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A Blunted Blood Plasmacytoid Dendritic Cell Response to an Acute Systemic Viral Infection Is Associated with Increased Disease Severity

Sathit Pichyangkul,* Timothy P. Endy,* SiriPen Kalayanarooj,† Ananda Nisalak,* Kosol Yongvanitchit,* Sharone Green,§ Alan L. Rothman,§ Francis A. Ennis,§ and Daniel H. Libraty‡

At least two distinct human dendritic cell (DC) subsets are produced in the bone marrow and circulate in the peripheral blood in infected hosts. Precursor myeloid DCs (pre-mDCs) and plasmacytoid DCs (PDCs). Both lineages of DCs are instrumental in antiviral innate immunity and shaping Th1 adaptive immune responses. PDCs are the most potent IFN-α-producing cells to viral pathogens. Dengue, an acute flavivirus disease, provides a model to study DC responses to a self-limited human viral infection. We analyzed circulating DC subsets in a prospective study of children with dengue across a broad range of illness severities: healthy controls; mild, non-dengue, presumed viral infections; moderately ill dengue fever; and, the most severe form of illness, dengue hemorrhagic fever. We also examined PDC responses in monkeys with asymptomatic dengue viremia and to dengue virus exposure in vitro. The absolute number and frequency of circulating pre-mDCs early in acute viral illness decreased as illness severity increased. Depressed pre-mDC blood levels appeared to be part of the typical innate immune response to acute viral infection. The frequency of circulating PDCs trended upward and the absolute number of circulating PDCs remained stable early in moderately ill children with dengue fever, mild other, non-dengue, febrile illness, and monkeys with asymptomatic dengue viremia. However, there was an early decrease in circulating PDC levels in children who subsequently developed dengue hemorrhagic fever. A blunted blood PDC response to dengue virus infection was associated with higher viremia levels, and was part of an altered innate immune response and pathogenic cascade leading to severe disease.

Dendritic cells (DCs) are a family of bone marrow-derived cells that comprise <1% of total cells in peripheral blood and have a pivotal role in the development of innate and adaptive immune responses (1). At least two distinct human DC subsets have been characterized: myeloid DCs (mDCs) and plasmacytoid DCs (PDCs). Immature mDCs reside in non-lymphoid organs and arise from peripheral blood CD11c+ mDC precursors (pre-mDCs) or CD14+ monocytes. Immature mDCs are characterized by effective Ag uptake. Following maturation, they acquire dendritic morphology, potent Ag presentation capacity, and marked ability to stimulate Ag-specific T cell responses (2, 3).

PDCs are CD123+ and found in the bone marrow, peripheral blood, and T cell areas of secondary lymphoid organs. With appropriate stimuli, they can migrate directly from the peripheral blood to lymph nodes and lymphoid organs. PDCs are characterized by strong Ag-presenting potential, lymphoid morphology, and high virus-induced and CD40-mediated IFN-α secretion (2, 3, 5, 6). Thus, mDCs, and particularly PDCs, are instrumental in antiviral innate immunity and shaping Th1 adaptive immune responses to viral pathogens.

The dengue viruses (DVs) are a group of four antigenically related, mosquito-borne flaviviruses that produce a self-limited systemic infection. Host immune responses play a significant role in determining the severity of illness. Dengue hemorrhagic fever (DHF) is the most severe, and potentially life-threatening, form of DV infection. DHF is characterized by the rapid onset of plasma leakage and coagulopathy near the time of defervescence and viremia resolution. DHF is associated with higher viremia levels than less severe disease (dengue fever (DF)) (7–9). Most DHF cases also occur after sequential, heterotypic, DV infections (10–12), suggesting an immune-mediated enhancement of disease severity.

Dengue provides a model to study the in vivo behavior of DC subpopulations in the response to an acute, self-limited, human viral infection. Earlier clinical studies examining in vivo innate antiviral immune responses have focused on circulating pre-mDCs and PDCs in HIV, a chronic progressive viral infection (13–17). We studied the in vivo behavior of circulating pre-mDCs and PDCs in a prospective study of children with acute febrile illnesses and dengue (18). Our study is novel in that we examined peripheral blood DC subsets early and across a broad range of illness severities, ranging from healthy controls to mild, non-dengue, presumed viral infections, to moderately ill DF and severe DHF. We were
able to combine in vivo human data along with in vitro and animal data on the PDC responses to DV infection, and found that a blunted blood PDC response to the acute systemic DV infection was a key early step in the pathogenic cascade toward DHF.

Materials and Methods

Abs and reagents

mAbs against CD3, CD11c, CD14, CD16, CD56, CD20, CD123, and HLA-DR were purchased from BD Biosciences (San Jose, CA). Culture media RPMI 1640 and gentamicin were obtained from Life Technologies (Gaithersburg, MD) and SoloPak Laboratories (Elk Grove Village, IL), respectively. FACS 

Generation, purification, and identification of DC subpopulations

Peripheral venous blood was obtained from healthy human donors. PBMC were obtained by centrifugation using Histopaque-1077. T cells were removed by rosetting with neuraminidase-treated sheep RBC. Pre-mDCs were purified by staining T cell-depleted populations with mAbs against lineage markers (CD3, CD14, CD16, CD56, and CD20) (FITC) and CD11c (PE). Cells that were negative for lineage markers, but strongly positive for CD11c expression, were sorted with FACS Vantage (BD Biosciences, Mountain View, CA). The sorted lin CD11c+ cells had typical myeloid morphology (see Fig. 1a). For PDC purification, T cell-depleted populations were stained with mAbs against CD123 (PE) and HLA-DR (FITC). CD123+HLA-DR cells were then stained with FACS Vantage. The sorted cells had typical plasma cell-like morphology (see Fig. 1b). Monoocyte-derived immature mDCs (mo-mDCs) were generated from flow cytometry sorted CD11c+ monocytes that had been cultured with rGM-CSF (50 ng/ml) and rIL-4 (500 U/ml) for 5–7 days. All DC preparations were ~99% pure.

To detect circulating pre-mDCs and PDCs, fresh whole blood from study subjects was aliquoted (200 µl) and then stained for 30 min at room temperature with two mAb mixture combinations: 1) lineage mixture (anti-CD3, CD14, CD16, CD56, and CD20) (FITC) + anti-HLA-DR (PerCP) + anti-CD11c (PE); or 2) lineage mixture (anti-CD3, CD14, CD16, CD56, and CD20) (FITC) + anti-HLA-DR (PerCP) + anti-CD123 (PE). Mouse isotype control mAbs were used as controls. The stained blood samples were treated with RBC lysing solution (BD Biosciences) for 10 min at room temperature in the dark. The remaining cells were washed in PBS and then reconstituted in 1% paraformaldehyde. For analysis, 100,000–200,000 cells were analyzed using FACS Vantage (BD Biosciences). lin HLA-DR CD11c+ and lin HLA-DR CD123+ cells were identified as pre-mDCs and PDCs, respectively.

DV infection of DC subpopulations

Dengue type 2 virus strain 16681 (D2V 16681) was grown and propagated from a germicidal lamp (60 min) or heat treatment (65°C). In some experiments, dengue 2 virus (D2V) was inactivated by UV irradiation (16681 (multiplicity of infection (MOI) ranging from 1 to 10) exposed to D2V 16681 (multiplicity of infection (MOI) ranging from 1 to 10) and CD20) (FITC) and CD123 (PE). Cells that were negative for lineage markers, but strongly positive for CD11c expression, were sorted with FACS Vantage. The sorted cells had typical plasma cell-like morphology (see Fig. 1a). For PDC purification, T cell-depleted populations were stained with mAbs against CD123 (PE) and HLA-DR (FITC). CD123+HLA-DR cells were then stained with FACS Vantage. The sorted cells had typical plasma cell-like morphology (see Fig. 1b). Monoocyte-derived immature mDCs (mo-mDCs) were generated from flow cytometry sorted CD11c+ monocytes that had been cultured with rGM-CSF (50 ng/ml) and rIL-4 (500 U/ml) for 5–7 days. All DC preparations were ~99% pure.

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Rhesus macaque study design

A placebo-controlled trial of rIFN-α treatment for dengue viremia was conducted in juvenile rhesus macaques (Macaca mulatta) at the Armed Forces Research Institute of Medical Sciences (Bangkok, Thailand). The animal use protocol was approved by the Institutional Animal Care and Use Committee at Armed Forces Research Institute of Medical Sciences and the U.S. Army Medical Research and Materiel Command (Fort Detrick, MD). In three rhesus monkeys who received placebo treatment, blood PDC levels were determined using multiparametric flow cytometry as previously described. The monkeys were DV and Japanese encephalitis virus neutralizing Ab seronegative at the start of the trial. On study day 0, the monkeys were bled and then inoculated (s.c.) with 1 × 107 PFU of D2V strain S16003. Blood was obtained daily over the next 11 days. A 0.5-ml aliquot of whole blood from each bleed (including study day 0) was used to measure the PDC levels. The onset and duration of viremia was determined by identifying D2V RNA in daily serum samples using a serotype-specific RT-PCR assay (19).

Clinical study design

Details of the investigational protocol have been published previously (18). Children included in this study were seen at the Queen Sirikit Institute of Child Health (Bangkok, Thailand) in 2000–2001. The investigational protocol was approved by the Institutional Review Boards of the Thai Ministry of Public Health, the Office of the U.S. Army Surgeon General, and the University of Massachusetts Medical School (Worcester, MA). Parents or guardians of all study subjects gave written informed consent.

Enrollment criteria were age 6 mo-14 years, a febrile illness with <72 h of symptoms, no hypotension or shock, and no other obvious source of infection. Children were observed in hospital until at least 1 day of defervescence. Venous blood samples were drawn daily up to the day of defervescence or for a maximum of 5 consecutive days, and 10 days after enrollment. Study day 1 was the calendar day of hospital presentation and study enrollment. Illness day 1 was the calendar day when fever began, ascertained by history. Blood pre-mDC and PDC levels were measured, as described above, in 0.5-ml fresh whole blood aliquots taken on study days 2 (corresponding to illness day 3 or 4) and 10. In a subset of these children with dengue, pre-mDC and PDC levels were also measured at a 1 year follow-up visit. Healthy control blood samples were obtained from previous study subjects (not involved in DC subset analysis) returning for 1 or 2 visits.

DV infection was identified by a serotype-specific RT-PCR assay (19) on study day 1 plasma samples. Primary or secondary DV infections were defined using previously established serologic criteria for IgM/IgG ELISAs and hemagglutination-inhibition assays in paired acute and convalescent samples (20, 21).

Clinical categories

1) Other, nondengue febrile illnesses (OFI): An acute febrile illness without evidence of DV infection, routine bacterial infection, or malaria, and presumed to be an acute viral infection. In our experience, other conditions such as scrub typhus or leptospirosis are rare in this Bangkok-based cohort. 2) Children with DV infections were classified into DF and DHF, according to our previously published (8) and World Health Organization (21) criteria.

Quantification of dengue viremia

The circulating level of DV RNA (viremia) was quantified in serial plasma samples using a serotype-specific fluorogenic RT-PCR assay (22). The RT-PCR products cloned into pNOT vectors were used as quantification standards (provided by Dr. H. S. Hought, Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, D.C.). Interassay precision was monitored by positive and negative controls on every 96-well plate. All samples were assayed in triplicate, in a blinded fashion, and quantified on the linear portion of the standard curve. Viremia levels were expressed as DV genome equivalent cDNA copies per milliliter (genome equivalent per milliliter). The maximum viremia level was defined as the highest plasma viremia level measured during illness.

Statistical analysis

We used the Student t test for comparisons between two normally distributed continuous variables and the Mann-Whitney U test for comparisons between two continuous variables not normally distributed. We used the Wilcoxon signed-rank test for paired comparisons and the Kruskal-Wallis test for multiple comparisons among continuous variables not normally distributed. χ2 analysis was used for comparisons among proportional data.
We analyzed the response of mo-mDCs, pre-mDCs, and PDCs to D2V exposure. mo-mDCs were generated from CD14+ monocytes, while circulating pre-mDCs and PDCs were identified and sorted by multiparametric flow cytometry. The sorted pre-mDCs (lin CD11c<sup>bright</sup>) had myeloid morphology (Fig. 1a), and, when reanalyzed, were found to be HLA-DR<sup>+</sup> and CD123<sup>bright</sup> (data not shown). The sorted PDCs (CD123<sup>bright</sup>HLA-DR<sup>-</sup>) had typical plasma cell-like morphology (Fig. 1b), and, when reanalyzed, were found to be lin<sup>-</sup> and CD11c<sup>-</sup> (data not shown). As previously reported (23–25), mo-mDCs were permissive to D2V infection (% D2V-infected mo-mDCs at 48 h = 60 ± 8%, mean ± SE, n = 4). By contrast, pre-mDCs and PDCs were poorly permissive or not permissive to D2V infection (% D2V-infected pre-mDCs at 48 h = 6 ± 2%, mean ± SE, n = 4) (%D2V-infected PDCs at 48 h = 0.4 ± 0.04%, mean ± SE, n = 4).

Secretion of IL-12 and IFN-γ by DCs are key steps in the development of effective cell-mediated immunity to viral infections (26). At MOI = 10, minimal amounts of IL-12p70 were detected in the 48-h cell culture supernatants of D2V-exposed mo-mDCs and pre-mDCs (data not shown). No IL-12p70 was detected in the 48 h cell culture supernatant of D2V-stimulated PDCs. Significant release of IFN-α was observed solely in D2V-exposed PDC cultures and showed a dose-dependent response (Fig. 2a). Even though PDCs were not productively infected with D2V, live virus was required to induce IFN-α secretion. UV- or heat-inactivated D2V failed to induce IFN-α release (Fig. 2b).

Table 1. Study population characteristics

<table>
<thead>
<tr>
<th>Parameter&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Healthy Controls</th>
<th>OFI</th>
<th>DF</th>
<th>DHF</th>
<th>Overall p Value</th>
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<tr>
<td>n</td>
<td>10</td>
<td>13</td>
<td>13</td>
<td>9</td>
<td>0.06</td>
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<tr>
<td>Age (y) median (range)</td>
<td>8.5 (4.8–16.8)</td>
<td>7.1 (4.4–10.3)</td>
<td>8.3 (5.0–13.7)</td>
<td>10.5 (5.9–13.3)</td>
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<td>Sex M:F ratio</td>
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<td>4:9</td>
<td>9:4</td>
<td>6:3</td>
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<td>Maximum temperature (°C)</td>
<td>39.7 ± 0.1</td>
<td>39.9 ± 0.1</td>
<td>40.5 ± 0.1</td>
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<td>Minimum pulse pressure (mm Hg)</td>
<td>32 ± 1</td>
<td>28 ± 1</td>
<td>28 ± 2</td>
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<tr>
<td>Maximum % hemocencentration</td>
<td>13 ± 2</td>
<td>13 ± 1</td>
<td>25 ± 4</td>
<td>0.002</td>
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<tr>
<td>Minimum platelet count (&lt;10&lt;sup&gt;7&lt;/sup&gt;/μl)</td>
<td>226 ± 20</td>
<td>122 ± 15</td>
<td>42 ± 25</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Minimum WBC count (&lt;10&lt;sup&gt;3&lt;/sup&gt;/μl)</td>
<td>5869 ± 830</td>
<td>2546 ± 237</td>
<td>2677 ± 407</td>
<td>&lt;0.001</td>
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<td>Maximum AST&lt;sup&gt;b&lt;/sup&gt; level (U/ml)</td>
<td>32 ± 3</td>
<td>79 ± 18</td>
<td>136 ± 35</td>
<td>0.002</td>
<td></td>
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<tr>
<td>Maximum ALT&lt;sup&gt;b&lt;/sup&gt; level (U/ml)</td>
<td>14 ± 1</td>
<td>54 ± 12</td>
<td>103 ± 72</td>
<td>&lt;0.001</td>
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<tr>
<td>Minimum albumin level (g/dl)</td>
<td>4.6 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>3.9 ± 0.3</td>
<td>0.006</td>
<td></td>
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</tbody>
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<sup>a</sup> Values are mean ± SE, unless otherwise stated.
<sup>b</sup> AST = aspartate aminotransferase; ALT = alanine aminotransferase.
Freshly isolated PBMC produced low amounts of IFN-α in response to D2V stimulation (5 × 10^6 PBMC infected with D2V at MOI = 10, cell culture supernatant IFN-α <10 and 111 pg/ml at 48 h, n = 2). Enrichment of PDCs, by removal of T cells from the PBMC, markedly increased D2V-induced IFN-α production (149 and 752 pg/ml, respectively). Positive selection and depletion of CD123 bright HLA-DR+ PDCs completely abrogated the D2V-induced IFN-α response in T cell-depleted PBMC (IFN-α <10 pg/ml at 48 h, n = 2). These results show that PDCs are the major IFN-α-producing cells of PBMC in response to D2V challenge.

Circulating pre-mDCs in acute DV infection

We analyzed the levels of circulating pre-mDCs in healthy controls (n = 10), and at an early time point (illness day 3 or 4) in children with acute nondenve (presumed viral) infections (OFI, n = 13) and acute DV infections (n = 22). The study population characteristics are summarized in Table I. Measures of illness severity generally increased in the order: healthy controls → OFI → DF → DFH. Among the children with dengue, there were 12 dengue 1 virus (D1V), 7 D2V, 2 dengue 3 virus (D3V), and 1 dengue 4 virus (D4V) infections (no difference in distribution of DV serotypes between DF and DHF, p = 0.3, data not shown). Seventy-seven percent (17 of 22) had secondary DV infections.

The total leukocyte count was lower on illness day 3 or 4 in the children with dengue compared with those with OFI or healthy controls. There was no difference in the mean total leukocyte or mononuclear cell counts early in dengue illness between DF and DHF (Fig. 3a and data not shown). Moving from healthy controls → OFI → DF → DHF, the percent of pre-mDCs in circulating leukocytes and the absolute number of circulating pre-mDCs progressively decreased (Fig. 3, b and c). At convalescence 1 wk later, the absolute number and frequency of circulating pre-mDCs returned to healthy control levels in all groups (data not shown).

Circulating PDCs in a nonhuman primate model of acute DV infection

We next examined circulating levels of PDCs in three rhesus macaques with acute DV infection. The only animals, besides man, that are naturally infected by DVs, and that can be experimentally infected by the parenteral route, are nonhuman primates (27). Many virologic, serologic, and clinical responses to DV infection are similar between rhesus macaques and humans (28). However, macaques or other nonhuman primates do not develop DHF with DV infection. They only exhibit mild or asymptomatic disease, and thus demonstrate good host control of DV infection. We identified the rhesus monkey equivalents of circulating human PDCs by using the same antihuman PDC Ab mixture (lin CD11c+CD123+HLA-DR+), as has been described in a previous study (29). We also demonstrated that rhesus monkey PDCs were functionally equivalent to human PDCs. Purified rhesus monkey PDCs (lin CD11c+CD123+HLA-DR+) produced IFN-α when stimulated by the human PDC-

FIGURE 3. The frequency and absolute number of circulating pre-mDCs decrease with increasing disease severity in acute viral infections. WBC (a), percent pre-mDC (b), and absolute circulating pre-mDC levels (c) were measured in acute illness blood samples (illness day 3 or 4) from four groups of children: 1) healthy controls, 2) OFI, 3) DF, and 4) DHF. Horizontal lines represent median values, WBC per microliter, percent pre-mDC, and pre-mDC per milliliter levels were significantly different among the four groups (p < 0.001, Kruskal-Wallis test for multiple group comparisons). *, p < 0.05 compared with healthy controls; †, p < 0.05 compared with OFI; ‡, p < 0.05 compared with DF (posthoc Dunnett C test for pairwise adjustment).

FIGURE 4. The frequency of circulating PDCs increases following D2V inoculation of rhesus monkeys. Three rhesus monkeys were inoculated with 1 × 10^9 PFU D2V strain S16803 (s.c.) on day 0 (arrows), after blood was taken for PDC determination. The PDC frequency and plasma D2V RNA levels were measured in serial daily blood samples for 11 days after D2V inoculation. Days where plasma D2V RNA was detectable are indicated with (+) signs.
specific stimulus, CpG ODN 2216 (data not shown). Positive selection and depletion of CD123 brightly HLA-DR+ PDCs from T cell-depleted rhesus monkey PBMC abrogated the CpG ODN-induced IFN-α response (from 892 to 24 pg/ml IFN-α at 48 h).

Shortly after s.c. inoculation with D2V, the percent of circulating PDC levels in rhesus macaques rose, peaked near the onset of detectable viremia, and slowly decreased to baseline levels thereafter (Fig. 4). We were unable to obtain enough whole blood to measure total leukocyte counts in the rhesus macaques, so absolute circulating levels of PDCs were not calculated. However, a previous study has shown that rhesus macaques, like humans, develop a transient leukopenia following D2V infection (28).

Circulating PDCs and dengue disease severity

In our study population (Table I), we measured circulating PDC levels early in acute illness, and at 1 wk and 1 year convalescence. As illness severity increased from healthy controls to OFI and DF, there was a trend of increasing the percent of circulating PDCs.
PBMC viability was 50–95% in healthy controls. An increased susceptibility to apoptosis might also develop in pre-mDCs with increasing illness severity and greater circulating levels of inflammatory mediators (32, 33). Underproduction of pre-mDCs by the bone marrow may also occur in acute viral infections. Depressed bone marrow production, migration to peripheral or lymphoid tissues, and apoptosis. We postulate that enhanced migration of pre-mDCs to peripheral tissue inflammatory sites is the predominant cause of a drop in blood levels. mDCs in nonlymphoid tissues become activated and develop into potent APCs as they take up virus (24, 25, 31). Inflammatory mediators (e.g., TNF-α, IL-6) and chemoattractive mediators (e.g., RANTES, MCP-1, and SDF-1) are produced so that pre-mDCs are recruited to the sites of inflammation where Ag-bearing mDCs are migrating to regional lymph nodes and lymphoid organs (32, 33). Underproduction of pre-mDCs by the bone marrow may also occur in acute viral infections. Depressed bone marrow release of pre-mDCs is unlikely to significantly affect the circulating pre-mDC levels, as pre-mDC levels dropped between DF and DHF, when concurrent total leukocyte and mononuclear cell counts remained unchanged. An increased susceptibility to apoptosis might also develop in pre-mDCs with increasing illness severity and greater circulating levels of inflammatory mediators (34).

Discussion

DC responses to viral pathogens are an important first step in shaping an effective innate antiviral response, and linking innate and adaptive immunity. In HIV, a chronic systemic viral infection, the absolute number and frequency of circulating pre-mDCs have been reported to decrease as disease severity increases (13, 16, 17). Early in dengue, a self-limited systemic viral infection, we also observed that the absolute number and frequency of circulating pre-mDCs decreased as disease severity increased (DF→DHF). When examined in the context of mild, nondengue, presumed acute viral infections (OFI) and healthy controls, our data suggest that depressed circulating pre-mDC levels reflect a typical innate immune response to acute viral infections. Circulating levels of DC subsets are determined by a balance between their bone marrow release of pre-mDCs is unlikely to significantly affect the circulating pre-mDC levels, as pre-mDC levels dropped between DF and DHF, when concurrent total leukocyte and mononuclear cell counts remained unchanged. An increased susceptibility to apoptosis might also develop in pre-mDCs with increasing illness severity and greater circulating levels of inflammatory mediators (34). Additional studies that address the bone marrow production, IFN-α secretion by PDCs from patients with acute DV infection

The functionality of circulating PDCs in dengue was assessed by measuring IFN-α release following exposure to a potent and PDC-specific stimulus, CpG ODN 2216 (30). No significant difference in CpG-stimulated PDC IFN-α secretion was seen between DF and DHF, despite a slightly higher number of PDCs in the DF PBMC. CpG-stimulated PDC IFN-α secretion was lower in children with dengue compared with healthy controls (Fig. 7). The viability of PBMC isolated from all children with acute febrile illnesses was lower than healthy control PBMC (50–80% viability at 48 h in OFI, DF, and DHF compared with >95% viability in healthy controls by trypan blue exclusion).

FIGURE 7. CpG ODN 2216 can stimulate IFN-α release from PBMC in all patient groups. PBMC (1 x 10^6) were stimulated with CpG ODN 2216 (10 μg/ml), a PDC-specific stimulus of IFN-α release (30). Supernatant IFN-α levels were measured at 48 h from healthy control PBMC cultures (n = 3), and PBMC obtained on illness day 3 from children with OFI (n = 4), DF (n = 5), and DHF (n = 4). By trypan blue exclusion, PBMC viability was 50–80% at 48 h in DF, and DHF compared with >95% viability in healthy controls. a, A value of p ≤ 0.03 compared with healthy controls.
migrate, and apoptotic mechanisms in pre-mDCs during viral infections are needed.
Although pre-mDCs and mDCs appear to play a primary role in host inflammatory responses (32), PDCs are more specialized effectors that play a key role in innate antiviral immunity (35). As with HSV (36), influenza virus (5), adenovirus (37), and HIV (38), PDCs were the most potent and significant source of IFN-α production in vitro following D2V exposure. Unlike inactivated HSV or influenza virus (13), inactivated D2V was unable to stimulate PDC IFN-α release. This suggests that dsRNA or nonstructural viral proteins are involved in D2V-induced IFN-α production from PDCs, even though a productive infection in PDCs was not seen at 48 h.
We observed an increase in the frequency of circulating PDCs soon after DV infection in nonhuman primates who developed very mild or asymptomatic disease. We also observed a similar pattern in children with mild, nondengue, presumed acute viral infections (OVI), and children with mild illness due to DV infection (DF). We postulate that an early increase in the frequency of circulating PDCs, or maintenance of absolute PDC blood levels in the face of leukemia, reflects an appropriate PDC response to viral infection. An increase in the frequency of circulating PDCs has also been reported in HIV-infected long-term nonprogressors compared with HIV-infected individuals with AIDS (14). An early rise in circulating PDC frequency was absent in the children who subsequently developed DHF, and suggests that the inability to maintain PDC levels in response to an acute systemic viral infection may lead to poor clearance of viral replication and severe disease. In secondary DV infections, depressed PDC levels early in illness contributing to higher viral burdens may result in enhanced activation of cross-reactive memory T lymphocytes and the development of DHF (39–41).
The possibility that a blunted increase in PDC frequency and a significant drop in PDC levels early in DHF is a consequence of higher viremia levels and increased disease severity seems unlikely. In the nonhuman primes, changes in circulating PDC frequency occurred very soon after DV inoculation and near the onset of detectable viremia. In the study subjects, we were able to measure PDC levels early in illness and antecedent to the maximal expression of clinical disease severity, but after the onset of viremia and peak circulating IFN-α levels (8). Thus, we likely missed the peak changes in PDC levels from baseline, and likely underestimated differences in PDC responses between groups.
PDCs from all patient groups could produce IFN-α in response to CpG ODN 2216, and no differences were seen between DF and DHF. CpG ODN 2216 stimulates PDC IFN-α release by signaling through Toll-like receptor (TLR)9 (30, 42). PDCs can also produce IFN-α in response to ligand binding to TLR7 (42). Whether TLR9-independent IFN-α release from PDCs is involved in DV responses, and differs between DHF and DF, is not known and deserves further study. Also, our IFN-α assay did not measure IFN-α production, and therefore may have underestimated total antiviral IFN levels.
In systemic viral infections like dengue, PDCs are migrating under chemotactic stimuli to lymphoid tissues (lymph nodes and spleen) and extravasating via high endothelial venules (5, 32, 43). To maintain circulating PDC levels, an increase in bone marrow production or a decrease in the rate of apoptosis must be occurring. Unstimulated healthy donor PDCs have a high rate of spontaneous apoptosis in vitro. IL-3 and IFN-α are both produced following viral infection, and are survival factors that can protect PDCs from apoptosis (44). However, our study cannot determine which factors may play a role in maintaining circulating PDC levels early in acute viral infections, and which factors might be impaired in patients who develop DHF following DV infection. Elucidation of host or viral mechanisms that impair PDC responses and contribute to DHF pathogenesis can expand our knowledge about the development of innate pathology in acute viral infections.

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