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Impaired T Cell Proliferation in Acute Dengue Infection

Anuja Mathew,* Ichiro Kurane,† Sharone Green,* David W. Vaughn,‡ Siripen Kalayanarooj,§ Saroj Suntayakorn,¶ Francis A. Ennis,* and Alan L. Rothman3*

Decreased proliferative responses to mitogens and recall Ags have been observed in PBMC obtained during several acute human viral infections. To determine whether cell-mediated responses are altered during acute dengue infection, we examined the proliferative responses of PBMC from children enrolled in a prospective study of dengue infections in Thailand. All responses of PBMC during acute illness were compared with the same patients’ PBMC obtained at least 6 mo after their infection. Proliferative responses to PHA, anti-CD3, tetanus toxoid, and dengue Ags were decreased significantly in PBMC obtained during the acute infection. The proliferative responses to PHA were restored by the addition of gamma-irradiated autologous convalescent or allogeneic PBMC. Cell contact with the irradiated PBMC was necessary to restore proliferation. Non-T cells from the acute PBMC of dengue patients did not support proliferation of T cells from control donors in response to PHA, but T cells from the PBMC of patients with acute dengue proliferated if accessory cells from a control donor were present. Addition of anti-CD28 Abs restored anti-CD3-induced proliferation of the PBMC of some patients. The percentage of monocytes was reduced in the acute sample of PBMC of the dengue patients. Addition of IL-2 or IL-7, but not IL-4 or IL-12, also restored proliferation of acute PBMC stimulated with anti-CD3. The results demonstrate that both quantitative and qualitative defects in the accessory cell population during acute dengue illness result in a depression of in vitro T cell proliferation.

S
everal acute viral infections such as measles, CMV, and HIV have been associated with transient immunosuppression of cell-mediated responses both in vivo and in vitro (1, 2). This immunosuppression has been characterized by decreased T cell proliferative responses to mitogens such as PHA or to specific recall Ags such as purified protein derivative and tetanus toxoid. A number of different mechanisms have been proposed to explain the immunosuppression seen during and following an acute viral infection (3). To date no single cell type or factor has been shown to be solely responsible for the defects seen during an acute viral infection. It is likely, however, that one mechanism may not fully explain the immunologic abnormalities observed and that a series of events in succession may result in the observed depression of cellular immune responses both in vivo and in vitro (4).

The in vitro proliferation of PBMC from patients with measles infection can be improved by the addition of rIL-2, which suggests inadequate production of this cytokine by T cells (5). PBMC from measles patients produce more IL-1 and less TNF than control patients in vitro, which may also contribute to the decreased proliferation seen (6, 7). The lymphopenia seen during measles includes reductions of both T and B cells, but the ratio of CD4/CD8 T cells is not altered (7, 8). Production of IFN-γ is low to normal, and IL-4 levels are elevated in vitro, implying that Th2 cells are activated (7, 9). These immunologic deficiencies occur in the context of significant in vivo immune activation, as evidenced by increases in plasma levels of soluble CD4 (sCD4), sCD8, sIL-2R, and β2 microglobulin.

The role of costimulatory molecules in mediating the immunologic unresponsiveness seen in acute viral infections has not been reported. CD28-B7 interactions have been shown to be essential for initiating immune responses, up-regulating cytokine production, and promoting T cell differentiation in various systems (10). Triggering of the TCR in the absence of costimulatory signals has been shown to inhibit T cell responses in several in vitro systems (11). On the other hand, elevations of the costimulatory molecules B7-1 and B7-2 on APCs have been associated with autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus (12, 13).

Patients with dengue hemorrhagic fever (DHF) have been shown to have elevated circulating levels of IFN-γ, IL-2, sCD4, sCD8, and sIL-2R, which are markers of T cell activation, similar to what has been reported in severe measles infection (14). Children are leukopenic for several days during acute dengue virus infection with a decrease in the absolute number of neutrophils and monocytes (15). The absolute T cell count and the CD4/CD8 ratios are not altered during acute dengue illness. The leukopenia is thought to be related to bone marrow suppression induced by dengue virus (16). This decrease is temporary, and monocyte counts return to normal within a few days. We studied the proliferative responses of PBMC to Ags and mitogens from children with acute dengue to determine whether T cell responses were restored.

4 Abbreviations used in this paper: s, soluble; DF, dengue fever; DHF, dengue hemorrhagic fever.
Our results indicate that in vitro proliferation of T cells to a wide variety of stimuli is suppressed during acute dengue infection. The accessory cells obtained from PBMC during acute infection do not provide an adequate stimulus to enable proliferation of the T cells, whereas the T cells from acute PBMC can proliferate if provided with competent accessory cells. The decreased responses of T cells in the samples of acute PBMC were also partially restored upon the addition of exogenous IL-2 and IL-7. Anti-CD28 Abs improved proliferative responses of CD3-stimulated acute PBMC in some subjects. The decrease in costimulatory and adhesion molecules due to the decreased number of monocytes may contribute to the immunosuppressive effects seen in vitro using PBMC obtained during acute dengue infection. These observations suggest that during acute dengue infection, the principal defect in the ability of T cells to proliferate is due to the accessory cell population.

Materials and Methods
Study subjects and blood samples
PBMC were obtained from children enrolled in a prospective study of dengue infections at the Queen Sirikit National Institute of Child Health (the Bangkok Children’s Hospital, Bangkok, Thailand) and the Kamphaeng Phet Provincial Hospital (Kamphaeng Phet, Thailand) (15, 17). Children were eligible to participate in the study if they were febrile and had no obvious source of infection for <72 h. A child was diagnosed as having acute dengue infection when the serologic tests were positive and/or dengue virus was isolated from the blood. Clinical diagnoses of dengue fever (DF) and DHF were assigned according to WHO criteria (18). Study day 1 was defined as the calendar day on which the subject was enrolled in the study. For these studies, a sample was considered to be an acute sample up to study day 11, although the subjects were not febrile at that time. Convalescent samples were obtained from the same patient 6 mo or later after their acute infection. All sample numbers in this manuscript are denoted by the study days on which the samples were obtained. Fever day 0 was defined as the calendar day during which the temperature fell and stayed <38°C, and days before and after this point were numbered consecutively (fever days −1, −2, etc., occurred before defervescence, and fever days +1, +2, etc., occurred after defervescence). Patient information and the diagnoses are given in Table I. Sample numbers indicate acute (A) or convalescent (C) PBMC and the study day on which they were obtained. The average age of the patients included in the study was 8 yr (SD 2.48). PBMC were separated, cryopreserved, and stored in liquid nitrogen until use. The PBMC were shipped to the University of Massachusetts Medical Center on dry ice for testing.

Proliferation assays
PBMC from both the acute and convalescent phase of infection (10^5/well) from each patient were thawed, resuspended in AIM V medium (Life Technologies, Gaithersburg, MD) supplemented with 10% HuAB serum (AIM/10%), and added to a V-bottom 96-well plate in the presence of PHA (1:1000), anti-CD3 (12F6; provided by Johnson Wong, Massachusetts General Hospital, Boston, MA) (0.1 μg/ml), or the indicated concentrations of other Ags. When cytokines were added to the wells, 10 U/ml rIL-2 (Collaborative Biochemical Products, Bedford, MA), 100 U/ml IL-4 (Genzyme, Cambridge, MA), 100 U/ml IL-7 (Genzyme), or 25 ng/ml IL-12 (Genetics Institute, Cambridge, MA) were added to PHA-stimulated or anti-CD3-stimulated cells. A total of 1 × 10^5 gamma-irradiated (3500 rad) autologous convalescent PBMC or allogeneic PBMC from control donors was added to wells containing PBMC from acute dengue patients, as indicated. The negative controls were cell suspensions in medium alone without any stimulation. Optimal concentrations of Ags to use were determined using control donors. A 1/40 dilution of noninfectious dengue Ags and control Vero cell Ag was used (19). Cells were incubated for 5 to 7 days,

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Serologic Response</th>
<th>Serotype</th>
<th>Acute/Conv. Bleed*</th>
<th>Study Day</th>
<th>Fever Day*</th>
</tr>
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<tbody>
<tr>
<td>1 (CHD94-090)</td>
<td>DF</td>
<td>Primary</td>
<td>D1</td>
<td>A</td>
<td>4, 11</td>
<td>−1, +6</td>
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<tr>
<td>2 (CHD94-067)</td>
<td>DF</td>
<td>Primary</td>
<td>D2</td>
<td>A</td>
<td>1.11</td>
<td>−2, +8</td>
</tr>
<tr>
<td>3 (CHD94-139)</td>
<td>DF</td>
<td>Secondary</td>
<td>D1</td>
<td>A</td>
<td>2.3</td>
<td>0, +1</td>
</tr>
<tr>
<td>4 (KPP94-017)</td>
<td>DF</td>
<td>Secondary</td>
<td>D2</td>
<td>A</td>
<td>1</td>
<td>−1</td>
</tr>
<tr>
<td>5 (CHD94-115)</td>
<td>DF</td>
<td>Secondary</td>
<td>D3</td>
<td>A</td>
<td>9</td>
<td>+8</td>
</tr>
<tr>
<td>6 (CHD94-089)</td>
<td>DF</td>
<td>Secondary</td>
<td>D3</td>
<td>A</td>
<td>2.10</td>
<td>−1, +7</td>
</tr>
<tr>
<td>7 (CHD94-118)</td>
<td>DF</td>
<td>Secondary</td>
<td>D4</td>
<td>A</td>
<td>2, 3</td>
<td>0, +1</td>
</tr>
<tr>
<td>8 (KPP94-013)</td>
<td>DF</td>
<td>Secondary</td>
<td>D4</td>
<td>A</td>
<td>367</td>
<td>−1</td>
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<tr>
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<td>DHF grade 1</td>
<td>Secondary</td>
<td>D4</td>
<td>A</td>
<td>1</td>
<td>−1</td>
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<tr>
<td>10 (CHD94-073)</td>
<td>DHF grade 2</td>
<td>Secondary</td>
<td>D4</td>
<td>A</td>
<td>336, 725</td>
<td>+5</td>
</tr>
<tr>
<td>11 (CHD94-020)</td>
<td>DHF grade 2</td>
<td>Secondary</td>
<td>D4</td>
<td>A</td>
<td>368</td>
<td>0, +1</td>
</tr>
<tr>
<td>12 (CHD94-138)</td>
<td>DHF grade 2</td>
<td>Secondary</td>
<td>D2</td>
<td>A</td>
<td>354</td>
<td>−2</td>
</tr>
<tr>
<td>13 (CHD94-095)</td>
<td>DHF grade 2</td>
<td>Secondary</td>
<td>D2</td>
<td>A</td>
<td>2</td>
<td>+7</td>
</tr>
<tr>
<td>14 (CHD94-134)</td>
<td>DHF grade 3</td>
<td>Secondary</td>
<td>D2</td>
<td>A</td>
<td>372</td>
<td>1</td>
</tr>
<tr>
<td>15 (KPP94-041)</td>
<td>DHF grade 3</td>
<td>Secondary</td>
<td>D1</td>
<td>A</td>
<td>2, 3, 5, 8</td>
<td>−4, −1, +2</td>
</tr>
<tr>
<td>16 (KPP94-035)</td>
<td>DHF grade 3</td>
<td>Secondary</td>
<td>D2</td>
<td>A</td>
<td>9</td>
<td>+7</td>
</tr>
<tr>
<td>17 (CHD94-081)</td>
<td>DHF grade 3</td>
<td>Secondary</td>
<td>D3</td>
<td>A</td>
<td>3</td>
<td>−1</td>
</tr>
</tbody>
</table>

* A, acute PBMC samples; C, convalescent samples.
* Fever day: 0, calendar day when the temperature fell and stayed <38°C.
as this was shown to be optimal for proliferation using 10^5 cells/well. The cells were pulsed with [3H]thymidine (on day 4 after the PHA or anti-CD3 stimulation, or on day 6 for dengue Ags and tetanus toxoid) for approximately 18 h before they were harvested and counted in a liquid scintillation counter (1205 Betaplate; Pharmacia, Wallac Oy, Finland).

**Cell separations**

Cryopreserved PBMC from patients with acute dengue or control healthy donors were thawed. Anti-CD4- and anti-CD8-coated magnetic beads (Dynal, Great Neck, NY) were used to isolate T cells from 2 x 10^6 PBMC. After the CD4- and CD8-coated beads were adhered to the magnet, the remaining cells were collected and used as a source of non-T cells. The non-T cell population was irradiated (3500 rad). As controls, unfractonated PBMC from both acute dengue patients and control healthy PBMC were stimulated with PHA.

**Transwell experiments**

PBMC from four patients with acute dengue infection and control allogeneic PBMC were resuspended at 1 x 10^6/ml in AIM/10% medium. The transwells (Costar, Cambridge, MA) consist of a lower and upper compartment that are separated by a polycarbonate-treated membrane with pores of 0.4 μm size. A total of 100 μl of acute or control PBMC was added to the upper well of the transwell, and 600 μl of media was added to the lower well with PHA at a final concentration of 1:1000. For coculture experiments, acute PBMC and gamma-irradiated allogeneic PBMC were cultured together in 100 μl in the upper well of the transwell as a positive control. To see whether cell contact was essential to increase proliferation, 100 μl of the acute PBMC was transferred to the upper well and 600 μl of gamma-irradiated, allogeneic PBMC was transferred to the lower well with PHA at 1:1000. The plate was incubated at 37°C. On day 4, 75 μl of the cell suspensions from the upper and lower wells was transferred to a 96-well plate in a final volume of 200 μl pulsed with 1.25 μCi of [3H]Tdr, and harvested approximately 18 h later.

**Monoclonal Abs**

FITC-conjugated mAbs to CD14 and CD19 were purchased from PharMingen (San Diego, CA). The FITC-conjugated CD3, CD4, and CD8 Abs were purchased from Becton Dickinson (Mountain View, CA). The anti-CD28 Abs were purchased from PharMingen and used at a concentration of 5 or 10 μg/ml together with the anti-CD3 Ab 12F6 (0.1 μg/ml) kindly provided by Johnson Wong. FACS analysis was performed on unstimulated PBMC.

**Results**

**PBMC from children with acute dengue infection have decreased proliferative responses to PHA, dengue Ags, and recall Ags**

We analyzed the proliferative responses of PBMC to mitogens and other Ags from patients with varying grades of severity of dengue illness. PBMC samples obtained from patients at different time points after they were admitted into the study (study day 1 up to study day 11) were tested in this assay (Table I). The responses during acute infection were compared with the proliferation of the same patient’s PBMC obtained 6–24 mo after their acute infection. In all 14 patients, there was a significant decrease in proliferation of acute PBMC in response to stimulation with PHA (Fig. 1). In the four patients tested, there was a decrease in the response to dengue Ag and also to a recall Ag tetanus toxoid in the acute samples of PBMC (Table II). Background cpm of acute PBMC were consistently much lower in all patients compared with their convalescent PBMC, but the stimulation indices (cpm of mitogen- or Ag-stimulated cells ÷ cpm of cells in medium) still indicated a substantial decrease in acute PBMC proliferation compared with convalescent PBMC (Table II and data not shown). The results indicate that in vitro proliferative responses of PBMC to mitogens and specific Ags are suppressed in all grades of acute dengue infection. Samples tested up to 11 days after the patient was admitted into the study showed decreased responses compared with the same individual’s convalescent samples.

**rIL-2 and irradiated PBMC restore proliferation of acute PBMC**

To characterize the immunologic unresponsiveness of the acute PBMC, we treated the PHA-stimulated cells with 10 U/ml of rIL-2. Inadequate production of IL-2 by T cells is thought to be one of the factors that contribute to the immunosuppression seen during acute measles and CMV infection (5, 9). In eight of nine patients tested, the decreased responses were restored by the addition of rIL-2 and irradiated PBMC. PBMC obtained during acute dengue infection do not respond to dengue Ags and recall Ags in [3H]Tdr proliferation assay

| Table II. PBMC obtained during acute dengue infection do not respond to dengue Ags and recall Ags in [3H]Tdr proliferation assay |
|---|---|---|---|---|
| Patient No. | Study Day | Dengue Ag | Tetanus Toxoid | No Ag |
| 9 | A1 | 141 | 140 | 128 |
| 11 | C336 | 14,166 | 45,391 | 387 |
| 14 | A4 | 78 | 55 | 58 |
| 15 | A5 | 83 | 80 | 75 |
| C366 | 2,344 | 5,376 | 856 |

* A total of 1 x 10^5 PBMC from acute and convalescent phases of infection from the same patient were stimulated with a 1/40 dilution of dengue Ag or a 1/5000 dilution of tetanus toxoid and incubated for 6 days in a 96-well plate at 37°C. Cells were pulsed with [3H]Tdr on day 6 for 18 h and harvested. The results presented are from four different experiments performed. Data are mean cpm of two or three replicates.
Table III. Decreased proliferative responses of acute PBMC are partially restored upon the addition of IL-2, gamma-irradiated autologous or allogeneic PBMC

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>PHA</th>
<th>PHA + IL-2</th>
<th>Autologous</th>
<th>Allogeneic</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (A1)</td>
<td>308</td>
<td>62,514</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3 (A3)</td>
<td>899</td>
<td>23,637</td>
<td>ND</td>
<td>23,146</td>
</tr>
<tr>
<td>5 (A9)</td>
<td>4,600</td>
<td>73,073</td>
<td>22,886</td>
<td>ND</td>
</tr>
<tr>
<td>7 (A3)</td>
<td>627</td>
<td>17,593</td>
<td>59,555</td>
<td>ND</td>
</tr>
<tr>
<td>9 (A1)</td>
<td>1,049</td>
<td>26,314</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10 (A9)</td>
<td>3,240</td>
<td>71,969</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>12 (A2)</td>
<td>102</td>
<td>128</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>13 (A11)</td>
<td>3,917</td>
<td>54,815</td>
<td>130,788</td>
<td>ND</td>
</tr>
<tr>
<td>16 (A9)</td>
<td>1,396</td>
<td>64,821</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* A total of 10 U/ml of rIL-2 or 1 x 10^5 gamma-irradiated autologous convalescent (for patients 5, 7, and 13) or allogeneic healthy PBMC (for patient 3) were added to acute PBMC stimulated with a 1/100 dilution of PHA and incubated for 5 days. Cells were pulsed overnight on day 4 with [3H]TdR and harvested. The results presented are from eight different experiments performed. Data are mean cpm of two or three replicates.

** Patient numbers are indicated in parenthesis.

** ND, not done.

of exogenous IL-2 (Table III). In one patient (number 12), addition of up to 100 U/ml of IL-2 did not restore proliferation (data not shown). Addition of 1 x 10^5 gamma-irradiated, autologous convalescent, or allogeneic control PBMC also improved proliferative responses of acute PBMC (Table III). Our data show that the unresponsive acute PBMC can be induced to proliferate by the addition of the cytokine IL-2 and by irradiated, control PBMC. These results, however, do not indicate whether soluble factors released from or cell contact with the irradiated PBMC are essential to restore the proliferation of the acute PBMC.

Acute PBMC require cell contact with gamma-irradiated control PBMC to increase proliferative responses

To determine whether cell contact with the irradiated PBMC is needed to mediate proliferation, we used a transwell system in which acute cells were separated from allogeneic control PBMC by a polycarbonate membrane. When acute and gamma-irradiated allogeneic cells were incubated together in the upper well, there was a substantial increase in proliferation compared with acute T cells alone stimulated with PHA (Fig. 2). When gamma-irradiated PBMC were separated from the acute cells in the upper well by a polycarbonate membrane, however, proliferation was much lower in all four patients' PBMC compared with when the cells were

Incubated together. In patient 6, acute cells proliferated somewhat when they were separated from the gamma-irradiated control cells by the membrane (approximately 30,000 cpm compared with 135,000 cpm), suggesting that both cell-soluble factors and cell contact were important in restoring proliferation. The results, however, suggest that cell contact with gamma-irradiated allogeneic PBMC is predominantly required to restore proliferation of acute cells.

APCs in PBMC obtained during acute dengue illness are unable to provide adequate stimuli to induce proliferation in T cells

To further demonstrate that there is a defect in the accessory cells of acute PBMC, both T and non-T cells were isolated from patients with acute dengue and mixed in proliferation assays with T and non-T cells from a control donor. When T cells from a control donor were added to gamma-irradiated non-T cells from the acute PBMC, responses were substantially reduced, indicating that the non-T cells in the acute PBMC sample could not support the proliferation of T cells from a control donor (Table IV). In contrast,
responses in children with acute dengue infection may be due to a defect in the APC population.

The results indicate that not all cytokines that share a common γ-chain receptor are able to restore the proliferation of acute PBMC obtained from patients with acute dengue illness.

To analyze whether costimulatory molecules may play a role in the cell-mediated suppression of acute phase cells, we treated the PBMC of six subjects with anti-CD28 and anti-CD3 Abs. In three patients (numbers 6, 13, and 15), addition of anti-CD28 restored proliferation of acute PBMC to levels comparable with stimulation with anti-CD3 + IL-2 (Fig. 3). The PBMC samples from these patients were obtained at least 8 days after enrollment, at which time the subjects were no longer febrile or viremic (17). PBMC from three other patients (numbers 3, 7, and 17) did not respond to costimulation with either 5 or 10 μg of anti-CD28. The PBMC were obtained from these patients when they had the most severe symptoms (days 2 or 3 after they were enrolled into the study) and were acutely ill in the hospital. Study days 2 or 3 also represent days when the absolute monocyte counts drop to the lowest levels.

The results suggest that anti-CD28 Abs can restore proliferation of PBMC samples when the patients are recovering from illness, but have no effect on PBMC obtained from the patients at the peak of their illness.

Acute PBMC have decreased numbers of monocytes, but normal levels of T and B cells

We found a significant decrease in the number and percentage of monocytes in the acute samples of PBMC compared with the convalescent samples (Table V); however, there was no decrease in either the CD4 or CD8 T cells in the acute PBMC. Therefore, a reduction in the number of T cells cannot be the primary reason for inadequate proliferative responses. There were also no differences in the percentage of B cells during acute infection. These results suggest that one of the reasons for the decrease in cell-mediated responses in children with acute dengue infection may be the decreased number of monocytes that would result in fewer total costimulatory ligands such as B7-1 and B7-2 and adhesion molecules available to activate the T cells in the acute samples.

IL-2 and IL-7, but not IL-4 and IL-12, improve proliferation of cells

Since receptors for the cytokines IL-2, IL-4, IL-7, and IL-15 are known to share a common γ-chain, we examined whether some of these cytokines would also help overcome the unresponsiveness of the acute phase PBMC. We chose IL-12 as a cytokine that did not utilize the γ-chain as a control. In four of the four patients tested, the acute samples of PBMC responded to IL-2 or IL-7, but not to IL-4 even at a concentration of 500 U/ml (Fig. 4 and data not shown). The results indicate that not all cytokines that share a common γ-chain receptor are able to restore the proliferation of the PBMC obtained during acute infection.

Discussion

This study is the first report of impaired cell-mediated responses of the PBMC of patients with acute dengue infection. The results demonstrate decreased in vitro proliferative responses of PBMC to mitogens and to several Ags during acute dengue infection. All of the responses of PBMC from children with acute dengue infection were compared with the responses of PBMC obtained from the same patients 6–24 mo after the infection.
Suppression of in vitro proliferation of acute phase PBMC was observed in subjects with either DHF, the more severe form of dengue illness, or DF, the milder form, in subjects experiencing either primary or secondary dengue infections, and in subjects infected with any of the four serotypes of dengue virus. A variety of mechanisms for virus-induced immunosuppression has been suggested, including abnormalities of T cells upon antigenic challenge in vitro and various defects in the APCs (3, 6, 20–22). Since the background cpm was lower in acute PBMC in all cases, it could imply that the acute cells cultured in media alone were more susceptible to apoptosis. However, the addition of gamma-irradiated feeders, IL-2, IL-7, or anti-CD28 Abs restored proliferation of the acute T cells, indicating that these cells could be rescued. T lymphocytes from patients with HIV or EBV, and in acute LCMV infection have been shown to undergo apoptosis in vitro upon stimulation (23–26). In EBV infection, T cell death could be prevented by the addition of IL-2; however, in acute LCMV infection, IL-2 sensitized non-LCMV-specific memory T cells for apoptosis upon subsequent stimulation (26). These studies, however, did not examine whether the accessory cell population contributed to the observed immunosuppression.

To test whether one cell type predominantly accounts for the unresponsiveness of the PBMC, we separated T and non-T cells and performed mixing experiments. The results demonstrate that the dominant abnormality lies in the accessory cells in the acute PBMC, because both acute and control T cells are incapable of responding to PHA in the presence of irradiated acute non-T cells. In contrast, proliferation of the T cells in the acute PBMC increased substantially when irradiated non-T cells from a control donor were present. The defect in the accessory cell population in the acute PBMC is not exclusively an Ag-processing defect because it was also observed with mitogens. The accessory cells in the acute PBMC sample might fail to provide an adequate stimulus or might provide a negative signal to the T cells from samples of acute or control PBMC. Incubation of acute PBMC with autologous convalescent PBMC did not suppress the proliferation of the convalescent PBMC (data not shown), suggesting that the acute phase PBMC do not present a negative signal. Therefore, we believe during acute dengue infection the accessory cells in the PBMC are unable to provide the necessary stimulus to activate the T cells. Accessory cells are necessary to help polyclonal activation of T cells with PHA by cross-linking of the TCR and by providing second signals or soluble factors to induce activation (27). Although we cannot entirely exclude the possibility that a small proportion of the acute T cells is triggered into activation-induced cell death, our results indicate that the primary defect is in the APC population.

Cell contact-dependent and soluble factors released from APCs have been shown to mediate unresponsiveness of T cells. The soluble factors include TNF-dependent release of arachidonic acid and PGE₂ by CMV-infected monocytes (22); release of IFN-α from monocytes followed by cell death of the mitogen-stimulated cells (28) in measles and an undescribed cytokine in suppressing Ag-specific proliferation by measles virus (29). Inhibition of proliferation of Ag-specific T cells by human rhinoviruses was shown to be dependent on virion binding to ICAM-1 on monocytes (30). Karp et al. have shown that cross-linking of the measles virus receptor CD46 inhibited monocyte production of IL-12, which is known to be important for the generation of cell-mediated responses (20). All of these studies have carefully analyzed the unresponsiveness of T cells following in vitro infection of either monocytes or PBMC with the respective viruses and then examined the effect of these infected monocytes/PBMC on T cell proliferation. How this relates to the observed suppression seen during or following in vivo infection with any of these viruses is still in question.

Our rationale for using the transwell system was to see whether irradiated autologous or allogeneic PBMC would restore proliferation of the T cells in the acute PBMC by the release of soluble factors. If this was the case, the acute cells should have proliferated when separated by a membrane from the irradiated allogeneic PBMC. Since the patients’ acute cells had significantly lower proliferation when separated by the membrane, these results suggest that the initial triggering of acute PBMC by irradiated control PBMC requires cell-cell contact. It is possible that soluble growth factors are produced by the acute T cells as a result of this initial cell contact-dependent stimulation, which can then restore proliferation. This may explain why cytokines such as IL-2 or IL-7, which are produced downstream of signaling events, increase the proliferation of the acute PBMC. The inability of the PBMC from one patient to respond to even high doses of IL-2 may reflect a more severe immunosuppression, and this patient did exhibit more severe disease manifestations (DHF grade 2). Since we had limited number of PBMC from this patient, it was not possible to further characterize this defect.

The absolute monocyte and absolute neutrophil counts are transiently decreased in patients with dengue illness compared with children with other febrile illnesses (15). The in vitro T cell responses we have observed are significantly depressed for at least 2 wk after the appearance of fever in these patients. Depletion of monocytes from PBMC of control donors decreases the anti-CD3 response, but not the PHA response, whereas depletion of B cells does not affect the proliferation of PBMC (data not shown). This suggests that decreases in the monocyte count in the acute PBMC of the dengue patients do not appear to solely account for the marked in vitro suppression observed.

Plasma levels of IL-10 are increased in patients with DF and DHF (S. Green et al., manuscript submitted). IL-10 is a cytokine with known immunosuppressive properties (31, 32). It down-regulates monocyte production of costimulatory molecules including B7-1 and B7-2, suppresses proliferation, and induces alloantigen-specific unresponsiveness of human CD8⁺ T cells (33). It is possible that increased levels of IL-10 in dengue patients in vivo alter the expression of costimulatory molecules on monocytes, making them incapable of activating T cells in the presence of mitogens.

Addition of CD28 Abs did not induce T cell proliferation of the acute samples of PBMC obtained on study day 2 or 3, when patients are typically viremic and have their most severe symptoms of disease. When the patients were recovering from their acute illness and the monocyte levels were slowly returning back to normal levels, the CD28 Abs improved proliferation of acute PBMC samples (Fig. 4). It is possible that the addition of costimulatory molecules at the earlier stage of illness cannot trigger sufficient production of IL-2 required for T cell proliferation, and therefore had no effect on the proliferation in the acute PBMC samples. Previous studies of patients with measles and CMV have not examined the effect of anti-CD28 Abs on acute T cell proliferation. It is interesting to speculate in acute dengue infection that decreased B7 molecules preferentially trigger the high affinity receptor CTL activation Ag-4 (instead of the low affinity receptor CD28), which has been shown to provide inhibitory signals and prevent proliferation (34, 35). Other interactions between T cells and APCs such as CD40/CD40 ligand and LFA-3/ICAM-1 may also be important to generate a good immune response.

The receptors for several cytokines, e.g., IL-2, IL-4, IL-7, IL-13, and IL-15, have common motifs and share the γc-chain (36). IL-2, IL-4, IL-7, IL-9, and IL-15 can all act as T cell growth factors, activate the same Jak family tyrosine kinases, but induce different
References


