REVIEW

Contributions of natural killer cells to the immune response against *Plasmodium*

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Abstract

Natural killer (NK) cells are important innate effector cells that are well described in their ability to kill virally-infected cells and tumors. However, there is increasing appreciation for the role of NK cells in the control of other pathogens, including intracellular parasites such as *Plasmodium*, the cause of malaria. NK cells may be beneficial during the early phase of *Plasmodium* infection—prior to the activation and expansion of antigen-specific T cells—through cooperation with myeloid cells to produce inflammatory cytokines like IFNγ. Recent work has defined how *Plasmodium* can activate NK cells to respond with natural cytotoxicity, and inhibit the growth of parasites via antibody-dependent cellular cytotoxicity mechanisms (ADCC). A specialized subset of adaptive NK cells that are negative for the Fc receptor γ chain have enhanced ADCC function and correlate with protection from malaria. Additionally, production of the regulatory cytokine IL-10 by NK cells prevents overt pathology and death during experimental cerebral malaria. Now that conditional NK cell mouse models have been developed, previous studies need to be reevaluated in the context of what is now known about other immune populations with similarity to NK cells (i.e., NKT cells and type I innate lymphoid cells). This brief review summarizes recent findings which support the potentially beneficial roles of NK cells during *Plasmodium* infection in mice and humans. Also highlighted are how the actions of NK cells can be explored using new experimental strategies, and the potential to harness NK cell function in vaccination regimens.

Keywords: Malaria, NK cells, Cytotoxicity, Cytokines

Background

Natural killer (NK) cells are lymphocytes of the innate immune system that participate in early defense against foreign cells and autologous cells undergoing various forms of stress, such as microbial infection or tumour transformation [1, 2]. NK cells are derived from a common lymphoid progenitor and develop primarily in the bone marrow [2]. Under homeostatic conditions, NK cells are abundant in blood, spleen, bone marrow, and liver, but they can migrate to inflamed or infected tissues in response to chemoattractants [3]. NK cells recognize Human Leukocyte Antigen (HLA) class I molecules via Killer Immunoglobulin Receptors (KIRs) that predominantly deliver signals that suppress, rather than activate NK cell function [2]. NK cell activation decisions are based on the balance of engaged inhibitory or activating receptors such as KIR, natural cytotoxicity receptors (NCRs), DNAX accessory molecule-1 (DNAM-1) and NK group 2 member D (NKG2D) [2, 4]. When inhibitory signals are reduced or absent, activating NK cell receptor ligation results in NK cell triggering and target cell death; this is referred to as natural cytotoxicity. In addition to natural cytotoxicity, NK cells can cooperate with the adaptive immune system by performing antibody-dependent cellular cytotoxicity (ADCC) upon recognition of opsonized targets via the Fc receptor—FcγRIIIa (CD16). Lastly, NK cells have the ability to induce apoptosis in target cells through Fas Ligand (FasL) and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) [5, 6]. Thus, NK cells exhibit a wide variety of methods to recognize and attack cells that are a danger to the host.

NK cells also respond to cytokine signals delivered by other immune cells, namely IL-2, IL-15, IL-18, and IL-12 [7]. Exposure allows NK cells to augment immune

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responses through rapid and robust production of pro-inflammatory cytokines, including interferon-gamma (IFN-γ) and tumour necrosis factor (TNF), among others. Much work has focused on this bolus of cytokine production which serves to amplify immune responses, particularly in the early hours after infection. However, NK cells may also contribute to overproduction of pro-inflammatory cytokines, which can result in immuno-pathology [8]. Perhaps to temper this danger, NK cells can also produce the anti-inflammatory cytokine IL-10, which limits immune responses [9–12]. Variation in the clinical symptoms associated with *Plasmodium* infection in humans, along with diverse characteristics in specific mouse models of infection, have made defining the protective immune response challenging. There is still not a consensus on whether NK cells are overall more harmful, helpful, or inconsequential to the immune response to *Plasmodium* (see review by Wolf et al. [13]). However, several recent studies have started to gain a better understanding of the mechanisms by which NK cells are activated during malaria infection and the downstream consequences of their activation. Here, findings are highlighted that relate to the potentially beneficial actions of NK cells during *Plasmodium* infection in mice and humans. These studies justify further evaluation of NK cells in the context of malaria disease.

NK cells during liver stage infection

After being bitten by a mosquito carrying *Plasmodium* parasites, a low number of sporozoites (on the order of 1–25) are transmitted [14]. The sporozoites travel through the blood stream to the liver and infect a small number of hepatocytes, where they replicate and differentiate into merozoites. Human trials with the RTS,S vaccine indicate that antibody against circumsporozo-ite protein (CSP) and $CD4^+$ T cell responses serve as good correlates of protection [15]. $CD8^+$ T cells are also implicated as critical effector cells in protection against pre-erythrocytic stage malaria [16, 17]. To obtain robust responses, $CD8^+$ T cells are primed by liver-infiltrating $CD11c^+$ cells that acquire *Plasmodium* antigens, traffic to the liver draining lymph nodes, and then present peptides to naive T cells [18]. NK and NKT cells are also abundant in the liver, and they are early producers of IFN-γ, which is an important effector molecule that could conceivably contribute to the activation of immune cells and indirectly lead to destruction of parasite-infected hepatocytes (Fig. 1) [19, 20].

Observational studies in humans have suggested that NK cells contribute to immunity against malaria during the liver stage of disease. However, human challenge studies are limited to showing that infection and increased protection correlated with decreased
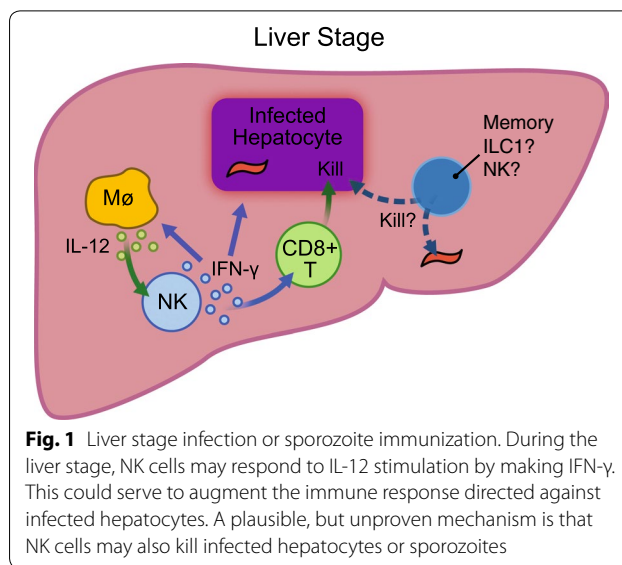

Fig. 1 Liver stage infection or sporozoite immunization. During the liver stage, NK cells may respond to IL-12 stimulation by making IFN-γ. This could serve to augment the immune response directed against infected hepatocytes. A plausible, but unproven mechanism is that NK cells may also kill infected hepatocytes or sporozoites

frequency and number of NK cells in the blood of subjects [21–23]. Although it is tempting to speculate that this could be due to increased trafficking to the infected liver, this is difficult to address experimentally in humans. Enhanced IFNγ production by human NK cells has been observed after RTS,S/AS01 malaria vaccination [20]. These improved responses could be due to either indirect activation of NK cells by cytokines or potentially, cognate antigen recognition. Regardless of the mechanism, NK cells in the liver might be sufficiently stimulated by vaccination to meaningfully contribute indirectly or directly to protective immune responses against *Plasmodium* [24].

Studies in mice have shown that NK and NKT cells both increase in number in the liver and produce increased amounts of IFNγ and TNF in response to *Plasmodium yoelii* infection [25, 26], which could be important to dampen the growth of schizonts in the liver and amplify the early immune response. Early work investigating the protective mechanisms of radiation-attenuated sporozoites used in vivo antibody depletion to conclude that, in addition to $CD8^+$ T cells, NK cells are required for vaccine-induced protection against *P. yoelii* challenge [27]. This was proposed to be the result of IL-12 activation of NK cells, which in-turn made IFNγ. Additionally, using $CD1d^{-/-}$ mice, Miller et al. showed that NKT cells play a significant role in lowering liver parasite burden [26]. Future work can reevaluate the importance of NK cell-specific cytokine production using new, more specific technical approaches. For example, the use of *Nkp46^{iCre}* mice [28] or NKp46-CreERT2 mice [29] bred with mice containing loxP sites flanking effector molecules (IFNγ, TNF) [30] would help to bolster or disprove the case that NK cell-derived cytokine production is important for *Plasmodium* control.

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Overall, additional studies are required, in both humans and mice, to determine the exact role, if any, NK cells play in the liver during *Plasmodium* infection. The presence of additional liver populations including resident NK cells and type 1 innate lymphoid cells (ILC1s) further complicates assignment of individual immune cell contributions to protection from the liver stage of disease. ILC1s are CD49a$^+$ CD49b$^-$ CXCR6$^+$ T-bet$^+$ Eomes$^-$ CD127$^{lo/-}$ and are predominantly tissue-resident [31]. Conventional NK cells are largely recirculating and can be identified based on the following phenotype: CD49a$^-$ CD49b$^+$ CXCR6$^-$ T-bet$^+$ Eomes$^+$ [31, 32].

To date, the contribution of NK-related cell types to protection in the liver has often relied on antibody depletion using shared markers (i.e., NK1.1). Thus, future experiments will need to precisely determine the individual impact of conventional NK cells and other similar immune cell types to pre-erythrocytic immunity.

NK cells during blood stage infection

Merozoites released from hepatocytes initiate the blood-stage of infection. In the bloodstream, free merozoites invade RBCs beginning a 48-h cycle of maturation followed by RBC rupture, and additional release of merozoites. Release of mature parasites from infected RBCs (iRBCs) results in symptomatic disease characterized by fever, headache, and lethargy. The most vulnerable are young children and pregnant women; severe disease takes the form of anaemia, placental disruption and miscarriage, acute respiratory distress, and cerebral manifestations [33]. With age and re-exposure, the risk of malaria goes down, and although hosts continue to carry parasite burdens, they usually do so without the associated symptoms.

Non-severe infection- human and humanized mouse studies

During uncomplicated malaria in *Plasmodium falciparum*-infected Kenyans, NK cell frequencies (CD56$^+$CD3$^-$) were reduced relative to asymptomatic and aparasitaemic people [34]. Similar results were found in a recent study of Mali subjects, but this report additionally showed that the CD56^{dim} subset was specifically reduced during malaria episodes, whereas the CD56^{bright} population was increased [35]. Other studies have investigated the potential protective role of NK cells during blood stage *Plasmodium* infection. In both mouse and human studies, IFNγ has been associated with control of parasitaemia, protection from malaria, and delayed reinfection [36–40]. NK cells rapidly produce IFNγ in the presence of many pathogens, including *Plasmodium* [37, 41]. However, in vitro assays indicate that IFNγ productio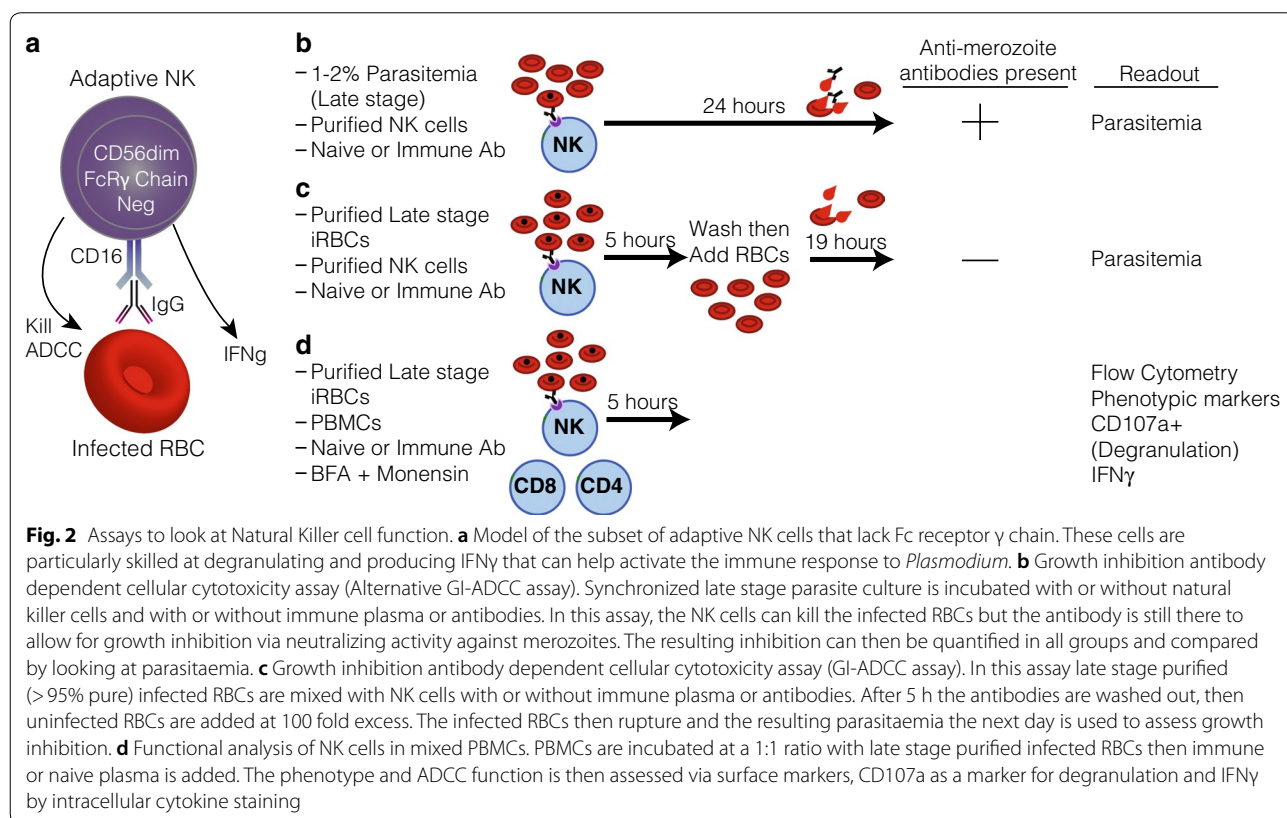n against *Plasmodium* requires direct contact between NK cells and iRBCs and a cellular source of IL-12 and IL-18, such as monocytes or DCs [42–44].

Evidence for direct NK cell natural cytotoxicity toward *P. falciparum*-infected erythrocytes was first demonstrated by Orago and colleagues via in vitro chromium release assay [34]. This was subsequently reproduced by other studies demonstrating antibody-independent lysis of infected red blood cells by NK cells, however the percent of natural cytotoxicity responders in studies is variable [45–47]. When PBMCs from malaria-naïve individuals are incubated with iRBCs, NK cells have been shown to increase expression of the cytotoxic molecules perforin, granzyme A, and CD107a (LAMP-1) [41]. It was subsequently shown that NK cells form stable conjugates with iRBCs leading to cytoskeletal actin rearrangement [41]. A recent study showed that human NK cells can perform natural cytotoxicity in vitro, and utilized a humanized mouse model of *Plasmodium* infection (reviewed in [48]) to demonstrate that NK cells were critical for the control of parasitaemia in vivo [49]. Humanized mouse models have several limitations; in this study, the mice exhibited low parasitaemia relative to what is observed in humans, and the ligand/receptor combinations required for NK cell recognition of iRBCs have not been fully elucidated. It has been shown that antibody-mediated inhibition of NKp30 and to a lesser extent NKp46 (both natural cytotoxicity receptors) reduced lysis of iRBCs [46]. Preincubation of NK cells with a peptide from the *P. falciparum* erythrocyte membrane protein-1 (*Pf*EMP-1) also reduced lysis, but other *Plasmodium* antigens did not have an effect [46]. These data suggest that NK cell natural cytotoxicity receptors can directly recognize *P. falciparum* PfEMP-1 proteins on iRBCs to mediate lysis. In a separate study, Saito and colleagues found that a subset of RIFIN proteins, which are expressed on the surface of iRBCs, bound to the inhibitory receptors LILRB1 or LAIR1 on NK cells and reduced NK cell cytotoxicity [50]. A recent report suggests that microvesicles from iRBCs can fuse with NK cells in vitro and cause activation and a reduction in parasitaemia [51]. The authors found that the innate receptor MDA5, which can bind uncapped RNA, could be induced in "iRBC responsive" NK cells, and that genetic knockdown of MDA5 resulted in the loss of parasitaemia control in vitro, suggesting that parasite RNA in the microvesicles stimulated NK cells to kill iRBCs. Ye et al. also showed that stimulation of "iRBC non-responsive" NK cells with a MDA5 agonist induced activation and improved control of parasitaemia, providing evidence of a molecular basis for variation in human NK cell responses to malaria [51]. However, the proportion of people with "responder" vs. "non-responder" NK cell phenotypes was not reported.

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A recent study showed that NK cell-mediated cytotoxicity required antibody; that is, NK cells killed iRBCs via ADCC and inhibited the growth of *Plasmodium* in vitro [52] **(**Fig. 2a). In this study the destruction of iRBCs was highly specific, as uninfected RBCs in the same culture were not lysed, and killing was dependent on Fc receptors. It was also shown that a monoclonal antibody with modifications to the Fc portion of the antibody—making it unable to bind to the Fc receptor CD16—abrogated the ADCC activity [53]. The in vitro growth inhibition assay was performed using two different approaches. The first approach added NK cells at a 3:1 effector (NK) to target (late stage infected RBC) ratio with naïve (USA) serum or hyperimmune serum (pooled Mali adult plasma containing antibodies against both the iRBC surface and merozoites), then let the culture grow. The addition of NK cells increased growth inhibition significantly over hyperimmune plasma alone (averaging 80% vs. 20% respectively), however anti-merozoite antibodies could account for some of the growth inhibition (Fig. 2b) [52]. In addition to this assay, purified late stage infected RBCs were incubated with NK cells at a 3:1 (Effector:Target) ratio for 5 h with USA or Mali plasma, the cells were washed to get rid of any antibodies, and fresh uninfected RBCs were added (Fig. 2c). Growth inhibition was still observed in this assay in the absence of anti-merozoite antibodies.

The contribution of complement was also removed by using plasma free system and purified IgG from the same naïve or immune plasma [52]. In total, these assays implicate NK cell-mediated ADCC as a potential mechanism of acquired immunity that effectively limits *Plasmodium* growth. Another study also found that in the presence of antibody FasL may be involved as a killing mechanism [45], however a comprehensive assessment of how growth is inhibited is still needed.

Recently, it was demonstrated that adaptive NK cells correlated with protective malaria phenotypes, including decreased parasitaemia and reduced symptoms [35]. Adaptive NK cells are long-lived and have enhanced functionality, but in the past were studied only in the context of human cytomegalovirus (HCMV) and human immunodeficiency virus (HIV) [54, 55]. In the context of *Plasmodium* infection, adaptive NK cells, which lacked Fc receptor γ chain [55, 56], performed enhanced ADCC function for both degranulation and IFN-γ production in an in vitro PBMC assay (Fig. 2d) [35]. Additionally, subjects that displayed increased frequencies of promyelocytic leukemia zinc finger protein (PLZF) negative adaptive NK cells (which lack a key transcription factor for Fc receptor γ chain expression) in the current season were significantly protected from malaria symptoms the following malaria season [35]. Increased frequencies

Fig. 2 Assays to look at Natural Killer cell function. **a** Model of the subset of adaptive NK cells that lack Fc receptor γ chain. These cells are particularly skilled at degranulating and producing IFNγ that can help activate the immune response to *Plasmodium*. **b** Growth inhibition antibody dependent cellular cytotoxicity assay (Alternative GI-ADCC assay). Synchronized late stage parasite culture is incubated with or without natural killer cells and with or without immune plasma or antibodies. In this assay, the NK cells can kill the infected RBCs but the antibody is still there to allow for growth inhibition via neutralizing activity against merozoites. The resulting inhibition can then be quantified in all groups and compared by looking at parasitaemia. **c** Growth inhibition antibody dependent cellular cytotoxicity assay (GI-ADCC assay). In this assay late stage purified (> 95% pure) infected RBCs are mixed with NK cells with or without immune plasma or antibodies. After 5 h the antibodies are washed out, then uninfected RBCs are added at 100 fold excess. The infected RBCs then rupture and the resulting parasitaemia the next day is used to assess growth inhibition. **d** Functional analysis of NK cells in mixed PBMCs. PBMCs are incubated at a 1:1 ratio with late stage purified infected RBCs then immune or naïve plasma is added. The phenotype and ADCC function is then assessed via surface markers, CD107a as a marker for degranulation and IFNγ by intracellular cytokine staining

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of PLZF negative adaptive NK cells also correlated with reduced parasitaemia and was found to be independent of age [35]. These studies provide the first evidence that one function of protective antibodies may be to "tag" iRBCs for removal by NK cells, leading to reduced parasitaemia and disease symptoms. The frequency of adaptive NK cells in the Malian cohort was 40% of the NK cells and most of the subjects were CMV positive. In non-malaria exposed subjects that are HCMV positive, frequencies of adaptive NK cells are generally not this high (< 10%) [55, 56]. It is unclear if HCMV co-infection with malaria further increases adaptive NK cell frequencies. Because mouse NK cells do not perform ADCC well [57, 58], additional studies using human NK cell and humanized mouse systems are needed to further investigate this mechanism. Since it was shown that NK cells killed iRBCs but not merozoites via ADCC [35], the data suggest that surface-expressed iRBC proteins may be good targets for vaccines designed to enhance NK cell function. These data also suggest that promoting the generation of antibodies that bind the CD16 Fc receptor—IgG1 and IgG3 isotypes, which are also complement-fixing antibodies—could benefit parasite clearance.
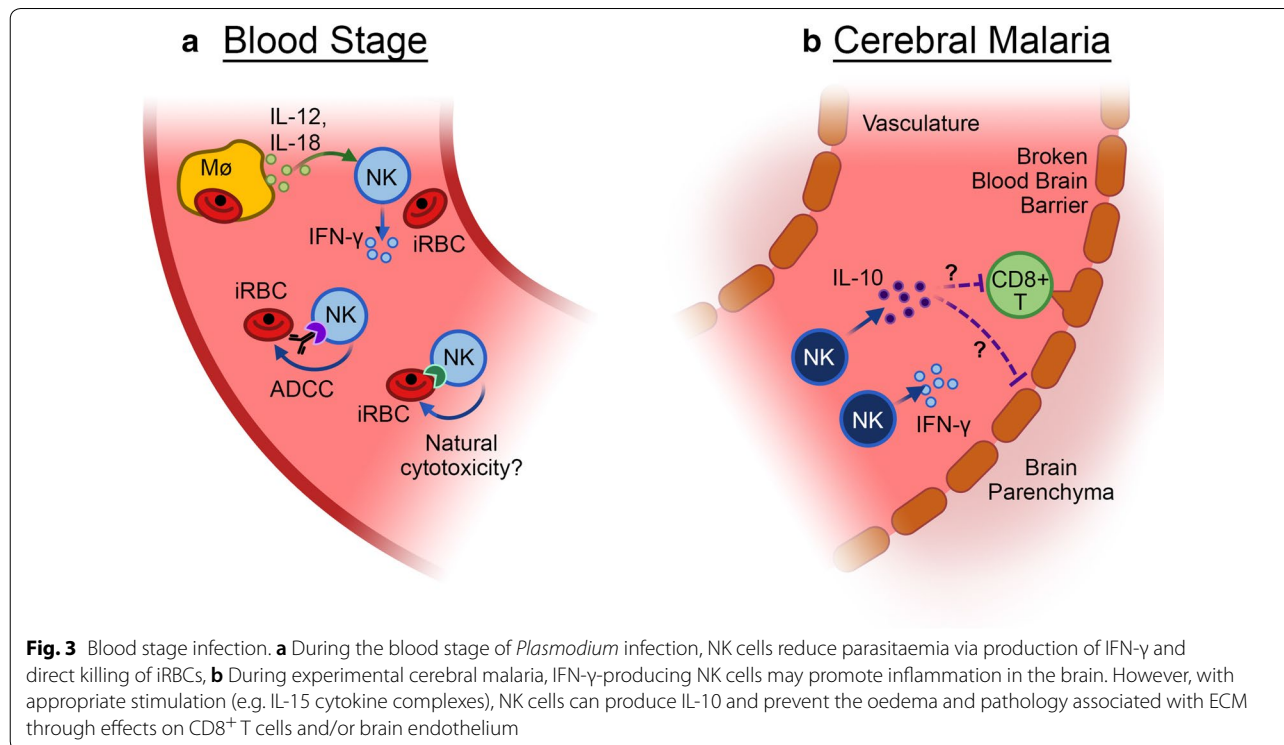
Non-severe infection- mouse studies

Plasmodium falciparum is restricted to humans, but several *Plasmodium* strains can infect mice and replicate features of human malaria disease. No one model recapitulates all features of human malaria disease states; however, depending on the strain of parasite and the genetic background of the mouse strain used, a wide range of outcomes can be mechanistically investigated, from parasitaemia to cerebral malaria [59]. Using *Plasmodium chabaudi* or *P. yoelii* 17X infection as models of blood stage infection, NK cell frequencies increase [60] as does their production of IFNγ and TNF [40]. Depletion of NK cells with anti-NK1.1 or anti-asialo GM1 Ab resulted in increased mortality, decreased IFNγ, and either slightly increased or no change in parasitaemia levels [22, 61, 62]. These data suggest that NK cells may contribute to control of parasitaemia and/or disease development during *P.c.* and *P.y.* infection models in mice, but the approaches used are not specific enough to definitively say that NK cells play a fundamental role in protection.

Severe infection

One severe result of blood stage *P. falciparum* infection is the development of neurological complications known as cerebral malaria (CM). CM mostly afflicts children under the age of five in sub-Saharan Africa and can be fatal [63]. Furthermore, over 25% of children that survive CM exhibit long-term cognitive impairment [64, 65]. There are no adjunctive therapies available for CM patients. CM pathogenesis is generally considered to be driven by two distinct processes: sequestration of iRBCs to vascular endothelium and inflammation [66, 67] (Fig. 3). The

Fig. 3 Blood stage infection. **a** During the blood stage of *Plasmodium* infection, NK cells reduce parasitaemia via production of IFN-γ and direct killing of iRBCs, **b** During experimental cerebral malaria, IFN-γ-producing NK cells may promote inflammation in the brain. However, with appropriate stimulation (e.g. IL-15 cytokine complexes), NK cells can produce IL-10 and prevent the oedema and pathology associated with ECM through effects on CD8+ T cells and/or brain endothelium

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sequestration and excessive inflammation lead to the loss of blood brain barrier integrity, ultimately resulting in oedema [68–70].

Infiltration of inflammatory immune cells into the brains of CM patients has not been extensively investigated due to the difficult nature of obtaining samples. A study from three decades ago found that CM patients had significantly reduced NK cell cytotoxicity against K562 cells compared with healthy controls or patients with uncomplicated *P. falciparum* infection [71]. A recent study compared the ability of NK cells purified from patients with uncomplicated malaria to those with severe malaria to control parasite growth in vitro; although the sample size was relatively small, the data suggest that NK cells from patients with severe malaria disease are less capable of controlling *P. falciparum* growth in vitro [51]. This could be due to immune suppression during severe disease or a 'hyper-immune' response that ultimately leads to NK cell functional exhaustion [72–74].

In contrast to the relative scarcity of studies on the role of NK cells during human CM, numerous studies have investigated the role of NK cells in the mouse model of CM, known as experimental cerebral malaria (ECM). The ECM mouse model replicates the neurological symptoms, parasite accumulation, and leukocyte infiltration observed in children with CM, including the induction of brain oedema and brain stem death as a predictor of severe disease [75–77]. Numerous studies in both mice and humans have demonstrated that an excessive pro-inflammatory response results in severe immunopathology during malaria [78–80]. IFNγ—which can be secreted in large amounts by NK cells—is a critical mediator of immune-mediated damage during ECM [81, 82]. An initial report using anti-NK1.1 Ab to deplete NK cells suggested that NK cells play no role in ECM [80]. More recent reports using anti-Asialo GM1 Ab suggested that NK cells actively contribute to ECM [83, 84]. This treatment reduced CD8^{+} T cell priming in the spleen [84] and upregulation of CXCR3 expression [83], a chemokine receptor important for T cell trafficking to the brain. However, treatment with NK1.1 can also deplete NKT cells and innate lymphoid cells (ILCs), whereas anti-Asialo GM1 treatment has been reported to deplete macrophages, basophils, and CD8 T cells among other immune cells. Other studies investigating the natural killer complex (NKC), a genetic region of highly inked genes encoding several receptors involved in the control of NK cell function, suggest that polymorphisms within the NKC modulate control susceptibility to the development of ECM and the type of immune response (TH1 vs. TH2) that unfolds [85, 86]. These data as a whole suggest that the pro-inflammatory nature of NK cells could contribute to immune-mediated damage during ECM,

but development of additional NK cell-specific depletion methods or use of adoptive transfer approaches [9, 87] need to be employed in future studies to definitively identify any pathological role of NK cells during ECM.

In contrast to a potential pathologic role for NK cells, it was recently shown that NK cells can be induced to produce IL-10, which inhibited the pathologic CD8^{+} T cell response and protected mice from ECM [9]. There is evidence in other disease models, including from other protozoan infections, that NK cells produce IL-10 [11, 12, 88], similar to CD4^{+} T cells that can produce both IFNγ and IL-10. It was also demonstrated that stimulation of NK cells with IL-15 cytokine complexes induced this "regulatory" NK cell phenotype. Additionally, primary human NK cells produced IL-10 following culture with IL-15 and IL-21 and increase production further with the addition of IL-12 [9]. Further studies are needed to determine if IL-10-producing NK cells contribute to the healthy resolution (or prevention) of inflammation during malaria disease in humans.

Other severe complications of *Plasmodium* infection include placental malaria and severe malaria anaemia (SMA). Placental malaria can result in placental disruption and lead to miscarriages, still birth, low birth rate, and in some cases death of the pregnant woman [89]. It is estimated that 25% of pregnant women in endemic areas are infected with *Plasmodium*, and that this is responsible for 20% of maternal deaths [89]. During this disease, iRBCs express a PFEMP1 protein called Var2CSA that facilitates adherence to chondroitin sulfate A expressed on the placenta [90, 91]. This infiltration and adherence of iRBC to the placenta is believed to be responsible for the observed clinical complications, however the mechanism of disruption is still unclear. Var2CSA is a large and variable gene however its amino acid variation is around 75–85% found in clinical isolates [89]. Women can become immune to placental malaria during successive pregnancies, showing that effective immune protection can be achieved (76, 77). It is unknown if NK cells provide protection against Var2CSA + infected RBCs through natural cytotoxicity or ADCC. Very few studies have investigated NK cells in the placenta and in the blood at various timepoints [92, 93]. It is known that specialized NK cells in the uterus are necessary for angiogenesis of the placenta that occurs mainly in the first trimester and that NK cells represent 70% of all leukocytes found in the decidua [94]. Furthermore, elevated numbers of IFNγ-producing NK cells and T cells in the placenta is associated with protection [95]. Severe malaria anaemia is characterized by haemoglobin levels less than 5 g/dl, poor erythrocytic output from the bone marrow, and often enlarged spleen [96]. SMA typically occurs in areas that have high exposure rates to *Plasmodium* or are

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genetically susceptible to the disease. FcγRIIIa is the lone Fc receptor expressed on NK cells that enables ADCC, and polymorphisms in FcγRIIIa are associated with SMA [97]. The cell responsible for this association is unknown, but lack of function mutations correlate with poorer outcomes. Whether or not NK cells in these subjects lack effector function has not been explored. In addition, the subset(s) of NK cells associated with placenta malaria or SMA are also unknown. Overall, there is a need for additional studies to indicate if there is a role for NK cells during placental malaria and SMA.

Conclusions

Although increased efforts toward prevention of malaria spread have resulted in significant declines in disease, there is still a need for greater understanding of how an effective immune response against *Plasmodium* unfolds. Given the mounting evidence of NK cell functional diversity and participation in the immune response to *Plasmodium*, NK cells represent a new opportunity for improving resistance to malaria—be it through therapeutics or as targets of vaccination approaches [24]. This review summarizes the evidence that NK cells can contribute to the immune response in the clearance of parasites, removal of infected hepatocytes and RBCs through natural cytotoxicity and ADCC, and dampen the adaptive immune response through production of regulatory cytokines. Therefore, NK cells may serve as a critical adjustment point for enhanced immunity to *Plasmodium* while still preventing immunopathology. However, the number of studies precisely detailing the role of NK cells during different stages of *Plasmodium* infection and during non-severe and severe forms of disease is sparse. Advances in the understanding of NK cell subsets along with technical improvements should now allow for further consideration of whether NK cells can and should be targeted to improve clinical outcomes during malaria.

Abbreviations

NK: natural killer cell; MHC: major histocompatibility; KIR: killer immunoglobulin-like receptors; NCRs: natural cytotoxicity receptors; DNAM-1: DNAX accessory molecule-1; NKG2D: natural killer cell group 2 member D; ADCC: antibody dependent cellular cytotoxicity; TRAIL: tumour necrosis factor-related apoptosis-inducing ligand; IFNγ: interferon gamma; TNF: tumour necrosis factor; CSP: circumsporozoite protein; ILC1s: type 1 innate lymphoid cells; RBCs: red blood cells; iRBCs: infected red blood cells; PfEMP-1: *P. falciparum* erythrocyte membrane protein-1; HCMV: human cytomegalovirus; HIV: human immunodeficiency virus; PLZF: promyelocytic leukaemia zinc finger protein; CM: cerebral malaria; ECM: experimental cerebral malaria; ILCs: innate lymphoid cells; SMA: severe malaria anaemia.

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