalan S, et al. Adaptive NK cells in people exposed to Plasmodium falciparum correlate with protection from malaria. The Journal of experimental medicine. 2019;216(6):1280-90.
89. Burrack KS, Hart GT, Hamilton SE. Contributions of natural killer cells to the immune response against Plasmodium. Malaria Journal. 2019;18(1):321.
90. Goodier MR, Jonjić S, Riley EM, Juranić Lisnić V. CMV and natural killer cells: shaping the response to vaccination. European journal of immunology. 2018;48(1):50-65.
91. Wilkinson GWG, Tomasec P, Stanton RJ, Armstrong M, Prod'homme V, Aicheler R, et al. Modulation of natural killer cells by human cytomegalovirus. J Clin Virol. 2008;41(3):206-12.
92. Sharma L, Shukla G. Placental Malaria: A New Insight into the Pathophysiology. Front Med (Lausanne). 2017;4:117-.
93. Oth T, Habets THPM, Germeraad WTV, Zonneveld MI, Bos GMJ, Vanderlocht J. Pathogen recognition by NK cells amplifies the pro-inflammatory cytokine production of monocyte-derived DC via IFN-γ. BMC Immunology. 2018;19(1):8.
94. Clark SE, Filak HC, Guthrie BS, Schmidt RL, Jamieson A, Merkel P, et al. Bacterial Manipulation of NK Cell Regulatory Activity Increases Susceptibility to Listeria monocytogenes Infection. PLoS Pathog. 2016;12(6):e1005708.

95. Othoro C, Moore JM, Wannemuehler KA, Moses S, Lal A, Otieno J, et al. Elevated gamma interferon-producing NK cells, CD45RO memory-like T cells, and CD4 T cells are associated with protection against malaria infection in pregnancy. Infect Immun. 2008;76(4):1678-85.
96. Moffett A, Colucci F. Uterine NK cells: active regulators at the maternal-fetal interface. The Journal of clinical investigation. 2014;124(5):1872-9.
97. Omosun YO, Blackstock AJ, Gatei W, Hightower A, van Eijk AM, Ayisi J, et al. Differential association of gene content polymorphisms of killer cell immunoglobulin-like receptors with placental malaria in HIV- and HIV+ mothers. PLoS One. 2012;7(6):e38617-e.
98. Parham P, Moffett A. Variable NK cell receptors and their MHC class I ligands in immunity, reproduction and human evolution. Nature reviews Immunology. 2013;13(2):133-44.
99. Colucci F. The role of KIR and HLA interactions in pregnancy complications. Immunogenetics. 2017;69(8-9):557-65.
100. Yindom LM, Forbes R, Aka P, Janha O, Jeffries D, Jallow M, et al. Killer-cell immunoglobulin-like receptors and malaria caused by Plasmodium falciparum in The Gambia. Tissue Antigens. 2012;79(2):104-13.
101. Korbel DS, Newman KC, Almeida CR, Davis DM, Riley EM. Heterogeneous human NK cell responses to Plasmodium falciparum-infected erythrocytes. Journal of immunology (Baltimore, Md : 1950). 2005;175(11):7466-73.
102. Wolf A-S, Sherratt S, Riley EM. NK Cells: Uncertain Allies against Malaria. Frontiers in immunology. 2017;8(212).
103. Hirayasu K, Ohashi J, Kashiwase K, Hananantachai H, Naka I, Ogawa A, et al. Significant association of KIR2DL3-HLA-C1 combination with cerebral malaria and implications for co-evolution of KIR and HLA. PLoS Pathog. 2012;8(3):e1002565-e.
104. Olaniyan SA, Amodu OK, Yindom L-M, Conway DJ, Aka P, Bakare AA, et al. Killer-cell immunoglobulin-like receptors and falciparum malaria in southwest Nigeria. Human immunology. 2014;75(8):816-21.
105. Norman PJ, Hollenbach JA, Nemat-Gorgani N, Guethlein LA, Hilton HG, Pando MJ, et al. Co-evolution of human leukocyte antigen (HLA) class I ligands with killer-cell immunoglobulin-like receptors (KIR) in a genetically diverse population of sub-Saharan Africans. PLoS genetics. 2013;9(10):e1003938-e.
106. Prakash S, Ranjan P, Ghoshal U, Agrawal S. KIR-like activating natural killer cell receptors and their association with complicated malaria in north India. Acta Trop. 2018;178:55-60.
107. Taniguchi M, Kawabata M. KIR3DL1/S1 genotypes and KIR2DS4 allelic variants in the AB KIR genotypes are associated with Plasmodium-positive individuals in malaria infection. Immunogenetics. 2009;61:717-30.
108. Artavanis-Tsakonas K, Eleme K, McQueen KL, Cheng NW, Parham P, Davis DM, et al. Activation of a Subset of Human NK Cells upon Contact with <em>Plasmodium falciparum</em>-Infected Erythrocytes. The Journal of Immunology. 2003;171(10):5396.
109. Korbel DS, Norman PJ, Newman KC, Horowitz A, Gendzekhadze K, Parham P, et al. Killer Ig-like receptor (KIR) genotype predicts the capacity of human KIR-positive CD56dim NK cells to respond to pathogen-associated signals. Journal of immunology (Baltimore, Md : 1950). 2009;182(10):6426-34.
110. Blackwell JM, Jamieson SE, Burgner D. HLA and infectious diseases. Clinical microbiology reviews. 2009;22(2):370-85.
111. Belachew EB. Immune Response and Evasion Mechanisms of Plasmodium falciparum Parasites. J Immunol Res. 2018;2018:6529681-.
112. Ghosh K. Evolution and selection of human leukocyte antigen alleles by Plasmodium falciparum infection. Human immunology. 2008;69(12):856-60.
113. Shankarkumar U, Devaraj JP, Ghosh K, Karnad D, Anand K, Mohanty D. HLA associations in P. falciparum malaria patients from Mumbai, western India. Indian J Malariol. 2002;39(3-4):76-82.

114. Hananantachai H, Patarapotikul J, Ohashi J, Naka I, Looareesuwan S, Tokunaga K. Polymorphisms of the HLA-B and HLA-DRB1 genes in Thai malaria patients. Jpn J Infect Dis. 2005;58(1):25-8.
115. Ndiaye M, Thiam A, Ndiaye R, Angel G, Seignot P, Roussilhon C, et al. Susceptibility to neuro-malaria and HLA-DR alleles in Senegal. Dakar Med. 1998;43(1):25-8.
116. Adrian VSH, Simon NRY, Catherine EMA, Gupta S, Gilbert SC, Lalvani A, et al. Human Leukocyte Antigens and Natural Selection by Malaria. Philosophical Transactions: Biological Sciences. 1994;346(1317):379-85.
117. Hill AV, Elvin J, Willis AC, Aidoo M, Allsopp CE, Gotch FM, et al. Molecular analysis of the association of HLA-B53 and resistance to severe malaria. Nature. 1992;360(6403):434-9.
118. Hirayama K, Zaidi ASM, Hakim SL, Kimura A, Ong KJ, Kikuchi M, et al. Molecular analysis of HLA-B in the Malaysian aborigines. Tissue Antigens. 1996;48(6):692-7.
119. Contu L, Carcassi C, Orrù S, Mulargia M, Arras M, Boero R, et al. HLA-B35 frequency variations correlate with malaria infection in Sardinia. Tissue Antigens. 1998;52(5):452-61.
120. Mehra NK, Jaini R, Rajalingam R, Balamurugan A, Kaur G. Molecular diversity of HLA-A*02 in Asian Indians: predominance of A*0211. Tissue antigens. 2001;57(6):502-7.
121. Hill AV, Allsopp CE, Kwiatkowski D, Anstey NM, Twumasi P, Rowe PA, et al. Common west African HLA antigens are associated with protection from severe malaria. Nature. 1991;352(6336):595-600.
122. Busson M, Vu Trieu A, Labelle P, Pham-Van K, Ho-Quang H, Bouteiller AM, et al. HLA-DRB1 and DQB1 allele distribution in the Muong population exposed to malaria in Vietnam. Tissue antigens. 2002;59(6):470-4.
123. Nakimuli A, Chazara O, Hiby SE, Farrell L, Tukwasibwe S, Jayaraman J, et al. A KIR B centromeric region present in Africans but not Europeans protects pregnant women from pre-eclampsia. Proceedings of the National Academy of Sciences of the United States of America. 2015;112(3):845-50.

## Figures

### Figure 1. KIR Haplotypes

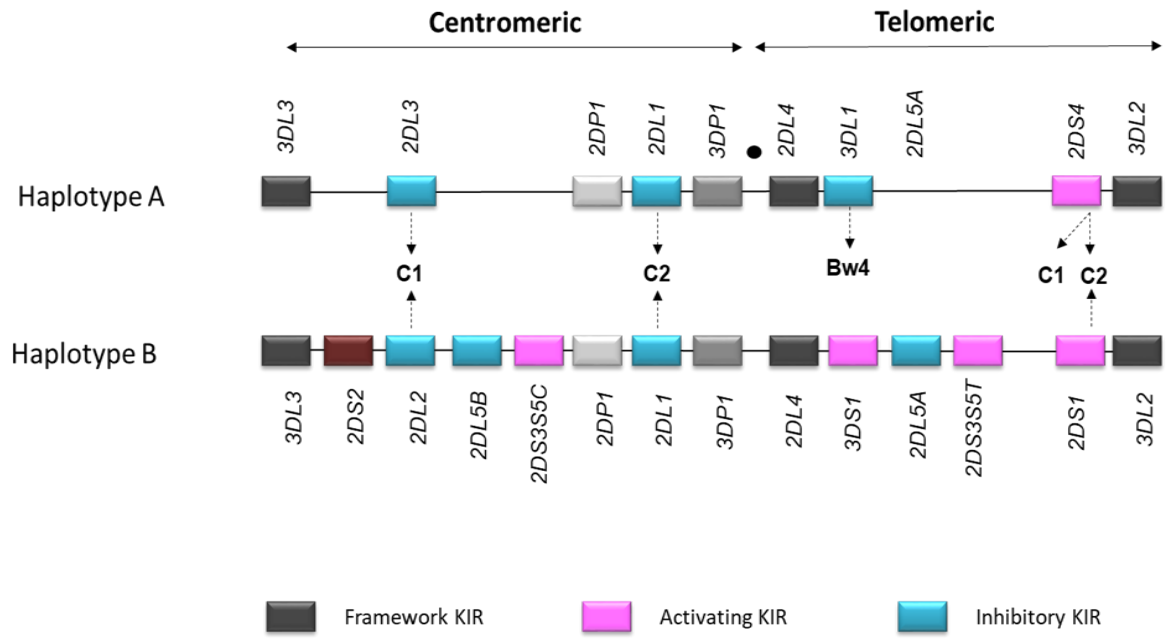


**Figure 1. *KIR* haplotypes.**

KIR haplotypes A and B are present in all populations worldwide. A recombination hotspot between KIR3DP1 and KIR2DL4 separates the Centromeric form the Telomeric end of both types of haplotype. KIR A haplotype is composed of mainly inhibitory *KIR* except for *KIR2DS4*. Allelic polymorphism is very high in the KIR A haplotype (*KIR3DL1*, *3DL2* and *3DL3* have > 100 alleles, *2DL1* and *2DL3* have ~ 50 alleles). Haplotypes B have several activating receptors, with variable number of genes (4-20) and less allelic polymorphisms. Some KIR B haplotypes are made up of combinations of haplotypes A and B (CenA-TelB, CenB-TelA). HLA epitopes bound by some KIR are known and indicated as C1, C2 or Bw4.

**Figure 2. NK receptors and responses to malaria**

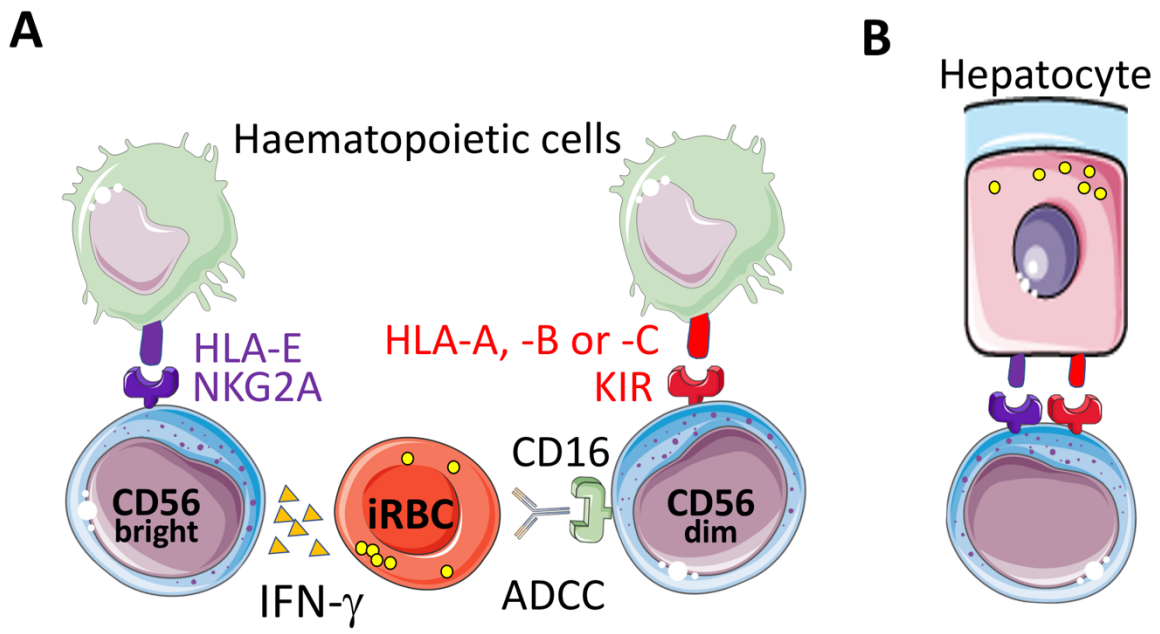


**Figure 2. NK receptors and responses to malaria.**

NK cell responses to malaria may be either beneficial if they target the parasite or detrimental if they contribute to immunopathology. The binding of HLA receptors (CD94/NKG2A or KIR) to self HLA molecules affects both how NK cells are primed for function in steady state and how they function during an immune response. In steady state, the binding of HLA molecules on haematopoietic cells by inhibitory NK-cell receptors such as NKG2A (binds HLA-E) and KIR (bind HLA-A, -B and -C) prime NK cells to become functionally competent. The process of acquiring functional competence through the binding of inhibitory receptors to self HLA molecules on haematopoietic cells is referred to as “NK cell education”. The binding of the same inhibitory receptors to HLA molecules on potential target cells during an immune response, however, suppresses NK cell function. **A)** For example, $CD56^{bright}$ NK cells that do not express KIR can be educated by NKG2A binding to HLA-E to produce IFN-γ during immune responses to malaria. On the other hand, $CD56^{dim}$ NK cells that express inhibitory KIR (e.g. KIR2DL1 or KIR3DL1 in a KIR A haplotype) for self HLA (e.g. HLA-C2 or HLA-Bw4, respectively) found on haematopoietic cells are educated to recognise and kill infected red blood cells that do not express HLA molecules and therefore cannot suppress KIR2DL1+ and KIR3DL1+ $CD56^{dim}$ NK cells during immune responses to blood stage malaria. $CD56^{dim}$ cells can also mediate ADCC because they express CD16, which binds antibody through its Fc portion. **B)** Liver NK cells are made up of different subsets, some of which express both KIR and NKG2A, and both these receptors can educate liver NK cells by binding HLA-A, -B, -C or -E on haematopoietic cells in steady state. On the other hand, during liver stage malaria, both receptors can suppress NK cell functions when NK cells interact with infected hepatocytes that express cognate HLA molecules.