MAJOR ARTICLE

Antibody-Dependent Cellular Cytotoxicity Mediated by Plasma Obtained before Secondary Dengue Virus Infections: Potential Involvement in Early Control of Viral Replication

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Background. Preexisting dengue virus (DV)–specific antibodies from prior heterologous DV infection may have several effects in secondary DV infection. These antibodies may mediate protective effects by means of antibody-dependent cellular cytotoxicity (ADCC), in which virus-specific antibodies bind to the surface of heterologous DV-infected cells and mediate natural killer cell lysis. In the present study, we examined the ability of plasma obtained before secondary DV infection to induce ADCC of DV-infected cells.

Methods. Plasma samples were obtained before DV2 or DV3 infection in a prospective cohort study of Thai schoolchildren. The ADCC activity in the plasma samples was measured by ^{51}Cr-release assay, using persistently DV2- or DV3-infected Raji cells as targets.

Results. ADCC activity in plasma obtained before secondary infection directly correlated with neutralizing antibody titers, anti-DV immunoglobulin G1 levels, and a multitypic 50% plaque reduction neutralization test pattern. ADCC activity in pre–secondary DV3 infection plasma samples inversely correlated with plasma viremia levels, but no such correlation was seen in pre–secondary DV2 infection plasma samples. ADCC activity did not correlate with disease severity in subsequent secondary DV2 or DV3 infection but was lowest in plasma from patients with dengue hemorrhagic fever due to secondary DV3 infection.

Conclusions. ADCC may contribute to the early control of secondary DV3 viremia in vivo.

Dengue fever (DF) is an acute flavivirus infection transmitted by several species of *Aedes* mosquitoes. Dengue virus (DV) has 4 antigenically related serotypes: DV1, DV2, DV3, and DV4. Infection with any 1 of the 4 serotypes can produce a broad spectrum of effects, including asymptomatic infection, mild febrile illness, classic DF, and the lethal dengue hemorrhagic fever (DHF)/dengue shock syndrome [1]. It is estimated that 50–100 million cases of DF and several hundred thousand cases of DHF occur each year in tropical countries. There is no specific treatment for DHF/dengue shock syndrome. The case fatality rate varies among countries from <1% to 15% [2]. Infection with one serotype confers lifelong homotypic immunity, with only short-term cross protection against heterotypic serotypes [3]. Preexisting DV antibodies may have both protective and pathogenic roles in secondary DV infection. Preexisting DV antibodies from prior heterologous DV infection have been proposed to play a role in disease severity by facilitating virus entry into Fcγ receptor–

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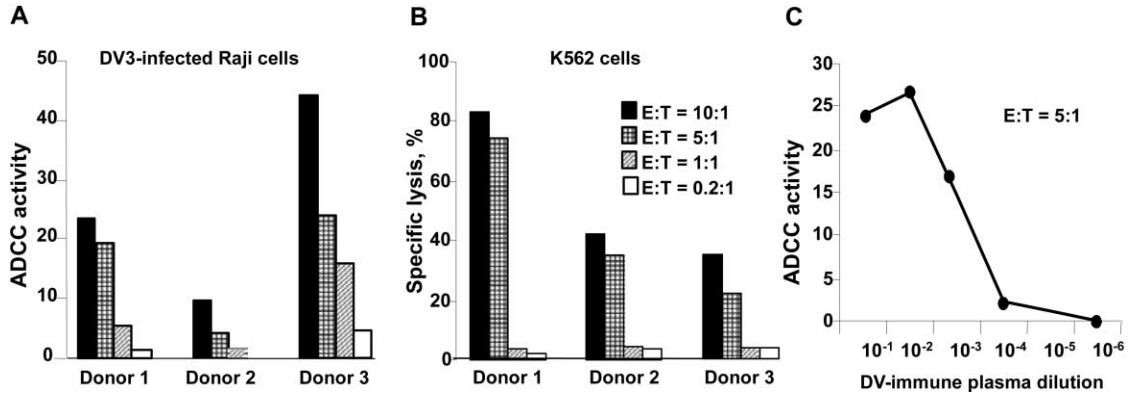

Figure 1. Antibody-dependent cellular cytotoxicity (ADCC) activity and NK cell cytotoxicity in 3 healthy NK cell donors. *A,* ADCC activity when dengue virus (DV) serotype 3 (DV3)–infected Raji cells are used as target cells. *B,* NK cytotoxicity as determined by K562 cell lysis. *C,* Mediation of ADCC activity (*Y*-axis) by DV antibody in a dose-dependent manner. DV-immune plasma was serially diluted (*X*-axis) before addition to DV3-infected Raji cells. Purified NK cells were added and incubated for 5 h. E:T, effector:target cell ratio.

bearing cells, leading to an increase in viral burden and disease severity (antibody-dependent enhancement) [4]. In a prospective cohort study of Thai schoolchildren, we showed that plasma obtained before DV2 or DV3 infection can enhance DV infection of K562 cells, but the percentages of DV-infected K562 cells and viral titers of DV-infected K562 supernatants did not correlate with either clinical severity or viral burden of subsequent secondary DV2 and DV3 infections [4]. In this same cohort, we found that preexisting antibody appeared to play a protective role in secondary DV3 infection, in that the levels of preexisting heterologous neutralizing antibody (NAb) negatively correlated with plasma viremia levels and disease severity [5].

Preexisting DV antibody may also mediate protective effects by other mechanisms, including antibody-dependent cellular cytotoxicity (ADCC), in which virus-specific antibodies bind to the surface of heterologous DV-infected cells and mediate lysis by NK cells. ADCC has been shown to be important in infections with other viruses, such as Epstein-Barr virus [6, 7], HIV [8–11], and hepatitis C virus [12]. Furthermore, ADCC responses may also have an impact on the efficacy of experimental vaccines against herpes simplex virus [13], influenza virus [14], and HIV [15].

Kurane et al. [16] previously showed that NK cell–mediated lysis of DV2-infected Raji cells was significantly higher when these cells were preincubated with DV-immune serum. Other studies have shown that NK cells are activated early in DV infection [17–19], which suggests that ADCC might also contribute to the immunopathogenesis of dengue disease. In the present study, we looked at the correlation of ADCC activity with both plasma viremia levels and disease severity in secondary DV infection, by using plasma samples collected prospectively before secondary DV infection.

MATERIALS AND METHODS

Study design and sample collection. Plasma samples were obtained from a prospective cohort study that has been published elsewhere [20]. More than 2000 primary-school students in Kamphaeng Phet, Thailand, were enrolled as part of an ongoing study of dengue pathogenesis. Blood samples were collected each January. Children were under daily active surveillance for school absences during peak dengue season, from 1 June to 15 November each year. Children with fever or a history of fever within 7 days of the first day of school absence were evaluated, and acute- and convalescent-phase blood samples were collected. We defined 3 categories of dengue disease severity: (1) "nonhospitalized DF" (nhDF), (2) "hospitalized DF" (hDF), and (3) "hospitalized DHF" (hDHF), as described elsewhere [20]. Signed consent was obtained from the parents or guardians of all subjects. The investigational protocol was approved by the institutional review boards of the Thai Ministry of Public Health, the Office of the US Army Surgeon General, and the University of Massachusetts Medical School.

Study population. For the present study, we selected subjects enrolled in the first 3 years of this ongoing prospective trial who had symptomatic secondary DV2 or DV3 infection and whose acute-phase plasma samples were collected within 3 days of fever onset [5, 21]. Of all 37 subjects with secondary DV2 infection and 45 subjects with secondary DV3 infection identified, 32 and 27 subjects were selected, respectively, on the basis of these criteria. For suspected cases of asymptomatic secondary DV3 infection, we randomly selected 10 asymptomatic subjects with serologic evidence of secondary DV infection from a school that had an isolated DV3 infection outbreak [22].

Virologic and serologic studies. Plasma viremia levels were determined by quantitative reverse-transcription polymerase

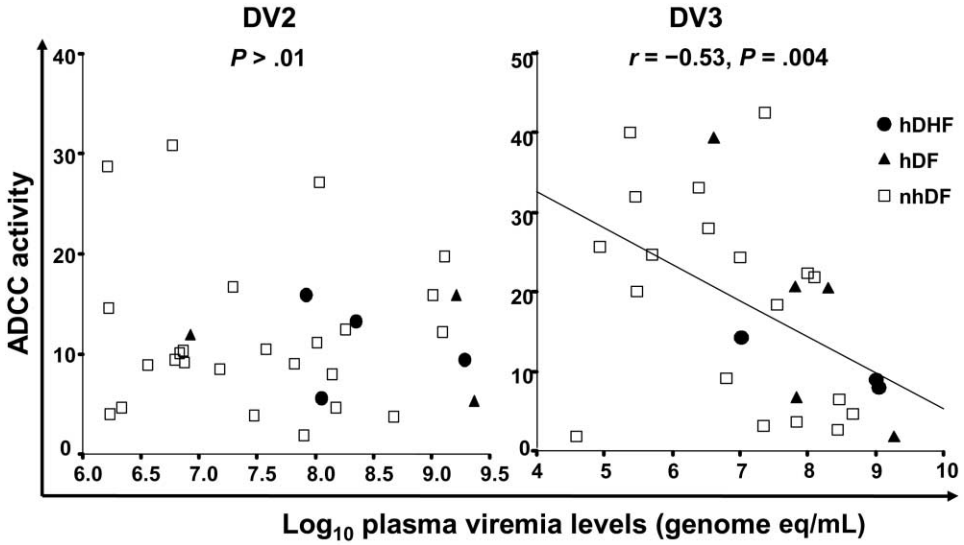

Figure 2. Correlation between antibody-dependent cellular cytotoxicity (ADCC) activity in preillness plasma samples and subsequent plasma viremia levels. ADCC activity in plasma samples from children who developed subsequent secondary dengue virus serotype 2 (DV2) and serotype 3 (DV3) infection is shown in the left and right panels, respectively. Log_{10} plasma viremia levels are shown on the *X*-axis. Data points represent individual subjects with hospitalized dengue hemorrhagic fever (hDHF), hospitalized dengue fever (hDF), or nonhospitalized dengue fever (nhDF). Spearman rank correlation was used to analyze data, with $P < .05$ considered to be significant. Genome eq, genome equivalents.

chain reaction [23]. Symptomatic secondary DV infections were defined by a DV IgM:IgG EIA ratio ≤1.8 [24]. Asymptomatic secondary DV3 infections were defined by a ≥4-fold increase in hemagglutination inhibition titer in serial plasma samples without a documented illness-related school absence during the period of DV infection [24, 25]. Isolation and serotype identification of plasma DVs were performed as described elsewhere [26, 27]. EIA was used for DV-specific IgG1 subclass assays [28]. NAbs were measured by 50% plaque reduction neutralization test ($PRNT_{50}$) in LLC-MK2 cells against prototype strains of all 4 DVs (DV1 16007, DV2 16681, DV3 16562, and DV4 1036), Japanese encephalitis virus (JEV) (Nakayama strain), and the patient's own DV isolate, as described elsewhere [29]. NAb data for this cohort have been published elsewhere [4].

Cell cultures. Raji cells (a human B cell lymphoma cell line) persistently infected with DV2 or DV3, uninfected Raji cells, and K562 cells (an erythroleukemia cell line) were used as target cells in ADCC assays [16]. All cell lines were cultured in RPMI 1640 containing 10% fetal calf serum (RPMI 10). The percentages of DV-infected Raji cells were determined by flow cytometry (described below) and were found to be 75% and 85% for DV2- and DV3-infected Raji cells, respectively.

NK cell separation. Whole blood was obtained from 1 healthy Filipino donor and 2 healthy Thai donors. NK cells for use as effector cells were negatively selected using tetrameric antibody complex (Rosette Sep NK cell enrichment; StemCell Technologies), in accordance with the manufacturer's recommendations. Purified NK cells consisted of >90% $CD56^+CD16^+$ lymphocytes. Less than 0.5% of cells were $CD14^+$ or $CD3^+$, and 3%–9% were $CD19^+$. The purity of the NK cells was adequate to ensure that target cell lysis was mediated by NK cells rather than by T cells.

ADCC assay. ADCC assays were performed in a total volume of 200 μL in triplicate. Plasma samples used in the ADCC assay were first heat inactivated for 30 min at 56°C. Flavivirus-naive and DV-immune plasma samples were included as negative and positive controls, respectively. DV-infected and -uninfected Raji cells (10^6 cells) were labeled with 25 μCi of ^{51}Cr in 1 mL of RPMI 10 at 37°C for 1 h, and 10^4 target cells in 50 μL of RPMI were added in triplicate to a 96-well U-bottom microtiter plate. Control or patient plasma (50 μL diluted 1:10; final dilution, 1:40) was added and incubated for 30 min at 37°C. For some assays, plasma was diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. NK cells (10^5 cells in 100 μL) were then added to each well. For maximum release, 100 μL of 5% Renex in PBS was added to target cells. Target cells with RPMI 10 alone were used for measurement of spontaneous release. Supernatants were collected after 5 h of incubation at 37°C in humidified 5% CO_2 and counted using a gamma counter. The percentage of specific lysis was calculated as $100 \times (\text{sample count} - \text{spontaneous release}) / (\text{maximum count} - \text{spontaneous release})$. Spontaneous release from DV-infected and -uninfected Raji cells was <15% of maximum release [16]. To minimize the effect of interassay variability, we performed ADCC assays for all pre–secondary DV2 or DV3 infection plasma samples concurrently and used the same effector NK cell donor in all assays. ADCC activity was calculated as the

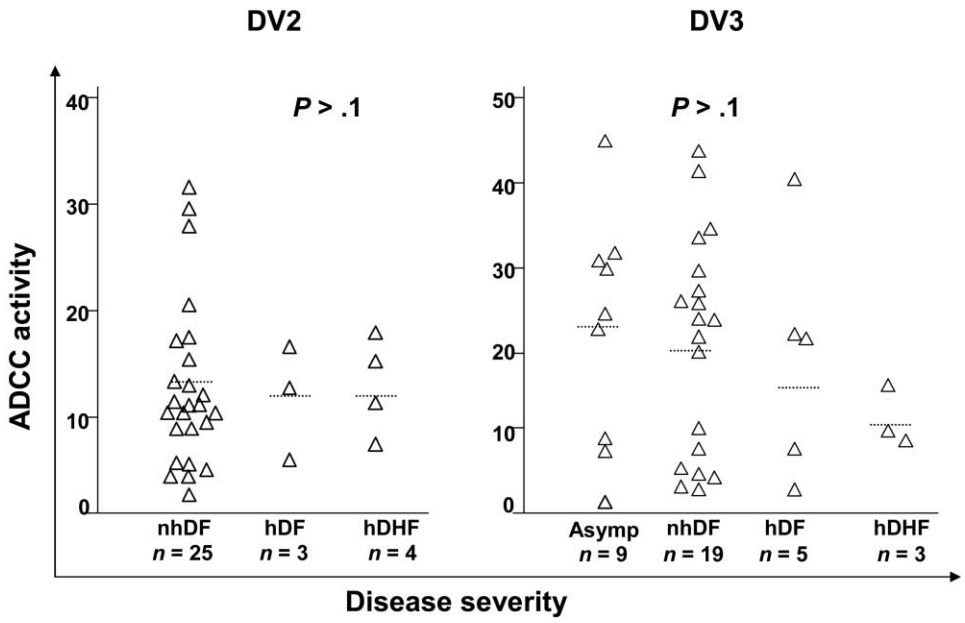

Figure 3. Relationship between antibody-dependent cellular cytotoxicity (ADCC) activity in preillness plasma and subsequent disease severity. ADCC activity in plasma samples from children with subsequent secondary dengue virus serotype 2 (DV2) and serotype 3 (DV3) infection is shown in the left and right panels, respectively. Disease severity is shown on the *X*-axis. Data points represent individual subjects with hospitalized DHF (hDHF), hospitalized DF (hDF), nonhospitalized DF (nhDF), or asymptomatic seroconversion (Asymp). Analysis of variance was used to analyze data, with $P < .05$ considered to be significant. Dotted lines represent mean values.

percentage of specific lysis of DV-infected Raji cells minus the percentage of specific lysis of uninfected Raji cells.

Flow-cytometric analysis. For analysis of NK cell purification, we used the following antibodies: phycoerythrin (PE)–Cy7–conjugated anti-CD56, fluorescein isothiocyanate (FITC)–conjugated anti-CD16, peridinin chlorophyll protein Cy5.5–conjugated anti-CD3, allophycocyanin-conjugated anti-CD14, and PE-conjugated anti-CD19 (BD Bioscience). Indirect staining was performed using mouse anti-DV2 and anti-DV3 monoclonal antibodies (American Type Culture Collection) to determine the percentage of DV2- and DV3-infected Raji cells, with FITC-conjugated sheep anti-mouse IgG (ICN/Cappel) used as a secondary antibody. Flow-cytometric analysis was performed on a FACSCalibur flow cytometer (BD Immunocytometry Systems) using FlowJo software (version 6.1; Tree Star).

Statistical analysis. Statistical analyses were performed using SPSS software for Windows (version 11.0; SPSS). Spearman rank correlation estimates were used to measure the association of continuous variables with a skewed distribution. Analysis of variance (ANOVA) was used to test mean ADCC activity among disease-severity groups and $PRNT_{50}$ patterns. Student's *t* test was used to compare ADCC activity between 2 groups. In all analyses, $P < .05$ was considered to be significant, $.05 < P < .10$ was considered to be a nonsignificant trend, and $P > .1$ was considered to be not significant.

RESULTS

Mediation of ADCC by NK cells and DV-immune plasma in a dose-dependent pattern. Kurane et al. [16] previously showed that NK cell–mediated lysis of DV2-infected Raji cells was significantly higher when cells were preincubated with DV-immune serum. We also found that target cell lysis was higher (38% for DV2-infected Raji cells and 60% for DV3-infected Raji cells) in samples with DV-immune plasma (positive control plasma) than with either media or flavivirus-naive plasma (<5% using both DV2- and DV3-infected Raji cells as targets). We also examined ADCC activity at different effector:target cell (E:T) ratios and at different DV-immune plasma dilutions. As expected, lower E:T ratios and higher dilutions of DV-immune plasma reduced ADCC activity (figure 1*A* and 1*C*). This confirms that ADCC is mediated by NK cells and anti-DV antibody in the plasma in a dose-dependent fashion.

Variation in NK cell effector function among NK cell donors. We wanted to determine the relationship between DV3-infected Raji cell lysis in ADCC assays and K562 cell lysis in NK assays. As was reported in previous studies [16], we found that the lysis of K562 and DV3-infected Raji cells differed among NK cell donors (figure 1*A* and 1*B*). Although numbers were small, we detected no correlation between the percentage of DV3-infected Raji cell lysis in ADCC assays and K562 cell lysis in NK cell assays ($n = 3$; $P > .1$, Spearman rank correlation).

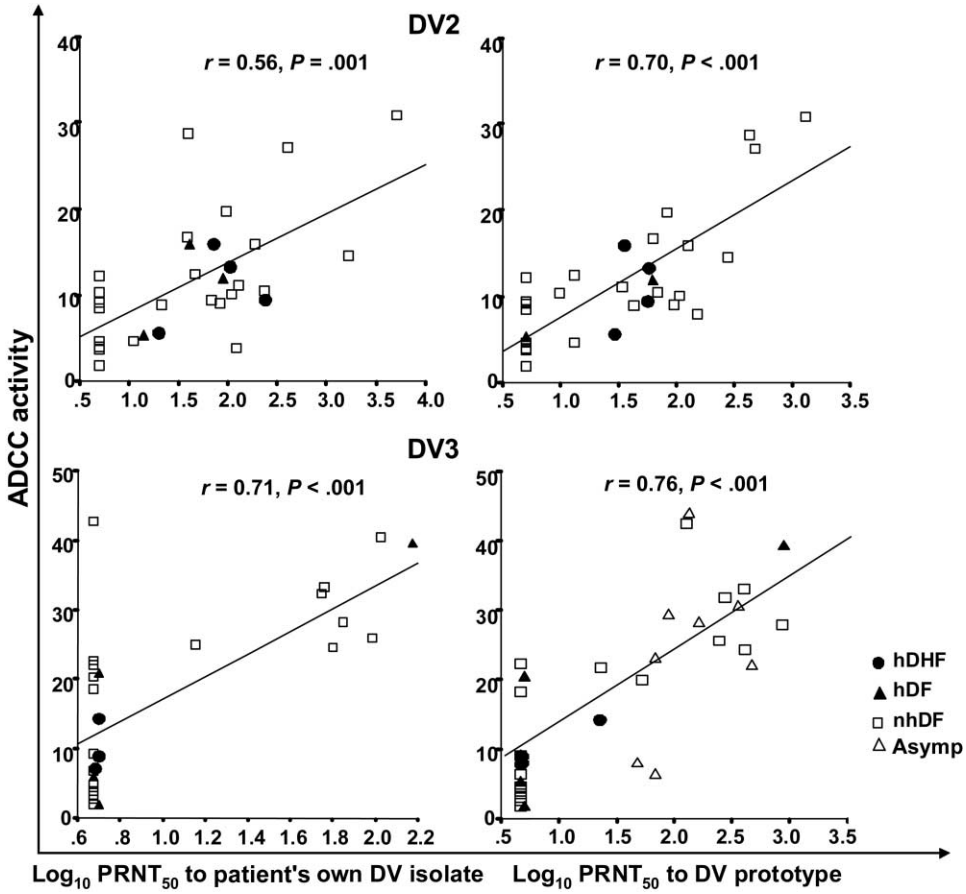

Figure 4. Correlation between antibody-dependent cellular cytotoxicity (ADCC) activity and neutralizing antibody titers. ADCC activity in plasma samples from children with subsequent secondary dengue virus (DV) serotype 2 (DV2) and serotype 3 (DV3) infection is shown in the top and bottom panels, respectively. Neutralizing antibody titers (50% plaque reduction neutralization test [$PRNT_{50}$] titer; *X*-axis) to the patient's own DV isolate are shown in the left panels, and titers to a prototype DV isolate are shown in the right panels. Data points represent individual subjects with hospitalized dengue hemorrhagic fever (hDHF), hospitalized dengue fever (hDF), nonhospitalized dengue fever (nhDF), or asymptomatic seroconversion (Asymp). Spearman rank correlation was used to analyze data, with $P < .05$ considered to be significant.

We next determined whether levels of expression of FcγRII (CD16), the antibody-binding receptors on NK cells, were associated with ADCC activity. Surprisingly, we found that CD16 expression was lower in donor 3 than in donors 1 and 2 (mean fluorescence intensity of 213, compared with 378 and 326, respectively), even though NK cells from donor 3 showed the highest lysis of DV-infected Raji cells in the presence of DV-immune plasma. We utilized NK cells from donor 3 for all further studies.

ADCC activity and plasma viremia level. We hypothesized that if DV-infected cells are killed by NK cells via ADCC, then ADCC activity should inversely correlate with plasma viremia level. We previously showed in this cohort that higher levels of NAb to DV3 but not to DV2 correlated with lower viremia levels [5]. Similarly, we found that ADCC activity in plasma obtained before secondary DV3 infection inversely correlated with DV3 viremia level ($r = .53$; $P < .01$, Spearman rank correlation), whereas no correlation of viremia level with ADCC activity was seen in plasma obtained before secondary DV2 infection ($r = .01$; $P > .1$, Spearman rank correlation) (figure 2).

ADCC activity and subsequent secondary dengue disease severity. We examined whether ADCC activity in plasma obtained before documented symptomatic secondary DV2 or DV3 infection or asymptomatic secondary DV seroconversion (presumed DV3 infection) correlated with subsequent disease severity in our cohort. We found that ADCC activity in plasma obtained before secondary DV2 infection did not correlate with subsequent DV2 disease severity ($P > .1$, ANOVA) (figure 3). Similarly, although DV3-infected Raji cell lysis was lowest when plasma obtained from the hDHF group before secondary DV3 infection was used, there was no statistically significant correlation between DV3-infected Raji cell lysis and subsequent disease severity ($P > .1$, ANOVA) (figure 3).

ADCC activity and DV NAb and IgG1 levels. We determined whether ADCC activity correlated with DV NAb levels. We found that ADCC activity in pre–secondary infection

plasma samples correlated with levels of NAb against both the patient's virus isolate and a prototype isolate for both DV2 infection and DV3 infection ($P < .01$, Spearman rank correlation) (figure 4).

IgG1 has been found to be more active than other IgG subclasses in NK cell–mediated ADCC [30]. In this study, we wanted to determine whether ADCC activity correlated with IgG levels. We found that ADCC activity was significantly correlated with anti-DV IgG1 levels in plasma samples obtained before both secondary DV2 infection and secondary DV3 infection ($P < .01$, Spearman rank correlation) (figure 5).

ADCC activity and NAb pattern. We previously showed that, in DV3 infection but not in DV2 infection, the proportion of DHF to DF was lower among patients who had a preexisting multitypic $PRNT_{50}$ pattern than among those with a preexisting monotypic $PRNT_{50}$ pattern [5]. In the present study, we examined the relationship between $PRNT_{50}$ pattern and ADCC activity in plasma samples obtained before secondary DV2 infection and DV3 infection. In both pre-secondary DV2 and DV3 infection plasma samples, ADCC activity did not differ among DV1, DV2, and DV3 monotypic $PRNT_{50}$ patterns (data not shown). When $PRNT_{50}$ patterns were classified into 3 groups—undetectable, monotypic, and multitypic—ADCC activity was significantly different among the 3 groups ($P < .05$, ANOVA). In both pre–secondary DV2 and DV3 infection plasma samples, ADCC activity was significantly higher in samples with a multitypic $PRNT_{50}$ pattern than in samples with a monotypic or undetectable $PRNT_{50}$ pattern (figure 6). In pre–secondary DV2 infection but not pre–secondary DV3 infection plasma samples, ADCC activity was also significantly higher in samples with a monotypic $PRNT_{50}$ pattern than in samples with an undetectable $PRNT_{50}$ pattern.

In secondary DV2 infection, ADCC activity was <5% in all (3/3) plasma samples with an undetectable $PRNT_{50}$ pattern. Surprisingly, in secondary DV3 infection, ADCC activity was >20% in 2 of 8 plasma samples with an undetectable $PRNT_{50}$ pattern. The $PRNT_{50}$ titer to JEV in these 2 cases was also undetectable (figure 6). In addition, ADCC activity was <5% in plasma samples with an undetectable $PRNT_{50}$ pattern but a detectable $PRNT_{50}$ titer to JEV ($n = 3$ each for pre–secondary DV2 infection and DV3 infection samples; all cases nhDF), which implies that monotypic antibody to any of the flaviviruses studied is not sufficient to mediate ADCC activity.

DISCUSSION

We have demonstrated that ADCC activity in plasma obtained before secondary DV2 or DV3 infection correlated with serotype-specific NAb titers, anti-DV IgG1 levels, and a multitypic $PRNT_{50}$ pattern. A higher level of ADCC activity measured before secondary DV3 infection was associated with lower subsequent viremia levels, which suggests a protective role for these antibodies. We did not see this association for secondary DV2 infection. Our previous study involving these same study subjects similarly showed that higher levels of preexisting NAbs to DV3 were associated with lower subsequent plasma viremia levels. We did not observe the same NAb association for DV2 infection [5]. Our results suggest that, in secondary DV3 in-

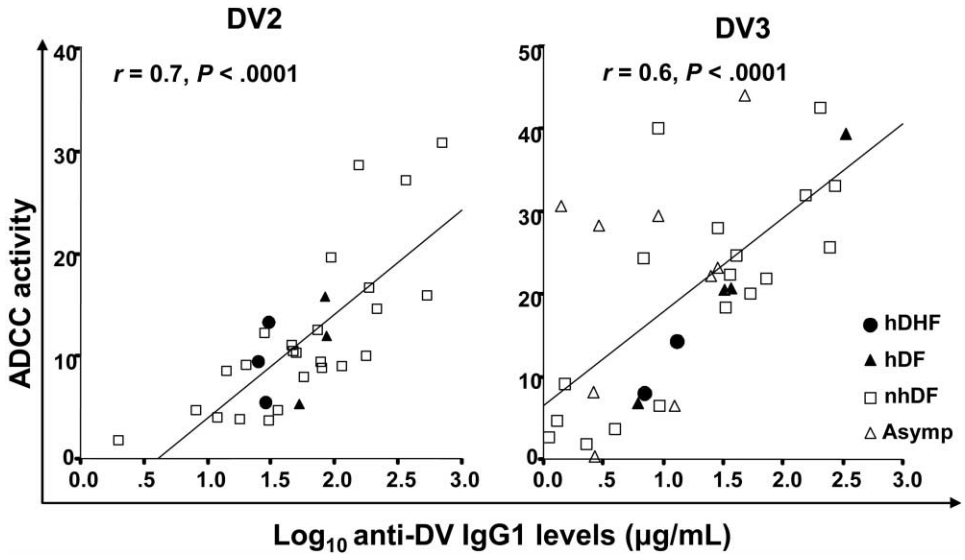

Figure 5. Correlation between antibody-dependent cellular cytotoxicity (ADCC) activity and anti–dengue virus (DV) IgG1 levels. ADCC activity (*Y*-axis) in plasma samples from children with subsequent secondary DV serotype 2 (DV2) and serotype 3 (DV3) infection is shown in the left and right panels, respectively. Log_{10} anti-DV IgG1 levels are shown on the *X*-axis. Data points represent individual subjects with hospitalized dengue hemorrhagic fever (hDHF), hospitalized dengue fever (hDF), nonhospitalized dengue fever (nhDF), or asymptomatic seroconversion (Asymp). Spearman rank correlation was used to analyze data, with $P < .05$ considered to be significant.

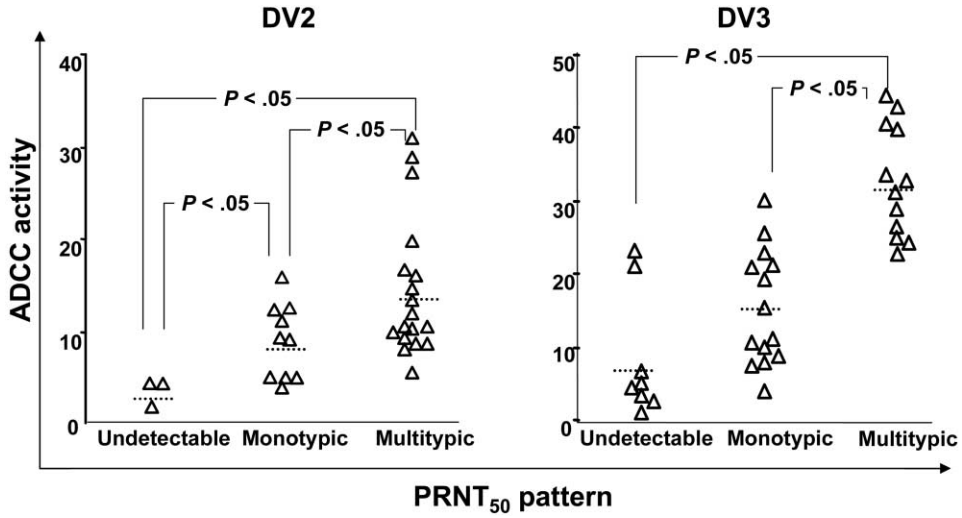

Figure 6. Relationship between antibody-dependent cellular cytotoxicity (ADCC) activity and neutralizing antibody patterns. ADCC activity (*Y*-axis) in plasma samples from children with subsequent secondary dengue virus (DV) serotype 2 (DV2) and serotype 3 (DV3) infection is shown in the left and right panels, respectively. Data points represent individual subjects. We defined 3 patterns of preillness DV $PRNT_{50}$ results (*X*-axis): undetectable ($PRNT_{50}$ <10 to all 4 DV serotypes), monotypic ($PRNT_{50}$ >10 to only 1 DV serotype or $PRNT_{50}$ >10 to >1 DV serotype but with a $PRNT_{50}$ ⩾80 to only 1 DV serotype), and multitypic ($PRNT_{50}$ >10 to >1 DV serotype with a $PRNT_{50}$ ⩾80 to >1 DV serotype). Analysis of variance and Student's *t* test were used to analyze data, with $P < .05$ considered to be significant. Dotted lines represent mean values.

fection, preexisting cross-reactive anti-DV antibodies contribute to the control of plasma viremia by neutralizing free DV3 (NAb) and clearing virally infected cells (ADCC). In symptomatic secondary DV2 infections, preexisting cross-reactive anti-DV antibodies do not appear to have the same effects.

The similar finding of NAb and ADCC activity at lower viremia levels in secondary DV3 infections may relate to their different mechanisms of action. NAbs bind circulating virions, whereas ADCC targets the virus-producing cells that have been found to be infected in vivo—B cells, monocytes/macrophages, and dendritic cells. Of note, the target cell used in our assay system was of B cell lineage. These 2 mechanisms that target different sites for DV (extracellular vs. intracellular) may play complementary roles in virus clearance.

Although ADCC activity was lowest in severe secondary DV3 infection (hDHF), we were unable to detect any significant correlation between ADCC activity and disease severity. It is possible that the difference between hDF and nhDF is not completely related to disease severity, but it is unlikely that the nhDF cases actually involved plasma leakage. Our preliminary analyses included comparisons between all hDF cases and nhDF cases, as well as between all DHF cases and all DF cases. In addition, the decision to hospitalize the subjects in this study was based on a standardized approach to case finding and evaluation. Therefore, we do not believe that our study design biases our final conclusions. In addition to ADCC, it is likely that other immune mechanisms (e.g., cytokine production) and plasma viremia levels play a role in shaping clinical outcomes. Our relatively small sample size ($n = 3$) for severe disease (hDHF) limits the strength of this conclusion.

We found that, when a single DV-immune plasma sample was used, ADCC activity differed among NK cell donors. Our study and other studies have shown that the level of NK cytotoxicity is dependent on effector cell donors [16, 31]. Although ADCC is mediated by FcγRIII (CD16), ADCC activity did not correlate with levels of surface CD16 expression on effector NK cells. This suggests that other NK cell receptors or signals (e.g., NKp46 receptor or interleukin-15) may also be involved in ADCC activity [19, 32, 33]. We also found that ADCC activity did not correlate with K562 cell lysis. This suggests that the mechanisms of target-cell lysis by NK cells in these 2 assays differ. K562 cells, which have no major histocompatibility complex (MHC) class I expression, are presumably lysed by NK cells using the missing self-recognition mechanism [34]. In contrast, DV-infected and -uninfected Raji cells express high levels of MHC class I (data not shown), so NK cell lysis activity is down-regulated by inhibitory receptors that interact with MHC class I. Previous studies [35–37] have also shown that flavivirus infection induces increased cell surface expression of MHC class I, which may result in a reduced susceptibility to lysis by NK cells. ADCC lysis of DV-infected cells may be a mechanism whereby NK cells can overcome these inhibitory signals.

In vivo, ADCC would be affected by both antibody and NK cell characteristics. Previous studies have shown that the percentages of NK and $CD8^+$ T cells expressing the early activation

marker CD69 were higher in children with DHF than in those with DF [18]. We hypothesize that, in early secondary DV infection, ADCC lysis of DV-infected cells [38–40] may lead to a decrease in the number of DV-infected cells and contribute to decreased plasma viremia levels. Limited NK cell–mediated ADCC activity in some secondary DV infections may contribute to a higher level of DV-infected antigen-presenting cells and cellular immune activation, leading to increased disease severity and DHF [41, 42]. Because the majority of secondary DV infections do not lead to DHF, it is likely that several NK/ADCC-related factors (e.g., killer immunoglobulin-like receptor polymorphisms) have a role in shaping the ability of NK cells to restrict virus spread.

Although we and others have demonstrated that DV-immune serum can mediate ADCC [19, 43], this is the first study, to our knowledge, to examine the relationship between preillness ADCC activity and subsequent dengue disease severity and viremia levels. ADCC correlated with serotype-specific NAb titers and anti-DV IgG1 levels in pre–secondary DV2 and DV3 infection plasma samples. In secondary DV3 infection, but not in secondary DV2 infection, ADCC activity inversely correlated with plasma viremia levels. At present, we are unable to conclude whether the same antibodies mediate virus neutralization and ADCC or whether different antibodies mediate these 2 protective immune responses. A dual role for an anti-DV antibody to mediate neutralization and ADCC has not, to our knowledge, been previously described. In fact, mutated anti-HIV NAbs with similar abilities to neutralize mediated significantly lower levels of ADCC lysis of infected target cells [44]. It is, therefore, important to pursue further studies to determine the precise mechanism of action of DV-specific antibodies and their role in mediating protection from heterologous DV infections.

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