# A Virus Hosted in Malaria-Infected Blood Protects against T Cell-Mediated Inflammatory Diseases by Impairing DC Function in a Type I IFN-Dependent Manner

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**ABSTRACT** Coinfections shape immunity and influence the development of inflammatory diseases, resulting in detrimental or beneficial outcome. Coinfections with concurrent *Plasmodium* species can alter malaria clinical evolution, and malaria infection itself can modulate autoimmune reactions. Yet, the underlying mechanisms remain ill defined. Here, we demonstrate that the protective effects of some rodent malaria strains on T cell-mediated inflammatory pathologies are due to an RNA virus cohosted in malaria-parasitized blood. We show that live and extracts of blood parasitized by *Plasmodium berghei* K173 or *Plasmodium yoelii* 17X YM, protect against *P. berghei* ANKA-induced experimental cerebral malaria (ECM) and myelin oligodendrocyte glycoprotein (MOG)/complete Freund's adjuvant (CFA)-induced experimental autoimmune encephalomyelitis (EAE), and that protection is associated with a strong type I interferon (IFN-I) signature. We detected the presence of the RNA virus lactate dehydrogenase-elevating virus (LDV) in the protective *Plasmodium* stabilates and we established that LDV infection alone was necessary and sufficient to recapitulate the protective effects on ECM and EAE. In ECM, protection resulted from an IFN-I-mediated reduction in the abundance of splenic conventional dendritic cell and impairment of their ability to produce interleukin (IL)-12p70, leading to a decrease in pathogenic CD4$^+$ Th1 responses. In EAE, LDV infection induced IFN-I-mediated abrogation of IL-23, thereby preventing the differentiation of granulocyte-macrophage colony-stimulating factor (GM-CSF)-producing encephalitogenic CD4$^+$ T cells. Our work identifies a virus cohosted in several *Plasmodium* stabilates across the community and deciphers its major consequences on the host immune system. More generally, our data emphasize the importance of considering contemporaneous infections for the understanding of malaria-associated and autoimmune diseases.

**IMPORTANCE** Any infection modifies the host immune status, potentially ameliorating or aggravating the pathophysiology of a simultaneous inflammatory condition. In the course of investigating how malaria infection modulates the severity of contemporaneous inflammatory diseases, we identified a nonpathogenic mouse virus in stabilates of two widely used rodent parasite lines: *Plasmodium berghei* K173 and *Plasmodium yoelii* 17X YM. We established that the protective effects of these *Plasmodium* lines on cerebral malaria and multiple sclerosis are exclusively due to this virus. The virus induces a massive type I interferon (IFN-I) response and causes quantitative and qualitative defects in the ability of dendritic cells to promote pathogenic T cell responses. Beyond revealing a possible confounding factor in rodent malaria models, our work uncovers some bases by which a seemingly innocuous viral (co)infection profoundly changes the immunopathophysiology of inflammatory diseases.

**KEYWORDS** CD4 T cell, RNA virus, autoimmunity, coinfection, dendritic cells, inflammation, malaria

**B**y extension of the "hygiene hypothesis" (1), the host immune system is "conditioned" by lifelong environmental encounters with micro- and macroorganisms (also known as the multibiome [2]), ranging from commensals to pathogens. In turn, this interplay is expected to influence, positively or negatively, the development and severity of inflammatory disorders, including allergy and autoimmune diseases (3).

With respect to multiple sclerosis (MS), an autoimmune demyelinating disease of the central nervous system (CNS), neurotropic virus infections were reported to confer an added risk of developing the disease in adolescents and young adults (4, 5). Although the mechanisms are still debated, it has been hypothesized that virus infections may lead to breakdown of self-tolerance in genetically predisposed individuals, in part by antigenic molecular mimicry and/or by bystander activation of autoreactive T cells via upregulation of major histocompatibility complex (MHC) and costimulatory molecules on dendritic cells (DC) (6). In experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, virus-specific brain-resident T cells generated in early life following CNS virus infection are indeed able to promote the activity of autoreactive T cells, resulting in brain autoimmune attacks (7). In contrast to viruses, helminthic parasites are known to play a protective role in MS, most likely because (i) they promote tissue repair and (ii) they induce regulatory T and B cells as well as anti-inflammatory cytokines (e.g., interleukin 10 [IL-10] and transforming growth factor $\beta$ [TGF-$\beta$]), which suppress pathogenic autoreactive granulocyte-macrophage colony-stimulating factor (GM-CSF)$^+$ CD4$^+$ T cell responses (reviewed in references 8 to 10).

Beside extracellular helminths, intracellular parasites such as *Plasmodium*, the malaria-causing parasite, which affects almost one-half of the world's population, can also elicit immune modulatory pathways, which could affect autoimmune reactions. Following an asymptomatic liver stage, the parasites reach the bloodstream and develop within red blood cells (RBC). Blood-stage malaria can be associated with mild to severe clinical symptoms, including anemia, acute respiratory distress syndrome, acidosis, renal failure, or cerebral malaria (CM), which is characterized by the sequestration of leukocytes and parasitized RBC (pRBC) in brain microcapillaries, leading to hypoxia and vascular damage. Interestingly, most of the reported modulatory effects of *Plasmodium*, which can affect DC and B and T cells, occur during blood stage. Examples of this modulation on DC include the increased apoptosis of blood-circulating DC (11, 12), an atypical/partial DC maturation profile (13), and a crippled ability to present antigens to CD4$^+$ and CD8$^+$ T cells (11, 14, 15). The impact of *Plasmodium* on DC may be direct, such as exposure to parasite effectors or by-products such as the heme crystal hemozoin (16), or indirect, such as the systemic activation by pattern recognition receptors such as Toll-like receptors (TLRs), which imprint a "refractory" state on DC (17), or by type I interferon (IFN-I), which impairs their Th1-promoting property (18). With regard to T cells, blood-stage malaria may cause T cell exhaustion, which can be restored by checkpoint inhibitor therapy (19). CD4$^+$ T follicular helper (Tfh) cells normally play a critical role in parasite control during blood stage, as they enhance the activation of germinal center B cell responses and enable long-lasting more-efficient humoral immunity (20, 21). Yet during severe malaria, a strong Th1-polarizing environment promotes the development of dysfunctional T-bet$^+$ "Th1-like" CD4$^+$ Tfh cells (22, 23), which exhibit poor help activity on B cell responses and lead to B cell apoptosis or differentiation into short-lived plasma cells and atypical memory B cells (24).

While such immune modulatory processes are thought to partially underlie the poor naturally acquired immunity to malaria observed in areas of endemicity, they may also have a beneficial impact on the course of autoimmune disorders. More than half a century ago, the incidence of two autoimmune diseases, rheumatoid arthritis and systemic lupus erythematosus, was found to be up to 6 times less frequent in Nigerians than in Europeans, and it was proposed that parasitic infections, in particular, malaria, were responsible for alleviating the development of autoimmunity (25). In accordance, experimental infection with *Plasmodium berghei* suppressed the spontaneous development of renal disease in a mouse lupus model (26). Intriguingly, the prevalence and incidence of MS has increased following malaria eradication in Sardinia (27), and work

using rodent-adapted *Plasmodium* strains has revealed an overall protective effect of malaria infection on EAE. Infection with *Plasmodium chabaudi chabaudi* AS (*Pcc*AS) reduced EAE severity, possibly due to the induction of regulatory CD4$^+$ T cells (Treg) and the production of IL-10 and TGF-$\beta$ (28). Moreover, transfer of DC incubated with extracts of *P. berghei* NK65 pRBC ameliorated EAE (29); although paradoxically, when induced in mice cured from that same parasite, EAE was aggravated (30). Currently, little is known about the molecular and cellular mechanisms by which *Plasmodium* infection influences CNS autoimmunity.

In addition, beside autoimmune contexts, the clinical evolution of malaria itself is influenced by coinfection with another *Plasmodium* species. In humans, the risk of developing symptomatic malaria seems to be lower in mixed *P. falciparum*/*P. malariae* or *P. falciparum*/*P. vivax* infections (31, 32). In mice, the development of experimental cerebral malaria (ECM), a deadly vascular pathology during which Th1 CD4$^+$ T cells promote the sequestration of pathogenic CD8$^+$ T cells in the brain vasculature (33), is inhibited by coinfection with *Plasmodium yoelii yoelii* 17X clone 1.1 (34) and by *P. berghei* K173 (35). In the former case, replication of the ECM-causing *P. berghei* ANKA parasites was found to be hampered by mixed infection, but in the latter case, there was no effect on parasite growth. Rather, protection was associated with an early production of IFN-$\gamma$ and IL-10 cytokines at 24 h postinfection. Yet, the exact protective mechanisms remain ill defined.

Here, in order to elucidate the bases of *Plasmodium*-mediated protection against T cell-mediated inflammatory diseases, we have investigated rodent malaria strains that block ECM and EAE. We show that live blood, as well as blood extracts, parasitized by *P. berghei* K173 or *P. yoelii* 17X YM confers full protection against *P. berghei* ANKA-induced ECM and myelin oligodendrocyte glycoprotein (MOG)/complete Freund's adjuvant (CFA)-induced EAE and that this is associated with a strong IFN-I signature. We report the identification of an RNA virus called lactate dehydrogenase-elevating virus (LDV) in the protective *Plasmodium* stabilates, and we find that LDV infection alone recapitulates all the protective effects. In brief, LDV infection triggers a massive IFN-I response, which leads to a decrease in the number and functional capacity of conventional dendritic cells (cDC), thereby preventing the provision of signal 3 cytokines that are responsible for the pathogenic polarization of CD4$^+$ T cells in ECM and EAE.

(This article was submitted to an online preprint archive [36].)

## RESULTS

***P. berghei* K173 infection does not cause ECM and elicits lower Th1 responses than *P. berghei* ANKA.** While the *P. berghei* ANKA isolate is widely used to induce ECM in C57BL/6 mice, the outcomes of *P. berghei* K173 infection vary. Some studies report its potential to induce ECM (37–39), while others suggest that it does not cause ECM (40) or that it even protects from this pathology (35). We first evaluated the immune responses and clinical outcomes following infections with our "in-house" *P. berghei* ANKA and *P. berghei* K173 stabilates. We infected mice intravenously (i.v.) with *P. berghei* ANKA- or *P. berghei* K173-infected pRBC and monitored the development of ECM and circulating parasitemia. Both parasites replicated *in vivo* (Fig. 1A), but only *P. berghei* ANKA led to ECM, characterized by brain edema and early death within 7 to 8 days of infection (Fig. 1B). At day 6 postinfection, the absence of ECM development upon *P. berghei* K173 infection was consistent with a lower number of brain-sequestered CD4$^+$ and CD8$^+$ T cells than with *P. berghei* ANKA (Fig. 1C). Moreover, the percentage of brain CD4$^+$ (Fig. 1D) and CD8$^+$ T cells (Fig. 1E) producing IFN-$\gamma$, an essential cytokine in ECM pathogenesis (33, 41, 42), was significantly decreased upon *P. berghei* K173 infection compared to that with *P. berghei* ANKA. In the spleen, the percentage of CD4$^+$ T cells producing IFN-$\gamma$ upon pRBC restimulation was reduced in *P. berghei* K173-infected mice (Fig. 1F), but no difference was observed in the percentage of IFN-$\gamma^+$ CD8$^+$ T cells (Fig. 1G). These data suggest that the inability of *P. berghei* K173 to elicit ECM is related to an impaired
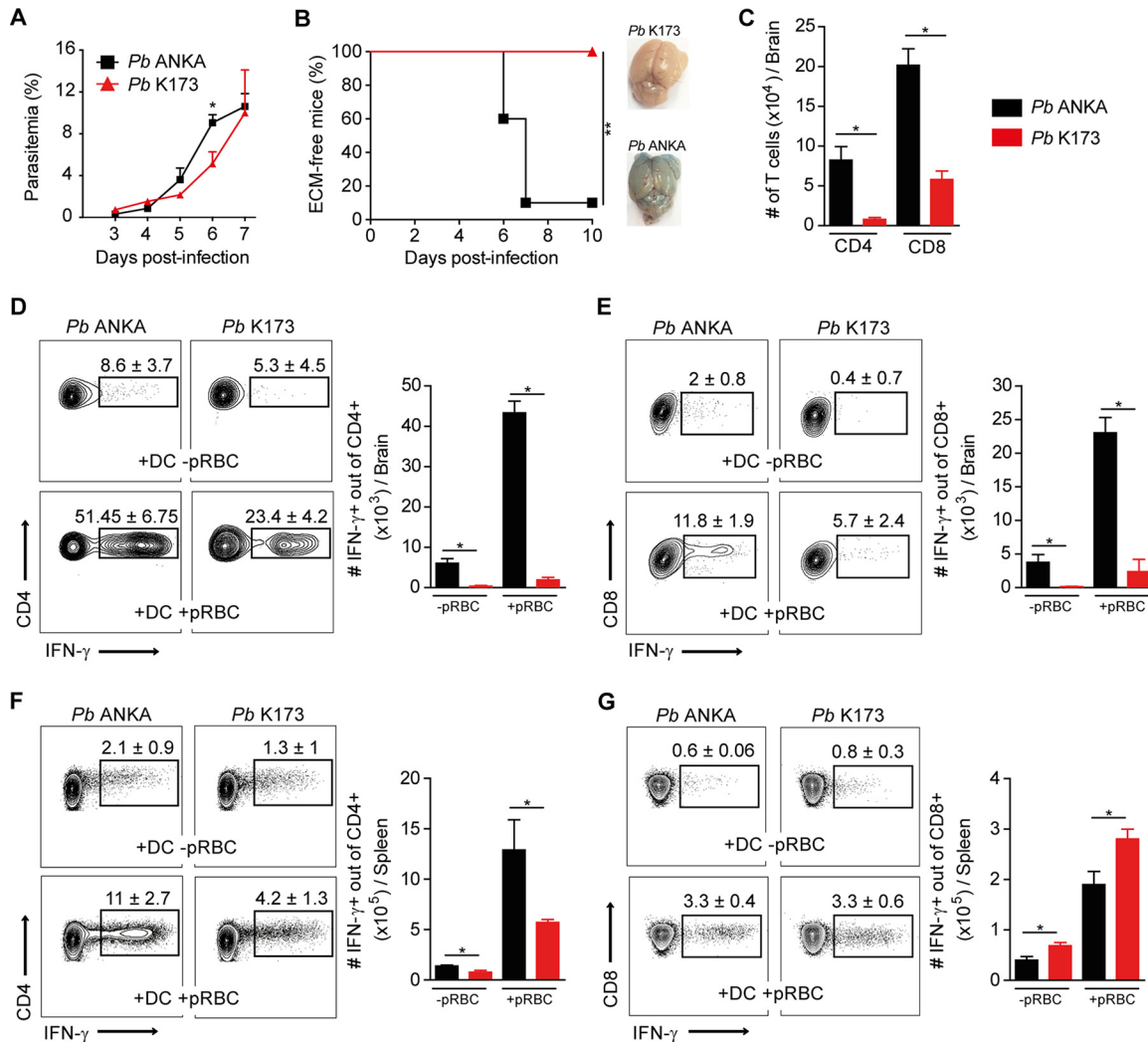
**FIG 1** Reduced Th1 responses and absence of ECM pathology following *P. berghei* K173 infection. C57BL/6 mice infected i.v. by injection of 10⁶ *P. berghei* ANKA (*Pb* ANKA) or *P. berghei* K173 (*Pb* K173) pRBC. Blood circulating parasitemia (A) and ECM development (B) monitored following infection. Brain edema was visualized by Evans blue coloration. (C) Total numbers of CD4⁺ or CD8⁺ T cells collected from brain at day 6 after infection. Cells collected from brain (D and E) and spleen (F and G) at day 6 after infection were restimulated *in vitro* with MutuDC preloaded or not with pRBC. IFN-γ production by CD4⁺ T cells (D and F) or CD8⁺ T cells (E and G) detected by intracellular staining. Percentages on the representative dot plots show the median percentages of IFN-γ⁺ cells of total CD4⁺ or CD8⁺ T cells ± IQRs. Bar graphs show the medians ± IQRs of absolute numbers. Data are representative of 2 independent experiments. *N* = 5 mice per group.

differentiation of CD4⁺ Th1 T cells in the spleen and a reduced sequestration of pathogenic CD8⁺ T cells in brain microcapillaries.

**P. berghei K173 infection triggers an early type I IFN response.** To investigate the molecular bases behind the lower Th1 responses in *P. berghei* K173-infected mice, we performed a kinetic analysis of several cytokines in the serum after infection. In accordance with the above findings, bioactive IL-12p70, a hallmark cytokine of Th1 responses, was induced exclusively by *P. berghei* ANKA but not by *P. berghei* K173 at day 3 postinfection (p.i.) (Fig. 2A). Instead, *P. berghei* K173 triggered an early production of IFN-α, CCL4, CCL5, tumor necrosis factor alpha (TNF-α), IL-6, and IL-12p40 at day 2 (Fig. 2A). In the spleen, *P. berghei* K173 infection induced a global activation of lymphocytes and DC, as exemplified by the upregulation of CD69 on T cells (Fig. 2B), B cells, and NK cells (not shown), and of CD86 on DC (Fig. 2C). These changes were suggestive of a systemic type I IFN (IFN-I) response (43). To confirm this hypothesis, we assessed whether they were dependent on IFN-I signaling. We analyzed the cytokine induction and T cell/DC activation in *P. berghei* K173-infected wild-type versus *Ifnar1*
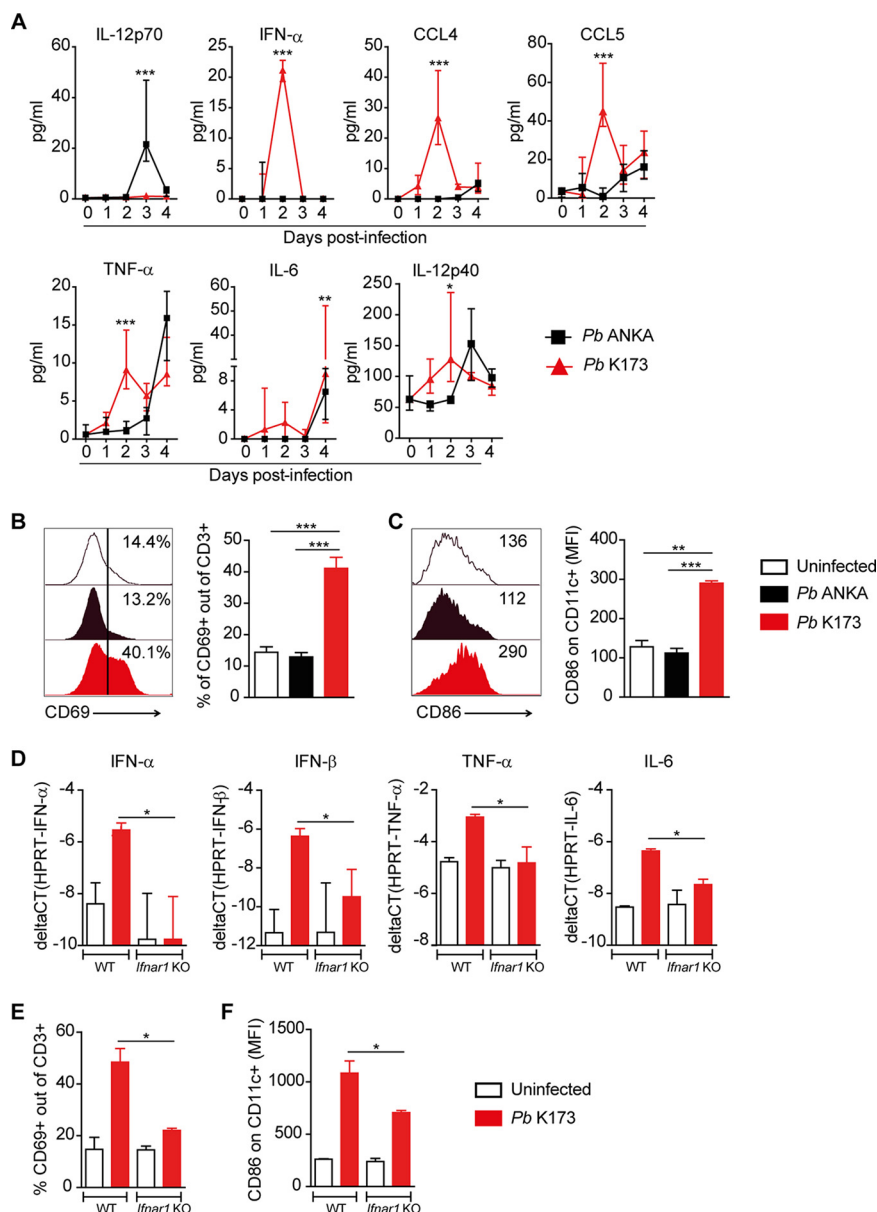
**FIG 2** *P. berghei* K173 but not *P. berghei* ANKA induces an early systemic type I IFN response. (A) Serum cytokines measured by Luminex assay at different time points after *P. berghei* (*Pb*) ANKA or *P. berghei* K173 infection. Proportions of CD69$^+$ of CD3$^+$ T cells (B) and geometric mean fluorescence intensities of CD86 expressed by CD11c$^+$ cells (C) in the spleen at day 2 after infection. Bars show the medians ± IQRs. WT and *Ifnar1* KO mice were infected with *P. berghei* K173 and analyzed at day 2 postinfection. (D) Cytokine gene expression in whole spleen analyzed by real-time qPCR. Proportions of CD69$^+$ of CD3$^+$ T cells (E) and geometric mean fluorescence intensities of CD86 on CD11c$^+$ cells (F) in the spleen. Bars show the medians ± IQRs. (A, D, E, and F) Data from 1 experiment with 5 to 7 mice/group. (B and C) Data representative of 4 independent experiments with $N = 4$ mice/group.

knockout (KO) mice. Both the induction of IFN-$\alpha$, IFN-$\beta$, TNF-$\alpha$, and IL-6 genes and the systemic activation of splenic T cells and DC were abrogated in *Ifnar1* KO mice (Fig. 2D to F). These data indicate that *P. berghei* K173- but not *P. berghei* ANKA-parasitized blood triggers a systemic IFN-I response, which correlates with defective Th1 differentiation and with ECM protection.

**Plasmodium-parasitized blood protects against ECM and EAE independently from live parasites.** We next investigated if upon *P. berghei* K173/*P. berghei* ANKA coinfection, *P. berghei* K173 had a "dominant" protective effect on ECM. Coinfected mice were indeed protected from ECM (see Fig. S1A in the supplemental material) and,
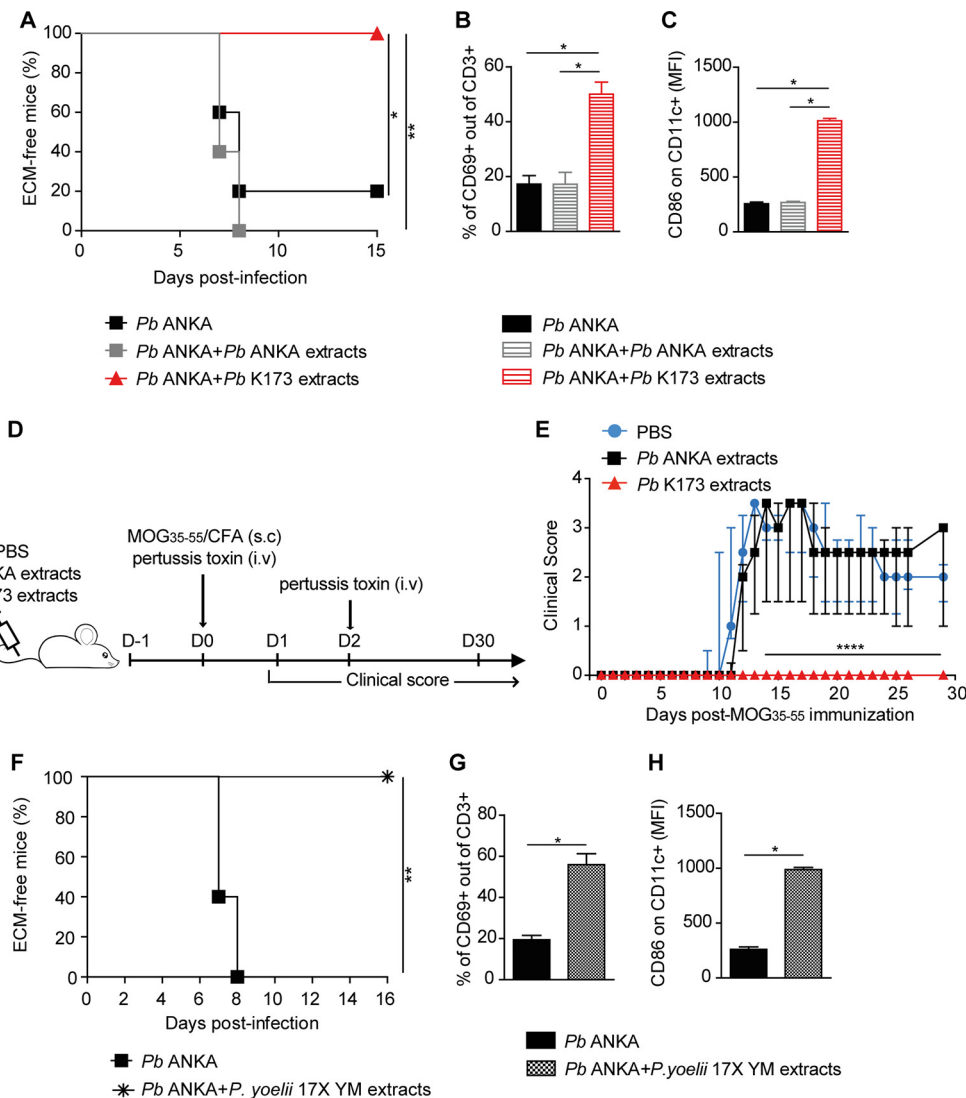
**FIG 3** *Plasmodium*-parasitized blood protects against ECM and EAE independently from live parasites. (A) ECM development following infection with $10^6$ *P. berghei* (*Pb*) ANKA pRBC administered with or without sonicated extracts of *P. berghei* ANKA or *P. berghei* K173. (B, C) Proportions of CD69$^+$ out of CD3$^+$ T cells (B) and geometric mean fluorescence intensities of CD86 on CD11c$^+$ cells (C) in the spleen at day 2 postinfection. Bars show the medians $\pm$ IQRs. (D) Experimental protocol. C57BL/6 mice were injected with *P. berghei* ANKA or *P. berghei* K173 pRBC sonicated extracts and immunized with MOG$_{35-55}$/CFA to induce EAE. (E) Clinical scores monitored up day 30 postimmunization. Dots show the medians $\pm$ IQRs. (F) ECM development following infection with $10^6$ *P. berghei* ANKA pRBC administered with or without sonicated extracts of $10^6$ *P. yoelii* 17X YM pRBC. (G, H) Proportions of CD69$^+$ out of CD3$^+$ T cells (G) and geometric mean fluorescence intensities of CD86 on CD11c$^+$ cells (H) in the spleen at day 2 postinfection. Bars show the medians $\pm$ IQRs. (A, B, and C) Data representative of 3 independent experiments with $N = 5$ mice/group. (E) Data representative of 2 experiments. $N = 6$ mice/group. (F, G, and H) Data from 2 experiments with $N = 5$ mice per group.

consistent with an impaired development of Th1 responses, blood CD4$^+$ T cells displayed lower production of IFN-$\gamma$ (Fig. S1B). To assess if live parasites were required for protection, we sonicated the *P. berghei* K173 stabilate, which precluded the parasites from replicating *in vivo* (not shown). Killed *P. berghei* K173 extracts still conferred full protection against ECM (Fig. 3A), and protection correlated with an early systemic activation of splenic T cells and DC (Fig. 3B and C). To examine if the protective potential of *P. berghei* K173 extracts could extend to other T cell-mediated inflammatory diseases, we evaluated their impact on experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis. EAE was induced by immunization with an MHC II peptide from the myelin oligodendrocyte self-antigen (MOG$_{35-55}$) combined with CFA. Control mice injected with *P. berghei* ANKA blood extracts 1 day

prior to MOG$_{35-55}$/CFA immunization developed a classical EAE disease characterized by a progressive ascendant paralysis, but mice that received extracts of *P. berghei* K173 blood showed no sign of disease (Fig. 3D and E). This indicates that inhibition of ECM and EAE by *P. berghei* K173-parasitized blood operates independently from live parasites.

It was previously reported that coinfection with certain clones of *P. yoelii* parasites can inhibit ECM (34) and that the *P. yoelii* 17X YM strain triggers an IFN-I response (44, 45). Thus, we tested whether *P. yoelii* 17X YM, obtained here from the MR4/BEI distributor, would phenocopy *P. berghei* K173. Although *P. yoelii* 17X YM does not elicit ECM, it causes hyperparasitemia and is known to be lethal around day 7. To mitigate this confounding factor, we sonicated the *P. yoelii* 17X YM stabile before coadministration with *P. berghei* ANKA. Akin to *P. berghei* K173 extracts, *P. yoelii* 17X YM extracts fully prevented the development of ECM (Fig. 3F) and induced T cell and DC activation at day 2 postinoculation (Fig. 3G and H). These data establish that at least two commonly used rodent malaria strains (*P. berghei* K173 and *P. yoelii* 17X YM) induce a systemic immune activation and protect from ECM.

**P. berghei K173 and P. yoelii 17X YM stabiles contain LDV virus, which alone, disrupts Th1 responses and protects from ECM.** Intriguingly, we noticed that the protective activity of *P. berghei* K173 blood was contained in the plasma (see Fig. S2A) and was disrupted by UV treatment (Fig. S2B), two observations suggestive of the presence of a viral element. To verify this hypothesis, we subjected *P. berghei* ANKA and *P. berghei* K173 stabiles to comprehensive PCR rodent infectious agent (PRIA) testing. We found the presence of a virus, the lactate dehydrogenase-elevating virus (LDV), in *P. berghei* K173 (titer of 3,600 particles per 100 $\mu$l plasma) and *P. yoelii* 17X YM (titer of 14,600 particles per 100 $\mu$l plasma) stabiles, but not in *P. berghei* ANKA stabiles.

LDV is an enveloped, single-stranded positive RNA virus of the family *Arteriviridae*, order *Nidovirales*, known to cause persistent asymptomatic infection in mice, with long-lasting circulating viremia (46). This virus has been reported to modulate host responses, thereby either alleviating (47, 48) or exacerbating (49) immune-mediated diseases. To assess the direct implication of LDV in ECM protection, we decontaminated *P. berghei* K173 stabiles by fluorescence-activated cell sorting (FACS) of erythrocytes. We verified by PRIA that these new *P. berghei* K173 pRBC stocks were LDV free. We also prepared parasite-free LDV-containing plasma by collecting the plasma from mice injected with *P. berghei* K173 sonicated blood extracts. *P. berghei* ANKA-infected mice coinjected with LDV-containing plasma were protected from ECM, while those coinfected with LDV-free *P. berghei* K173 were no longer protected (Fig. 4A). Strikingly, the LDV-free *P. berghei* K173 stabile was now causing ECM in the majority of mice (Fig. 4A), and these effects were not due to faster parasite growth (Fig. 4B). Since we had observed that ECM protection was linked to a reduced Th1 response, we analyzed the activation and production of Th1 cytokines by parasite-specific CD4$^+$ T cells at day 6 postinfection. While there was no major difference in the percentages of activated (CD11a$^+$ CD49d$^+$) splenic CD4$^+$ T cells between the groups (Fig. 4C), activated CD4$^+$ T cells were unable to produce IFN-$\gamma$ and TNF-$\alpha$ upon restimulation with the I-A$^b$-restricted ETRAMP$_{272-288}$ antigenic peptide (Fig. 4D). This inhibition was also observed in the total CD4$^+$ T cell population upon anti-CD3 stimulation (Fig. 4E). The results demonstrate that LDV is necessary and sufficient for ECM protection, most likely by abrogating the Th1 effector activity of CD4$^+$ T cells.

**LDV induces *Ifnar1*-dependent depletion and functional impairment of splenic cDC.** To study how LDV impedes the Th1 CD4$^+$ T cell response, we analyzed splenic conventional DC (cDC), which are primarily responsible for Th1 priming and polarization in this context (50–52). Two days following *P. berghei* ANKA/LDV coinfection, we observed a strong reduction in the proportion and numbers of total CD11c$^+$ MHC II$^+$ cDC, which was not observed in *Ifnar1* KO mice (Fig. 5A to C). In proportion, the decrease was more pronounced for the CD8$\alpha^+$ cDC1 subset, but the CD172a$^+$ cDC2 subset was also affected (Fig. 5D to F). In addition, we analyzed the capacity of the remaining cDC to make the pro-Th1 cytokine IL-12p70, which is composed of the
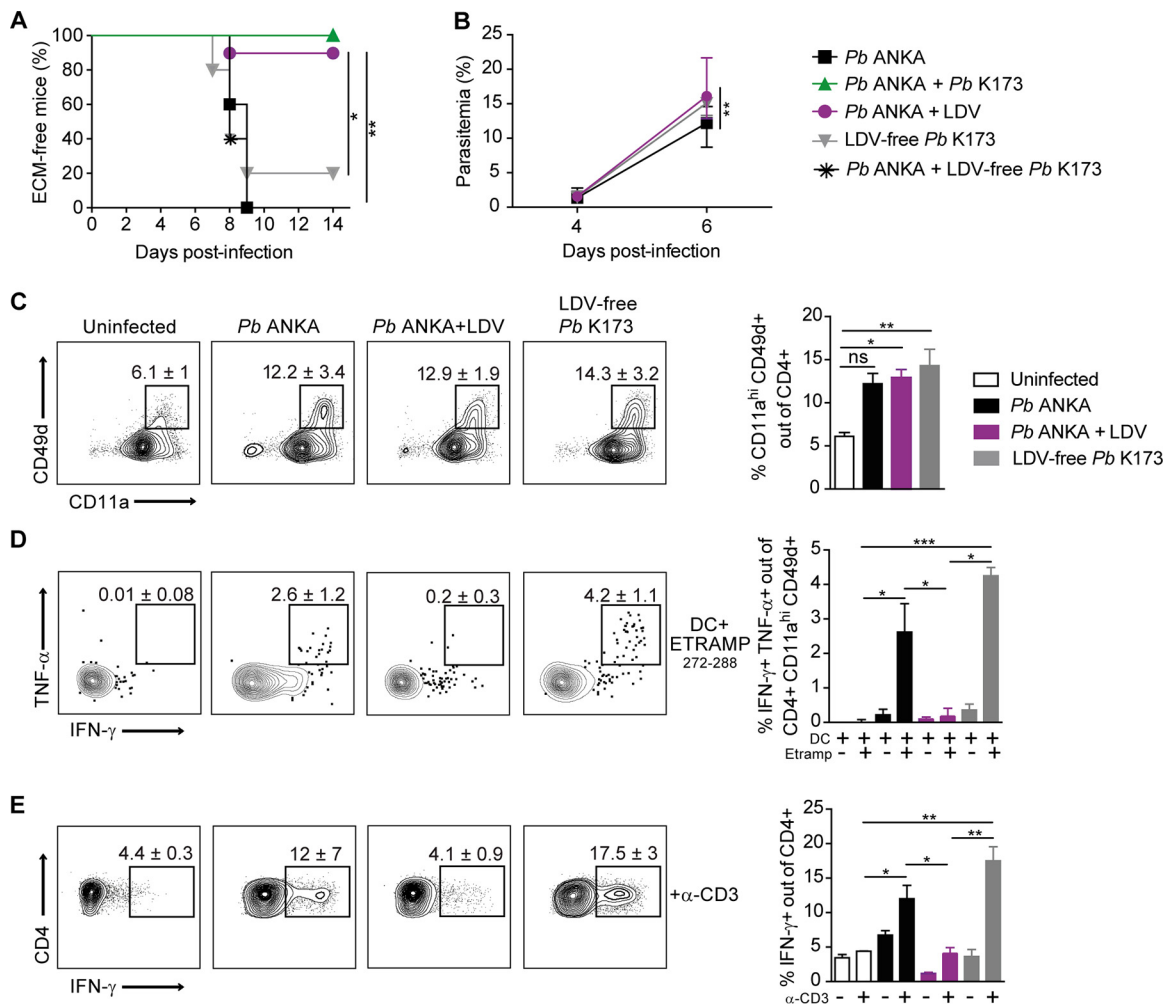
**FIG 4** LDV alone, but not LDV-free *P. berghei* K173, impedes Th1 responses and prevents ECM development. ECM development (A) and blood circulating parasitemia (B) after infection or coinfection of C57BL/6 mice with the indicated inocula: *P. berghei* (*Pb*) ANKA pRBC, *P. berghei* ANKA plus *P. berghei* K173 pRBC, *P. berghei* ANKA pRBC plus LDV, LDV-free *P. berghei* K173 pRBC, *P. berghei* ANKA pRBC plus LDV-free *P. berghei* K173 pRBC. (C) Proportions of activated CD11a$^+$ CD49d$^+$ of spleen CD4$^+$ T cells collected 6 days after infection. Cytokine production by spleen CD4$^+$ T cells restimulated *in vitro* with MutuDC loaded with ETRAMP$_{272-288}$ peptide (D) or with anti-CD3 (E). (D) Proportions of double IFN-$\gamma^+$ TNF-$\alpha^+$ cells of activated CD4$^+$ T cells upon ETRAMP$_{272-288}$ restimulation. (E) Proportions of IFN-$\gamma^+$ cells out of activated CD4$^+$ T cells upon anti-CD3 restimulation. Numbers on the dot plots and bar graphs show median percentages $\pm$ IQRs. Data are representative of 2 independent experiments with $N = 5$ mice/group.

IL-12p35 and IL-12p40 subunits. Upon day 2 of infection, *P. berghei* ANKA induced the upregulation of the IL-12p35 and IL-12p40 genes, which was blocked by LDV coinfection but rescued in *Ifnar1* KO mice (Fig. 5G and H). Logically, this resulted in the lack of bioactive IL-12p70 in the serum at day 3 postinfection, an effect that was reversed in *Ifnar1* KO mice (Fig. 5I). Taken together, our data establish that IFN-I signaling induced by LDV infection causes a quantitative reduction in cDC and a functional impairment in their ability to polarize Th1 CD4$^+$ T cell responses.

Type I IFNs can affect multiple cell types, including CD4$^+$ T cells. Therefore, to determine to which extent the dysfunctional cDC compartment was responsible for LDV-mediated ECM protection, we tested if the transfer of functional cDC could restore disease in *P. berghei* ANKA/LDV coinfected mice. cDC were isolated from *P. berghei* ANKA-infected mice and transferred into LDV/*P. berghei* ANKA coinfected mice on days 3, 4, and 5 (Fig. 6A). This procedure restored ECM in only 3 of 10 transferred mice. We reasoned that even if fully functional at the time of injection, the transferred cDC may immediately be negatively conditioned by IFN-I in the LDV-infected environment. To overcome this effect, we isolated "IFN-I-insensitive" cDC from *Ifnar1* KO *P. berghei*
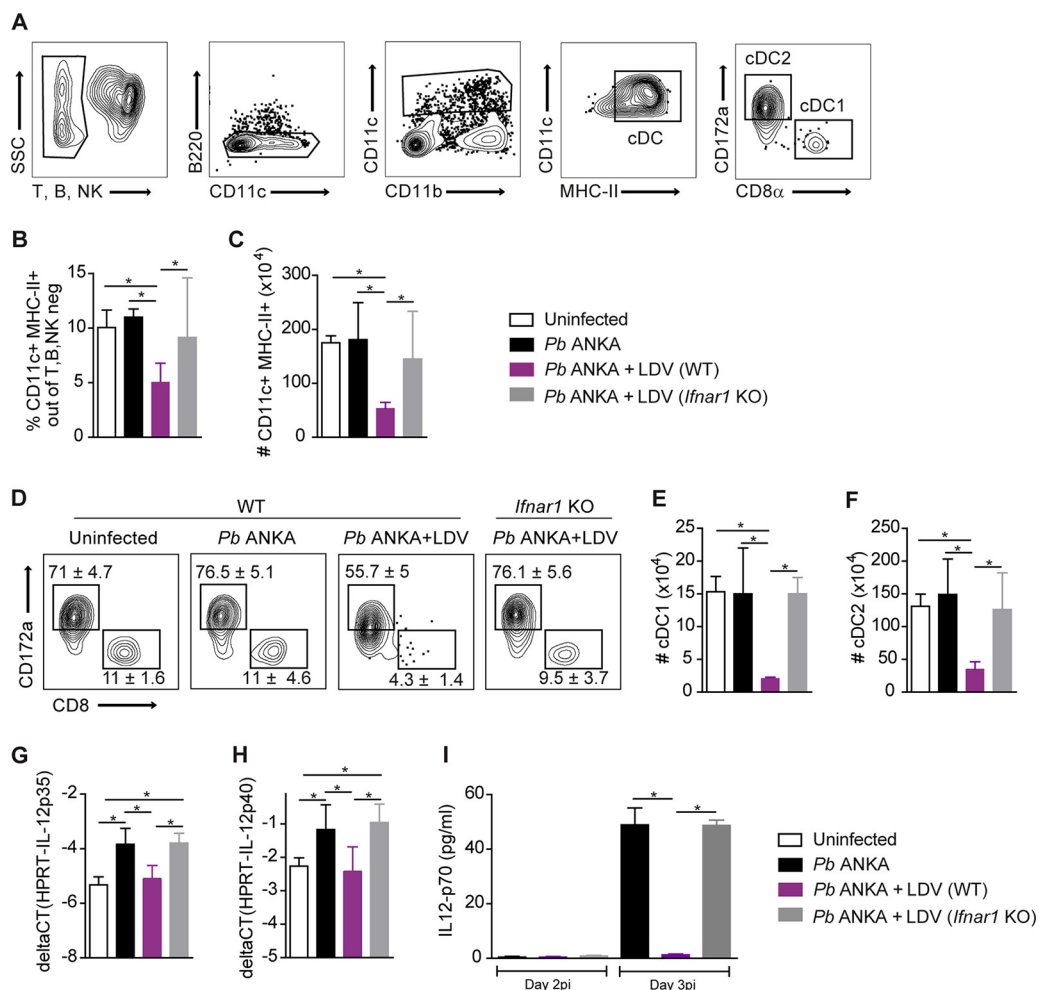
**FIG 5** LDV causes an IFNAR-dependent decrease in number and IL-12p70 production of splenic conventional DC. C57BL/6 WT and *Ifnar1* KO mice were infected i.v. with 10⁶ *P. berghei* (*Pb*) ANKA pRBC or 10⁶ *P. berghei* ANKA pRBC plus LDV. (A) Gating strategy used for cDC analysis 2 days after infection. Proportions of CD11c⁺ MHC II⁺ cDC of non-T, non-B, non-NK cells (B) and absolute numbers of cDC (C). (D) Representative dot plots showing the median percentages ± IQRs of cDC1 and cDC2 subsets. Absolute numbers of splenic cDC1 (E) and cDC2 (F). Real-time qPCR analysis of IL-12p35 (G) and IL-12p40 (H) gene expression by sorted CD11c⁺ cDC at day 2 after infection. (I) Plasmatic level of IL-12p70 measured by ELISA at day 2 and 3 after infection. (A to H) Data representative of 4 independent experiments with $N = 5$ mice/group. (I) Data representative of 2 independent experiments with $N = 5$ mice/group.

ANKA-infected mice and transferred them into LDV/*P. berghei* ANKA coinfected wild-type (WT) mice. This procedure restored ECM in 8 of 10 mice (Fig. 6B), indicating that crippling of cDC by IFN-I signaling is the predominant mechanism that underlies the LDV-mediated protection against ECM.

**LDV prevents EAE by disrupting the polarization of pathogenic autoreactive CD4⁺ T cell responses.** Finally, we investigated to which extent the protective mechanisms of *P. berghei* K173 extracts against EAE were analogous to those described in ECM. We first confirmed that LDV infection alone was protective against EAE. Mice injected with phosphate-buffered saline (PBS) or with sonicated extracts of LDV-free *P. berghei* K173 prior to MOG₃₅₋₅₅/CFA immunization developed EAE, while mice that received LDV-containing plasma were fully protected (Fig. 7A and B). Upon day 2 of MOG₃₅₋₅₅/CFA immunization, the IL-12p40, p35, and p19 subunit genes were induced in cDC isolated from draining lymph nodes (dLN), but this upregulation was blocked by LDV infection in an interferon α/β receptor (IFNAR)-dependent manner (Fig. 7C to E). These findings show that IFN-I signaling blocks the production by cDC of IL-23 (composed of IL-12p40 and p19 subunits), a key cytokine in the polarization of
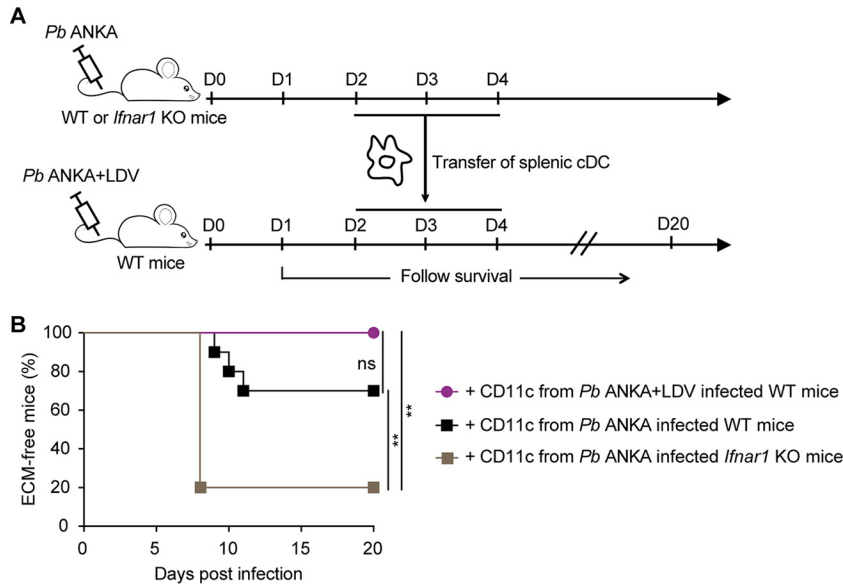
**FIG 6** cDC from *P. berghei* ANKA-infected *Ifnar1* KO mice restore ECM upon transfer into *P. berghei* ANKA/LDV-coinfected mice. (A) Experimental protocol. WT or *Ifnar1* KO donor mice were i.v. infected with 10⁶ *P. berghei* (*Pb*) ANKA pRBC, with or without LDV. At days 2, 3, and 4 of infection, CD11c⁺ DC were magnetically sorted from spleen and transferred into *P. berghei* ANKA plus LDV coinfected WT mice. (B) ECM development. Data pooled from 2 independent experiments with N = 10 mice receiving WT DC and N = 5 mice receiving *Ifnar1* KO DC.

encephalitogenic CD4⁺ T cells (53). Accordingly, while the proportions of antigen-experienced (CD44⁺) CD4⁺ T cells were similar in the spleen and dLN 15 days after MOG₃₅₋₅₅/CFA immunization (see Fig. S3A and C), the proportion of autoreactive CD44⁺ CD4⁺ T cells producing IFN-γ, IL-17, and GM-CSF in response to MOG₃₅₋₅₅
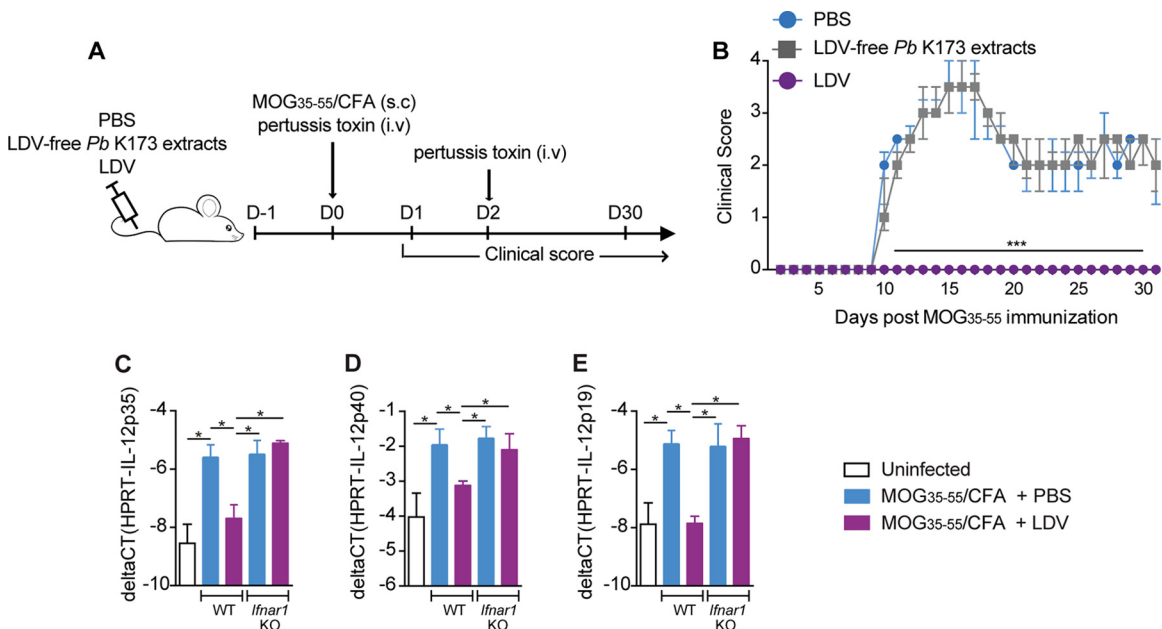


**FIG 7** LDV alone, but not LDV-free *P. berghei* K173, prevents EAE development. (A) Experimental protocol. C57BL/6 mice were injected with PBS, LDV-free *P. berghei* (*Pb*) K173 pRBC sonicated extracts or LDV-containing plasma, and immunized with MOG₃₅₋₅₅/CFA to induce EAE. (B) Clinical scores up to day 30 postimmunization. Dots show the medians ± IQRs. Real-time qPCR analysis of IL-12p35 (C), IL-12p40 (D), and IL-12p19 (E) gene expression on CD11c⁺ DC magnetically sorted from dLN of C57BL/6 WT or *Ifnar1* KO mice, injected with PBS or infected with LDV 1 day prior to MOG₃₅₋₅₅/CFA immunization. Analysis at day 1 postimmunization. (B) Data representative of 3 independent experiments with N = 5 mice/group. (C to E) Data representative of 2 independent experiments with N = 5 mice/group.
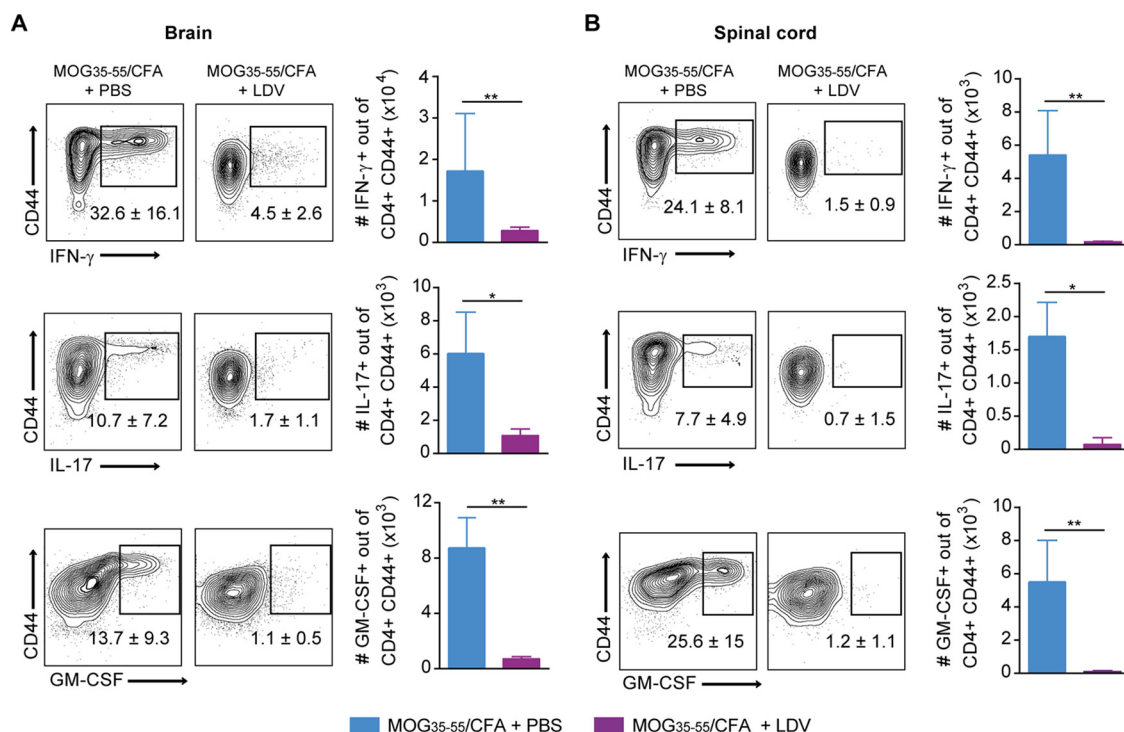
**FIG 8** LDV alone protects against EAE by blocking the encephalitogenic CD4+ T cell response. C57BL/6 mice were injected with PBS or LDV 1 day before MOG$_{35-55}$/CFA immunization. Mononuclear cells from CNS and spinal cord harvested 15 days after MOG$_{35-55}$/CFA immunization were restimulated with 100 $\mu$g MOG peptide for 24 h. Production of IFN-$\gamma$, IL-17, and GM-CSF visualized by intracellular staining in CD4+ T cells from brain (A) and spinal cord (B). Numbers on the representative dot plots show the median percentages of cytokine-positive cells of CD44+ CD4+ T cells ± IQRs. Histograms show the absolute numbers. Data representative of 2 independent experiments with $N = 7$ mice/group.

stimulation was lower in LDV-infected mice (Fig. S3B and D). This decrease was confirmed by dosing the 3 cytokines in the supernatants of MOG$_{35-55}$-stimulated spleen cells (Fig. S3E). In parallel to the impaired CD4+ T cell functional polarization in the spleen and dLN, we observed a major drop in the percentages and numbers of CD4+ T cells producing IFN-$\gamma$, IL-17, and GM-CSF in the brain (Fig. 8A) and spinal cord (Fig. 8B), in line with the absence of disease. In conclusion, our data show that LDV-mediated protection against EAE is mediated by an IFN-I signaling-dependent blockade of IL-23-dependent polarization of autoreactive GM-CSF-producing encephalitogenic CD4+ T cells.

## DISCUSSION

The host immune status is shaped by continuous exposure to commensal as well as potentially pathogenic microorganisms. Depending on whether they exacerbate or alleviate immunopathology, the surrounding microbes may be harmful or beneficial. In this study, we initially sought to address why malaria infection influences the clinical outcome of a concurrent *Plasmodium* infection and autoimmune reactions. To investigate these mechanisms, we took advantage of rodent *Plasmodium* lines that prevent the development of ECM and EAE, two inflammatory diseases mediated by T cells. Unexpectedly, we found that the beneficial effects of the protective *Plasmodium berghei* K173 strain are entirely due to LDV, a nonpathogenic mouse virus, and that this virus is cohosted in stabilates of other parasite strains (e.g. *P. yoelii* 17X YM) commonly used by the community. We demonstrated that protection against ECM results from an IFN-I signaling-dependent depletion and functional impairment of splenic DC, which become incapable of producing IL-12p70 and polarizing pathogenic Th1 CD4+ T cell responses. Analogous to this mechanism, we found that the protection against EAE is linked to an IFN-I-dependent reduction in the production of encephalitogenic IL-23 and GM-CSF cytokines.

Based on the facts that (i) in our experiments, CD8$^+$ T cell accumulation in the CNS was reduced (see Fig. 1C and E) despite a stronger induction of parasite-specific CD8$^+$ T cell responses in the spleen (see Fig. 1G) and (ii) Th1 CD4$^+$ T cells are known to enhance CD8$^+$ T cell sequestration in the brain through the induction of CXCL9/10 (33), we hypothesize that the defective Th1 responses underlie ECM protection. However, because IL-12p70 may also heavily influence CD8$^+$ T cells, we do not rule out the possibility that dysfunctional cDC also directly cripple the pathogenic CD8 T cell response.

This work reiterates the importance of screening products that are injected into animal models for the presence of unsuspected contaminants. LDV was initially discovered as a companion virus of transplantable tumor cell lines more than half a century ago (54). Our data establish that the virus is carried over in commonly used rodent *Plasmodium* stocks. Although LDV should not be an issue for *Plasmodium* strains that are regularly passaged through mosquitoes, the results of some studies on innate immune sensing and/or IFN-I responses performed with blood-passaged *Plasmodium* strains may need to be reinterpreted if these strains are LDV positive. So far, the timing of the contamination of certain *Plasmodium* stocks by LDV and the causes remain unclear. As LDV can be, though very poorly, transmitted to cage mates (46, 55), one may speculate that at times when the confinement of cages in animal facilities was less stringent, some *Plasmodium*-infected mice may have been exposed to LDV-contaminated bedding. Another possibility is that at some point, mice used to expand *Plasmodium* stocks have been injected with a biological product containing LDV. LDV is indeed a known contaminant of mouse-passaged ascitic fluids (56). In any case, we are confident that the contamination of strains used in this study occurred prior to their manipulation in our facility for various reasons: (i) for *P. yoelii* 17X YM, quantitative PCR (qPCR) detection of LDV was performed immediately on one-half of the obtained cryovials, before any mouse passage, (ii) all cages were kept isolated from each other on ventilated racks, (iii) for stock preparation, mice infected with different parasite strains were never mixed in the same cage, (iv) our "clean" *Plasmodium* stocks have remained LDV free in the course of this study, (v) the data on *P. berghei* K173 are fully consistent with earlier observations of ECM inhibition by this strain (35).

LDV is a mouse-specific virus that causes limited pathology but persists lifelong with detectable circulating viremia (46). Only one study from 1978 examined the outcome of LDV/malaria coinfection. It was observed that LDV exacerbates *P. yoelii* virulence by increasing parasitemia, leaving open the possibility that the phenotype of the lethal and nonlethal *P. yoelii* 17X substrains may be in part determined by the presence of LDV (57). Why, in this case, LDV aggravated the pathology remains unclear, but one could speculate that with malaria strains that do not cause cerebral malaria, the suppression of Th1 responses is deleterious for parasite control. Concerning autoimmunity, the beneficial role of LDV has been reported in various contexts such as lupus (58), diabetes (59), and EAE (47). In lupus, LDV-mediated protection was correlated with a decrease in IFN-$\gamma$ production in the serum (60), but no mechanism was provided to explain the suppression of diabetes and EAE.

Beyond the importance of our findings for the community working on malaria models, our work has fundamental relevance to understand how viruses shape inflammatory diseases. We indeed reveal some of the molecular bases that underlie the protective effects of LDV in two settings involving T cell-mediated immunopathology. In the case of ECM, protection results from the functional paralysis and the strong depletion of splenic cDC, both of which are caused by IFN-I signaling. It was previously shown that LDV induces a rapid systemic IFN-I production by pDC in a TLR7-dependent manner (61). IFN-I signaling has been reported to play a dual role on the IL-12 pathway. On the one hand, IFN-I drives DC activation and maturation (62) and production of IL-12 (63), which may augment Th1 CD4 responses. On the other hand, IFN-I can selectively suppress IL-12p70 (64). It is likely that IFN-I dosage determines the outcome of IFN-I signaling on Th1 responses (65). While low levels of IFN-I signaling positively regulate Th1 responses, higher doses of IFN-I, as observed here upon massive viral replication in

the first 2 days, rather impede the development of Th1. In addition to the functional alterations of cDC, we reported a major drop in the number of splenic cDC that is dependent on IFN-I signaling. It will be interesting to analyze whether the reduction in cDC is due to apoptosis induced by BH3-only proteins, such as the proapoptotic factor Bim (66).

In the case of EAE, LDV infection prior to MOG/CFA immunization blunted the production of IL-12 and IL-23 by cDC in the dLN, resulting in the absence of IFN-$\gamma$, IL-17, and GM-CSF production by MOG-specific CD4$^+$ T cells, both in the periphery and in the CNS. It has now become clear that IL-12p35 (67), IFN-$\gamma$, and IL-17 (68) are dispensable for the development of MOG-induced EAE, while the key pathogenic cytokines are GM-CSF produced by CD4$^+$ Th cells and IL-23, which may be produced by multiple sources (68, 69). Also, in contrast to ECM pathology in which cDC are required for the priming and polarization of Th1 CD4$^+$ T cells (50–52), cDC are not necessary for the priming of encephalitogenic CD4$^+$ Th cells in MOG protein-induced EAE (70), and mice lacking cDC even display an aggravated disease (71). Based on this, we propose that LDV-mediated protection against EAE is due to the global effect of IFN-I signaling, which inhibits IL-23 production by several different cell types of the dLN, including cDC, and that this prevents the differentiation of GM-CSF-secreting CD4$^+$ T cells. In human DC, IFN-$\beta$ has been shown to inhibit IL-23p19 production by DC, possibly through a STAT3-dependent induction of the negative regulator SOCS3 (72). The exact transcriptional and/or epigenetic reprogramming that cripples the IL-12 and IL-23 synthesis pathways in response to IFN-I remains to be fully unraveled in this context.

Type I IFNs are powerful signals that can modify the functionality of many cell types, including CD4$^+$ T cells, which play a major role in the two diseases. For example, in EAE, IFN-I signaling in T cells can directly impair Th17 differentiation (73). During blood-stage malaria, CD4$^+$ T cell-intrinsic IFN-I signaling induces T-bet and Blimp-1 expression, thereby promoting IL-10$^+$ Tr1 responses in mice (74) as well as in humans (75). Therefore, it was possible that CD4$^+$ T cells impacted by IFN-I may contribute to protection. Because ECM pathology in LDV-infected animals could be restored by transferring activated "IFN-I-insensitive" (*Ifnar1* KO) DC from *P. berghei* ANKA-infected mice, we infer that the role, if any, of non-DC in the protection phenotype should be modest.

There have been other examples of viruses modifying the clinical course of a parasitic disease. In experimental malaria, coinfection with the chikungunya arbovirus also prevented ECM but through a distinct mechanism involving the IFN-$\gamma$-mediated retention of CXCR3-expressing pathogenic CD8$^+$ T cells in the spleen (76). Another striking example is the discovery of a double-stranded RNA virus in *Leishmania* parasites (LRV), which is responsible for the exacerbation of the severity of mucocutaneous leishmaniasis via a TLR3/IFN-I-dependent proinflammatory response (77, 78). A notable difference is that LRV is an endogenous viral element of the parasite itself, while LDV is an exogenous mouse-specific virus that is cohosted in *Plasmodium*-parasitized blood. Nevertheless, it was observed that the aggravation and metastasis of *Leishmania* lesions were not exclusively associated with LRV and that they could also be enhanced by coinfection with exogenous viruses (79). Interestingly, a new RNA virus called Matryoshka RNA virus 1 (MaRNAV-1) was recently identified in *P. vivax*-infected blood samples (80). Like LRV, which infects the *Leishmania* parasite, MaRNAV-1 is thought to be a virus of the *Plasmodium* parasite. Future investigations should reveal whether this virus modulates the pathogenicity of *P. vivax*, as *P. vivax* malaria is associated with lower severity than *P. falciparum* malaria (81) and was suggested to be protective in cases of coinfection (32).

In conclusion, our data emphasize the notion that viral infections, even with viruses that are seemingly innocuous, can have dramatic consequences on a concurrent infectious or autoimmune disease. LDV is a mouse-specific virus, but the IFN-I-mediated modulatory mechanisms highlighted here may be fully relevant in humans. As constant exposure to an immense variety of commensal and noncommensal microbes profoundly shapes our health, it appears essential to actively and systematically charac-

terize environmental microbial agents. From a fundamental standpoint, one could foresee the discovery of new languages in the host-pathogen cross talk. From a medical perspective, this research could suggest novel therapeutic immune modulatory molecules.

## MATERIALS AND METHODS

**Animals.** Animal care and use protocols were carried out under the control of the National Veterinary Services and in accordance with the European regulations (EEC Council Directive, 2010/63/EU, September 2010). Protocols inducing pain were approved under code APAFIS 4318-2016022913333485 v4, by the local ethical committee for animal experimentation (Comité d'Ethique sur l'Experimentation Animale, CEEA number [no.] 122 of US006/CREFRE) affiliated with the Comité National de Réflexion Ethique sur l'Expérimentation Animale. C57BL/6J (B6) mice were purchased from Envigo or Janvier (France) with similar results. *Ifnar1* KO mice with at least 5 backcrosses on the C57BL/6 background were a gift from D. Hudrisier and E. Meunier (IPBS Toulouse). All mice were housed and bred under specific-pathogen-free conditions at the Centre Régional d'Exploration Fonctionnelle et de Ressources Expérimentales (CREFRE-Inserm UMS006). Mice used in experiments were male mice aged 8 to 10 weeks. The number of mice and experimental replicates are indicated in the respective figure legends.

**Parasites and experimental infections.** *Plasmodium berghei* ANKA and *Plasmodium berghei* Kyberg 173 parasites were gifts from S. Picot (University of Lyon, France) and I. Landau (National Museum of Natural History, Paris, France), respectively. The identity of these parasites was confirmed by whole-genome sequencing. *Plasmodium yoelii* subs *p. yoelii*, strain YM (*P. yoelii* 17X YM, MRA-755) was obtained through BEI Resources, NIAID, NIH, in 2017, contributed by David Walliker. All *Plasmodium* strains were propagated in C57BL6/J mice. To prepare pRBC stocks, mice were bled with heparin, and the proportion of pRBC was evaluated by blood smear. The pRBC concentration was adjusted to $10^7$ pRBC/ml in Alsever's solution with 10% glycerin. One-milliliter aliquots were stored at $-80°C$. All infections were conducted by intravenous inoculation of $10^6$ pRBC. For Evans blue staining, mice were injected i.v. with a solution of 1% Evans blue dissolved in 0.9% NaCl, and brain coloration was examined 1 h after dissection. To prepare the sonicated blood extracts, total blood was sonicated using an ultrasonic liquid processor (Branson, Sonifier) under the following parameters: 40 mA, pulse 2, 1 min. Blood was sonicated twice with a 1-min rest. For UV treatment, plasma was exposed for 40 min.

**Parasitemia quantification.** Parasitemia was measured by blood smear or flow cytometry with similar results. For flow cytometry, 3 $\mu$l of tail blood was collected using an EDTA-coated Microvette (CB 300 K2E; Sarstedt) and diluted in 500 ml PBS. The diluted blood was labeled with antibodies directed against Ter119 (fluorescein isothiocyanate [FITC], TER-119, 1/30; Miltenyi Biotec), CD71 (phycoerythrin [PE], C2, 1/300; BD Pharmingen), and CD41–PE–Cy7 (MWReg30, 1/100; BioLegend), fixed, and permeabilized with 4% paraformaldehyde (PFA) with 0.6% saponin for 10 min, followed by 4',6-diamidino-2-phenylindole (DAPI) staining.

**LDV detection and dosage and decontamination of *P. berghei* K173 blood.** For the initial detection of LDV, *P. berghei* ANKA and *P. berghei* K173 stabilates were subjected to a mouse/rat comprehensive clear panel for PCR infectious agent testing (PRIA; Charles River). Further detection and quantitation of LDV particles were conducted with a simple PCR LDV test (Charles River) on mouse plasma.

To free *P. berghei* K173 blood from the virus, C57BL6/J mice were injected with $10^6$ *P. berghei* K173 pRBC; 10 days after injection, intracardiac blood was collected with heparin and washed with PBS. RBC and platelets were stained with anti-Ter119 (allophycocyanin [APC], REA847, 1/50; Miltenyi Biotec) and CD41 (PE, MWReg30, 1/300; BD Biosciences), and then RBC were separated from platelets by magnetic sorting using anti-PE microbeads (Miltenyi Biotec). The Ter119$^+$ CD41$^-$ fraction was stained again with the same anti-Ter119 and CD41 antibodies, and RBC were FACS sorted with an Aria cell sorter (BD Biosciences). New B6 mice were injected with $10^6$ sorted pRBC. Five days later, a new stock of pRBC was prepared as indicated above. Confirmation of the absence of LDV was performed by PCR (Charles River).

**Induction of EAE and clinical score.** The MOG$_{35-55}$ (MEVGWYRSPFSRVVHLYRNGK) peptide was purchased from Polypeptide Laboratories (San Diego, CA, USA). Mice were immunized subcutaneously with 50 $\mu$g of MOG$_{35-55}$ peptide emulsified in complete Freund's adjuvant (CFA; BD Difco, Franklin Lakes, NJ, USA) containing 500 $\mu$g of killed *Mycobacterium tuberculosis* (strain H37a; Difco). Mice were injected intravenously with 200 ng of pertussis toxin (List Biological Laboratories, Campbell, CA, USA) at days 0 and 2 postimmunization. Clinical scores were recorded daily by an experimenter blinded to the experimental groups. Scores were assigned as follows: 0, no sign of disease; 1, loss of tone in the tail; 2, hind limb paresis; 3, hind limb paralysis; 4, tetraplegia; 5, moribund.

**Real-time quantitative PCR for gene expression.** Total mRNA was extracted from tissue and cells by standard TRIzol-chloroform precipitation. For reverse transcription, the iScript cDNA synthesis kit (Bio-Rad) was used. Quantitative PCR (qPCR) reactions were prepared with LightCycler 480 DNA SYBR green master reaction mix (Roche Diagnostics). Primers were used at 0.2 $\mu$M, and sequences are available upon request. qPCRs were run in duplicates on a LightCycler 480 System (Roche diagnostics), and cDNA abundance was normalized to the reference gene *Hprt*. Results are expressed as the threshold cycle difference ($\Delta C_T$) (*Hprt* minus gene of interest).

**Isolation of spleen, lymph node, and CNS leukocytes.** Mice were anesthetized with ketamine-xylazine and perfused intracardially with cold PBS. Spleens, lymph nodes, spinal cord, and brains were collected in complete RPMI (Gibco) supplemented with 10% (vol/vol) fetal calf serum (FCS; Gibco). Splenocytes were mashed through a 100-$\mu$m cell strainer (Falcon). Lymph node cells were mashed in a Potter and filtered through a 100-$\mu$m cell strainer (Falcon). Brain and spinal cord were collected

separately, homogenized, and digested with collagenase D (2.5 mg/ml; Roche), DNase I (100 $\mu$g/ml; Sigma-Aldrich), and N$\alpha$-p-tosyl-L-lysine chloromethyl ketone (TLCK) (1 $\mu$g/ml, Roche) for 30 min at 37°C. Cells were then washed, suspended in 37% Percoll, and layered on 70% Percoll. After a 20-min centrifugation at 800 $\times$ g, the mononuclear cells were collected from the interface. In both cases, erythrocytes were lysed using ACK buffer (100 $\mu$M EDTA, 160 mM NH$_4$Cl, and 10 mM NaHCO$_3$).

**Ex vivo T cell restimulation.** In the ECM model, $10^6$ splenocytes and $3 \times 10^5$ brain leukocytes were incubated for 5 h at 37°C in the presence of brefeldin A (BfA) with MutuDC, a C57BL/6-derived DC line obtained from H. Acha-Orbea (82). MutuDC were loaded overnight with pRBC (ratio, 1 MutuDC to 10 P. berghei ANKA or P. berghei K173 pRBC) or just incubated with anti-CD3 or 5 $\mu$M of ETRAMP$_{272-288}$ peptide (52) during the in vitro restimulation. Cells were stained with CD4 (APC-Cy7, GK1.5, 1/300; BD Pharmingen), CD8$\alpha$ (BV421, 53-6.7, 1/300; BD Biosciences), CD11a (PE, 2D7, 1/400; BD Biosciences), and CD49d (BV786, R1-2, 1/300; BD Biosciences). Intracellular IFN-$\gamma$ (APC, XMG1.2, 1/200; eBioscience) and TNF-$\alpha$ (Alexa Fluor 700, MP6-XT22, 1/300; BD Pharmingen) were detected with an intracellular fixation and permeabilization buffer Set (eBioscience) according to the manufacturer's instructions.

In the EAE model, $6 \times 10^6$ cells from spleen, $3 \times 10^6$ from dLN, $3 \times 10^5$ from brain, and $3 \times 10^5$ from spinal cord were incubated for 24 h at 37°C with MutuDC (ratio, 1 MutuDC to 10 cells) and 100 $\mu$g of MOG$_{35-55}$. BfA was added for the last 5 h. Cells were stained with CD4 (BV510, RM4-5, 1/200; BD Horizon) and CD44 (BV605, IM7, 1/300; BD Horizon). Intracellular IFN-$\gamma$ (AF700, XMG1.2, 1/300; BD Pharmingen), IL-17 (AF700, TC11-18H10, 1/200; BD Pharmingen), and GM-CSF (PE, MP1-22E9, 1/300; BioLegend) were detected with the same kit as described above.

**Flow cytometry analysis of dendritic cells.** Spleens and dLN were treated with collagenase D (1 mg/ml; Roche) and DNase I (100 $\mu$g/ml; Sigma-Aldrich), minced into small pieces, and incubated at 37°C for 30 to 45 min. Cell preparations were filtered using 100-$\mu$m cell strainers. For DC identification, cells were stained for CD3 (PE, 145-2C11, 1/300; BD Biosciences), CD19 (PE, 1D3, 1/300; BD Biosciences), and NK1.1 (PE, PK136, 1/300; BD Biosciences) to exclude T, B, and NK cells. A mix containing CD11c (PE-Cy7, N418, 1/400; BioLegend), B220 (BV510, RA3-6B2, 1/900; BD Horizon), CD11b (PE-CF594, M1/70, 1/3,000; BD Horizon), MHC II (AF700, M5/114.15.2, 1/400; BD Pharmingen), CD8$\alpha$ (FITC, 53-6.7, 1/200; BD Biosciences), and CD172a (APC-Cy7, P84, 1/200; BioLegend) was used to characterize the different DC subsets. In some experiments, an anti-XCR1 antibody (PE, REA707, 1/200; Miltenyi Biotec) was used to confirm the identity of cDC1. All flow cytometry samples were run on a Fortessa (BD Biosciences) and analyzed using FlowJo software.

**Sorting and transfer of splenic dendritic cells.** Spleens were injected with 1 ml of PBS supplemented with Liberase TL (0.125 mg/ml; Roche) and DNase I (100 $\mu$g/ml; Sigma-Aldrich), incubated for 30 min at 37°C, and mashed through a 100-$\mu$m cell strainer. RBC were lysed and cells washed in magnetically activated cell sorting (MACS) buffer. For DC sorting, cells were incubated 10 min with CD11c microbeads (Miltenyi Biotec), and CD11c$^+$ cells were magnetically sorted according to the manufacturer's instructions. Postsorting flow cytometry analysis showed that less than 0.5% of transferred CD11c$^+$ cells were CD8$^+$ CD3$^+$ T cells. After sorting, $10^6$ CD11c$^+$ cells in 100 $\mu$l PBS were transferred to recipient mice by i.v. injection.

**Measurements of soluble cytokines.** Enzyme-linked immunosorbent assay (ELISA) was used to measure cytokines in plasma (50 $\mu$l) and culture supernatants (72 h after restimulation). For IL-12p70, the ELISA MAX Deluxe Set (BioLegend) was used according to the manufacturer's instructions. For the other cytokines, 96-well plates (Nunc Maxi Sorp; BioLegend) were coated for 2 h at 37°C with anti-IFN-$\gamma$ (AN18), anti-IL-17 (TC11-18H10), or anti-GM-CSF (MP1-22E9) antibodies. Culture supernatants or standards (Peprotech) were incubated for 2 h at 37°C. The plates were then incubated for 2 h with a secondary biotinylated antibody, anti-IFN-$\gamma$ (XMG1.2), anti-IL-17 (TC11-8H4), and anti-GM-CSF (MP1-31G6), followed by a 20-min incubation with streptavidin-phosphatase alkaline (Sigma-Aldrich) at 37°C. Plates were visualized by using phosphatase alkaline substrate (Sigma-Aldrich), and absorbance was measured at 450 nm to 540 nm. Luminex analyses were performed by Genotoul Anexplo platform using the kits EP0X10 and EPX020 (eBioscience), according to the manufacturer's instructions.

**Statistical analyses.** Statistical evaluation of differences between the experimental groups was done by using two-way analysis of variance followed by a Bonferroni's posttest for clinical monitoring and parasitemia, Kaplan-Meier test for monitoring of ECM-free mice, Mann-Whitney U test for group versus group comparison, and Kruskal-Wallis test for multiple-group comparisons. All tests were performed with GraphPad Prism 5.00 (GraphPad Software Inc., San Diego, CA, USA). All data are presented as medians $\pm$ interquartile ranges (IQRs).

## SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

**FIG S1**, PDF file, 0.3 MB.

**FIG S2**, PDF file, 0.3 MB.

**FIG S3**, PDF file, 0.2 MB.

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