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**The C-Type Lectin Receptor DCIR Is Crucial for the Development of Experimental Cerebral Malaria**

Maha Maglino,* Robert Klopfleisch, ‡ Peter H. Seeberger,* ‡ and Bernd Lepenies* ‡

Cerebral malaria (CM) is the most severe complication of malaria. The murine *Plasmodium berghei* ANKA (PbA) infection model has helped to identify crucial players in the pathogenesis of CM. However, the role of pattern recognition receptors in innate immunity to CM induction is still poorly understood. C-type lectin receptors (CLRs) represent a family of pattern recognition receptors that recognize carbohydrate structures on pathogens and self-Ags often in a Ca²⁺-dependent manner. In this study, we investigated the role of the CLR dendritic cell immunoreceptor (DCIR) in the genesis of CM. Using the murine PbA infection, we show in this article that DCIR is essential for the development of CM. Although PbA infection led to 80% CM in wild-type C57BL/6 mice, DCIR-deficient mice were highly protected with only 15% CM development. In accordance with the reduced CM incidence in DCIR⁻/⁻ mice, CD8⁺ T cell sequestration was markedly reduced in brains of PbA-infected DCIR⁻/⁻ mice, which was accompanied by reduced brain inflammation. Reduced T cell sequestration in the brain was caused by decreased TNF-α in sera, as well as a modulated activation of CD4⁺ and CD8⁺ T cells in spleen of PbA-infected DCIR⁻/⁻ mice. This study indicates that DCIR is critically involved in CM induction, thus highlighting the importance of this CLR in innate immunity during malaria infection. *The Journal of Immunology*, 2013, 191: 000–000.

**Malari**a is one of the main global causes of death from infectious diseases, resulting in >200 million clinical cases and 655,000 deaths per year with 86% of those infected children <3 y of age (1). The vast majority of severe malaria cases and deaths are caused by the protozoan parasite, *Plasmodium falciparum*, which is endemic mainly in sub-Saharan Africa and in tropical regions of the world. Infection with *P. falciparum* can lead to severe pathology, which includes anemia, metabolic acidosis, impaired circulation, and cerebral malaria (CM) (2).

Host defense to malaria infection involves multiple strategies. The role of T cells and cytokines in the course of experimental CM (ECM) has helped to identify crucial players in the pathogenesis of CM. However, the role of pattern recognition receptors in innate immunity to CM induction is still poorly understood. In *Plasmodium berghei* ANKA (PbA) infection of mice, an established model of ECM (3, 4), sequestration of CD8⁺ T cells into the brain (5), accompanied by the release of the cytolytic molecules granzyme B (GrB) and perforin (6), promotes CM development. CD8⁺ T cells also mediate the accumulation of PbA-infected RBCs (pRBCs) in the brain (7). ECM is associated with high levels of proinflammatory cytokines (8, 9), with TNF-α and IFN-γ playing a crucial role in CM induction (10, 11). Consistently, IFN-γ-producing CD4⁺ T cells were shown to trigger the enhanced CD8⁺ T cell accumulation in the brain, thus inducing ECM (12). In contrast, the immune modulatory cytokine IL-10 plays a key role in protection against severe malaria (13).

Although adaptive immunity during the course of malaria has been investigated intensively, still little is known about the role of innate immunity in CM induction. Innate immunity is of crucial importance as a first line of defense against infections (14), with dendritic cells (DCs) playing a pivotal role in Ag presentation and the initiation of a protective immune response (15). A number of studies indicate that DC functions such as maturation and the ability to cross-present malaria Ags are compromised by the interaction with pRBCs (16–18), whereas other studies report that DCs from malaria-infected mice are still efficient in presenting pRBC-derived Ags to CD4⁺ T cells (19, 20). In recent years, the contribution of DC subsets to immunity but also malaria-associated pathology has started to be unraveled (21–23). Nonetheless, little is known about which DC-expressed pattern recognition receptors (PRRs) are involved in host defense to malaria on the one hand and how they might contribute to CM development on the other hand.

Two major families of PRRs predominantly expressed by cells of the innate immune system are TLRs and C-type lectin receptors (CLRs). TLRs have been implicated to play a role in innate immunity to malaria and CM development (24, 25). However, the role of TLRs in malaria is still under debate (26–28). Besides TLRs, members of the TNF and TNFR superfamilies were also shown to affect the outcome of malaria infection such as the interaction of LIGHT (TNFSF14) with the lymphotoxin β receptor that is involved in CM induction (29, 30). CLRs recognize specific carbohydrate structures on the surface of pathogens and self-Ags (31, 32), and orchestrate innate responses to a number of pathogens including bacteria, viruses, fungi, and helminths (32). In the case
of malaria, DCs expressing the CLR Clec9a are critically involved in CM development. Ablation of Clec9a−/− DCs resulted in complete resistance to ECM and reduction of CD8+ T cell sequestration in the brain (33). In another study, the role of the caspase recruitment domain-containing protein 9 (CARD9) was investigated. CARD9 is an adaptor protein involved in signaling of CLRs such as Dectin-1 or Dectin-2. CARD9 deficiency did not affect CM induction, indicating that CLR signaling through the adaptor protein CARD9 plays a limited role in CM development (34).

In this study, we sought to elucidate the role of the CLR DC immunoreceptor (DCIR) in CM induction. DCIR is mainly expressed by DCs, but also by macrophages, monocytes, and B cells, and its signaling is mediated through an immunoreceptor tyrosine-based inhibitory motif (ITIM) (35, 36). We demonstrate in this article that DCIR is crucial for CM development. DCIR deficiency resulted in a dramatically reduced incidence of CM in the murine PbA infection model compared with wild-type control mice. Parasite loads were similar in wild-type and DCIR−/− mice, indicating that DCIR deficiency did not affect parasite replication directly. Protection of DCIR−/− mice was associated with a reduced number of sequestered IFN-γ+ and GrB+ CD8+ T cells in the brain, indicating that DCIR impacts T cell migration to the brain during the course of malaria. DCIR deficiency also led to decreased serum TNF-α level and to modulated CD4+ and CD8+ T cell activation in the spleen. These findings suggest that DCIR impacts T cell priming in spleen, which finally influences T cell sequestration in the brain. In conclusion, DCIR is crucial for the genesis of CM, underscoring the importance of this CLR in immune pathology during malaria.

Materials and Methods

Ethics statement

Animal experiments were performed in strict accordance with the German regulations of the Society for Laboratory Animal Science and the European Health Law of the Federation of Laboratory Animal Science Associations. The protocol was approved by the Landesamt für Gesundheit und Soziales Berlin (Permit No. G0053/10). All efforts were made to minimize suffering.

Mice and parasites

DCIR−/− mice and the respective C57BL/6 control mice were housed and bred in the animal facility of the Federal Institute for Risk Assessment in a pathogen-free environment. DCIR−/− (031932-UCD, DCIR KO/Mmcd) mice were obtained from the National Institute of Health-sponsored Mutant Mouse Regional Resource Center (MMRRC) National System (Supplemental Fig. 1). The PbA stably mateB-31 was obtained through the MR4, deposited by Thomas McCutchen, and fresh stably mates were used. Mice were monitored daily for early signs of CM. Parasitemia was determined by examining Wright-stained tail blood samples. The cytokines TNF-α, IFN-γ, IL-10, IL-12p70, IL-1β, IL-4, and IL-6 were measured by flow cytometry brain lysate array according to the manufacturer’s instructions (BD Pharmingen). For the cytokine IL-12p40, indirect sandwich ELISA was performed according to the manufacturer’s instructions (PeproTech, Hamburg, Germany).

Analysis of cytokine production

For cytokine quantification, sera from infected mice were obtained from tail blood samples. The cytokines TNF-α, IFN-γ, IL-10, IL-12p70, IL-1β, IL-4, and IL-6 were measured by flow cytometry brain lysate array according to the manufacturer’s instructions (BD Pharmingen). For the cytokine IL-12p40, indirect sandwich ELISA was performed according to the manufacturer’s instructions (PeproTech, Hamburg, Germany).

Binding studies with mouse DCIR-IgGl-Fc

Total RNA was isolated from C57BL/6 mouse spleen cells and was reversely transcribed into cDNA using RevertAid Transcriptionase (New England Biolabs, Ipswich, MA). cDNA encoding the extracellular part of mouse DCIR (mDCIR) (Clec4a2) was PCR-amplified using the primers DCIR forward primer (5′-GACATCTTTAGCTTCTTCAACCAGTTAAA-3′) and reverse primer (5′-AGATCTTTAGCTTCTTCAACCAGTTAAA-3′). For expression of the mDCIR-IgGl fusion protein, the cDNA encoding the DCIR extracellular part was ligated into the pFuse-human IgGl (hIgGl-Fc) expression vector (Invivogen, Ipswich, MA).
Reduced T cell sequestration in brain of DCIR<sup>−/−</sup> mice

Numerous studies have demonstrated that leukocytes in the brain, particularly CD8<sup>+</sup> T cells, are important for CM induction in the murine PbA infection model. To determine whether the absence of DCIR influenced the sequestration of CD8<sup>+</sup> T cells into the brain, CD4<sup>+</sup> leukocytes in the brain were stained and the number of activated CD45<sup>+</sup>CD8<sup>+</sup> T cells (which were CD62L<sup>lo</sup>) was analyzed by flow cytometry (Fig. 2A). Indeed, upon PbA infection, the number of CD45<sup>+</sup> cells in the brain of DCIR<sup>−/−</sup> mice was significantly reduced compared with infected wild-type mice (Fig. 2B). This was mainly due to a reduced sequestration of activated CD8<sup>+</sup> T cells into the brain of DCIR<sup>−/−</sup> mice (Fig. 2C). To determine the kinetics of T cell migration into the brain, we analyzed the number of brain-sequestered T cells from day 2 p.i. on. Cells started to migrate into the brain on day 6 p.i. (data not shown), but numbers of brain-sequestered CD8<sup>+</sup>CD62L<sup>lo</sup> T cells were significantly different between PbA-infected wild-type and DCIR<sup>−/−</sup> mice not until day 7 p.i. (Fig. 2C). Because neurologic symptoms were observed only from day 8 p.i. on, this finding indicates that CD8<sup>+</sup> T cell sequestration in the brain precedes the occurrence of CM. This observation is in agreement with a recently published study showing that there is indeed no direct correlation between the number of specific T cells in the brain and the clinical condition (40). Our results demonstrate that the reduced incidence of CM among DCIR<sup>−/−</sup> mice is accompanied by a decreased migration of activated CD8<sup>+</sup> T cells into the brain compared with wild-type mice.

Next, to evaluate effector functions of the brain-sequestered T cells, we determined the production of IFN-γ by intracellular flow cytometry and the expression of Grb by immunohistochemistry because both have been demonstrated to be necessary for CM development (6, 40, 41). As a control for the immunohistochemistry staining, the expression of the endothelium marker CD31 on brain sections of PbA-infected wild-type and DCIR<sup>−/−</sup> mice was determined (Fig. 3). Because CD31 is an integral marker for endothelial cells, no difference in the total CD31 expression by wild-type, PbA-infected wild-type, and infected DCIR<sup>−/−</sup> mice was observed (Fig. 3A, upper panel). In contrast, CD3 staining showed a significantly higher number of intravascular and extravascular lymphocytes in PbA-infected wild-type mice than in DCIR<sup>−/−</sup> mice (Fig. 3A, middle panel, 3B). A substantial portion of the brain-sequestered T cells were IFN-γ<sup>+</sup> (10%) and Grb<sup>+</sup> (between 10 and 40%; Fig. 3A, lower panel, 3C). We did not observe

FIGURE 1. PbA-infected DCIR<sup>−/−</sup> mice are significantly protected from CM induction. DCIR<sup>−/−</sup> mice (n = 38) and the respective C57BL/6 control mice (n = 38) were infected i.p. with 1 × 10<sup>6</sup> PbA-infected RBCs. (A) Mice were monitored daily for early signs of CM and were euthanized in case of clinical symptoms such as ataxia, convulsions, or coma. Survival was determined using log-rank test (****p ≤ 0.0001). The survival curves represent a summary of 5 independent experiments with 5–10 mice each. (B) Parasitemia was determined on days 7 and 11 p.i. On day 11 p.i., the number of PbA-infected wild-type mice used for determination of parasitemia was significantly lower (n = 8) because ~80% of mice had experienced development of CM by that time point. Data are expressed as mean ± SEM for each group. Statistical analysis was performed using Student t test. n.s., Not significant.
a difference in the frequency of IFN-\(\gamma\)CD8\(^+\) T cells in the brain between PbA-infected wild-type and DCIR \(^{-/-}\) mice (Fig. 3C). However, as the absolute number of CD8\(^+\) T cells in the brain of DCIR \(^{-/-}\) mice was markedly reduced, DCIR deficiency resulted in a significantly reduced absolute number of IFN-\(\gamma\)+CD8\(^+\) T cells in the brain as well. A markedly reduced percentage of GrB+ brain-sequestered T cells was found in DCIR \(^{-/-}\) mice compared with wild-type control mice (Fig. 3A, lower panel, 3C). This finding suggests that DCIR not only influences T cell migration to the brain, but also impacts effector functions of the brain-sequestered T cells.

To corroborate the effect of DCIR deficiency on brain pathology, we performed histologic examination of brain sections using a clinical scoring system as described in Materials and Methods. Brain sections were analyzed for the presence of vessel wall hyalinization, endothelial activation, and intravascular leukocytes (Fig. 4A). A significantly increased eosinophilic thickening of intracerebral vessel walls, a distinct feature characterizing vessel wall hyalinization, was observed in PbA-infected wild-type mice compared with DCIR \(^{-/-}\) mice (Fig. 4A, upper panel, 4B). Consistently, there was also an enlargement of endothelial nuclei indicating endothelial activation that was predominantly observed in PbA-infected wild-type mice, but only to a significantly lower level in DCIR \(^{-/-}\) mice (Fig. 4A, middle panel, 4C). In addition, the abundance of leukocytes in vessels observed was also higher among PbA-infected wild-type mice compared with DCIR \(^{-/-}\) mice, although differences did not reach statistical significance (Fig. 4A, bottom panel, 4D). These findings demonstrate that brain inflammation is indeed alleviated in PbA-infected DCIR \(^{-/-}\) mice.

Reduced TNF-\(\alpha\) levels in serum of DCIR \(^{-/-}\) mice

ECM is associated with high levels of proinflammatory cytokines with TNF-\(\alpha\) and IFN-\(\gamma\) playing a crucial role in CM induction (10, 11). Thus, we examined whether the levels of IL-1\(\beta\), IL-12p70, IL-12p40, TNF-\(\alpha\), IL-10, and IFN-\(\gamma\) in sera of PbA-infected wild-type and DCIR \(^{-/-}\) mice differed. Indeed, the level of the proinflammatory cytokine TNF-\(\alpha\) in serum was significantly lower in PbA-infected DCIR \(^{-/-}\) mice than in wild-type mice (Fig. 5A). Interestingly, no significant differences in the levels of IFN-\(\gamma\), IL-10, and IL-12p40 were observed (Fig. 5B, 5C, and data not shown). The levels of IL-1\(\beta\), IL-12p70, IL-6, and IL-4 were at or below detection level for both groups (data not shown). Thus, DCIR

**FIGURE 2.** Decreased sequestration of CD8\(^+\) T cells in the brain of DCIR \(^{-/-}\) mice upon PbA infection. C57BL/6 \((n=18)\) and DCIR \(^{-/-}\) mice \((n=18)\) were infected i.p. with \(1 \times 10^6\) PbA-infected RBCs. On day 7 p.i., brains were homogenized and cells were stained with anti-CD45-PerCP-Cy5.5, anti-CD8-allophycocyanin-H7, and anti-CD62L-PE, and analyzed by flow cytometry. Cells were gated on CD45\(^+\) cells in the brain. (A) Representative dot plots are shown for uninfected wild-type mice (top panel), PbA-infected wild-type mice (middle panel), and infected DCIR \(^{-/-}\) mice (bottom panel). (B) Bar diagrams showing the frequency of CD45\(^+\) cells and (C) CD45\(^+\)CD8\(^+\)CD62L\(^{low}\) T cells per \(1 \times 10^7\) cells in the brain of PbA-infected wild-type and DCIR \(^{-/-}\) mice. Dashed lines represent cell numbers in brain of uninfected DCIR \(^{-/-}\) and wild-type mice. A summary of three independent experiments is shown with six to eight mice each. Data are expressed as mean \(\pm\) SEM for each group. Statistical analysis was performed using unpaired Student \(t\) test (*\(p<0.05)\). Results indicate a significantly reduced sequestration of CD45\(^+\) leukocytes and CD8\(^+\)CD62L\(^{low}\) T cells in the brain of PbA-infected DCIR \(^{-/-}\) mice.
The activation of CD4+ and CD8+ T cells in spleen is important for the frequency of activated CD4+ and CD8+ T cells in spleen during CM induction (5, 42). Thus, we determined the frequency of activated CD4+ and CD8+ T cells in spleen expressing the early activation marker CD69 was significantly lower in PbA-infected DCIR−/− mice compared with wild-type control mice. (B and C) Data are presented as mean ± SEM for each group. Statistical significance was determined by unpaired Mann–Whitney U test (***p < 0.01).

**FIGURE 3.** Decreased number of activated T cells in the brain of PbA-infected DCIR−/− mice. Brain sections from PbA-infected C57BL/6 and DCIR−/− mice were taken on day 7 p.i. These sections were then stained for CD31, CD3, and GrB. (A) Representative figures of uninfected wild-type, PbA-infected wild-type, and infected DCIR−/− mice are shown for CD31 expression, CD3+ T cell sequestration, and GrB expression, respectively. All mice were positive for the presence of endothelial cells as confirmed by CD31 staining (top panel; scale bar, 20 μm). In the case of CD3 staining (middle panel; scale bar, 40 μm), a significantly higher number of intravascular and extravascular lymphocytes was observed among PbA-infected wild-type mice (black arrow) compared with DCIR−/− mice, which exhibited only rare CD3+ cells in few cerebral and cerebellar vessels. A higher number of lymphocytes in the brain of PbA-infected wild-type mice were GrB+ compared with DCIR−/− mice (bottom panel; scale bar, 40 μm). (B) Analysis was performed using a scoring system to determine the degree of lymphocyte presence. A summary of three independent experiments is shown with six to eight mice each. Dashed lines represent scores of uninfected DCIR−/− and wild-type mice. (C) The frequency of IFN-γ+ brain-sequestered CD8+ T cells was measured by intracellular flow cytometry (n = 4). The frequency of GrB+ lymphocytes in the brain was determined by immunohistochemistry (n = 5). A markedly reduced percentage of GrB+ brain-sequestered T cells was found in DCIR−/− mice compared with wild-type control mice. (B and C) Data are presented as mean ± SEM for each group. Statistical significance was determined by unpaired Mann–Whitney U test (***p < 0.01).

**Modulated T cell activation in spleen of DCIR−/− mice**

The activation of CD4+ and CD8+ T cells in spleen is important for these leukocyte populations to migrate to the brain and to contribute to CM induction (5, 42). Thus, we determined the frequency of activated CD4+ and CD8+ T cells in spleen during PbA infection (Fig. 6). Although whole splenic composition was unaltered in uninfected as well as PbA-infected wild-type and DCIR−/− mice (Fig. 6A and data not shown), the frequency of activated CD4+ and CD8+ T cells in spleen expressing the early activation marker CD69 was significantly lower in PbA-infected DCIR−/− mice compared with infected wild-type mice (Fig. 6B, 6C). This finding indicates that DCIR deficiency affects T cell priming in spleen, which may finally impact T cell sequestration in the brain.

DCIR is an attachment factor for HIV-1 (43) but no other ligands have yet been identified. To examine whether DCIR interacts directly with a parasitic glycan ligand, we expressed the extracellular part of mDCIR as a chimeric mDCIR-IgG1-Fc fusion protein (see Supplemental Fig. 2). In a glycan array provided by the CFG including >600 different carbohydrates, as well as in our own glycan array containing several P. falciparum glycan structures, no DCIR ligand was detected (data not shown). We also did not observe binding of mDCIR-Ig to pRBCs (data not shown). These findings suggest that DCIR does not bind to parasitic glycans directly but might rather interact with danger-associated molecular pattern molecules released during the course of malaria, or that DCIR influences the activation state of DCs directly.

**Discussion**

In this study, we used the murine PbA infection model to determine whether the CLR DCIR has a functional role in the control and/or genesis of CM. We show in this study that DCIR is a key player in CM development as DCIR−/− mice were significantly protected from clinical symptoms and brain inflammation, thus highlighting the contribution of this CLR to malaria-associated pathology.

To date, numerous studies focus on the role of TLRs in immunity to malaria and in the pathogenesis of severe malaria/CM, whereas studies on the relevance of CLRs are lacking. TLRs are involved in the recognition of parasitic pathogen-associated molecular patterns such as TLR2/4 in binding to P. falciparum glycosylphosphatidylinositol (44, 45) or TLR9 in the interaction with hemozoin/P. falciparum DNA (46, 47). The TLR/MyD88 pathway was shown to be crucial for CM development (48), and targeting of nucleic acid–sensing TLRs protected mice from clinical signs of CM (49). In Plasmodium chabaudi infection, malaria-induced priming of TLR responses correlated with the increased expression of TLR mRNA, and TLR9−/− mice were protected from LPS-induced lethality during acute infection (50). However, in other studies, only a limited role for TLRs in the genesis of CM was found (26, 28). A recent study indicated that the impact of TLR signaling on pathology might depend on the genetic background of the mice (51). In contrast to TLRs, CM genesis is independent of the Nod pathway. Although cytokine levels such as IL-1β or IFN-γ were decreased in Nod1/Nod2−/− mice during...
PbA infection, there was no difference in survival or parasitemia compared with wild-type mice (52).

On the contrary, no role for a specific CLR in CM induction has yet been described. The CLR DCIR is mainly expressed by DCs, macrophages, monocytes, and B cells, but currently no ligand for DCIR is known. The extracellular part of DCIR is homologous to other members of the CLR superfamily and contains an extracellular carbohydrate-recognition domain (32). Human DCIR acts as an attachment receptor for HIV-1 and promotes virus uptake into cells (43); however, these results were obtained by DCIR

FIGURE 4. Reduced inflammation in the brain of PbA-infected DCIR−/− mice. Brain sections from PbA-infected C57BL/6 and DCIR−/− mice were taken on day 7 p.i. Brain histology of uninfected and infected mice was assessed. (A) Representative figures of uninfected wild-type mice, as well as PbA-infected wild-type and DCIR−/− mice, are shown to depict the histologic differences among the groups. Infected wild-type mice exhibited vessel wall hyalinization characterized by eosinophilic thickening of intracerebral vessel walls (black arrow), which was significantly reduced in infected DCIR−/− mice (top panel). Enlargement of endothelial nuclei (black arrow), indicative of endothelial activation, was observed in most PbA-infected wild-type mice but only a few infected DCIR−/− mice (middle panel). The presence of intravascular leukocytes was observed (black arrow), with higher tendency, in PbA-infected wild-type mice than DCIR−/− mice (lower panel). Scale bar, 40 μm. Histologic analysis was performed using a scoring system to determine the degree of vessel wall hyalinization (B), endothelial activation (C), and presence of intravascular leukocytes (D). A summary of six independent experiments is shown with four to eight mice each. Data are presented as mean ± SEM for each group. Dashed lines represent scores of uninfected DCIR−/− and wild-type mice (0 for all parameters analyzed). Statistical significance was determined by unpaired Mann–Whitney U test (*p < 0.05, **p < 0.01). n.s., Not significant.

FIGURE 5. Decreased TNF-α levels in sera of PbA-infected DCIR−/− mice. Sera from PbA-infected wild-type and DCIR−/− mice were taken on day 7 p.i. Levels of the cytokines TNF-α (A), IFN-γ (B), and IL-10 (C) were evaluated using cytometric bead array. The production of TNF-α was markedly reduced in PbA-infected DCIR−/− mice compared with wild-type mice. Data are expressed as mean ± SEM for each group. The summary of six independent experiments is shown with five to six mice each. The dashed lines represent cytokine levels in sera of uninfected wild-type and DCIR−/− mice. Statistical analysis was performed using unpaired Student t test (**p < 0.01).
transfection experiments and were not based on direct interaction studies with purified ligands. We have tried to identify a carbohydrate ligand of DCIR using a recombinant DCIR-Ig fusion protein. However, in a glycan array provided by the CFG, as well as in our own glycan array containing Plasmodium glycans such as glycosylphosphatidylinositols, no specific ligand was detected (data not shown). Although this finding does not formally exclude an interaction with carbohydrate ligands, DCIR binding to glycoprotein or protein ligands might be more likely. Binding to protein ligands was also shown for other CLRs such as Clec9a that binds to the F-actin component of the cellular cytoskeleton (53, 54). We also performed binding studies between DCIR-Ig and pRBCs; however, we did not observe a specific interaction. Moreover, parasitemia levels in blood were unaltered in PbA-infected DCIR \(^{−/−}\) mice compared with control mice. These findings indicate that DCIR might not bind to parasitic pathogen-associated molecular patterns directly but rather suggest a role for DCIR in the recognition of self-Ags released by the rupture of pRBCs during malaria progression, DC activation, or both. Indeed, several CLRs such as Mincle or Clec9a sense danger-associated molecular pattern molecules such as necrotic cells highlighting the dual role of CLRs in the recognition of pathogens and self-Ags (32). Thus, the nature of the DCIR ligand still remains to be identified. The scope of this study, however, was to unravel the role of DCIR in immune pathology during malaria.

We show in this article that DCIR deficiency impacted T cell activation in the spleen and modulated TNF-\(\alpha\) production. Previous studies demonstrate the importance of PRRs expressed by APCs for cell activation and T cell priming. TLRs are essential players in DC activation and cross-priming during malaria infection because DCs become refractory to TLR-mediated IL-12 and TNF-\(\alpha\) production during the course of the disease (55). Moreover, PbA infection leads to an inhibited cross-priming of CD8\(^{+}\) T cells by activated DCs (18). In a recent study using murine Plasmodium yoelii infection, TLR9 and MyD88 were shown to be critical in the regulation of cytokine production, Th1/Th2 development, as well as cellular and humoral responses (25). So far, there is a knowledge gap regarding the role of CLRs in these processes.

Because signaling of a number of CLRs is mediated through the adaptor protein CARD9, the susceptibility of CARD9 \(^{−/−}\) mice to PbA infection was analyzed (34). Although CARD9 expression
was upregulated in the brains of PbA-infected wild-type mice, PbA-infected CARD9+− mice succumbed to neurologic signs and blood–brain barrier disruption similar to wild-type mice (34).

In conclusion, we establish a crucial role for DCIR in CM development during PbA infection of mice. To the best of our knowledge, this is the first report showing the importance of a specific DC-expressed CLR in malaria-associated pathology. The only CLR that was reported to be associated with placental malaria is the complement protein mannamin-binding lectin (58). We provide evidence that DCIR affects T cell priming in spleen, contributes to the proinflammatory cytokine TNF-α, and triggers the pathogenesis of CM. Thus, interference with DCIR signaling might be a means to modulate APC function during the course of malaria.

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Disclosures
The authors have no financial conflicts of interest.

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