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REVIEW ARTICLE

# Variations in killer-cell immunoglobulin-like receptor and human leukocyte antigen genes and immunity to malaria

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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 (KIRs) and human leukocyte antigens (HLAs). Therefore, variations in *KIR* and *HLA* genes can have a direct impact on NK cell functions. Understanding the role of KIRs and HLAs in immunity to malaria can help to better characterize antimalarial immune responses. In this review, we summarize the different KIRs and HLAs associated with immunity to malaria thus far.

**Keywords:** Genetic variation; Human Leukocyte Antigen; Innate immunity; Killer-cell immunoglobulin-like receptor; Malaria; Natural killer cells

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## INTRODUCTION

Malaria represents one of the most serious infectious disease challenges in the world.[1] Malaria burden is greatly affected by immunity,[2,3] especially in populations with moderate to high-transmission intensity.[4] Partial immunity to malaria develops over years of exposure.[5] Although this partial immunity does not provide complete protection, it reduces the risk of parasitemia progressing to clinical illness and 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 that a number of human genetic variants are associated with protection against uncomplicated and severe *P. falciparum* malaria. Hemoglobin S heterozygous individuals are protected against uncomplicated and severe malaria.[8,9] Hemoglobin C (HbAC) heterozygotes[10,11] and α-thalassemia heterozygotes (_α/αα) and homozygotes (_α/_α) are protected against severe malaria.[8] However, these well-characterized polymorphisms only partially explain the 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 (KIRs) and human leukocyte antigens (HLAs) are strongly associated with the risk of infectious diseases,[13] autoimmune disorders,[13–15] success in cell transplantation for the treatment of hematopoietic malignancies,[16] certain cancers,[17] and pregnancy outcomes.[18] The *KIR* and *HLA* genes segregate independently on chromosomes 19 and 6, respectively; both gene families are 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 partly driven the high *KIR* and *HLA* genetic diversity in Africa.[20,21] The 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 the natural killer (NK) cell response to malaria infections, these genes remain good candidates for elucidating the role of immune cells in malaria.

Despite recent reports indicating improvement in the control of malaria in some populations and the potential for the 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 exerted in vector control using insecticides and malaria treatment and chemoprevention using antimalarial drugs.[23] However, these approaches have faced challenges arising from both insecticide and drug resistance.[24] Antimalarial drug discovery is challenging and costly,[24] and parasite resistance develops easily.[25] Given the limitations of insecticides and antimalarial drugs, a highly effective malaria vaccine would significantly contribute to malaria control.[26] The major challenges to the development of vaccines against malaria include a failure to induce strong innate immune responses and a 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

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vaccine that provides long-lived protection against 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 short-term protection,[31,32] and the efficacy of this vaccine varies with the malaria transmission intensity.[27] Other approaches are under study, but none have 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 development. In this review, we provide a concise overview of the evidence for associations between *KIR* and *HLA* genetic variants and susceptibility to or protection against malaria.

## 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 leukocyte receptor complex on human chromosome 19q13.4.[36] The *KIRs* are the second most genetically diverse family in the mammalian genome after *HLA* genes, and they differ between individuals at three main 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 both 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 *KIRs* have two or three extracellular immunoglobulin domains (D), they are designated as *KIR2D* or *KIR3D*.[42] Functionally, *KIRs* with short (S) intracytoplasmic tails activate NK cells by pairing with the immunoreceptor tyrosine-based activation motif-containing adapter protein DAP12, while those with long (L) intracytoplasmic tails inhibit NK cell functions because they contain one or two immunoreceptor tyrosine-based inhibitory motifs that recruit the 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 short intracytoplasmic tails are designated as *KIR2DS* or *KIR3DS*, and specific genes are identified by a suffix (e.g., *KIR2DS2*, *KIR2SD4*, or *KIR3DS1*).[45] *KIR* genes with two or three extracellular immunoglobulin domains and long intracytoplasmic tails are designated as *KIR2DL* or *KIR3DL*, and specific genes are identified by a suffix (e.g., *KIR2DL1*, *KIR2DL2*, *KIR3DL1*, or *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; because it often carries a 22 bp deletion, haplotype *A* is thought to be mostly inhibitory. Approximately half of the individuals in any population studied to date exhibit 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 *KIRs*, such as *KIR2DS1, KIR2DS2, KIR2DS5*, and *KIR3DS1*, in addition to *KIR2DL5*.[46] The 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, allowing crossover to 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 LD, many disease association studies with *KIR* genes have actually analyzed the association with haplotypes because it is difficult to isolate the role of single *KIR* genes.[48]

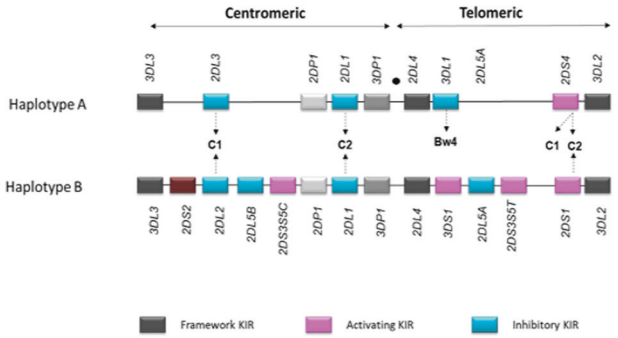


**Fig. 1** *KIR* haplotypes. KIR haplotypes A and B are present in all populations worldwide. A recombination hotspot between *KIR3DP1* and *KIR2DL4* separates the centromeric region from the telomeric end of both types of haplotypes. The KIR A haplotype is mainly composed of inhibitory *KIRs*, except for *KIR2DS4*. Allelic polymorphism is very high in the KIR A haplotype (*KIR3DL1*, *3DL2*, and *3DL3* exhibit >100 alleles, and *2DL1* and *2DL3* exhibit ~50 alleles). Haplotype B has several activating receptors, with variable numbers of genes (from 4 to 20 genes) and fewer allelic polymorphisms. Some KIR B haplotypes are composed of combinations of haplotypes A and B (*CenA-TelB*, *CenB-TelA*). The HLA epitopes bound by some KIRs are known and are indicated as C1, C2, or Bw4

A well-supported hypothesis is that *KIR A* specializes in fighting infectious pathogens,[49] while *KIR B* is important for successful reproduction.[50] How inhibitory receptors in haplotype *A* may protect against infectious diseases is unclear; however, there are at least two possibilities. The first is related 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 impacts the 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 leukocyte 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 encode 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] However, HLA class I molecules are also major ligands for *KIRs*,[59] and as such, they play a role in the regulation of NK cell activity.[59] KIR binding to HLA class I molecules involves four epitopes, A3/A11, Bw4, C1, and C2, found in some HLA-A allotypes, some HLA-B allotypes and all HLA-C allotypes (Fig. 1). The nonclassical HLA class I molecule HLA-G[60] is expressed only on placental cells; HLA-G may play roles in reproduction[61] and binds to *KIR2DL4* and leukocyte Ig-like receptor B. The other nonclassical HLA class I molecule 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 the control of parasite growth and inflammation, as the 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 the attenuation of inflammation[66] and the generation of antimalarial antibodies that play an essential role in the control of blood-stage malaria infection.[67,68] Antimalarial antibodies play an essential role in immunity to malaria in areas with a 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 the control of parasitemia require years of repeated exposure to malaria parasites and remain incomplete.[70–72]

It has been observed that humans are protected against 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 (iRBCs). This induces antibody-dependent cell-mediated cytotoxicity (ADCC) exerted by NK cells,[73] phagocytosis by monocytes, and the engagement of effector cells via Fc-mediated interactions to mediate the 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[75] and can be further subdivided based on CD56 expression.[76] Typically, $CD56^{dim}$ NK cells constitute the majority (about 90%) of peripheral blood NK cells,[77] whereas $CD56^{bright}$ NK cells are more abundant in secondary lymphoid tissues.[78] $CD56^{dim}$ NK cells express high levels of the low-affinity Fc receptor CD16, display heterogeneous expression of inhibitory KIRs for HLA class I, and express high levels of perforin.[79] In contrast, $CD56^{bright}$ NK cells express no or low levels of CD16, exclusively express the inhibitory receptor CD94/NKG2A and show no KIR expression and present tenfold lower perforin expression than $CD56^{dim}$ NK cells.[80] Therefore, the $CD56^{dim}$ and $CD56^{bright}$ NK cell subsets are considered to perform different functional roles (Fig. 2a). Another subset of NK cells referred to as adaptive or memory NK cells develops with age in people with certain infections. For example, in $HCMV^{+}$ individuals, adaptive NK cells that can be found in blood are identified by the expression of the CD94/NKG2C receptor and the CD57 marker.[81] Adaptive NK cells are differentiated from other types of NK cells by the absence of the transcription factor promyelocytic leukemia zinc finger (PLZF) and the signaling Fc receptor γ-chain (FcRγ), which are lost through epigenetic modifications.[82]

NK cells play a vital role in the innate immune response to falciparum malaria infections by the production of IFN-γ, the inhibition of parasite growth, and the cytotoxic killing of intraerythrocytic parasites.[73] NK cells are the first cells in peripheral blood to produce IFN-γ in response to *P. falciparum* infection[83] and participate in the initiation and development of adaptive immune responses.[84] 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.[85] NK cell activities can be triggered by three different but complementary pathways: cytokine activation,[73] ADCC,[73] and loss of inhibitory signaling due to the downregulation of HLA class I ligands, also known as missing self recognition.[73] 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 in individuals who live in malaria-endemic areas through ADCC (Fig. 2a). This is important during blood-stage *P. falciparum* malaria infection.[73] Liver NK cells also express KIR, which may modulate the NK cell response to liver-stage malaria infection through interactions with HLA class I molecules expressed on the surface of hepatocytes (Fig. 2b).[86]

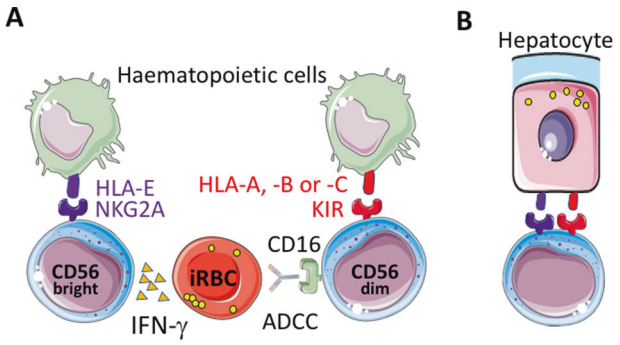


**Fig. 2** NK receptors and responses to malaria. NK cell responses to malaria may be either beneficial, if they target the parasite, or detrimental, if they contribute to immunopathology. The binding of HLA receptors (CD94/NKG2A or KIR) to self HLA molecules affects both how NK cells are primed for function in a steady state and how they function during an immune response. In the steady state, the binding of HLA molecules on hematopoietic cells by inhibitory NK cell receptors such as NKG2A (which binds HLA-E) and KIR (which binds HLA-A, -B, and -C) primes NK cells to make them functionally competent. The process of acquiring functional competence through the binding of inhibitory receptors to self HLA molecules on hematopoietic 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 the immune response to malaria. On the other hand, $CD56^{dim}$ NK cells that express inhibitory KIRs (e.g., KIR2DL1 or KIR3DL1 in a KIR A haplotype) for self HLA (e.g., HLA-C2 or HLA-Bw4, respectively) found on hematopoietic cells are educated to recognize 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 antibodies through its Fc domain. **b** Liver NK cells are composed of different subsets, some of which express both KIR and NKG2A, and both of these receptors can educate liver NK cells by binding HLA-A, -B, -C, or -E on hematopoietic cells in a 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

In a recent study in Mali, multiparameter flow cytometry showed an increase in the proportion of adaptive NK cells that were associated with lower parasitemia and protection against malaria infection.[87] Indeed, these cells enhanced the ADCC response to *P. falciparum*-iRBCs in the presence of naturally acquired antibodies from malaria-resistant individuals.[87] Malaria-susceptible individuals exhibiting a higher proportion of PLZF- and FcRγ-negative NK cells during the transmission season showed improved odds of getting protected against malaria infection during the subsequent season.[87] Although the most prominent cytokines produced by NK cells are IFN-γ and tumor necrosis factor-α, they also secrete other cytokines, including IL-10, the growth factor GM-CSF, and the chemokines MIP-1α, MIP-1β, IL-8, and RANTES. It has been demonstrated that the production of the regulatory cytokine IL-10 by NK cells prevents overt pathology and death from cerebral malaria in mouse models.[88]

Some studies have demonstrated that individuals may exhibit a varying ability to elicit an innate immune response to malaria infection, with clear implications for disease manifestations.[4] This variation may arise as a result of several factors, including the strength of the cytokine and costimulatory signals relayed 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 by engaging inhibitory receptors without triggering activating surface receptors.[89] HCMV also leads to the suppression of NK cell receptor recognition by the corresponding HLA class ligands.[90] The heterogeneity of NK cell responses to plasmodial infection may be attributed to interactions between NK cell receptors and their HLA class I molecule ligands.[83]

### NK cells and placental malaria

Placental malaria results from the 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.[91] The cytolysis of *P. falciparum*-iRBCs by peripheral NK cells may be important in defense against placental malaria.[92,93] 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.[94]

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 remodeling.[95] Decidual NK cells may also provide first-line innate immune defense against infectious diseases, including placental malaria.[96] Decidual NK cells mainly express inhibitory *KIR2DL1*, *KIR2DL2*, and *KIR2DL3*, activating *KIR2DS1* and *KIR2DL4*, which may exhibit inhibitory or activating functions.[96] *KIR* diversity may be driven by balanced selection between pregnancy success and defense against pathogens.[50] Certain specific combinations of *KIR* and *HLA* genes affect the susceptibility to certain pregnancy complications.[95] Other combinations may be associated with susceptibility to or protection against placental malaria. In a study of 688 placental malaria and HIV-coinfected individuals in Kenya, *KIR BB* homozygosity was associated with protection against placental malaria in HIV-negative pregnant women but with malaria susceptibility in HIV-positive pregnant women.[96] This reverse association between *KIR* genes and placental malaria remained only in women with high CD4 cell counts and was not observed in those with low CD4 cell counts.

### KIRs and immunity to *P. falciparum* malaria

The activity of NK cells in immunity against *P. falciparum* infection depends on a fine balance between the strength of the activating and inhibitory signals induced by KIR molecules.[97] It has been clearly shown that KIR molecules regulate the NK cell-mediated production of IFN-γ in response to *P. falciparum* infection and consequently influence the pathogenesis and severity of malaria.[98] For example, NK cells from individuals with *KIR AB* haplotypes produce stronger IFN-γ responses in vitro in response to *P. falciparum* infection compared with the responses observed in either *KIR AA* or *KIR BB* homozygous individuals.[99]

A study of 477 malaria cases in Thailand showed an association of the *KIR2DL3* gene (which identifies haplotype *A*) and its ligand, *HLA-C1*, with cerebral malaria. Moreover, this receptor-ligand pair was found at a lower frequency in highly malaria-endemic populations, suggesting the existence of selection due to unhelpful NK cell responses, which may increase susceptibility to cerebral malaria.[100] However, a comparison of the *KIR* genotypes of 321 children with severe and uncomplicated malaria with those of 314 control children showed that the *Cen-AB* haplotype was more frequent in malaria cases in the Gambia, with more activating KIR genes (*KIR2DS2* and *KIR2DS5*) being found in very ill children, suggesting that exaggerated NK cell activation may contribute to the pathogenesis of severe malaria.[97] Furthermore, parasitemia was higher in *KIR AA* homozygous individuals and more frequent in controls, suggesting that a more inhibitory *A* haplotype may not favor parasite clearance but may protect against severe malaria.[97]

In Southwest Nigeria, the *KIR2DL5, KIR2DS3*, and *KIR2DS5* genes were observed to be overrepresented in individuals with asymptomatic *P. falciparum* parasitemia compared with those with either uncomplicated or severe malaria.[101] Furthermore, the frequency of *KIR2DS3* and *KIR2DS5* was higher in individuals with uncomplicated malaria than in those with severe malaria.[101] These results suggest a protective role of inhibitory *KIR2DL5* and activating *KIR2DS3* and *KIR2DS5* genes against severe malaria. Heterozygosity at the *KIR Cen* region, *Cen-AB2*, has also been shown to be more frequent in asymptomatic controls compared with individuals with severe malaria.[101] This implies that KIR heterozygous *AB* haplotypes may be associated with protection against 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 *Cen* region of the *KIR* locus, which encodes *HLA-C* receptors, is highly diverse, whereas the *Tel* region encoding Bw4-specific receptors (*KIR3DL1*) lacks diversity.[102] These characteristics of *KIR* are consistent with the ongoing selection imposed by malaria pathogens in Africa.[102]

In northern India, individuals possessing the *KIR2DS2, KIR2DL1*, and *KIR2DL3* genes were shown to be susceptible to severe malaria.[103] In the same study, a combination of *KIR2DS2-HLAC1, KIR2DL1-HLAC2*, and *KIR2DL3-HLAC1* was found to be associated with an increased risk of severe malaria.[103] In a study of 77 Melanesian individuals with a history or symptoms of malaria in the highly malaria-endemic Solomon Islands, a higher frequency of *KIR AB* heterozygosity was found among 37 *Plasmodium*-positive individuals than among 40 negative individuals.[104] The nondeleted allele of *KIR2DS4* (*001) was also more frequent in malaria-positive individuals.[104] Other studies have focused on the functional responses of lymphocytes from healthy donors to *P. falciparum*-iRBCs and found heterogeneity in donor NK cell IFN-γ production that was significantly associated with *KIR* genotypes,[105] showing that KIR *AB* heterozygosity was associated with strong responses of CD56dim cells.[106] It is difficult to draw a conclusive picture from the available evidence. While *KIR AB* heterozygosity may favor a larger number of educated $CD56^{dim}$ NK cells, which can respond more effectively to the infection, individuals with numerous activating KIRs may mount responses that are too vigorous and may be deleterious in infected individuals. Individual *KIR* genes as well as the *KIRs* and the corresponding *HLA* class I ligands that have been shown to be associated with susceptibility to or protection against severe malaria are summarized in Table 1.

### 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 the need to fight multiple, frequent deadly infectious pathogens.[107] HLA class I molecules are major ligands for *KIRs*, and as such, they play a role in the 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 the stimulation of T helper cells.[108] Some studies have clearly shown that HLA molecules influence antibody titers against malaria antigens, including glutamate-rich protein and merozoite surface antigens.[109]

**Table 1.** Combinations of KIR genetic variants and HLA ligand linked to susceptibility to and protection against *P. falciparum* malaria

| Reference | Study | Findings |
|---|---|---|
| [105] | NK cell responses of healthy donors to *P. falciparum* iRBCs ($n = 27$) | Individuals with *Tel-AA KIR* exhibited a strong NK cell response to *P. falciparum* among donors of European, Asian, and African descent |
| [104] | *Plasmodium*-positive and negative malaria patients ($n = 77$) | *KIR-AB* heterozygotes were more frequent in *Plasmodium*-positive Melanesians |
| [106] | NK cell responses of healthy donors to infected RBCs ($n = 81$) | *KIR-AB* heterozygosity favored IFN-γ production by $CD56^{dim}$ cells in response to *P. falciparum*-infected RBCs |
| [97] | Children with severe malaria ($n = 133$) or uncomplicated malaria ($n = 188$) and control ($n = 314$) children | In the Gambia, parasitemia was higher in children with *KIR-AA*, but *Cen-AA* was more frequent in the controls. *Cen-AB* was more frequent in malaria patients with *KIR-BB*, and *HLA-C1* was more frequent in very ill children |
| [100] | Cerebral and noncomplicated malaria cases ($n = 477$) | In Thailand, *KIR2DL3* and its ligand *HLA-C1* showed a significant association with cerebral malaria. In addition, *KIR2DL3* and *HLA-C1* were less frequent in highly malaria-endemic populations |
| [96] | Placental malaria in 479 HIV-positive and 209 HIV-negative pregnant women | In Kenya, *KIR-BB* homozygosity was associated with protection against placental malaria in HIV-negative pregnancies but with susceptibility in HIV-positive pregnancies |
| [101] | Severe malaria ($n = 201$), uncomplicated malaria ($n = 153$), and asymptomatic malaria ($n = 200$) | In Southwest Nigeria, *Cen-AB* was less frequent in severe malaria patients, while *KIR-BB* was more frequent in severe and uncomplicated malaria patients compared with asymptomatic individuals |
| [103] | Cerebral malaria ($n = 213$)<br>Uncomplicated ($n = 87$) | In North India, cerebral malaria was associated with *KIR2DS2* (*B*) *KIR2DL1* (*A*) and *KIR2DL3* (*A*). *KIR-BB* was associated with cerebral malaria. *AB* was associated with uncomplicated malaria |

**Table 2.** HLA class I and HLA class II genetic variants associated with susceptibility to or protection against *P. falciparum* malaria

(A) HLA class I genetic variants associated with susceptibility to or resistance against *P. falciparum* malaria

| Reference | Country | HLA class I variants associated with susceptibility to severe malaria | HLA class I variants associated with protection against severe malaria |
|---|---|---|---|
| [115] | The Gambia | – | *B*53* |
| [116] | Malaysia | – | *B*1513* |
| [110] | Mumbai, India | *HLA-A1, HLA-B27* and *HLA-B49* | *HLA-A19* |
| [111] | Thailand | *HLA-B46* | *HLA-B56* |
| [117] | Sardinia, Italy | – | *B*35* |
| [118] | New Delhi, India | – | *A*0211* |
| [97] | The Gambia | – | *HLA-Cw*16:01* frequency (a C group 1 allele) |

(B) HLA class II genetic variants associated with susceptibility to or resistance against *P. falciparum* malaria

| Reference | Country | HLAs associated with susceptibility to malaria | HLAs associated with protection against malaria |
|---|---|---|---|
| [112] | Senegal | *DR*3, DR*10* (cerebral malaria) | – |
| [119] | The Gambia | – | *DRB1*1302-DQB1 *0501* |
| [120] | Vietnam | – | *HLA-DQ1*0502* |
| [110] | Mumbai, India | *HLA-DRB1*0809* | *HLA-DQB1*0203* |
| [111] | Thailand | – | *DRB1*1001* |

Several studies have shown some *HLA* genetic variants to be associated with susceptibility to or protection against severe malaria. However, the results have been inconsistent (Table 2). The first study on the role of *HLA* in malaria described protection against cerebral malaria and severe malarial anemia conferred by the *HLA* class I allele *HLA-Bw53* and the *HLA* class II haplotype *HLA-DRBl*1302-DQAl*0102-DQBl*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 no significant associations of malaria antigens other than LSA-1 with HLA molecules have 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 malaria than in uncomplicated malaria. *HLA-B49, -A1, -B27*, and *HLA-DRB1*0809* were shown to be strongly associated with severe malaria in a study conducted in Mumbai in India.[110] *HLA-A19* and *HLA-DQB1*0203* were associated with protection against severe malaria in the same population.[110] In a study conducted in Thailand in patients with severe cerebral malaria, *HLA-B46* was significantly associated

with the risk of cerebral malaria, while *HLA-B56* and *HLADR1*1001* were associated with protection against cerebral malaria.[111] In Senegal, *HLA-DR3* and *HLA-DR10* were strongly associated with cerebral malaria.[112] In the Gambia, *HLA-B53* and *HLA-DRB1*1302* alleles were found to be strongly associated with protection against severe malaria.[113]

## 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 been established, and it is therefore important to decipher how *KIR* and *HLA* immunogenetics regulate susceptibility to or protection against malaria to understand the heterogeneous responses of populations to malaria infection. This may in turn help understand malaria transmission and severity in specific populations and inform specific malaria control interventions.

Several studies have suggested that certain *KIR* and *HLA* variants are associated with malaria, but there are challenges at different levels to obtaining 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, some of the relevant studies were conducted with small sample sizes, highlighting the need to study larger populations in Africa and other malaria-endemic populations, particularly to accommodate allelic diversity. Different *KIR* and *HLA* variants may have been selected in different populations. Other infectious pathogens that are 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 coinfections should be considered in studies involving *KIR* and malaria, especially in populations affected by many infectious pathogens. Different species of parasites may infect different populations, and unique antigenic drift may occur in parasites in different populations. How the binding between certain KIR and HLA molecules regulates 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 the binding affinities of these receptors to equally diverse *HLA* ligands are still being sought. KIRs can either inhibit or enhance NK cell effector functions. Combinations of *KIR* and *HLA* genes determine both NK cell education (the priming of effector function) and effector function itself. However, because inhibitory KIRs 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 reconstruct how *KIR* and *HLA* immunogenetics determine the biology of NK cells during malaria infection. For example, inhibitory interactions may be necessary to educate NK cells to mediate ADCC required to eliminate iRBCs but may not be required or could even be counterproductive for the activation of IFN-γ 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 has been well documented for sickle cell disease and other hemoglobinopathies. For example, the high frequency of certain *KIR* and *HLA* variants associated with pregnancy complications in sub-Saharan Africa[114] may be the result of a trade-off for the selection of genetic variants that protect against malaria.

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## ADDITIONAL INFORMATION

**Competing interests:** O.C. had started in a role as an employee of AstraZeneca, UK, at the time of manuscript preparation. Other authors have declared that no competing conflicts of interest exist.

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