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Inflammatory Flt3L is essential to mobilize dendritic cells and for T cell responses during *Plasmodium* infection

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Summary

Innate sensing mechanisms trigger a variety of humoral and cellular events that are essential to adaptive immune responses. Here we describe an innate sensing pathway triggered by *Plasmodium* infection that regulates dendritic cell (DC) homeostasis and adaptive immunity via Flt3L release. *Plasmodium*-induced Flt3L release requires toll-like receptor activation and type I interferon production. We find that type I interferon supports the up-regulation of xanthine

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dehydrogenase, which metabolizes the xanthine accumulating in infected erythrocytes to uric acid. Uric acid crystals trigger mast cells to release soluble Flt3L from a pre-synthesized membrane-associated precursor. During infection Flt3L preferentially stimulates expansion of the CD8 α ⁺/CD103⁺ DC subset or its BDCA3⁺ human DC equivalent and has a significant impact on the magnitude of T cell activation, mostly in the CD8⁺ compartment. Our findings highlight a new mechanism that regulates DC homeostasis and T cell responses to infection.

Introduction

The Flt3 receptor tyrosine kinase and its ligand Flt3L, regulate DC development in the steady state^{1–6}. Flt3 is first expressed in multipotent haematopoietic progenitors (CD150⁺Flt3⁺) that develop into lymphoid and myeloid cells^{7, 8}, but expression is lost by all lineages except DCs during development.

In the steady state, DC development proceeds from precursors that give rise to monocytes and DCs (MDP)^{4, 9}, to common DC progenitors that lack monocyte potential (CDP)^{5, 6, 10, 11}, and give rise to circulating pre-DCs^{11–13}. All of these cell types are dependent on Flt3L in the steady state. During inflammation, pattern recognition receptors, such as Toll-Like Receptors, RIG-like helicases and the inflammasome, mediate pathogen sensing and induce the maturation of differentiated DCs, a process controlling the initiation of immune responses. However, little is known about the mechanisms that regulate the development of DCs in response to infection and microbial innate sensing.

In this report we examine how the DC pathway is regulated during acute malaria infection. We find that *Plasmodium* infection is associated with increased serum levels of Flt3L and increased DC production in both mice and humans. Moreover, DC expansion in response to *P. chabaudi* infection requires Flt3L, which has a predominant effect on the CD8 α ⁺ DC subset in mice and BDCA3⁺ DC subset in humans. We identify mast cells (MCs) as an important source of the Flt3L, and show that they produce it in response to uric acid accumulation. This response is facilitated by TLR9-dependent type I IFN induction of Xanthine dehydrogenase, which supports hyper-uricemia during *Plasmodium* infection. Ultimately, the DCs induced by inflammatory Flt3L help activate T cell responses to the parasites.

Results

Systemic Flt3L increase and DC expansion during *P. chabaudi* infection

To examine DC regulation during *Plasmodium* infection, we infected C57BL/6 mice with blood stage *P. chabaudi* and measured Flt3L^{1–3}. We found that soluble Flt3L was increased from 300 to 1000 pg.ml^{−1} in serum as early as d2 and that it remained elevated up to six days after infection (Fig.1a). Consistent with previous reports^{14, 15}, we found that spleen DCs (DEC205⁺CD8 α ⁺ and 33D1⁺CD8 α [−]) undergo transient contraction and then expansion (Fig.1b, c, d, Supplementary Fig.1a, b). To determine if DC expansion during *P. chabaudi* infection was dependent on Flt3L and its receptor Flt3, we infected wild type or *Flt3*^{−/−}¹⁶ or *Flt3L*^{−/−} mice and quantified spleen DCs (Fig.1c, d). As previously noted, both subsets of spleen DCs were reduced in naive *Flt3*^{−/−} and *Flt3L*^{−/−} mice¹. In addition, expanded DC populations, especially CD8 α ⁺ DCs, remained *Flt3*- and *Flt3L*-dependent (Fig. 1c, d).

To determine which aspect of DC development is dependent on Flt3 during *P. chabaudi* infection, we analyzed DC progenitors in the BM. Haematopoietic stem cell (HSC)-containing fraction (Lin[−] CD117(c-kit)^{high}Sca-1⁺) undergoes a progressive expansion as early as two days after infection (Fig.1e, Supplementary Fig.2a)¹⁷. Both Lin[−]CD117(c-

kit)^{high}Sca-1⁺CD150⁺ and CD150⁻ subsets expand, but only Flt3-expressing CD150⁻ cells are critically regulated by Flt3 (Fig.1f, Supplementary Fig.2b)^{7, 8}. Similar to DCs in the periphery, MDP and CDP undergo a transient contraction followed by expansion as determined by analyzing Lin⁻CD117⁺Sca1⁻CD115⁺ cells^{4, 9, 10}(Fig.1g, Supplementary Fig. 2c). Importantly, the infection-induced MDP/CDP expansion is compromised in *Flt3*^{-/-} and *Flt3L*^{-/-} mice (Fig.1h, Supplementary Fig.2d) demonstrating the entire DC developmental pathway responds to infection by a Flt3-dependent mechanism.

Role of Flt3L responsive CD8 α ⁺/CD103⁺ DCs in *P. chabaudi* and *P. berghei* infection

We used langerin-DTR mice to deplete CD8 α ⁺/CD103⁺DCs^{18, 19}, which is the DC subset most affected by acute increase in Flt3L (Fig.2a). *P. chabaudi* infection triggers activation of both CD4⁺ and CD8⁺ T cells as measured by CD44^{high}CD62L^{low} effector/memory T cells 7 days after infection (Fig.2b–g). Depletion of spleen CD8 α ⁺/CD103⁺ DCs did not modify the percentage of activated CD4⁺ T cell in the spleen (Fig.2b,c), nor their phenotype (Supplementary Fig.3d), however their number was reduced (Fig. 2d). In contrast, CD8 α ⁺/CD103⁺ DC depletion impaired CD8⁺ T cell activation both at the level of percentages and absolute numbers (Fig.2e,f,g). We conclude that Flt3L-regulated CD8 α ⁺ DCs support T cell responses most importantly in the CD8⁺ T cell compartment. Depletion of CD8 α ⁺/CD103⁺DCs increased parasitemia and compromised survival to *P. chabaudi* infection (Supplementary Fig.3a–c).

In contrast to *P. chabaudi*²⁰, CD8⁺ T cell responses to *P. berghei* ANKA induce lethal brain immunopathology^{21–23}. Depletion of CD8 α ⁺/CD103⁺ DCs during to *P. berghei* ANKA infection using langerin-DTR mice delayed lethality without impacting parasitemia (Supplementary Fig. 4a), reduced brain pathology (Supplementary Fig.4b) and CD8⁺ T cell accumulation (Supplementary Fig.5a). Similar results were found in *Flt3L*^{-/-} mice (Supplementary Fig.6). We conclude that Flt3L-regulated CD8 α ⁺/CD103⁺ DCs are required for the onset of CD8⁺ T cell-dependent brain immunopathology during *P. berghei* ANKA infection.

MCs release Flt3L in response to *Plasmodium* infection

To determine the source of inflammatory Flt3L we produced reciprocal BM chimeras between WT and *Flt3L*^{-/-} mice (Fig.3a). Analysis of Flt3L serum levels revealed that the increase in serum Flt3L in response to *P. chabaudi* infection was dependent on the radio-resistant compartment. Lethally irradiated *Flt3L*^{-/-} recipients of WT or *Flt3L*^{-/-} BM were defective in their serum Flt3L responses to *P. chabaudi* infection, whereas WT recipients of WT or *Flt3L*^{-/-} BM were not (Fig. 3a). We conclude that a radio-resistant cell type releases Flt3L during *P. chabaudi* infection.

Analysis of expression database (<http://biogps.gnf.org>) indicate that radioresistant²⁴ mast cells (MCs, and Supplementary Fig 7), express high levels of Flt3L in the steady state. To analyze the potential contribution of MCs to Flt3L increase, we analyzed the membrane bound precursor of Flt3L on BM-derived MC (BMMC) before and after degranulation (Supplementary Fig.8). MC degranulation (probed by lamp1 upregulation), decreases surface Flt3L levels while soluble Flt3L increases dramatically in supernatants (Supplementary Fig.8). Thus, BMMCs express Flt3L precursor, which is released as a soluble cytokine upon degranulation. To address the relevance of this process *in vivo*, we measured serum Flt3L upon Fc ϵ RI-triggered MC degranulation (Supplementary Fig. 9a–d). Naïve peritoneal MCs express high levels of membrane bound Flt3L (Supplementary Fig. 9a), but other immune cells do not (Supplementary Fig.9b). Upon administration of anti-IgE, MCs degranulate (lamp-1 up-regulation) and show decreased cell surface Flt3L (Supplementary Fig. 9a). Concomitantly, Flt3L serum levels rapidly increase in anti-IgE but

not in MC-deficient Kit^{W-sh} mice²⁵ (Fig.3f) or control Ig-treated mice (Supplementary Fig. 9c–d). We conclude that MC constitutively display cell surface Flt3L and that MC degranulation triggers the release of soluble Flt3L.

We next examined peritoneal MCs from *P. chabaudi*-infected mice. MCs underwent degranulation as measured by increased surface lamp-1 (Supplementary Fig.9e), and decreased membrane Flt3L (Fig. 3b, quantified in 3c) concomitantly with Flt3L increase in the serum (Fig.1a, 3d). Furthermore, the increase in serum Flt3L in response to *P. chabaudi* (and *P. berghei* ANKA) was dependent on MCs since it was nearly absent in MC-deficient Kit^{W-sh} mice²⁵ despite normal levels of steady state Flt3L (Fig.3d, Supplementary Fig.9f). We conclude that MCs are activated during malaria infection and they release membrane Flt3L into the circulation.

In order to determine whether MCs are required for the observed increases in DCs during malaria infection, we analyzed spleen DC populations in MC-deficient Kit^{W-sh} mice (Fig. 3e–g). Although steady state numbers of CD8 α ⁺ DCs are similar in WT and Kit^{W-sh} mice, CD8 α ⁺ but not CD8 α [−] DCs fail to increase in the spleen of *P. chabaudi*-infected Kit^{W-sh} mice (Fig.3e–g). Importantly, administration of recombinant Flt3L to *P. chabaudi*-infected Kit^{W-sh} mice restored CD8 α ⁺ DC expansion (Fig.3e,f) showing that the Kit^{W-sh} mutation does not impair DC responsiveness to Flt3L. MC deficiency also lead to a significant impairment in CD8⁺ T cell activation (Fig.3h,i) but not had no effect on CD4⁺ T cells (Fig. 3j). Again, this defect is circumvented by administration of recombinant Flt3L, thus excluding a default in Kit^{W-sh} CD8⁺ T cells. Taken together, these results show that MCs promote CD8⁺ T cell activation by controlling Flt3L serum levels and spleen CD8 α ⁺/CD103⁺ DCs expansion during *P. chabaudi* infection.

Uric acid triggers Flt3L release from MCs

P. chabaudi infection might induce Flt3L release from MCs by inflammasome-dependent cytokines (IL-1/IL-18), or ATP-sensing receptor P2X₇R, but we found no evidence that these pathways mediate Flt3L release from MCs (Supplementary Fig.10a–c). An alternative possibility is that MC activation is mediated directly by Uric Acid. It has been shown *in vitro* that hypoxanthine released from *Plasmodium*-infected RBCs can be converted to uric acid by phagocyte-derived xanthine dehydrogenase^{26, 27} (Xdh) and uric acid crystals can in turn be sensed by various immune cells by a mechanism that requires direct activation of Syk^{28–30}. To test the possibility that uric acid induces Flt3L, we injected mice with uric acid crystals (Fig.4a–c). Whereas serum Flt3L increased rapidly after the injection, MCs showed decreased levels of membrane bound Flt3L (Fig.4a–c). Control, allopurinol crystals had no effect on MC activation (Supplementary Fig.11a). By contrast, oxonic acid, a specific inhibitor of uricase inducing hyper-uricemia *in vivo* also increases serum Flt3L (Supplementary Fig.11b). We conclude that uric acid induces rapid increases in circulating Flt3L through MC activation.

To determine whether uric acid triggers MC release of Flt3L during *Plasmodium* infection, we measured serum uric acid levels during *P. chabaudi* infection (Fig.4d). We found that *P. chabaudi* infection (but not control injection, Supplementary Fig.11c) induced a significant and transient hyperuricemia, which is also a hallmark of experimental cerebral malaria in mice^{31, 32} and severe malaria in humans^{33, 34}. To test the contribution of uric acid to serum Flt3L responses, we blocked uric acid accumulation by administration of allopurinol (an inhibitor of Xdh) and uricase (Supplementary Fig.11d). Consistent with the idea that uric acid triggers Flt3L release from MCs, Flt3L serum levels were significantly decreased in treated mice (Fig.4e). MC activation was also decreased (Lamp1 exposure and Flt3L release: Fig.4f). Uric acid inhibition also led to a significant decrease in CD8 α ⁺ DCs expansion in the spleen of *P. chabaudi* infected mice (Fig.4g) and CD8⁺ T cell activation

(d8, Fig.4h). We conclude that uric acid participates in MC activation and Flt3L release during malaria infection, which results in DC expansion and promotes T cell activation.

TLR9 induced Type I IFN signaling supports Uric Acid production during *Plasmodium* infection

Toll like receptor (TLRs) are of paramount importance in innate sensing of *Plasmodium* parasites^{35–38}. We found that Myd88^{-/-}/TRIF^{-/-}, Myd88^{-/-} and TLR9^{-/-} mice but not TRIF^{-/-} mice had an impaired Flt3L response to *P. chabaudi* blood stage infection (Fig.5a). In accordance with TLR9 involvement^{35–38}, we found that *P. chabaudi* infection triggers a transient and systemic IFN- α response that was fully abrogated in Myd88^{-/-}/TRIF^{-/-} mice (Fig.5b) (and pDCs-depleted, Supplementary Fig 12a,b). Mice deficient in type I IFN receptor (*IFN α / β RI*^{-/-}) failed to produce a systemic increase in Flt3L in response to *P. chabaudi* (Fig.5c) or *P.berghei* ANKA (Supplementary Fig.12c). However, this effect was indirect because type I IFN failed to induce Flt3L secretion from BMMCs (Supplementary Fig.10a,b), and the requirement for type I IFN receptor was on radiosensitive cells (Fig.5d), which are not the main source of inflammatory Flt3L (Fig.3a). Since uric acid sensing participates to MC activation and Flt3L release during *Plasmodium* infection (Fig.4), we asked whether type I IFN signaling was involved in regulating uric acid production. To test this possibility, we measured the uric acid producing enzyme Xanthine dehydrogenase (Xdh). We found that *Xdh* mRNA, its enzymatic activity and lung uric acid levels (Fig.5e) were highly up-regulated in the first day after *P. chabaudi* or *P.berghei* ANKA (Sup. Fig. 12d) infection in a manner that was *IFN α / β RI*-dependent.

Consistent with the role of uric acid in triggering MC activation and Flt3L release (Fig.4), we found that MC activation was impaired in *IFN α / β RI*^{-/-} both in terms of Lamp1 exposure and Flt3L release (Fig.5f). As a result of impaired Flt3L release, both type I *IFN α / β RI*^{-/-} (Fig.5g) and Myd88^{-/-}-TRIF^{-/-} (Supplementary Fig.13a) showed impaired spleen CD8 α ⁺ DC expansion in response to *P. chabaudi* infection despite normal responsiveness to recombinant Flt3L (Supplementary Fig.13b). CD8 α ⁺, but not CD4 α ⁺ T cell activation was also impaired in *IFN α / β RI*^{-/-} mice (Fig.5h). We conclude that type I IFN signaling downstream TLR-dependent type I IFN secretion controls XdH production by radiosensitive cells. This acute increase in XdH synthesis supports uric acid production, which in turn induces MCs to release Flt3L in response to *Plasmodium* infection (Supplementary Fig. 14 for diagram).

Flt3L supports BDCA3⁺ DC expansion and correlates with the magnitude of CD8⁺ T cell activation during *P. falciparum* infection

Severe human malaria is associated with increased numbers of circulating BDCA3⁺ DCs³⁹, which correspond to murine CD8 α ⁺/CD103⁺ DCs^{18, 40–42}. To determine whether Flt3L is elevated in humans infected with *P. falciparum* we measured levels of this growth factor in the blood of Kenyan children with mild or severe malaria. Flt3L was not detectable in the serum of age matched un-infected controls (n=47), but was present in n=5/27 cases of mild and n=32/92 cases of severe malaria (Fig. 6a). The increase in Flt3L plasma concentration with increasing disease severity was statistically significant (Kruskal Wallis test p<0.0001). For a subgroup of patients, we had previously determined the frequency of peripheral blood DC subsets³⁹. Patients with detectable levels of Flt3L in plasma had elevated frequencies of circulating BDCA3⁺ but not BDCA1⁺ or BDCA2⁺ DCs (Fig. 6b–d).

To determine whether human Flt3L increases the numbers of circulating BDCA3⁺ DCs we analyzed the effect of human Flt3L administration in mice reconstituted with human HSCs (Fig. 6e,f, Supplementary Fig15a). We found that Flt3L administration was sufficient to induce an increase the human DC compartment in blood (Fig6e) and the spleen (Fig.6f).

Importantly, the effect was more pronounced in the BDCA3⁺ DC subset in both blood and spleen compartments (Fig. 6e,f, Supplementary Fig15b).

To determine whether the increased levels of Flt3L and DCs are also associated with enhanced levels of CD8⁺ T cell activation in infected individuals, we analyzed T cell activation status by measuring CD69 expression levels by flow cytometry. We found that Flt3L positive patients showed a similar proportion of activated CD4⁺ T cells (Fig.6g) and an increased proportion of activated CD8⁺ T cells (Fig. 6h). We conclude that the host response to human malaria includes a systemic increase in Flt3L, BDCA3⁺ DC expansion and CD8⁺ T cell activation.

Discussion

Here we demonstrate that innate sensing by MCs during *Plasmodium* infection leads to a systemic elevation of Flt3L levels which impacts on host responses to the parasite by increasing the number of DCs and promoting T cell activation.

How is inflammatory Flt3L produced?

Flt3L is an essential growth factor for DCs *in vitro* and *in vivo*^{1, 4, 43} that can have tolerogenic effects in the absence of systemic inflammation^{44, 45}. During MCMV infection, Flt3L production is upregulated⁴⁶. We have shown that Flt3L levels in serum also increase in response to *P. chabaudi* and *P. berghei* ANKA infections in mice, and *P. falciparum* in humans. Having found that inflammatory Flt3L originates from the radio-resistant compartment, we identified radio-resistant MCs as major producers of soluble Flt3L.

MCs and their products have been implicated in regulating DCs^{47, 48}. MCs release of pre-synthesized TNF- α during inflammation promotes DC maturation and migration from skin to lymph nodes^{47–49}. Our experiments uncover a previously unappreciated TNF- α –independent role for MCs in regulating serum Flt3L and DC populations during inflammation. MCs express high levels of the membrane Flt3L, which is rapidly released into the circulation upon degranulation resulting in preferential expansion of CD8 α ⁺CD103⁺ DCs. This new function for activated MCs in adaptive immunity is complementary their role in DC migration^{47–49} and down-regulation of Treg function⁵⁰.

Mechanism of MC activation during *Plasmodium* infection

Our experiments show that Flt3L release by radio-resistant MCs in response to *Plasmodium* infection requires type I IFN signaling by radio-sensitive cells. Type I IFN is produced in response to TLR-9/Myd88 sensing during *Plasmodium* infection^{35–38}. Moreover, mice deficient for the type I IFN receptor or TLRs display impaired immune responses to *P. chabaudi* infection³⁶ and are more resistant to *P. berghei* immunopathology^{51, 52}. Type I IFN mediates most of its effects on Flt3L indirectly, *i.e.*, by acting on the radiosensitive compartment. Type I IFN induces Xanthine dehydrogenase (Xdh), an enzyme that converts hypoxanthine and xanthine that accumulates in *Plasmodium*-infected erythrocytes to uric acid^{26, 27}. In addition uric acid can also precipitates inside infected RBCs providing an inflammatory stimulus for CD80/CD86 upregulation and MHC II down-regulation in DCs³⁰. Uric acid, released from dead cells³⁶ or *Plasmodium*infected erythrocytes^{26, 27, 30} is known to be pro-inflammatory and hyper-uricemia is a hallmark of malaria infection^{31, 32} including severe malaria in humans^{33, 34}. Our experiments reveal that, in addition to direct DC activation^{29, 30, 53}, uric acid contributes to Flt3L release from MCs leading to increased DCs numbers.

Consequences of inflammatory Flt3L production

In mice, the CD8 α ⁺CD103⁺ subset is the more sensitive to acute increases in serum Flt3L. This phenomenon is mirrored by the increase of their human equivalent, BDCA3⁺ DCs^{18, 40–42} in the circulation during *P. falciparum* infection³⁹. Although, only limited data was available for infected children, the association between increased Flt3L and increased numbers of human BDCA3⁺ DCs was confirmed by mechanistic experiments in humanized mice showing Flt3L alone is able to preferentially increase the number of circulating BDCA3⁺ DCs.

Despite toxic effects of infection⁵⁴, DC function is relevant for protective immunity to *Plasmodium* blood stage infection⁵⁵. Experiments with CD11c-DTR mice where CD11c-expressing cells were depleted with DT show that these cells are essential for protective immunity to *P. yoelii* liver phase⁵⁶ and for induction of experimental cerebral malaria in mice infected with the blood stage of *P. berghei* ANKA⁵⁷. Our experiments with Langerin-DTR mice show that the loss of CD8 α ⁺CD103⁺ DCs, which are acutely responsive to inflammatory Flt3L, results in impaired CD8⁺ T cell activation^{19, 58, 59}. In addition, the same CD8 α ⁺CD103⁺ DC subset is essential to induce pathogenic CD8⁺ T cells that are responsible for lethal brain inflammation during *P. berghei* ANKA infection. Finally we show that, the proportion of activated CD8⁺ T cells (but not CD4⁺ T cells) in peripheral blood correlates with the magnitude of the Flt3L response in *P. falciparum* infection in severe malaria.

Taken together, our data uncover an evolutionarily conserved pathway that regulates DC development and T cell activation during infection.

Online Methods

A detailed version of methods is available in the Supplementary Online Materials including detailed cellular preparation protocols and flow cytometry analysis.

Mice and parasites and infections

C57BL/6 mice were purchased from Jackson. *Flt3*^{-/-} mice were a kind gift of I. Lemischka¹⁶. *Flt3L*^{-/-} mice were obtained from Taconics¹. *Kit*^{W-sh/W-sh} (*Kit*^{W-sh}/HNIhrJaeBsmJ)²⁵ and all other strains are described in S.O.M. Langerin-DTR mice⁶⁰ were obtained from B. Malissen and hosted at the Mount Sinai Immunology Institute. BM chimeric mice were produced as previously described⁴. irradiated NOD Rag1^{-/-} IL2R γ ^{null} (the Jackson Laboratory) were reconstituted by injecting human CD34⁺ fetal liver-derived hematopoietic stem cells. Human fetal livers were obtained from Advanced Bioscience Resources (ABR), Inc. (Alameda, CA) or the Human Fetal Tissue Repository (HFTR, Bronx, NY) and hematopoietic stem cells were isolated as detailed in the S.O.M. All mice were maintained in specific pathogen-free conditions, and all mouse protocols were approved by the Rockefeller University Institutional Animal Care and Use Committee (IACUC). *P. chabaudi chabaudi* clone AS and *P. berghei* ANKA was obtained through the Malaria Research Reagents and Resource Center (MR4, <http://www.mr4.org>). Infections with *Plasmodium berghei* ANKA were performed as described previously²³. See S.O.M for more details.

Study population

We analyzed the concentration of FLT3L in plasma by ELISA from children presenting with severe malaria at the Kilifi District Hospital on the Kenyan Coast between 2003 and 2007, those presenting with mild malaria in 2004–2009 and in healthy children identified during cross-sectional surveys in 2004 and 2009. Children admitted to Kilifi District Hospital with

severe malaria had a parasite-positive blood film by microscopy and suffered from one or more of the following symptoms: impaired consciousness (Blantyre Coma Score <5), coma (Blantyre Coma score <3) severe anemia (Hb <5 g.dl⁻¹ and at least 10,000 parasites.ml⁻¹ blood) or severe respiratory distress (deep breathing and chest recession or nasal flaring). Children with mild malaria were identified in cohorts under active surveillance for acute malaria episodes and had a parasite-positive blood film by microscopy and temperature >37.5°C. Healthy children had no fever or history of fever over the last 7 days and were negative for *Plasmodium* spp. by microscopy and rapid diagnostic test (Optimal). For 21 healthy children and 27 children with severe malaria, data on the frequency of peripheral blood DC subsets were available as reported in *Urban et al.*³⁹. Parents or Guardians of the children provided informed consent before participation in the studies. The studies received ethical approval from the Kenyan National Ethics Review Committee (SSC nos. 669, 883 and 1131) and the Oxford Tropical Research Ethics Committee (protocol nos. 005-02 and 30-06).

Flt3L, IFN- α , Uric acid and Xanthine dehydrogenase assays

Mouse serum or culture supernatants Flt3L or IFN- α and human plasma Flt3L were measured by ELISA kit (RnD systems Cat#MFK001 and cat#DFK00, interferon source Cat# 42120-1). Serum uric acid or from lung extracts (prepared by smashing lungs in sucrose 250mM, tris 50mM pH7.4, followed by two cycle of freeze/thaw before to be centrifuged 15mn at 15000rpm at 4degC to get rid of debris) was assessed using a recombinant Xdh-based colorimetric assay (Invitrogen, Cat# A22182). Xdh was assayed using hypoxanthine as a substrate using a colorimetric assay (Invitrogen, Cat# A22181). For Xdh PCR, lungs from infected mice were chopped in Trizol LS reagent (Invitrogen, Cat# 10296-028), frozen on dry ice and stored at -80°C. RNA was purified according manufacturer's protocol. cDNA was prepared using SuperScript II Reverse Transcriptase (Invitrogen, Cat# 18064-014) with random 6-mer primers according manufacturer's protocol. Quantitative PCR (Q-PCR) was performed using Brilliant SYBR Green QPCR Master Mix (Agilent Technologies, Cat# 600548) according manufacturer's protocol. Reactions were performed in triplicate and analyzed with an MX3000P Q-PCR machine (Stratagene). Results were normalized to GAPDH. Primers used were as follows: Xdh forward, 5' TGCTATTTCTCTGATCATCTGC 3', Xdh reverse, 5' CCAGGACACTTTCCACCAAG 3, GAPDH forward, 5' TGA AGC AGG CAT CTG AGG G 3', GAPDH reverse, 5' CGA AGG TGG AAG AGT GGG AG 3'.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Systemic Flt3L increase, Flt3-dependent BM activation and DC expansion during *P. chabaudi* infection

a : Curve shows serum Flt3L (pg.ml⁻¹) in wild type mice on d0-d6 after *P. chabaudi* infection.

b : Changes in spleen leukocyte (black) and DC numbers (white:CD8 α^- , grey CD8 α^+) during the first 8 days of *P. chabaudi* infection in CD57BL/6 mice. See supplementary figure for DC gating. Absolute numbers are normalized to naïve mice. (3–7 mice analyzed independently for each time point).

c, d : Bar graphs show absolute numbers of CD8 α^+ (**c**), CD8 α^- (**d**) DCs in the spleens of wild type (WT) or Flt3^{-/-} mice normalized to WT controls before and 7 days after infection with *P. chabaudi*. Each dot represents an individual mouse. Mean, SEMs, and t-tests are shown.

e, f : *P. chabaudi*-induced alterations in early, multipotent progenitors in WT (**e, f**) and *Flt3*^{-/-} mice (**f**).

e : Bar graph shows the percentage of Lin⁻(CD3, CD4, CD8, CD19, B220, CD11b, CD11c, NK1.1, Ter119) CD117⁺/Sca1⁺ HSC-containing fraction in the BM during the first 5 days of *P. chabaudi* infection (mean+/- SEM, t-tests are shown) of a representative experiment with 3 mice analyzed independently at each time point. **f** : Bar graph indicate the percentage (mean+/- SEM of 4 mice analyzed independently for each group) of Lin⁻CD117⁺Sca1⁺ CD150⁺ and Lin⁻CD117⁺Sca1⁺ CD150⁻ in the BM of WT or *Flt3*^{-/-} mice at d6 after *P. chabaudi* infection.

g, h : *P. chabaudi*-induced alterations in monocyte/DC committed progenitors in WT (**g, h**), *Flt3*^{-/-} and *Flt3L*^{-/-} mice (**h**). Bar graphs show the percentage of Lin⁻CD117⁺Sca1⁻CD115⁺ (MDP and CDP) in the BM (mean+/- SEM, t-test are shown) of a representative experiment with 3 mice analyzed independently at each time point.

Figure 2. Role of Flt3L responsive CD8 α ⁺/CD103⁺ langerin positive DCs in T cell activation during *P. chabaudi* infection

Control (DT-treated WT) or langerin-DTR transgenic mice were infected with *P. chabaudi*. Depletion of CD8 α ⁺ spleen DCs during *P. chabaudi* infection using Langerin-DTR mice was measured at d7 (**a**). DT had no effect on T cell activation (Supplementary Fig.3e). T cell activation was assessed at d7 in the spleen by measuring the percentage of CD62L^{low}/CD44^{high} cells inside TCR β ⁺CD8⁺ (**b**, upper panel) or TCR β ⁺/CD4⁺ (**d**, lower panel). Quantitation is shown in (**c,d,f,g**). Relative (**c, f**) and absolute (**d, g**) accumulation of activated TCR β ⁺CD4⁺ (**c, d**) and TCR β ⁺CD8⁺ (**f, g**) cells in the spleen are shown. Each dot represents an individual mouse, mean, SEM and some t-tests are shown.

Figure 3. MCs release inflammatory Flt3L during *Plasmodium* infection

a : Production of soluble Flt3L by radioresistant cells during *P. chabaudi* infection. Serum Flt3L levels in BM chimeras ($Flt3L^{+/+}$ or $Flt3L^{-/-}$ donor BM and recipient irradiated $Flt3L^{+/+}$ or $Flt3L^{-/-}$ host), naïve (left) or at d4 after *P. chabaudi* infection (right).
b, c : MC express high levels of surface Flt3L that is lost during *P. chabaudi* infection.
b : Histogram shows surface levels of membrane Flt3L in FcεRI⁺CD117⁺ peritoneal MCs from naïve mice before (black) or after *P. chabaudi* infection (red, d2) or $Flt3L^{-/-}$ mice (green).
c : Histogram shows surface levels of membrane Flt3L in FcεRI⁺CD117⁺ peritoneal MCs from naïve mice before (black) or after *P. chabaudi* infection (red, d2) or $Flt3L^{-/-}$ mice (green).

c : Quantification of membrane Flt3L fluorescence in individual mice before (black) or after *P. chabaudi* infection (red, d2 and d3). Absolute values are normalized to 1 in *Flt3L*^{-/-} MCs used as a negative control. Each dot is an individual mouse.

d : Deficient Flt3L serum increased levels in Kit^{W-sh} MC-deficient mice infected with *P. chabaudi* (d2,d4,d6). Each dot represents an individual mouse.

e–g: Deficient CD8⁺ DC expansion in Kit^{W-sh} MC-deficient mice infected with *P. chabaudi*. **e** : Lin⁻ (CD3,CD19,Ter119,Nk1.1, Gr1) CD11c^{high}MHCII^{high} DC compartment in spleen of WT or Kit^{W-sh} mice, before or after *P. chabaudi* infection (d8) with or without Flt3L injection. **f, g** : Absolute numbers per spleen of CD8⁺ (**f**) or CD8⁻ (**g**) DCs, values are normalized to WT naïve. Each dot is an individual mouse.

h–j: Deficient CD8⁺ T cell activation in Kit^{W-sh} MC-deficient mice infected with *P. chabaudi*. T cell activation was assessed at d8 in spleen of WT or Kit^{W-sh} mice during *P. chabaudi* infection (d8, with or without Flt3L injection) by measuring the percentage of CD62L^{low}/CD44^{high} cells inside TCR^β⁺CD8⁺ (**h**, upper panel) or TCR^β⁺/CD4⁺ (**h**, lower panel). Absolute (**i, j**) accumulation of activated TCR^β⁺CD4⁺(**i**) and TCR^β⁺CD8⁺(**j**) cells in the spleen are shown. Each dot represents an individual mouse, mean, SEM and some t-tests are shown.

Figure 4. Role of uric acid sensing by MCs leads in Flt3L release, DC expansion and T cell responses during *Plasmodium* infection

a–c. Uric acid triggers MCs to release Flt3L. PBS or preformed uric acid crystals in PBS (200ug or 2000ug) were injected i.p. into WT mice. Mice were sacrificed 2 hours post injection and CD117⁺FcγRI⁺ peritoneal MCs were analyzed for surface Flt3L levels (**a**). Histogram shows Flt3L levels with *Flt3L*^{-/-} MCs as a negative control. (**b**), as is (**a**) but bar graph summarizes results for 3 mice for each condition. (**c**) Serum Flt3L levels from the same mice is shown in (**b**).

d. Hyperuricemia during the early phases of *Plasmodium* blood stage infection. Uric acid levels in the serum of mice before or after infection with *P. chabaudi* (d1, d2, d4).

e. Uric acid inhibition (UAI) decreases Flt3L release during *P. chabaudi* infection. WT mice infected with *P. chabaudi* were treated with allopurinol and uricase (d0 and d1, here termed as UAI) or a control vehicle injection. Serum Flt3L was measured at d2 post infection.

f. Uric acid inhibition (UAI) decreases partially MC activation and Flt3L release during *P. chabaudi* infection. Histogram plots show examples of peritoneal MCs phenotypes at d1 post- *P. chabaudi* infection (surface Flt3L, left, surface Lamp1, right). Mice were treated with allopurinol and uricase (d0 and d1, here termed as UAI) or control (vehicle). Quantification of Lamp1 surface upregulation (left bar graph panel) or Flt3L surface staining (right bar graph panel) in various experiments. Each dot represents an individual mouse, mean, SEM and some t-tests are shown.

g. Deficient CD8a⁺ DC expansion in Uric Acid Inhibited (UAI treatment) mice infected with *P. chabaudi*. Lin (CD3,CD19, Ter119, Nk1.1, Gr1) CD11c^{high}MHCII^{high} DC compartment in spleen of WT or UAI-treated mice (allopurinol injection, daily), before or after *P. chabaudi* infection (d8). Absolute numbers per spleen of CD8a⁺ (left bar graph panel) or CD8a⁻ (right bar graph panel) DCs, values are normalized to WT naïve. Each dot is an individual mouse, mean, SEM and some t-tests are shown.

h. Deficient T cell activation in UAI in Uric Acid Inhibited (UAI treatment) mice infected with *P. chabaudi*. T cell activation was assessed at d8 in the spleen by measuring the percentage of CD62L^{low}/CD44^{high} cells inside TCRβ⁺CD8⁺ (upper flow plot panel) or TCRβ⁺/CD4⁺ (lower flow plot panel). Absolute accumulation of activated TCRβ⁺CD8⁺ (left bar graph panel) and TCRβ⁺CD4⁺ (right bar graph panel) cells in the spleen are shown. Each dot represents an individual mouse, mean, SEM and some t-tests are shown.

Figure 5. Role of TLR and type I IFN-dependent signaling in uric acid up-regulation, Flt3L release, DC expansion and T cell activation during *Plasmodium* blood stage infection

a : Impaired serum Flt3L response in Myd88^{-/-}/TRIF^{-/-}, Myd88^{-/-}, TLR9^{-/-} but not TRIF^{-/-}. WT or TLR mutant mice were infected with *P. chabaudi* and serum Flt3L measured at d2 after infection.

b : Impaired serum IFN-α response in Myd88^{-/-}/TRIF^{-/-}. **c** : Decreased serum Flt3L response in type I IFN receptor deficient mice. WT or IFNα/βRI^{-/-} mice were infected with *P. chabaudi* and serum Flt3L measured at d2 and d4 after infection.

d : Type I IFN signaling is required in radiosensitive cells for an efficient Flt3L response. Irradiated WT or IFNα/βRI^{-/-} recipients were injected with WT or IFNα/βRI^{-/-}

BM and infected at least 8 weeks after with *P. chabaudi*. Flt3L serum levels were measured after 4d.

e : Type I IFN receptor mediates increased Xanthine dehydrogenase production and uric acid levels upregulation. XdH mRNA was measured by qPCR on total lung mRNAs in WT or *IFN α / β RI^{-/-}* mice, before or after *P. chabaudi* infection (d1 and d2, left panel). XdH activity (middle panel) and Uric acid levels (right panel) were measured in total lung extracts at d1.5 after infection with *P. chabaudi* in WT or *IFN α / β RI^{-/-}* mice.

f : Decreased MC activation and Flt3L release in type I IFN receptor deficient mice. Histogram plots show examples of peritoneal MCs phenotypes at d1 post- *P.chabaudi* infection (surface Flt3L, left, surface Lamp1, right) in WT or type I IFN receptor deficient mice. Quantification of Lamp1 surface upregulation (left bar graph) or Flt3L surface staining (right bar graph) in various experiments.

g : Decreased spleen CD8 α ⁺ DC expansion in type I IFN receptor deficient mice. Lin⁻ (CD3,CD19,Ter119,Nk1.1) CD11c^{high}MHCII^{high} spleen DCs from WT or *IFN α / β RI^{-/-}* mice, naïve or after *P. chabaudi* infection (d8). Absolute numbers of CD8 α ⁺ DCs (left bar graph panel), and CD8 α ⁻ DCs (right bar graph panel) in the various groups as indicated, values are normalized to WT naïve for comparison.

h : Deficient T cell activation in type I IFN receptor deficient mice. T cell activation was assessed at d8 in the spleen by measuring the percentage of CD62L^{low}/CD44^{high} cells inside TCR β ⁺CD8⁺ (upper panel) or TCR β ⁺/CD4⁺ (lower panel). Absolute accumulation of activated TCR β ⁺CD4⁺(left bar graph panel) and TCR β ⁺CD8⁺(right bar graph panel) cells in the spleen are shown. Each dot represents an individual mouse, mean, SEM and some t-tests are shown.

Figure 6. Systemic Flt3L increase, BDCA3⁺ DCs expansion and CD8⁺ T cell responses in patients infected by *P. falciparum*

a. Plasma Flt3L concentration in healthy children and children suffering from mild or severe malaria. Numbers indicate the numbers of Flt3L positive patients for each group. Each dot indicates a patient. Mean and Kruskal Wallis significance are shown.

b–d. Circulating BDCA1⁺ (**b**), BDCA2⁺(**c**), BDCA3⁺(**d**) DCs for a subgroup of patients (experimental values taken from³⁹ plotted against plasma Flt3L concentration (measured in this study). Each dot indicates a patient. Median and interquartile range is shown.

e,f. Circulating (**e**) or spleen (**f**) BDCA1⁺, BDCA2⁺, BDCA3⁺ DCs in the blood of humanized mice treated or not 8 days with recFlt3L (10µg/day) after gating on human

CD45⁺, lin⁻, CD14⁻, CD16⁻ (Supplementary Fig.15a). Results are quantified as percent of humanCD45⁺ positive cells (**e,f**). A representative experiment is shown.

g,h. Activation status of circulating CD4⁺ (**g**) and CD8⁺ (**h**) T cells in patients classified upon their Flt3L levels. Median and interquartile range is shown.