Cytokine-Mediated Loss of Blood Dendritic Cells During Epstein-Barr Virus–Associated Acute Infectious Mononucleosis: Implication for Immune Dysregulation

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Acute infectious mononucleosis (IM) is associated with altered expression of inflammatory cytokines and disturbed T-cell homeostasis, however, the precise mechanism of this immune dysregulation remains unresolved. In the current study we demonstrated a significant loss of circulating myeloid and plasmacytoid dendritic cells (DCs) during acute IM, a loss correlated with the severity of clinical symptoms. In vitro exposure of blood DCs to acute IM plasma resulted in loss of plasmacytoid DCs, and further studies with individual cytokines showed that exposure to interleukin 10 could replicate this effect. Our data provide important mechanistic insight into dysregulated immune homeostasis during acute IM.

Keywords: herpesvirus; acute infection; cytokines; immune regulation; virus; dendritic cells; immune dysregulation; anti-viral immunity.

Epstein-Barr virus (EBV) infection commonly occurs in childhood, but delayed onset tends to manifest as infectious mononucleosis (IM), a clinical form of EBV infection [1, 2]. It is not known why some individuals are more likely than others to develop clinical symptoms from delayed infection [3]. Previous studies in healthy asymptomatic virus carriers have shown that EBV-specific CD8+ T cells play a crucial role in controlling latent EBV infection [4]. However, during acute IM, despite massive expansion of EBV lytic antigen-specific CD8+ T cells, viral replication is poorly controlled [5]. During the last 3 decades, while much emphasis has been placed on the role of antiviral T-cell immunity in controlling EBV infection, studies in other viral infections have shown that early immune priming plays an important role in controlling primary viral infection [6–9]. Of specific importance has been the realization that innate immune responses, particularly those mediated through the activation and maturation of dendritic cells (DCs) and the production of proinflammatory cytokines, can have a qualitative impact on the development of adaptive immune responses [7, 8, 10].

To investigate the possibility that immune dysregulation during acute IM may be linked to the early events of host-virus interactions, we performed cross-sectional and longitudinal analyses of circulating DCs in a cohort of human subjects that included patients with acute IM, and healthy EBV carriers. Our analyses demonstrated the almost complete absence of circulating plasmacytoid DCs (pDCs) and myeloid DCs (mDCs) that may be linked to the increased expression of proinflammatory cytokines including interleukin 10 (IL-10) during acute IM. Data presented here provide novel insight into dramatic changes in DC populations during primary EBV infection that probably have significant implications for adaptive immune regulation.

MATERIALS AND METHODS

Study Cohort
This study included 32 adult volunteers from Brisbane, Australia; Minneapolis, Minnesota; and Birmingham, United Kingdom, of whom 22 had a diagnosis of acute IM. Longitudinal samples were collected at the time of diagnosis (all 22 individuals), during the early recovery phase (<6 months after IM; 12 individuals), or long term after recovery (>6 months after IM; 17 individuals). At each time point, 40 mL of peripheral blood was collected, and peripheral blood mononuclear cells (PBMCs) were prepared using a Ficoll-Paque gradient. Ten healthy EBV-seropositive individuals were recruited as controls. The age range was 16.1–32.1 years (median, 19.7 years; mean, 20.7 years) for patients with acute IM, and 22–28 years (median, 24.5; mean, 25.3 years) for asymptomatic healthy carriers.

The diagnosis of acute IM was confirmed by the presence of anti-viral capsid antigen immunoglobulin M antibodies, and the clinical symptoms included sore throat, cervical lymphadenopathy, upper respiratory tract symptoms, headache, fever,
body aches, and abdominal pain. Each patient was scored based on the severity of illness, as described elsewhere, and these scores ranged from 0 (asymptomatic) to 6 (essentially bedridden) [11].

**Ethics Statement**
This study was approved by the QIMR Berghofer, University of Birmingham, and University of Minnesota human research ethics committees. Each volunteer participating in this study signed a written consent form that was approved by the committee for the relevant institution.

**Flow Cytometric Analysis of DC Subsets**
The frequency of DC subsets during EBV infection was studied using the 4-color dendritic value bundle (BD Biosciences). PBMCs were stained with a cocktail of lineage-specific antibodies and anti–HLA-DR, anti-CD11c, and anti-CD123 antibodies. The lineage cocktail included FITC-labeled antibodies to CD3, CD14, CD16, CD19, CD20, and CD56 to eliminate T cells, B cells, natural killer cells, and monocytes.

Two subsets of peripheral blood DCs (mDCs and pDCs) were identified; mDCs were characterized as lineage-negative (CD3<sup>−</sup>CD14<sup>−</sup>CD16<sup>−</sup>CD19<sup>−</sup>CD20<sup>−</sup>CD56<sup>−</sup>) populations expressing high levels of HLA-DR (peridinin-chlorophyll protein) and CD11c (allophycocyanin), and pDCs were characterized as lineage-negative HLA-DR<sup>hi</sup> cells expressing CD123 (phycoerythrin or BV421). The data were acquired and analyzed using flow cytometry and FlowJo software (version 9.6.4) (TreeStar). The proportion of each DC population in total PBMCs was then determined, and the absolute number of mDCs and pDCs per

![Figure 1](https://academic.oup.com/jid/article-abstract/212/12/1957/2911946)

**Figure 1.** Acute Epstein-Barr virus infection is associated with reduction of peripheral blood dendritic cell (DC) populations. Peripheral blood mononuclear cells (PBMCs) from patients with infectious mononucleosis (IM) and healthy virus carriers were assessed for the frequency of plasmacytoid DCs (pDCs) and myeloid DCs (mDCs), using the 4-color dendritic value bundle from BD Biosciences as outlined in “Materials and Methods” section. A, Representative analysis of the gating strategy for CD123<sup>+</sup> pDCs and CD11C<sup>+</sup> mDCs from a patient with IM during acute infection and after convalescence and from a latent virus carrier. B, C, Box-and-whisker plots represent the mean and range of either pDCs (B) or mDCs (C) in PBMCs during acute infection, during convalescence of <6 or >6 months, and in latent virus carriers. Dots represent data from individual donors. D, The frequency of DC populations was assessed in patients with acute infection based on the severity of infection (SOI) score (low [0–3] vs high [4–6]). Statistical analysis was performed using the non-parametric Kruskal–Wallis test for multiple comparisons or the non-parametric Mann–Whitney test for single comparisons. *P<.05; †P<.01; ‡P<.001.
liter of blood was extrapolated based on the total number of PBMCs per liter.

To test the effect of cytokines on blood DCs, PBMCs from healthy virus carriers were cultured in serum-free OpTmizer medium in the presence of interleukin 3 (IL-3) and lipopolysaccharide (LPS). These cells were incubated either with 50% vol/vol plasma from patients with acute IM and healthy virus carriers or with different cytokines, interleukin 4 (10 ng/mL), interleukin 6 (IL-6; 10 ng/mL), IL-10 (500 pg/mL), interleukin 12 (IL-12; 10 ng/mL), interferon (IFN) γ (100 IU/mL), tumor necrosis factor (TNF; 50 ng/mL), and transforming growth factor (TGF) β (10 ng/mL) for 24 hours at 37°C. The cells were then analyzed using flow cytometry with DC-specific surface markers and annexin V staining. The relative mean fluorescence intensity for HLA-DR was calculated based on mock-treated control samples.

**Statistical Analysis**

Statistical analysis was performed using Prism 6 software (GraphPad Software). Statistical significance was determined the nonparametric Kruskal–Wallis 1-way analysis of variance with Dunn multiple comparisons test or with the nonparametric Mann–Whitney test for single comparisons. Data were considered statistically significant at \( P < .05 \).

**RESULTS AND DISCUSSION**

To characterize blood DC subsets during acute EBV infection and after convalescence, lineage-negative (CD3−CD14−CD16−CD19−CD20−CD56−) HLA-DR+ PBMCs were assessed for the expression of CD11c and CD123 [12, 13]. Representative data presented in Figure 1A show the gating strategy for DC subset analysis. Our analysis of different DC subsets showed a significant reduction in the frequency of HLA-DRhi CD123+ pDCs and CD11c+ mDCs during acute IM (Figure 1B and 1C). The reduction in pDCs was more pronounced than that of mDCs, with the majority of patients with IM showing a complete loss of this subset. Analysis of the absolute number of pDCs and mDCs confirmed the significant reduction in peripheral

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**Figure 2.** Inflammatory cytokines in plasma of patients with infectious mononucleosis (IM) impairs dendritic cell (DC) activation and survival. A, Box-and-whisker plots showing the mean and range of frequency of plasmacytoid DCs (pDCs) after incubation with acute IM and healthy Epstein-Barr virus (EBV) carrier plasma samples. B, Summary of the data on HLA-DR expression on lineage-negative CD123+ cells after exposure to multiple acute IM and healthy EBV carrier plasma samples. C, Box-and-whisker plots showing the mean and range of frequency of pDCs after incubation with recombinant human interleukin 10 (IL-10). D, Summary of the data on HLA-DR expression on lineage-negative CD123+ cells after exposure to recombinant human IL-10. Relative mean fluorescence intensity (ΔMFI) for HLA-DR was calculated based on mock-treated control samples. Statistical analysis was performed using the non-parametric Mann–Whitney test for single comparisons. * \( P < .05 \); † \( P < .01 \); ‡ \( P < .001 \). Abbreviation: PBMCs, peripheral blood mononuclear cells.
DC populations during acute EBV infection (Supplementary Figure 1A and 1B).

Furthermore, patients with acute IM with clinical severity scores of 4–6 showed fewer pDCs and mDCs than patients with IM who displayed milder clinical symptoms (clinical severity score, 0–3) (Figure 1D). The frequency of pDC and mDC populations gradually increased during convalescence, with long-term post-IM values similar to those seen in healthy virus carriers (Figure 1B and 1C). Interestingly, recovery of pDCs was much slower than that of mDCs. These results indicate that patients with acute EBV infection show dramatic loss of all circulating DC subsets, and these observations are consistent with previously published data showing a lack of IFN-α in plasma samples from patients with IM [11].

We then set out to delineate a potential mechanism for the dramatic reduction in circulating DC subsets. Because our initial studies showed that the reduction in DCs was not due to direct infection of these cells with EBV (data not shown), we hypothesized that the DC loss may be related to the inflammatory response during acute EBV infection. Previous studies by Balfour et al [11] had shown significantly elevated levels of inflammatory cytokines, including IL-6, IL-10, TNF, IL-12, IFN-γ, IFN-β, and TGF-β. To explore the possibility that inflammatory cytokines in peripheral blood may be contributing to DC loss, we first exposed DCs from healthy virus carriers to plasma samples from patients with acute IM. As shown in Figure 2A, although treatment with plasma samples from healthy virus carriers had minimal impact on circulating DCs after incubation with IL-3 and LPS, we observed a significant reduction in the lineage-negative HLA-DRhi CD123high pDC population after exposure to acute IM plasma, whereas mDCs were not significantly affected (data not shown).

We next assessed HLA-DR expression in the total lineage-negative CD123high population and observed reduced HLA-DR expression in these cells (Figure 2B and Supplementary Figure 2A). To identify the potential cytokine(s) responsible for the observed effect, we tested each cytokine individually to assess its effect on different DC subsets. PBMCs from healthy individuals were incubated with IL-3 and LPS in the presence of IL-6, IL-10, TNF, IL-12, IFN-γ, or TGF-β. Although IL-6, TNF, IL-12, IFN-γ, and TGF-β had minimal impact on circulating DC populations (Supplementary Figure 2B), exposure to IL-10 was associated with a significant loss in pDCs and reduced HLA-DR expression in lineage-negative CD123high cells (Figure 2C and 2D and Supplementary Figure 2C). Furthermore, these pDCs also showed significantly increased apoptosis (data not shown). Although we observed a direct effect of IL-10 on DC survival, other host-derived and/or viral derived mediators may also contribute to changes in DC populations during IM.

Taken together, these studies provide important insight into a previously unappreciated mechanism of immune dysregulation during acute EBV infection. Previous studies have shown that DCs are critical mediators of the adaptive and immune responses and proinflammatory signals, including IL-12 and IFN-α, that are critical for the differentiation of naive and memory T cells. One unique aspect of primary EBV infection is the lack of IFN-α in the plasma during symptomatic infection despite elevated levels of other inflammatory cytokines [11]. This correlates with our findings demonstrating the almost total absence of HLA-DRhi peripheral pDCs during acute IM, because pDCs are the main producers of IFN-α during viral infection [14]. Future studies into the kinetics of DC loss before the onset of symptomatic IM should provide further insight into the role of DC loss in uncontrolled viremia and disease manifestation during acute IM.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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