Oxidative Stress Enhances Dendritic Cell Responses to Plasmodium falciparum

Anton Götz, Maureen C. Ty and Ana Rodriguez

ImmunoHorizons 2019, 3 (11) 511-518
doi: https://doi.org/10.4049/immunohorizons.1900076
http://www.immunohorizons.org/content/3/11/511

This information is current as of January 29, 2021.

Supplementary Material  http://www.immunohorizons.org/content/suppl/2019/11/05/3.11.511.DCSupplemental

References  This article cites 43 articles, 13 of which you can access for free at:
http://www.immunohorizons.org/content/3/11/511.full#ref-list-1

Email Alerts  Receive free email-alerts when new articles cite this article. Sign up at:
http://www.immunohorizons.org/alerts
Oxidative Stress Enhances Dendritic Cell Responses to Plasmodium falciparum

Anton Götz, Maureen C. Ty, and Ana Rodriguez

Department of Microbiology, New York University School of Medicine, New York City, NY 10016

ABSTRACT

Malaria is a highly inflammatory disease caused by the protozoan parasite Plasmodium. During the blood stage of infection, patients exhibit fever with high levels of inflammatory cytokines in their blood. However, when cells of the immune system are incubated with the parasite in vitro, their cytokine response is low. In particular, human primary dendritic cells (DCs) respond to Plasmodium falciparum–infected erythrocytes by upregulating maturation markers and chemokines but lack a substantial cytokine response. Because oxidative stress is a trigger of inflammatory cytokines in malaria and synergizes with P. falciparum to induce IL-1β secretion by macrophages, we assessed whether oxidative stress has an impact on DC maturation and function in response to P. falciparum. Using xanthine oxidase, a reactive oxygen species (ROS)–producing enzyme that is increased during malaria, we observed that exposure to extracellular ROS potentiated DC maturation in response to the parasite. Xanthine oxidase–derived ROS increased parasite-induced cytokine secretion and CD80 surface expression in DCs. This enhanced maturation phenotype boosted the DCs’ ability to prime autologous naive CD4+ T cells, resulting in higher T cell proliferation in vitro. Xanthine oxidase–derived ROS did not have an effect on the cytokines produced by primed T cells. We propose that oxidative stress during malaria contributes to the inflammatory response by enhancing the magnitude of DC and CD4+ T cell responses without changing the quality.


INTRODUCTION

Malaria, caused by the protozoan parasite Plasmodium, is a major global health burden, with >200 million cases and >400,000 deaths annually, with children in sub-Saharan Africa being the most vulnerable population affected. Despite continued efforts, no efficient, long-lasting vaccine is available, and the declining trend in malaria cases and deaths observed in the last decade has recently stalled (1). Malaria is characterized by an intense inflammatory response during the blood stage of the infection, which can contribute to severe forms of the disease (2). Despite this inflammatory response, malaria fails to induce sterile immunity and only induces clinical immunity, meaning infection progresses in the absence of symptoms. This clinical immunity is short-lived and only acquired...
after years of exposure to the parasite (3). A detailed understanding of how the human immune system responds to the parasite is critical to achieving the ultimate goal of eradication.

How human dendritic cells (DCs) respond to *Plasmodium falciparum*, the cause of most severe cases of the disease, is of particular interest because DCs are essential for the initiation and orchestration of immune and vaccine responses (4). As sentinels of the immune system, DCs are constantly sampling their surroundings and are found among the first cells to respond to pathogens. Pathogen recognition causes DCs to undergo a rapid maturation process, which is characterized by the upregulation of costimulatory surface markers in addition to secretion of immunomodulatory cytokines. DCs can be divided into two major subsets, myeloid DCs (mDCs) and plasmacytoid DCs (pDCs), each having distinct functions during immune responses to diverse pathogens. pDCs produce high levels of type I IFN upon activation through TLR9 by pathogen-derived nucleic acids and play an important role during viral infections (5). mDCs upregulate costimulatory markers when activated and are efficient APCs, which activate and polarize naïve T cells (4, 6). In the case of CD4+ T cells, activation by DCs leads to the development Th cell subsets, like Th1, Th2, or Th17, characterized by IFN-γ, IL-4, or IL-17 secretion, respectively (6).

Previous studies investigating DC responses to *Plasmodium* in both humans and mice have been contradictory (7, 8), possibly because of differences in experimental design, host species, parasite preparation and concentration, and DC subsets used in these studies. Although some authors suggest DCs mature and function efficiently in response to the parasite (9–11), others propose an inhibition of activation and/or apoptosis induced by the parasite (12–14). We have previously used freshly isolated human primary DCs from malaria-naïve donors incubated with clinically relevant amounts of whole *P. falciparum*–infected RBCs (iRBCs) and observed an unusual maturation phenotype. When primary DCs are activated with the well-described TLR4-ligand LPS, they respond by marker upregulation and secretion of inflammatory cytokines, like TNF and IL-6. The DC response to the parasite, however, was characterized by the lack of significant cytokine secretion but upregulation of maturation markers and secretion of chemokines. Despite the lack of a measurable cytokine response, DCs were able to induce a robust primary autologous CD4+ T cell response in vitro (15).

This seemingly counterintuitive observation that the in vitro system did not mimic the inflammatory responses observed in vivo has been described in other studies with DCs (9, 16) and macrophages (17–20). The differential in vitro versus in vivo inflammatory response suggests that host factors play a modulatory role. We hypothesize that oxidative stress, a host factor that is absent from most in vitro systems, could account for this difference. Malaria is a highly oxidative disease, as evidenced by high levels of plasma malondialdehyde, an indicator of lipid peroxidation (21). Sources of oxidative stress in malaria include the infected erythrocyte (22), the oxidative burst of macrophages (23), and elevated levels of oxidative host enzymes (24). One of these host enzymes, xanthine oxidase (XO), produces extracellular reactive oxygen species (ROS) and synergizes with *P. falciparum* to activate human macrophages. Accordingly, plasma levels of XO in malaria patients correlate with disease severity and inflammatory cytokine levels (20).

In the current study, we examined the effect of XO-derived ROS on DC maturation and function in the context of malaria. We used an established in vitro model using primary DCs incubated with intact *P. falciparum*–iRBC and autologous naïve CD4+ T cells (15). This in vitro system is the most physiologically accessible because it avoids the divergent phenotypes that are developed during in vitro DC differentiation (25).

**MATERIALS AND METHODS**

**P. falciparum culture and isolation**

Asexual blood stage cultures of the *P. falciparum* strain 3D7 were maintained at 5% hematocrit in RPMI 1640, 25 mM HEPES supplemented with 10 μg/ml gentamicin, 250 μM hypoxanthine, 25 mM sodium bicarbonate, and 0.5% Albumax II (pH 6.75) under atmospheric conditions of 5% oxygen, 5% carbon dioxide, and 90% nitrogen using purified RBCs of unknown blood type. Late-stage *P. falciparum*–iRBCs (trophozoites and schizonts) were isolated using MACS Cell Separation LS Columns (Miltenyi Biotec). Parasite cultures were mycoplasma free as determined using the MycoAlert Mycoplasma Detection Kit (Lonza).

**Cell enrichment and culture**

Peripheral blood (450 ml) from healthy malaria-naïve donors was obtained by venipuncture on the day of the experiment at the New York University blood bank or Clinical and Translational Institute of New York University School of Medicine with sodium citrate as anticoagulant. Institutional Review Board approval was obtained at New York University School of Medicine. RBCs form these blood draws were used for parasite culture. PBMCs were enriched from buffy coat using Ficol-Paque PLUS (GE Life Sciences). The Dynabeads Human DC Enrichment Kit (Invitrogen) was used to negatively enrich primary peripheral blood DCs from PBMCs, following the manufacturer’s protocol. For subset enrichment, the enriched DCs were further purified using the Diamond Plasmacytoid Dendritic Cell Isolation Kit II (Miltenyi Biotec) with positive selection. Unlabeled cells were either used directly as mDC subset or further enriched using HLA-DR Magnetic Beads (Miltenyi Biotec). Purity was assessed by staining for HLA-DR and the pDC marker CD303 by flow cytometry (FACS). Primary DCs and DC subsets were cultured with RPMI 1640 supplemented with 10% heat-inactivated human AB serum (Valley Biomedicals) at 37°C and 5% CO2. Untouched naïve CD4+ T cells were enriched by negative selection using the Naive CD4+ T Cell Isolation Kit II (Miltenyi Biotec). T cells and DC–T cell cocultures were cultivated with chemically defined, serum-free XVIVO-15 (Lonza) medium at 37°C and 5% CO2.

**DC activation**

Enriched DCs were seeded at 1.5 × 10^5 per well in 96-well tissue culture–treated plates and cultured with intact, freshly isolated
**P. falciparum**-iRBCs at a ratio of 1:3 (DC/iRBC) for 24 h. DCs alone and uninfected RBCs were used as a negative control. XO from bovine milk, lyophilized (Sigma-Aldrich), was used at a concentration of 0.25 U/ml, corresponding to levels reported in uncomplicated symptomatic malaria (24). One microgram per milliliter of LPS (Sigma-Aldrich) was used as a positive control for cytokine secretion. H$_2$O$_2$ and N-acetyl-l-cysteine (used at 30 mM) were obtained from Sigma-Aldrich. Febuxostat (Tocris) was used at 200 µM in DMSO, and the same concentration of DMSO was added to controls. For XO heat inactivation, the enzyme was incubated at 95°C for 1–3 min in RPMI 1640 prior to adding it to the culture. DCs were then harvested for FACS analysis, and supernatants were stored at −80°C until cytokine/chemokine analysis.

**Autologous CD4⁺ T cell activation**

The naive CD4⁺ T cell activation assay used in this study has been described before and was adapted to the use of primary DCs instead of monocyte-derived DCs (15, 26). DCs were plated together with **P. falciparum**-iRBCs at a ratio of 1:3 (DC/iRBC) with or without XO and incubated for 3 h. Uninfected RBCs were used as negative control. DCs were then harvested and replated together with CFSE-labeled naive CD4⁺ T cells from the same donor at a ratio of 1:30 (DC/T cell) and cultured together in tissue culture–treated 96 well plates (3.2 × 10⁵ T cells per well) in duplicate or triplicate for the indicated days. T cell proliferation was measured by CFSE dilution, and supernatants were stored at −80°C until cytokine analysis.

**Flow cytometry**

Flow cytometry was performed using a FACScalibur (BD Biosciences), and data were analyzed with FlowJo (Tree Star). DCs were identified using HLA-DR and costimulatory markers CD86 and CD80. CD4⁺ T cells were gated on CD4 and CFSE for proliferation. All Abs were purchased from BioLegend.

**Cytokine and chemokine analysis**

To quantify cytokines secreted by DCs and characterize T cell polarization, the following BD Cytometric Bead Arrays (BD Biosciences) were used: Human Inflammatory Cytokine Kit, Human Chemokine Kit, Human Th1/Th2 Cytokine Kit, or Human Th1/Th2/Th17 Kit. The arrays were acquired on a FACScalibur (BD Biosciences) and analyzed with FCAP Array Software (BD Biosciences). Cytokine levels (picograms per milliliter) were expressed in fold change over the negative control in all cytokine/chemokine graphs.

**Statistical analysis**

Statistical analyses were performed using Prism 8 (GraphPad Software). Shapiro-Wilk test was used to test for normality. Not normally distributed data were log transformed to achieve normal distribution. For normally distributed data and multiple comparisons, a repeated measure (RM) one-way ANOVA or mixed-effects analysis (for missing values) with Geisser-Greenhouse correction was used. Paired Student t test was performed for the comparison of two groups when normally distributed. For not normally distributed data (despite transformation), the following nonparametric tests were used: Friedman test or Kruskal-Wallis test (for missing values) with Dunn test for multiple comparisons. The statistical test and p values are described in the figure legends, with *p < 0.05, **p < 0.01, and ***p < 0.001.

**RESULTS**

**XO exposure enhances cytokine secretion and CD80 levels in P. falciparum–activated DCs**

To address if XO-produced ROS modify DC activation in the context of a malaria infection, we first analyzed maturation marker upregulation on primary human DCs. To mimic the presence of oxidative stress in malaria patients, DCs (lineage negative and HLA-DR positive) were enriched by negative selection from peripheral blood of healthy malaria-naïve donors and incubated with intact **P. falciparum**-iRBC with or without XO at a concentration typically found in uncomplicated malaria patients (24). Intact uninfected RBCs were used as negative control, and bacterial LPS was used as positive control. We observed that incubation of DCs with XO (in the presence of control RBC) resulted in upregulation of the maturation markers HLA-DR, CD80, and CD86 in most donors, compared with the uninfected RBC alone, suggesting that XO activity triggers DC maturation. Addition of other stimuli, **P. falciparum**-iRBC and LPS, also induced upregulation of the maturation markers in most donors (Fig. 1). We also observed that addition of XO to DC cultures incubated with the parasite did not further increase marker expression of HLA-DR and CD86. However, CD80 expression increased significantly when XO was present in **P. falciparum**/DC cultures, even above the levels induced by LPS (Fig. 1).

We next examined the effect of XO on **P. falciparum**-iRBC–induced chemokine and cytokine secretion by DCs. We found that XO alone induced increased chemokine and cytokine secretion in primary DCs, similarly to previous findings in monocyte-derived DCs and macrophages (20, 27). Depending on the cytokine, we observed a 10- to 1000-fold increase of secretion when XO was added to the uninfected RBC negative control (Fig. 2B). This XO-induced cytokine secretion was dose dependent (Supplemental Fig. 1A) and could not be replicated by addition of a short-lived source of ROS, such as H$_2$O$_2$ (Supplemental Fig. 1B). This suggests that DC activation may need elevated levels of ROS continuously, as provided by XO. We also observed that XO-induced cytokine secretion by DCs was reduced by incubation with Febuxostat, an XO-specific inhibitor (Supplemental Fig. 2A); the antioxidant N-acetyl-l-cysteine (Supplemental Fig. 2B); and heat inactivation of the enzyme (Supplemental Fig. 2C). These findings confirm that XO-produced ROS are responsible for the induction of cytokine secretion in DCs and exclude the possibility of enzyme contaminants triggering the observed inflammatory response.

Additional supplementation with XO did not further increase parasite-induced chemokine levels in supernatants of DC cultures (Fig. 2A). **P. falciparum**-iRBC alone failed to induce IL-1β and IL-10 secretion by DCs and triggered only low IL-6 and TNF production compared with LPS-induced levels (Fig. 2B). When DCs were
incubated with both XO and P. falciparum–iRBC, however, cytokine secretion for all four of the measured cytokines increased significantly compared with stimulation with parasite alone (Fig. 2B). When XO was added to P. falciparum/DC cultures, TNF, IL-1β, IL-10, and IL-6 secretion was enhanced by 10-, 20-, 34-, and 100-fold, respectively, indicating that the presence of XO is able to supersede the low inflammatory cytokine response to the parasite in vitro. To determine whether XO can boost DC cytokine responses to other stimuli, we activated DCs with the TLR4-ligand LPS together with XO. We observed that XO does not further increase LPS-induced cytokine secretion by DCs (Supplemental Fig. 2D).

Taken together, these results suggest that in the context of a malaria infection, prolonged exposure of human primary DCs to ROS may lead to increased CD80 surface expression and cytokine secretion, contributing to an inflammatory response.

**DC subsets are differentially activated by XO and P. falciparum**

Experiments described so far analyzed primary DCs consisting primarily of two major subsets: mDCs and pDCs. To address if XO had specific effects on either one of the subsets, we further enriched mDCs and pDCs. The purity of each subset was 90–95% (Supplemental Fig. 3A). As positive controls, TLR4-ligand LPS was used for mDCs, and TLR9-ligand CpG was used for pDCs. Analysis of marker expression revealed that XO together with the parasite did not further increase HLA-DR or CD86 on the surface of mDCs (Fig. 3A). Although CD80 expression in mDCs was enhanced by XO, the levels were similar to incubation of uninfected RBC and mDCs with XO. Comparable to results obtained with DCs and mDCs (Figs. 1, 3A), HLA-DR levels on pDCs were not boosted by the addition of XO to P. falciparum/pDC cultures. CD80 and CD86 on pDCs, however, increased significantly by 1.5-fold when XO and the parasite were added to the culture (Fig. 3B). LPS and CpG enhanced expression of HLA-DR, CD80, and CD86 comparable to or higher than XO and the parasite (Fig. 3).

Cytokine levels in mDC and pDC culture supernatants were also examined. Overall, cytokine secretion was similar between mDCs, pDCs, and when both subsets were cultured together (Figs. 1, 2) in contrast to surface marker expression, which was found to be different between the subsets (Figs. 3, 4). For all cytokines tested, except for TNF in mDC and IL-1β in pDC supernatants, addition of XO significantly enhanced the levels compared with the parasite alone (Fig. 4). Analysis of mDC-derived IL-10 revealed a synergistic effect of XO and the parasite. IL-10 secretion in these cultures increased by 175-fold in response to the parasite when XO was added. P. falciparum–iRBC and XO together enhanced IL-10 levels 6-fold compared with uninfected RBC and XO (Fig. 4A). We detected significant levels of IL-1β and IL-10 in response to XO in pDC culture supernatants (Fig. 4B). The pDC positive control CpG induced significantly higher TNF levels than XO and the parasite but did not induce IL-1β or IL-10 (Fig. 4B). IFN-α expression in response to the parasite was not further boosted or induced by XO alone (Supplemental Fig. 3B).

**XO exposure enhances the priming efficacy of P. falciparum–activated DCs**

DCs incubated with P. falciparum–iRBC are able to activate naive autologous CD4+ T cells in an MHC class II–dependent way to proliferate and become Th1-like effector cells characterized by secretion of IL-2, TNF, IFN-γ, IL-5, and IL-10 ([15]; Fig. 5, Supplemental Fig. 4). Because the quality of DC cytokine responses dictates the polarization of T helper cells, we asked whether DC cytokine secretion boosted by the presence of XO-derived ROS would have an impact on priming of naive autologous CD4+ T cells. To address this question, DCs were incubated with uninfected RBC or P. falciparum–iRBC with XO for 3 h to allow phagocytosis and ROS exposure. DCs were washed and plated with naive autologous CD4+ T cells. T cell proliferation was assessed, and secreted cytokines were measured in the supernatants at day 11 of the culture (Fig. 5). Priming CD4+ T cells with DCs that had been in contact with P. falciparum–iRBC (and were secreting more cytokines due to XO-derived ROS exposure) resulted in a significantly higher percentage of proliferated T cells compared with coculture with DCs presenting parasite Ags without prior XO exposure (Fig. 5B). However, levels of IL-2, TNF, IFN-γ, IL-10
and IL-5 (Supplemental Fig. 4) in the supernatants remained unchanged, and IL-4 and IL-17A were not detected (Supplemental Fig. 4). As expected, when DCs were incubated with uninfected RBC and XO, presumably secreting cytokines without presenting foreign Ag, they did not induce T cell proliferation or cytokine secretion (Fig. 5). Taken together, our findings indicate that XO-derived ROS increase DC activation and, in the presence of parasite Ags, enhance CD4+ T cell priming.

DISCUSSION

Given the highly inflammatory nature of malaria and the strong correlation of disease severity with increased inflammation (28), it is critical to gain detailed insight into the processes involved in the development of the host inflammatory response. Mouse models of malaria have contributed greatly to our understanding of the immune response and pathology during malaria (8); however, caution is required when translating conclusions derived from mouse models to the human disease (29). This is particularly true in the case of malaria in which infections in mice induce sterile immunity, whereas this is not acquired in naturally occurring Plasmodium infections in humans (3, 30).

Therefore, the analysis of human immune cells either ex vivo from infected patients or in vitro using cultured parasite is required for understanding the mechanisms contributing to the symptoms and lack of sterile immunity we observe in patients. One obvious caveat when incubating immune cells in vitro is the lack of host factors that are present in circulation or tissues. The observation that P. falciparum–iRBC induce little to no cytokines when incubated in vitro with different immune cell types (9, 15–19) suggests a role for host factors in malaria inflammatory response. We propose that oxidative stress could be an important host factor triggering inflammation in malaria.

FIGURE 2. Chemokines and cytokines secreted by DCs incubated with P. falciparum and XO.
Chemokines (A) or cytokines (B) were analyzed in supernatants of DCs incubated for 24 h with P. falciparum–iRBC or uninfected RBC at a ratio of 1:3 (DC/iRBC) with or without XO. LPS was used as a positive control. Fold change relative to the control (RBC) is shown. Each symbol represents results from one individual donor and experiment. Line depicts grand mean. *p < 0.05, **p < 0.01, ***p < 0.001 by RM one-way ANOVA (A and B) IL-6 and TNF or Friedman test (B) IL-1β and IL-10.

FIGURE 3. Costimulatory marker expression on primary DC subsets incubated with P. falciparum and XO.
mDCs (A) or pDCs (B) were incubated with P. falciparum–iRBC or uninfected RBC at a ratio of 1:3 (DC/iRBC) with or without XO for 24 h and analyzed for surface marker expression. LPS or CpG were used as a positive control. Fold change of median fluorescence intensity (MFI) of HLA-DR, CD80, and CD86 is shown. Each symbol represents results from one individual donor and experiment. Line depicts grand mean. *p < 0.05 by RM one-way ANOVA (A) HLA-DR and CD86, Friedman test (A) CD80, or mixed-effects analysis (B).
Results of a clinical trial conducted to test the effect of allopurinol (an inhibitor of XO) when given in addition to a classical malaria treatment suggest an important role for oxidative stress in malaria patients. The patients who received additional allopurinol showed a much faster decrease in inflammation (fever) and splenomegaly when compared with patients who were treated with the antimalarial alone, suggesting that inhibition of XO decreased inflammation in malaria (31). These findings are in agreement with the observation that, in malaria patients, high levels of XO activity correlate with plasma inflammatory cytokines and the development of cerebral malaria. Consistent with what we observed in the current study when adding XO to DCs, XO activity in the plasma of these patients also induced cytokine secretion in human macrophages (20).

In the same study, XO-derived ROS were shown to synergize with *P. falciparum*–iRBC activating the inflammasome, which resulted in efficient secretion of IL-1β. However, the synergistic effect was only observed for IL-1β, not for TNF, IL-6, and IL-10. This differs from our observations in DCs, which show that XO exposure together with the parasite triggers significantly higher levels of IL-10 than XO together with the uninfected RBC control. Similarly, the chemokine response to the parasite and/or XO was different in DCs compared with macrophages. Although *P. falciparum*–iRBC alone did not induce significant amounts of chemokine secretion in macrophages (20), high levels of CXCL9 and CXCL10 were secreted by DCs in response to the parasite (15).

XO in addition to the parasite did not further increase chemokine secretion by DCs, as observed in the current study, whereas in macrophages, XO together with *P. falciparum*–iRBC induced synergistic production of IL-8, CCL2, and CCL5. The different effects of XO on macrophages and DCs in the context of malaria suggest the involvement of different mechanisms in each cell type. These observations also highlight the different roles these two cell types play during an immune response and the importance of studying more than one “innate” cell type for a given observation or phenotype.

It is likely that the XO-induced cytokine and chemokine secretion in DCs is mediated by ROS acting as a second messenger for the activation of NF-κB, a common signaling pathway in inflammatory responses (32). Additionally, oxidative stress could increase DC activation through the oxidation of parasite DNA. Oxidation of DNA increases its immunogenicity and enhances signaling through STING (33), which was proposed to be involved in immune cell responses to genomic DNA from *Plasmodium* (34, 35). However, it is still unclear which specific pathways are involved in activation of primary human DCs by *P. falciparum* (15, 36).

Our observations suggest that XO exposure boosts the CD80 surface expression in DCs induced by the parasite. Conversely, CD86 surface expression was not impacted by XO when analyzing both pDCs and mDCs, which could be explained by the differences in regulation between CD80 and CD86. Upon activation, CD80 is expressed de novo, whereas CD86 can be transported to the cell surface independent of protein neosynthesis (37). The trigger to induce CD80 gene expression likely benefits from the additional stimulatory signals XO can provide.

Surprisingly, we detected significant levels of IL-1β and IL-10 in response to XO in pDC culture supernatants. Both cytokines are typically not expressed by pDCs, which are known to secrete large amounts of type I IFNs and TNF when activated by viral DNA (5). Accordingly, the TLR9-ligand CpG induced significantly higher TNF levels than XO and the parasite but no IL-1β or IL-10.
production of IL-1β in this context is particularly unusual because primary human pDCs lack caspase-1 and only have a decreased inflammasome response (38). Our findings might indicate that XO-derived ROS induce IL-1β production through a caspase-1–independent pathway, as seen in other contexts (39).

Although DCs are typically not the main producers of inflammatory cytokines during infections, they might contribute to the “cytokine storm” observed during malaria infections directly or through the activation of T cells. Our observations indicate that this contribution could be enhanced by the presence of XO. It is noteworthy that primary DCs only produce minimal amounts of IL-12p70 in vitro (15, 40, 41). In this study, XO did not induce noteworthy that primary DCs only produce minimal amounts of IL-12p70 in vitro (15, 40, 41). In this study, XO did not induce inflammatory cytokines during infections, they might contribute to the “cytokine storm” observed during malaria infections directly or through the activation of T cells. Our observations indicate that this contribution could be enhanced by the presence of XO. It is noteworthy that primary DCs only produce minimal amounts of IL-12p70 in vitro (15, 40, 41).

Activation of T cells by parasite-loaded DCs incubated with XO did not change the polarization of the CD4+ T cell response, suggesting that XO-derived ROS do not change the cytokine profile secreted by DCs. However, we observed that DCs loaded with parasite and exposed to XO were more efficient in inducing CD4+ T cell proliferation than DCs incubated with the parasite alone, which may be a consequence of higher levels of cytokines secreted and/or increased CD80 expression by the DCs. Surprisingly, we did not observe a difference in IL-2 secretion in the supernatants of T cell cultures. This may be due to differences in IL-2 levels during early time points that were not measured in our study (before 5 d) or to IL-2–independent T cell proliferation, as observed in other contexts (42, 43). A higher number of Ag-specific activated CD4+ T cells could contribute to parasite elimination; however, it may also increase the severity of malaria. At least in mice, CD4+ T cells induce pathology during cerebral malaria (44).

Taken together, our results indicate that ROS have a strong effect in the DC response to *P. falciparum* and suggest that malaria-induced inflammation is shaped by the levels of oxidative stress in patients. These findings emphasize the role of host factors in malaria-induced inflammatory response and are evidence of the need for a better understanding of the host response to malaria.

**DISCLOSURES**

The authors have no financial conflicts of interest.

**ACKNOWLEDGMENTS**

We thank the New York University School of Medicine Clinical and Translational Science Institute and the blood donors at New York University School of Medicine for participating in this study. We thank the National Institute of Health Fellows Editorial Board for editorial assistance.

**REFERENCES**


