A malaria protein factor induces IL-4 production by dendritic cells via PI3K–Akt–NF-κB signaling independent of MyD88/TRIF and promotes Th2 response

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Dendritic cells (DC) and cytokines produced by DC play crucial roles in inducing and regulating pro-/anti-inflammatory and Th1/Th2 responses. DC are known to produce a Th1-promoting cytokine, interleukin (IL)-12, in response to malaria and other pathogenic infections, but it is thought that DC do not produce Th2-promoting cytokine, IL-4. Here, we show that a protein factor of malaria parasites induces IL-4 responses by DC. Malaria parasite–activated DC induced IL-4 responses by T cells both in vitro and in vivo, favoring Th2, and IL-4–deficient DC were unable to induce IL-4 expression by T cells. Interestingly, lethal parasites, Plasmodium falciparum and Plasmodium berghei ANKA, induced IL-4 response primarily by CD8α+ DC, whereas nonlethal Plasmodium yoelii induced IL-4 by both CD8α+ and CD8α− DC. In both P. berghei ANKA- and P. yoelii-infected mice, IL-4–expressing CD8α− DC did not express IL-12, but a distinct CD8α− DC subset expressed IL-12. In P. berghei ANKA infection, CD8α+ DC expressed IL-12 but not IL-4, whereas in P. yoelii infection, CD8α+ DC expressed IL-4 but not IL-12. These differential IL-4 and IL-12 responses by DC subsets may contribute to different Th1/Th2 development and clinical outcomes in lethal and nonlethal malaria. Our results for the first time demonstrate that a malaria protein factor induces IL-4 production by DC via PI3K–Akt–NF-κB signaling, revealing signaling and molecular mechanisms that initiate and promote Th2 development.

Malaria caused by the Plasmodium family of parasites inflicts enormous morbidity and mortality (1). Malaria is an inflammatory response–driven disease (2). Early during the blood stage infection, malaria induces high levels of pro-inflammatory cytokine responses, leading to Th1 development (3–5). These responses are necessary for clearing infections by promoting appropriate cellular and humoral immunity (6). However, excessive inflammatory mediators contribute to pathogenesis (7). Usually, as infection progresses, pro-inflammatory cytokine responses are counter-regulated by increased production of anti-inflammatory cytokines, leading to Th2 development (8). These coordinated immune responses result in balanced pro-/anti-inflammatory and Th1/Th2 responses, avoiding pathogenesis (3–5).

Dendritic cells (DC)2 are central to the initiation and regulation of innate and adaptive immunity (9). In response to infections, DC produce IL-12, which in turn activates NK cells to produce IFN-γ. The activated DC and signals induced by cytokines together regulate Th1/Th2 development (9). In malaria, DC are the major early responders that produce pro-inflammatory cytokines, leading to NK and T-cell activation and IFN-γ production (10–12). IFN-γ contributes to parasitemia control by priming phagocytes for clearance of parasites (13). However, excessive inflammatory responses are harmful, especially if parasites sequester in organs (7, 14–16). Normally, as infection progresses, DC produce reduced levels of pro-inflammatory cytokines, concomitantly increasing IL-10 production, which suppresses pro-inflammatory and Th1 responses (3, 4). Also, DC are switched from Th1- to Th2-inducing phenotype (3, 8, 9). Several malaria parasite factors, including DNA, RNA, glycosylphosphatidylinositol, and microparticles and heme, released by IRBCs, activate macrophages and DC via TLR9, TLR7, TLR2, TLR4, and MyD88 signaling and induce pro-inflammatory responses, promoting Th1 responses (17–25). Uric acid activates DC via MyD88-independent signaling (26). However, how the activated DC are programmed to induce Th2 development and whether parasites have factor(s) that induce Th2-promoting IL-4 responses by DC

2 The abbreviations used are: DC, dendritic cell(s); GM-DC, DC obtained by the differentiation of mouse bone marrow cells with GM-CSF; FL-DC, DC obtained by the differentiation of mouse bone marrow cells with fit3 ligand; FLT3, FMS-like tyrosine kinase 3; Gm–DC, GM-DC obtained by the differentiation of human monocytes with human GM-CSF; RBC, red blood cell; IRBC, infected red blood cell; IL-4GF, GFP expression under the control of IL-4 promoter; Pf, P. falciparum; Pba, P. berghei ANKA; Py, P. yoelii 17XNL; Sup, supernatant; A5, ammonium sulfate; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; NF-κB, nuclear factor κB; IL, interleukin; IFN-γ, interferon-γ; TLR, Toll-like receptor; MyD88, myeloid differentiation factor 88; TRIF, TIR domain-containing adapter inducing interferon β; TIR, Toll-interleukin-1 receptor; GM-CSF, granulocyte/macrophage colony-stimulating factor; PI3K, phosphatidylinositol 3-kinase; NK, natural killer; APC, allopurinol; PE, phycoerythrin; DME, Dulbecco’s modified Eagle’s medium.
Results

Malaria parasites induce IL-4 production by DC

To determine whether DC produce IL-4 in response to malaria parasites, we tested IL-4 and IL-12 production in response to *Plasmodium falciparum* IRBCs ( Pf-IRBCs) by WT GM-DC and FL-DC. Both DC types produced IL-4 and IL-12 in an IRBC dose–dependent manner (Fig. 1A). We next analyzed cytokine responses to soluble and insoluble portions of Pf-IRBC lysates by GM-DC and FL-DC. The soluble portion (IRBC-Sup) induced IL-4, but not IL-12, production by both DC types (Fig. 1B). By contrast, the insoluble portion (IRBC-pellet) containing the parasite’s nuclear material efficiently induced IL-12, but not IL-4, production. The merozoites could induce IL-12, but not IL-4, production, suggesting that a soluble factor that was exported to IRBC cytoplasm induces IL-4 production by DC. Pf-IRBCs and IRBC-Sup induced IL-4 production by hGM-DC, whereas IRBCs, but not IRBC-Sup, induced IL-12 production by hGM-DC (Fig. 1C). Also, we tested IRBCs of nonlethal *Plasmodium yoelii* 17XNL (Py) and lethal *Plasmodium berghei* ANKA (Pba), the parasite that causes cerebral malaria in mice (27). IRBCs and IRBC-Sup of both Pba and Py induced IL-4 production by GM-DC and FL-DC, whereas IRBC-pellet induced IL-12, but not IL-4 (Fig. 1D). The Pf-IRBCs could also induce IL-4 production by basophils and mast cells (Fig. 1E).

To confirm the above findings, we analyzed IL-4 and IL-12 responses by GM-DC of IL-4GFP transgenic mice expressing GFP under the control of IL-4 promoter. A significant population of CD11c<sup>hi</sup>MHCII<sup>+</sup>CD49b<sup>−</sup>CD3ε<sup>−</sup>CD19<sup>−</sup>FceRI<sup>−</sup> DC (Fig. S1) expressed IL-4GFP in response to IRBCs, but not to IRBC-Sup (Fig. 2, A–C). A distinct population of CD11c<sup>hi</sup>MHCII<sup>+</sup>CD49b<sup>−</sup>CD3ε<sup>−</sup>CD19<sup>−</sup>FceRI<sup>−</sup> DC expressed IL-12, but not IL-4GFP, in response to Pf-, Pba-, and Py-IRBCs and IRBC-Sup, but not to IRBC-pellet (Fig. 2, A–C). The above results demonstrated that IL-4 production by DC in response to parasites is independent of TLR- mediated IL-4 production by DC. Both IL-4 and IL-12 production in response to Pf-IRBCs by trf-deficient GM-DC was similar to that by WT GM-DC (Fig. 4B). The above results demonstrated that IL-4 production by DC in response to parasites is independent of TLR-signaling.

To identify the downstream signaling in IRBC-induced IL-4 production, we tested the effect of inhibitors of several signaling pathways, including NF-κB (PDTC), ERK (PD98059), p38 (SB203580), JNK (SP600125), PI3K (wortmannin and LY294002), and Akt (Afuerserib and ARQ-092). ERK and p38 inhibitors had no effect, but NF-κB, PI3K, and Akt inhibitors efficiently reduced the production of IL-4 (Fig. 4C), indicating that the PI3K–Akt–NF-κB signaling axis mediates the parasite-induced mediated IL-4 production by DC. Interestingly, IL-4 production was significantly enhanced upon JNK inhibition (Fig. 4C). This observation agrees with a previous report that inhibition of JNK augments Th2 responses (30), and it suggests a reciprocal regulation between PI3K–Akt–NF-κB and JNK signaling.

To characterize the nature of the parasite factor that induces IL-4 expression by DC, Pf-IRBCs were treated with DNase, RNase, or uricase or heated to 100 °C. The enzymes had no effect on IL-4 production, but heating at 100 °C markedly
Figure 1. Malaria parasites induce IL-4 production by DC. A–D, WT GM-DC and FL-DC and hGM-DC (each 1 × 10^5 cells/well/200 μl) in 96-well plates were treated with Pf-IRBCs, PbA-IRBCs, Py-IRBCs, and pellets and supernatants (Sup) of Pf-, PbA-, and Py-IRBC lysates or Pf merozoites (MZs) as outlined under "Materials and methods." Unstimulated DC and DC stimulated with RBCs and CpG ODN were used as negative and positive controls. IL-4 and IL-12 secreted into the culture medium were measured by ELISA in duplicates. A, DC were treated with the indicated doses of Pf-IRBCs or RBCs. B and C, WT GM-DC and FL-DC (B) and hGM-DC (C) were treated with 2 × 10^6/ml Pf-IRBCs, IRBC-Sup, and IRBC-pellet (2 × 10^6/ml equivalent) or 20 × 10^6/ml Pf merozoites. D, WT GM-DC and FL-DC were treated with 2 × 10^6/ml PbA-IRBCs and Py-IRBCs and 2 × 10^6/ml IRBC equivalent of IRBC-Sup or IRBC-pellet. E, basophils and mast cells (1 × 10^5 cells in 96-well plates) were treated with 2 × 10^6/ml of Pf-IRBCs. A–E, data are mean values ± S.E. (error bars) of 3–5 independent experiments. Statistical analyses of data were performed by two-way ANOVA followed by the Newman–Keuls test compared with the unstimulated cells. *, p ≤ 0.05; **, p ≤ 0.01; ***, p ≤ 0.001.
The activity was trypsin-resistant, but treatment with proteinase K abolished IL-4 production, suggesting that the activity was by a protein factor. In contrast to IL-4, treatment of Pf-IRBCs with DNase, trypsin, or proteinase K or heating at 100 °C abolished IL-12 production by DC. This was due to DNA being the prominent immunostimulatory factor in Pf, and DNA complexing with proteins is essential for DNA uptake and recognition by TLR9 in endosomes (21). In agreement with these findings, RNase and uricase had no effect on IL-12 production (Fig. 4D).
Figure 3. Spleen DC from malaria-infected mice express IL-4. A–C, IL-4GFP mice (A) and WT mice (B and C) were infected with either PbA or Py. After 5 days, splenocytes were stained with antibodies against DC surface marker proteins, IL-4, and IL-12 and analyzed by flow cytometry. DC were gated as shown in Fig. S2. Shown are IL-4GFP (A) or IL-4 and IL-12 (B and C) expression by gated spleen CD11c+MHCII+CD49b+CD3+CD19+FcεRI+CD8α+ and CD8α− DC from PbA- and Py-infected mice. A–C, left panels show contour diagrams representative of DC samples, and right panels represent an individual mouse from 2 (A) to 4 (B and C) independent experiments. Statistical analysis was by one-way ANOVA followed by the Newman–Keuls test (A) or by unpaired two-tailed t test (B and C). Mean values ± S.D. (error bars) are shown. *, p ≤ 0.05; **, p ≤ 0.01; ***, p < 0.001.
The IL-4–inducing factor of malaria parasites is protein(s)

The IL-4–inducing activity of PF-IRBC-Sup was completely precipitated with AS, and AS-supernatant (AS-Sup) had no activity. Treatment of the AS-precipitated proteins (AS-precipitate) with proteinase K abolished the activity, but treatment with trypsin had negligible effect, confirming

Figure 4. The malaria parasite factor that induces IL-4 expression by DC is protein(s).

A-D, GM-DC (1 × 10⁵ cells/well in 96-well plates) derived from WT, TLR, and MyD88 knockout mice (A), TRIF⁻/⁻ mice (B), and WT mice (C and D) were stimulated with PF-IRBCs (2 × 10⁶/ml). In C, cells were treated with inhibitors 1 h before IRBC stimulation, and in D, IRBCs were treated with the enzymes or heated to 100 °C. E, schematic diagram of PF-IRBC-Sup fractionation. The IRBC-Sup was subjected to either AS precipitation or filtration using Centricon filters with the indicated molecular weight cutoff membranes. F and R, filtrate and retentate from Centricon filtration, respectively. F, WT GM-DC were stimulated with PF-IRBCs, untreated AS-precipitate, AS-precipitate treated with either proteinase K (ProK) or trypsin, and untreated AS-Sup. G, WT GM-DC stimulated with PF-IRBCs, PF-IRBC-Sup, or Centricon filtrates (F) and retentates (R) of PF-IRBC-Sup. A–D, F, and G, cells were stimulated with 2 × 10⁶/ml PF-IRBCs or the equivalent of PF-IRBCs. IL-4 and IL-12 in culture medium were measured by ELISA. Statistical analysis was performed by one-way ANOVA followed by the Newman–Keuls test. Mean values ± S.D. (error bars) of individual samples from three or four independent experiments are shown. *** p ≤ 0.001.
that the IL-4–inducing protein(s) is trypsin-resistant (Fig. 4F). These results confirmed that the IL-4–inducing factor is protein(s).

Centrifugation of Pf-IRBC-Sup (see Fig. 4E) in Centrifuge tubes fully retained the IL-4–inducing protein(s) on 30-kDa membrane (Fig. 4G). On 100-kDa membrane, ~50% of IL-4–inducing protein(s) were retained and ~50% filtered through, suggesting that the active protein factor has a molecular mass of 30–300 kDa.

To determine the role of IL-4–inducing malaria protein(s) in DC orchestrating T-cell responses, we analyzed OT-11 T-cell responses by coculturing with either WT or IL-4−/− GM-DC treated with Pf-IRBCs, IRBC-Sup, or IRBC-pellet, followed by stimulation with OVA(323–339) peptide. WT DC treated with IRBCs induced significant IL-4 and IFN-γ responses by OT-11 T cells, DC treated with IRBC-pellet induced IFN-γ but not IL-4 response, and DC treated with IRBC-Sup induced significant IL-4, but not IFN-γ, response (Fig. 5A). In contrast, OT-11 T cells cocultured with IL-4−/− GM-DC stimulated with Pf-IRBCs, IRBC-Sup, or IRBC-pellet showed no IL-4 response but expressed IFN-γ in cocultures in which IL-4−/− DC were treated with IRBCs and IRBC-pellet (Fig. 5B). Further, FACS-sorted spleen CD11c+MHCIId+CD3ε−CD49b−CD19−FceRI−CD8α− DC from Py-infected WT mice at 5 days postinfection induced significantly higher levels of IL-4 expression by OT-11 T cells than CD8α− DC from infected IL-4−/− mice (Fig. 5C). In contrast, compared with WT CD8α− DC, CD8α− DC from IL-4−/− mice at both 3 and 5 days postinfection induced significantly higher levels of IFN-γ by OT-11 T cells (Fig. 5C). These results suggested that DC activated by malaria parasites have the ability to induce IL-4 response by T cells, promoting Th2 responses.

**Discussion**

Here, we report a novel finding that CD11c+MHCIId+CD3ε−CD49b−CD19−FceRI− classical DC produce IL-4 in response to a protein factor of malaria parasites via PI3K–Akt–NF-κB signaling axis independent of TLR-MyD88/TRIF. An earlier study has reported that GM-DC derived from bone marrow cells of BALB/c mice produce IL-4 in response to Rauscher leukemia virus (31). Another study has reported that mouse fetal skin myeloid DC and mouse spleen DC produce IL-4 in response to *Candida albicans* hyphae (32). However, in both studies, DC were not fully characterized, and the nature of the stimulatory factors was not investigated. A recent study has reported that mouse spleen CD11c+CD49b+FceRI+ basophil-like DC from mice infected with *Schistosoma japonica* expressed IL-4 (33). However, these cells closely resembled basophils by being positive to both CD49b and FcεRI and were not classical DC. Hence, to date, it is thought that DC do not produce IL-4. Thus, our study is the first one to show that classical DC produce IL-4 in response to a malaria protein factor.

Our results show that CD8α− and CD8α− DC differentially produce IL-4 and IL-12 in PbA and Py infections that may lead to differential Th1/Th2 responses and clinical outcomes. A previous study in *P. chabaudi*-infected mice (8) found that, although both CD8α+ and CD8α− DC promote Th1 response by inducing IFN-γ production in CD4 T cells, only CD8α− DC induce CD4 T cells to produce IL-4 and IL-10, promoting Th1–Th2 switching at the acute stage of infection. Also, as infection progressed, CD8α+ DC numbers markedly decreased due to their increased apoptotic death. These changes paralleled the gradual decrease in Th1 response, suggesting that CD8α+ DC primarily induce Th1 responses, whereas CD8α− DC induce both Th1 and Th2 responses, promoting Th1-to-Th2 switching at late stages of infection (8). However, whether DC produce IL-4 or how DC were programmed to induce IL-4 by T cells was not investigated (8). These observations and the general notion that CD8α+ DC primarily guide Th1 responses (34) are consistent with PbA infection efficiently driving Th1 responses. In contrast, in Py infection, CD8α− DC barely produce IL-12 but produce IL-4, suggesting that the Py-induced Th1 response is not as robust as in PbA infection. Thus, these results suggest that the IL-4–inducing malaria protein factor enables DC to program and gain capacity to initiate and promote Th2 responses.

The conclusion here that, by producing IL-4 in response to a malaria protein factor, DC are programmed for Th2 priming is supported by the findings that (i) WT DC, but not il-4−/− deficient DC, treated with IRBCs or IRBC-Sup induce IL-4 response by OT-11 T cells; (ii) DC treated with IRBC-pellet containing parasite nuclear material induce IFN-γ, but not IL-4 response, by OT-11 T cells; and (iii) DC from infected WT mice, but not from IL-4−/− mice, efficiently induce IL-4 response by OT-11 cells. Thus, our findings support the conclusion that...
IL-4 production by DC is involved in Th2 polarization. Low levels of IL-4 production by DC and low abundance of the IL-4–inducing factor in malaria parasites may be the consequence of host–parasite adaptation during their evolution for co-existence.

Importantly, this study provides a rational explanation for how Th2 response is initiated in the face of ongoing strong pro-inflammatory and Th1 responses early during malaria infection. Previously, we and others have demonstrated that nucleic acids are the dominant immunostimulatory factors of malaria parasites (6, 17, 20, 21, 23, 25). Sponaas et al. (8) have shown that, at the acute stage of malaria infection, DC induce IL-4 response by T cells. These results together with our findings that DC produce IL-4 and the activated DC induce IL-4 responses by T cells suggest the following. The nucleic acids of malaria parasites dominantly activate DC via TLR signaling to produce IL-4, contributing to a Th1 development, whereas the IL-4–inducing factor triggers a low IL-4 response by DC via PI3K–Akt–NF-κB signaling, leading to the initiation of Th2 response. The interaction of IL-4–inducing DC with NK and T cells amplifies Th2 responses. This results in DC gradually losing the capacity to induce Th1 while increasingly gaining the ability to promote Th2 development (8), eventually leading to a balanced Th1/Th2 response.

**Materials and methods**

**Reagents**

The reagents were purchased from the following companies: ELISA kits from R&D systems; Cpg ODN-1826 from Coley Pharmaceutical; Macaloid from Axner Pottery supply (Oviedo, FL); and RNase from Promega Corp. Trypsin, proteinase K, DNase I, uricase, and lipopolysaccharide were from Sigma; SB203580 and PD98059 were from LC Laboratory (Woburn, MA); PDTC was from Enzo Life Sciences (Farmingdale, NY); SP600125 was from Ascent Scientific (Princeton, NJ); wortmannin, LY294002, aferesertib, and ARQ-092 were from Cayman (Ann Arbor, MI); human GM-CSF, IL-4, and mouse IL-3 were from Peprotech; OVA(323–339) was from InvivoGen; mouse CD11c and CD90.2 microbeads were from Miltenyi Biotech; and Pf (strain 3D7), Pb (strain ANKA), and Py (strain 17XNL) were from Bei Resources-ATCC. Human blood and plasma were from adult healthy volunteers and the Blood Bank, Hershey Medical Center, respectively. The institutional review board of the Pennsylvania State University College of Medicine approved the protocols for using human blood (Hy03-261), and the study abides by the Declaration of Helsinki Principles.

**Mice and malaria infection**

TLR2−/−, TLR4−/−, TLR9−/−, MyD88−/− mice (C57BL/6) were provided by Dr. Shizuo Akira. OT- II, TRIF−/−, IL-4−/−, and IL-4GFP mice (mice that express GFP under the control of the IL-4 promoter) were from Jackson Laboratory.

The mice were housed in a pathogen-free environment. The care and experimental protocols were as per the approval by institutional animal care and use committee of the Pennsylvania State University College of Medicine (protocol 46661). The studies were not blinded.

**Preparation and fractionation of IRBC lysates**

IRBCs were lysed by alternative freezing and thawing, and the lysates were centrifuged at 13,000 × g for 15 min. Pellets containing most of the nuclear material were reconstituted with DMEM to volumes corresponding to the original IRBC suspensions. IRBC-Sup was used for cell stimulation, fractionation on Centricon filters, and precipitation of proteins with AS.

**Characterization of IL-4–inducing factor**

Proteins in Pf-IRBC-Sup were precipitated by adding solid AS to 80% concentration. The AS-precipitated proteins were collected by centrifugation. The AS-precipitate and AS-Sup were dialyzed against PBS.

To characterize the nature of IL-4–inducing factor, Pf-IRBCs and AS-precipitated proteins in PBS were treated with 100 µg/ml trypsin, 50 µg/ml proteinase K, 100 units/ml DNase, 100 µg/ml RNase, or 1 units/ml uricase at 37 °C for 1 h. The proteases were inactivated by fetal bovine serum. DNase was inactivated by adding EDTA to 2.5 mM and heating at 65 °C for 10 min. RNase was removed by adding macaloid (which binds RNase) followed by centrifugation.
**Preparation of DC, basophils, and mast cells**

FL-DC and GM-DC were prepared by culturing mouse bone marrow cells with complete DMEM supplemented with 15% FLT3 ligand-containing and 10% GM-CSF-containing conditioned medium, respectively, for 7–8 days (10, 21, 35). Basophils and mast cells were prepared by culturing bone marrow cells in complete DMEM supplemented with 10 units/ml mouse IL-3 for 10 and 28 days, respectively (36, 37). hGM-DC were prepared by culturing adherent blood monocytes in complete RPMI medium containing 25 ng/ml human GM-CSF and 10 ng/ml recombinant human IL-4 for 7–8 days (38).

**Isolation of spleen DC and T cells**

Spleen cells were prepared as described previously (21). DC and OT-II T cells were isolated using CD11c and CD90.2 microbeads (21). CD11c+ cells were further purified to obtain CD11c+MHCII+CD49b+CD3ε+CD19-FceRI- DC by FACS sorting.

**Cell stimulation and cytokine analysis**

DC (untreated or inhibitor-treated) and mast cells and basophils (each 1× 10^5/well) in 96-well plates in 200 μl of complete DMEM (mouse cells) or RPMI (human cells) were stimulated for 24 h with IRBCs, enzyme-treated IRBCs, IRBC lysate fractions, or AS fractions (2 × 10^6/ml IRBCs or 2 × 10^6/ml IRBC equivalent amount) or with Pf-merozoites (~20 × 10^6/ml). DC treated with inhibitors (see Fig. 4C) were fully viable as tested by trypan blue exclusion. Cytokines in culture supernatants were measured by ELISA.

**Cell stimulation and flow cytometry**

IL-4GFP GM-DC (5 × 10^6 cells/well in 1 ml) in 24-well plates and FACS-sorted naïve spleen IL-4GFP DC (2 × 10^5/well in 200 μl) in 96-well plates were stimulated with 2 × 10^6/ml Pf-, PbA-, and Py-IRBCs or 2 × 10^6/ml IRBC equivalent of IRBC-Sup and IRBC-pellet. Cells were stained with antibodies against surface markers followed by intracellular staining using anti-IL-12 and anti-GFP antibodies (for enhanced detection limit of GFP) and analyzed along with fluorescence minus one controls. In the case of infected WT mice, spleen cells were treated with GolgiPlug for 6 h, and DC were stained and analyzed similarly.

**Coculturing**

GM-DC (1 × 10^5/well) in 96-well V-bottomed plates were stimulated with IRBCs or IRBC lysate fractions. After overnight culturing, OT-II T cells (1.5 × 10^5/well) were added and cultured in the presence of 2 μg/ml OVA(323–339) peptides for 42 h. Then cultures were treated with GolgiPlug for 6 h, and IL-4 and IFN-γ responses by T cells were analyzed by flow cytometry.

**Statistical analysis**

Statistical significance of results was determined by one-way or two-way ANOVA followed by the Newman–Keuls test or unpaired two-tailed t test using GraphPad Prism version 6.01. p values ≤ 0.05 were considered statistically significant.

### References


ACCELERATED COMMUNICATION: Malaria-induced IL-4 responses by DC


