Cutting Edge: Conventional Dendritic Cells Are the Critical APC Required for the Induction of Experimental Cerebral Malaria


*J Immunol* 2007; 178:6033-6037; doi: 10.4049/jimmunol.178.10.6033

http://www.jimmunol.org/content/178/10/6033

**References**

This article cites 23 articles, 8 of which you can access for free at: http://www.jimmunol.org/content/178/10/6033.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publishations/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
CUTTING EDGE

Cutting Edge: Conventional Dendritic Cells Are the Critical APC Required for the Induction of Experimental Cerebral Malaria


Cerebral malaria (CM) is a serious life-threatening complication in Plasmodium falciparum infection, affecting mainly young children in sub-Saharan Africa (1). The host immune response to P. falciparum is thought to contribute to the development of CM (2). Studies in experimental CM (ECM) caused by infection of C57BL/6 mice with Plasmodium berghei ANKA (PbA) have identified the spleen as a key site for the initiation of pathogenic immune responses (3), which includes the activation of CD4+ and CD8+ T cells that contribute to the onset of ECM (4). In addition, proinflammatory cytokines, such as IFN-γ (5), TNF (6), and lymphoxygenin α (7), produced in response to PbA infection play critical roles in ECM pathogenesis.

Dendritic cells (DCs) are a heterogeneous APC population with a key role in the initiation and regulation of cell-mediated immune responses (8). They can be broadly divided into plasmacytoid DC (pDC) and conventional DC (cDC), based on the expression of a variety of cell surface markers and their responses to pathogen molecules (8, 9). In this study, we demonstrate that the depletion of cDC ablates parasite-specific CD4+ T cell responses early in infection and prevents ECM, whereas the depletion pDC has no effect on ECM development.

Materials and Methods

Mice and parasites

Female C57BL/6 mice (5–6 wk of age) were obtained from the Animal Resources Centre (Canning Vale, Western Australia). C57BL/6 mice expressing the human diphtheria toxin receptor (DTR) under the CD11c promoter (B6.CD11c-DTR mice) (10) were bred in-house. Chimeric mice were generated with bone marrow (BM) cells from either C57BL/6 (controls) or B6.CD11c-DTR donor mice as previously described (7), and were used in experiments 12 wk after BM transplantation. All procedures were approved by the Queensland Institute of Medical Research Animal Ethics Committee. PbA was used in all experiments and mice were monitored as previously described (7).

RNA isolation and real-time RT-PCR

Real-time RT-PCR was performed as previously described (7). IFN-α (5′-TG CAACCCCTCTAGACTCTCTC-3′ (forward) and 5′-CCAGCAGGCC GTCTTCTCT-3′ (reverse)) and IFN-β (5′-GAAAGGGAAATTCTCAAAC TCACTTA-3′ (forward) and 5′-CTTCTGCTACTTCTCCGTCA-3′ (reverse)) mRNA levels were measured and normalized against the expression of hypoxanthine phosphoribosyltransferase (7).

Antibodies

All Abs for FACS and histology were purchased from Biolegend, BD Biosciences, or Miltenyi Biotec. The pDC-depleting Ab produced by the 120G8 hybridoma (11) was purchased from AbCys. Rat IgG was purchased from Sigma-Aldrich.

DC depletion

pDC were depleted by administering 1 mg of 120G8 mAb i.p. into C57BL/6 mice on days −1, 2, and 4 postinfection (p.i.). To deplete cDC in B6.CD11c-DTR mice and chimeric mice, 100 ng of diphtheria toxin (DT) (Sigma-Aldrich) was administered i.p. 48 h before PbA infection.

Materials and Methods

Received for publication January 30, 2007. Accepted for publication March 15, 2007.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This work was supported by grants from the Australian National Health and Medical Research Council.

Address correspondence and reprint requests to Dr. Christian Engwerda, Queensland Institute of Medical Research, 300 Herston Road, Herston, QLD 4006, Australia. E-mail address: Christian.Engwerda@qimr.edu.au

Abbreviations used in this paper: CM, cerebral malaria; ECM, experimental CM; pRBC, parasitized RBC; nRBC, naive RBC; PbA, Plasmodium berghei ANKA; DC, dendritic cell; cDC, conventional DC; pDC, plasmacytoid DC; DTR, diphtheria toxin receptor; BM, bone marrow; p.i., postinfection; DT diphtheria toxin; CBA, cytometric bead array.

Copyright © 2007 by The American Association of Immunologists, Inc. 0022-1767/07/$2.00

www.jimmunol.org

The Journal of Immunology

Cerebral malaria (CM) is a serious complication of Plasmodium falciparum infection, causing significant morbidity and mortality among young children and non-immune adults in the developing world. Although previous work on experimental CM has identified T cells as key mediators of pathology, the APCs and subsets therein required to initiate immunopathology remain unknown. In this study, we show that conventional dendritic cells but not plasmacytoid dendritic cells are required for the induction of malaria parasite-specific CD4+ T cell responses and subsequent experimental CM. These data address: Christian.Engwerda@qimr.edu.au

Institute of Medical Research, 300 Herston Road, Herston, QLD 4006, Australia. E-mail address: Christian.Engwerda@qimr.edu.au

1 Address correspondence and reprint requests to Dr. Christian Engwerda, Queensland Institute of Medical Research, 300 Herston Road, Herston, QLD 4006, Australia. E-mail address: Christian.Engwerda@qimr.edu.au

2 Address correspondence and reprint requests to Dr. Christian Engwerda, Queensland Institute of Medical Research, 300 Herston Road, Herston, QLD 4006, Australia. E-mail address: Christian.Engwerda@qimr.edu.au

Inflammation Kit (BD Biosciences). After 72 h of culture, cells were pulsed was collected after 72 h and cytokines were measured using the CBA Mouse

Pathology during ECM (4). Soluble schizont extracts from

activated CD4

DCs are critical for priming naive Ag-specific T cells (8), and pDC produce type I IFNs during PbA infection, but do not mediate ECM

Results

pDC produce type I IFNs during PbA infection, but do not mediate ECM

DCs are critical for priming naive Ag-specific T cells (8), and activated CD4^+ and CD8^+ T cells are principal mediators of pathology during ECM (4). Soluble schizont extracts from P. falciparum parasites bind TLR 9 on human pDC and stimulate production of type I IFNs (13). To determine whether pDC were activated following PbA infection, we measured IFN-α and IFN-β mRNA accumulation within splenic DC subsets early in the course of disease. As shown in Fig. 1A, pDC were activated within 3 days of malaria infection, as determined by increased levels of type I IFN mRNA. cDC were also characterized by increased type I IFN mRNA accumulation, albeit it at much lower levels. However, cDC were a more significant source of IL-12p40 mRNA than pDC at this time point (data not shown), indicating they were also activated following PbA infection. The number of splenic cDC and pDC in PbA-infected mice at day 3 p.i. did not increase relative to naive mice (data not shown). To determine whether pDC played any role in the pathogenesis of ECM, we depleted these cells throughout the course of infection as indicated. Plots show CD11c^+ splenocytes. The percentage of pDC is indicated above the gate. Survival (C) and blood parasitemia (D) were measured in five mice per group of control (■) and 120G8-treated (▲) mice over the course of PbA infection (one representative experiment of two performed). Shaded areas indicate the time when mice displayed ECM symptoms.

Interestingly, we found a 50–60% reduction in serum IFN-γ levels in PbA-infected mice lacking pDC at days 3 and 5 p.i., suggesting that they may contribute to T cell priming, albeit redundant to subsequent pathology.

cDC play a key role in ECM pathogenesis following PbA infection

Since pDC were not critical for the generation of ECM, we next examined whether cDC played a role in pathogenesis in this disease model. To test this, we used a well-established transgenic mouse strain which carries a DTR-GFP construct linked to a CD11c promoter (10). Administration of DT to these mice results in the depletion of cells expressing high levels of CD11c (i.e., cDC; Fig. 2A). Because of a relatively low CD11c expression by pDC, DT administration did not deplete these cells, thus the ratio of splenic cDC:pDC changed from ~4:1 to 1:7 by 24 h after DT treatment (Fig. 2A). In addition, we confirmed that the recently reported IFN-producing killer DC (14, 15) were also unaffected by DT treatment, defined as CD11c<sup>hi</sup>/B220<sup>-</sup>/PDCA<sup>hi</sup> (data not shown). Consistent with this, both pDC and IFN-producing killer DC populations were negative for the enhanced GFP transgene. The DT-treated mice had significantly reduced cDC numbers for 48–72 h (10) (Fig. 2A). We also confirmed that irradiated whole spleen cells from DT-treated mice were impaired in their ability to drive
allogeneic CD4+ T cell proliferation and cytokine production (TNF, IFN-γ, and IL-2), relative to cells from control mice, as previously shown (10). DT treatment of C57BL/6 mice the day before PβA infection had no effect on blood parasitemia, serum cytokine levels, or the development of ECM (data not shown), indicating that any differences observed in DT-treated B6.CD11c-DTR mice could be attributed to the depletion of cDC and not other effects of DT.

B6.CD11c-DTR mice were administered with a single dose of either saline (control) or DT on the day before PβA infection. Following PβA infection, the DT-treated mice had reduced serum levels of IFN-γ at days 3 and 5 p.i. (Fig. 2B), indicating that cDC are involved in the induction of IFN-γ in this disease model. Control mice developed ECM on days 5–8 p.i., and died within 2 days of ECM onset (Fig. 2C). In contrast, mice lacking cDC early in PβA infection did not develop any symptoms of ECM and survived significantly longer than control animals (p < 0.0001). Furthermore, histological examination of the brain showed no cerebral hemorrhages in ECM-resistant mice that received DT, unlike those routinely seen in control animals (data not shown). Blood parasitemia in cDC-depleted mice was initially lower than that in control mice; however, these animals eventually developed hyperparasitemia and anemia and were killed according to ethical guidelines around day 18 p.i. (Fig. 2, C and D). These data confirm a critical role for cDC in the pathogenesis of ECM.

cDC prime CD4+ T cell responses and induce cerebral inflammation early after PβA infection

We next investigated whether CD4+ T cell priming was impaired in cDC-depleted mice following PβA infection. Purified splenic CD4+ T cells isolated from naive animals and mice at day 3 p.i. were restimulated with parasite Ag (pRBCs) in vitro in the presence of naive APC. No CD4+ T cell activation was found in the presence of medium alone (data not shown) or nRBC (Fig. 2E). Furthermore, all CD4+ T cells, irrespective of treatment, proliferated well in response to Con A stimulation (all cpm >70,000), indicating that all CD4+ T cells under study were viable and capable of proliferation. CD4+ T cells from control-treated, PβA-infected B6.CD11c-DTR mice responded strongly to parasite Ag as measured by proliferation (Fig. 2E) and IFN-γ production (Fig. 2F). In contrast, CD4+ T cells isolated from cDC-depleted PβA-infected B6.CD11c-DTR mice failed to respond to parasite Ag, indicating that cDC are critical for the effective priming of parasite-specific CD4+ T cell responses early in PβA infection.

The increased expression of cell adhesion molecules on cerebral microvasculature endothelial cells is a common feature of ECM and is used as a marker for inflammation in the brain (16). The number of cerebral vessels expressing ICAM-1 and VCAM-1 was assessed by histological examination of brain tissue sections harvested when control PβA-infected B6.CD11c-DTR mice succumbed to ECM. Expression levels of both cell adhesion molecules were found to be significantly increased (p < 0.001) in both control and cDC-depleted, PβA-infected B6.CD11c-DTR mice, compared with naive animals (Fig. 2G). However, cDC-depleted B6.CD11c-DTR mice had significantly lower numbers of ICAM-1- and VCAM-1-positive cerebral vessels than control-treated B6.CD11c-DTR mice (p < 0.01), confirming a reduction in cerebral inflammation in these animals. These data demonstrate that transient cDC depletion prevented early T cell activation, attenuated cerebral inflammation, and eliminated mortality due to ECM.

Resident splenic DC do not control malaria parasitemia

Previous reports and our own observations indicate that cDC are absent for 48–72 h after DT administration, after which
FIGURE 3. Continued depletion of cDC fails to improve control of blood parasitemia, but prevents the development of ECM following PbA infection. B6.CD11c-DTR chimeric mice were treated with 100 ng of DT (gray columns and closed triangles) or saline (black columns and closed squares) on days $-1, +1, +3,$ and $+5$ after PbA infection. A. Serum IFN-γ levels following PbA infection (mean ± SEM of five mice per group at each time from one representative experiment of two performed). B. Development of ECM following infection (pooled data from two experiments, $n = 10$ mice/group). C. Blood parasitemia levels (mean ± SEM of five mice per group from one representative experiment of two performed). Shaded areas indicate the time when mice displayed ECM symptoms. Significant differences of $p < 0.01$ are indicated by **.

time cDC numbers in the spleen reconstitute from immature precursors (10) (Fig. 2A). The ratio of splenic cDC:pDC changes from ~1:7 at 24 h after DT treatment to 1:1 at 72 h, indicating the emergence of new cDC expressing high levels of CD11c, as well as more immature DC expressing lower levels of CD11c (Fig. 2A). The failure of animals to develop ECM following temporary depletion of cDC and PbA infection (Fig. 2C) may therefore be related to the potentially tolerogenic effects of emerging immature cDC (17). To exclude this possibility, we next depleted cDC throughout the entire period of PbA infection before the development of ECM. To enable prolonged depletion by multiple DT treatments, we generated BM chimeric mice by engrafting lethally irradiated C57BL/6 recipients with B6.CD11c-DTR BM cells, thus ensuring that only BM-derived cells would express DTR. These animals received DT on days $-1, +1, +3,$ and $+5$ p.i. Again, PbA-infected mice depleted of cDC had reduced levels of serum IFN-γ (Fig. 3A) and failed to develop ECM, unlike control mice (Fig. 3B). In contrast to PbA-infected B6.CD11c-DTR mice (Fig. 2D), there was no difference in blood parasitemia between cDC-depleted and control chimeric mice (Fig. 3C), suggesting that the reduced blood parasitemia observed following temporary depletion of cDC may have been the result of more efficient antiparasitic immunity being generated by immature cDC that reconstitute early in the course of infection. Importantly, this result also indicates that the reduced blood parasitemia in B6.CD11c-DTR mice that received a single dose of DT (Fig. 2D) was not the reason for their failure to develop ECM. Instead, qualitative and/or quantitative changes to the evolving immune response in these animals prevented disease. Together, these data indicate that the protection of DT-treated B6.CD11c-DTR mice from ECM was due to the transient elimination of resident cDC only and that cDC alone are critical for priming pathogenic T cell responses early in PbA infection and for subsequent development of ECM.

Discussion

The relative contribution of APC subsets to the initiation of ECM has previously been unknown due largely to the lack of appropriate reagents. Mice lacking B cells develop ECM following PbA infection (4), excluding a role for this APC subset in initiating disease. The effect of selective macrophage depletion early in PbA infection has not been possible to date but it is important to note that macrophages are not depleted following the administration of DT to B6.CD11c-DTR mice (10). Thus, it appears unlikely that macrophages are capable of initiating ECM in isolation. In contrast, our data indicate for the first time that a single APC population, the cDC, can induce CD4+ T cell activation and ECM. Recent studies on Plasmodium chabaudi chabaudi (AS) infection in mice indicate that both CD8+ and CD8- splenic cDC can activate parasite-specific CD4+ T cell responses, albeit at different times after infection, with distinct functional outcomes (18). The striking feature of our data was that early, but transient, depletion of cDC protected mice from ECM. These cells begin to repopulate lymphoid tissue 48–72 h following depletion (10) (Fig. 2A). Therefore, the cDC appears to condition the evolving immune response to become pathogenic in the first 2 days of PbA infection. After cDC repopulate lymphoid tissue following DT depletion, they are capable of priming immune responses during PbA infection, as indicated by increased serum IFN-γ levels at day 5 p.i. in DT-treated B6.CD11c-DTR mice (Fig. 2B) and increased expression of cell adhesion molecules on blood vessels in the brains of these animals (Fig. 2G). Significantly, the reduced blood parasitemia in these mice (Fig. 2D) raises the possibility that newly differentiated cDC generate more efficient antiparasitic immunity than resident cDC at the time of infection. Alternatively, a nonhemopoietic cell expressing CD11c (and thus present in naive CD11c-DTR mice but not BM chimeras) may suppress the control of parasitemia, although this would appear less likely.

Despite mice being protected from ECM when cDC were absent early in PbA infection, they did go on to die with severe anemia and hyperparasitemia, as has been reported for all mouse strains that are resistant to ECM induced by PbA (19). Moreover, resident cDC did not appear to modify levels of parasitemia unless they were actively reconstituting. The control of blood parasitemia in rodent malaria infections requires T cell cytokines and cytotoxicity (20). Parasite-mediated modulation of the host immune response is likely to inhibit the immunological control of PbA growth. Parasite-specific CD4+ T cells are depleted via an IFN-γ-dependent mechanism during PbA infection (21), and CD8+ cDC become unresponsive after PbA infection and fail to cross-prime CD8+ T cells (22). In addition, rodent malaria infections require parasite-specific Ab for final clearance of parasites (23). Thus, the improved control of parasitemia in mice transiently depleted of cDC suggests the subversion of one or more of these inhibitory mechanisms and/or the enhancement of primary immunity by cDC reconstitution early during PbA infection.

In summary, we identify cDC as a key cell population in the pathogenesis of ECM. Significantly, this is the first report of a specific APC subset promoting disease in malaria and highlights the fine balance that exists between the induction of immunity and pathology in infectious disease.

Acknowledgments

We thank Paula Hall and Grace Chojnowski for assistance with flow cytometry, Michelle Gatton for advice on statistical analysis of data, and Mariapia Degli-Espositi for helpful discussions.

Disclosures

The authors have no financial conflict of interest.
References


