An observational study of children vertically infected with human immunodeficiency virus type 1 (HIV-1) was performed to determine the role of CD38 expression in CD8+ T cells as a prognostic marker of virological failure in children receiving HAART. We studied 42 children who were receiving antiretroviral therapy and who had an undetectable virus load (uVL), and we found a negative correlation between CD38 expression in CD8+ T cells and the duration of uVL. We selected 17 HIV-1–infected children with CD38 values close to the baseline level (i.e., the first uVL achieved), and we distributed the children into 2 groups on the basis of median CD38 value in CD8+ T cells. Children with CD38 values in CD8+ T cells that were higher than the median had a higher incidence and relative risk of virological failure than did those with values lower than the median. In conclusion, we demonstrate for the first time that CD8+CD38+ T cell count is a good prognostic marker of therapeutic failure in HIV-1–infected children.

Infection with HIV-1 induces lymphoid activation, resulting in an increase in T cell activation–associated antigens, such as CD38 [1–3]. Also, CD38 could represent HIV-1–specific or cytokine-activated cells [4–6]. Several studies have shown that such increased CD8+CD38+ T cell expression is a strong predictive marker for disease progression in HIV-1 infection [7–10]. The CD8+CD38+ T cell count not only predicts progression of HIV disease to AIDS and death, but it also offers additional independent predictive value for evaluation of plasma virus load (VL) and CD4+ T cell count [7–9], suggesting that additional factors may be involved in the pathogenesis of HIV-1 infection and thus contribute to high levels of CD38 cells.

In recent years, the introduction of HAART has led to the suppression of HIV-1 replication, with a dramatic decrease in VL in many HIV-1–infected children [11, 12]. It has also been shown that the initiation of antiretroviral therapy is associated with a reduction in CD8+CD38+ T cell count [13–15]. However, not all HAART-based treatments are successful [11, 16]. Therefore, there is a need for adequate prognostic markers that are capable of predicting the lack of response to HAART in advanced or early phases of treatment. CD8+CD38+ T cell count has been proposed as a useful tool for monitoring viral replication in HIV-1–infected patients [14].

Thus, CD8+CD38+ T cell count could be an early marker of either viral replication and HAART failure. However, to date, to our knowledge, there have been no studies on its predictive value with regard to virological failure that have been performed with survival analysis. For that purpose, we performed an observational study to determine the role of CD8+CD38+ T cell count as a prognostic marker of virological failure in HIV-1–infected children receiving HAART.
PATIENTS, MATERIALS, AND METHODS

Patients. Forty-two children vertically infected with HIV-1 were recruited for an observational study performed at the Pediatric Department of the Hospital General Universitario “Gregorio Marañón” (Madrid, Spain) from December 1997 through September 2002. For the purpose of our study, inclusion criteria were receipt of antiretroviral therapy and undetectable VL (uVL) and CD8+CD38+ T cell counts. The first uVL value achieved was considered to be the baseline value. For the survival analysis, we selected 17 HIV-1–infected children who presented with CD8+CD38+ values that were close to the baseline value (time since first detected uVL, 0–2.5 months).

HIV-1 infection was diagnosed on the basis of positive results of both DNA PCR and viral culture assays, as described elsewhere [17]. Clinical classification of the children was based on the 1994 revised guidelines of the Centers for Disease Control and Prevention (CDC) [18]. Our study was conducted in accordance with the Declaration of Helsinki and was approved by the institution’s Ethics Committee. “Combination therapy” was defined as antiretroviral therapy consisting of ≥2 nucleoside analogues, excluding protease inhibitors and nonnucleoside analogues. There was no strict approach to antiretroviral treatment. Instead, each pediatrician administered the appropriate HAART regimen and changed the drugs according to his or her interpretation of the patient’s data and on the basis of CDC and European guidelines [19, 20]. For each patient in our study, we gathered data to assess the moment when an uVL was achieved. Changes in treatment were made by the pediatrician if the patient did not respond to antiretroviral therapy. For each patient, such changes were made on the basis of clinical, immunological, and virological evolution and in accordance with CDC and European (Paediatric European Network for the Treatment of AIDS) guidelines for pediatric treatment [19, 20].

Response to therapy was evaluated every 3 months by serial assessment of CD4+ cell percentage, CD8+ T cell percentage, VL, and clinical data, in accordance with published guidelines [19, 20]. T lymphocyte subsets were quantified by flow cytometry (Becton-Dickinson Immunocytometry Systems). HIV-1 RNA levels were measured using a quantitative RT-PCR assay (Amplicor Monitor; Roche Diagnostic Systems).

Quantification of CD8+CD38+ T cell counts in peripheral blood. For analysis of CD8+ T cell subsets, the following monoclonal antibodies were used: CD8-PerCP (Becton-Dickinson), HLA-DR-PE (Becton-Dickinson Immunocytometry Systems), and CD38-FITC (Immunotech). Three-color phenotypic characterizations of lymphocytes were performed by flow cytometry with whole, lysed, and washed blood. Acquisition was performed in a FACSscan cytometer (Becton-Dickinson) using the Lysis II acquisition program (Becton-Dickinson), as described elsewhere [2]. We calculated the median CD38-relative fluorescence intensity (MFI) using single-parameter histograms with no cursor sets.

Statistical analysis. The relationship between variables was investigated using a partial correlation coefficient, which describes the linear relationship between 2 variables while controlling for the effects of age. Seventeen HIV-1–infected children were observed from the first recorded uVL (VL, <400 copies/mL) until they had experienced virological failure (rebound of VL) or until their last available VL measurement. Kaplan-Meier analysis was performed. The exposure variable was the activation of the immune system, which was measured as the expression of CD38+ in CD8+ T cells. We performed the survival analysis according to the median CD8+CD38+ percentage (median, 70.6%), CD8+HLA-DR+ percentage (median, 30.05%), CD8+HLA-DR+CD38+ percentage (median, 19.8%), CD8+CD38+ MFI (median, 14.1), and CD8+HLA-DR+ MFI (median, 8.06) at baseline. The outcome variable was virological failure with a rebound in the VL to >400 copies/mL or >5000 copies/mL. Cox regression analyses were performed to calculate the relative risk (RR) of a rebound in the VL. The variable used for the statistical adjustment was age at baseline.

RESULTS

Clinical, immunological and virological characteristics of the 42 vertically HIV-1–infected children prior to baseline (i.e., the first uVL measurement) are illustrated in table 1. Table 2 shows the characteristics of HIV-1–infected children, according to antiretroviral treatment. One-half of the study population had AIDS. Children with AIDS had higher CD8+CD38+ MFI values than did children without AIDS (mean ± SEM, 15.83 ± 1.15 vs. 11.54 ± 0.92; P = .008). Also, the CD8+CD38+ percentage was higher in children with AIDS (mean ± SEM, 70.53% ± 2.88%) than in children without AIDS (mean ± SEM, 61.67% ± 3.47%; P = .054).

Correlation between decrease in CD38 expression in CD8+ T cells and duration of follow-up with uVL. After the attainment of uVL, CD8+CD38+ T cell counts decreased markedly with duration of uVL (figure 1). We found a negative correlation between CD8+CD38+ percentage, CD8+HLA-DR-CD38+ percentage, and CD8+CD38+ MFI value and the duration of follow-up with VL (figure 1A and 1B). CD8+CD38+ percentage presented the strongest negative association.

CD8+CD38+ T cell count as prognostic marker of virological failure. Standard survival techniques, including Kaplan-Meier analysis and Cox proportional hazard models, were used to evaluate data for 17 children. HIV-1–infected children with CD8+CD38+ levels of ≤70.6% had a lower incidence of virological failure than did HIV-1–infected children with CD8+CD38+ levels of >70.6% (figures 1A, 1B, and 2). During the entire follow-up period, 33.3% children with CD8+CD38+
Table 1. Baseline immunological and virological parameters and characteristics of HIV-1–infected children receiving antiretroviral treatment.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of children</td>
<td>42</td>
</tr>
<tr>
<td>Age, years Mean ± SEM</td>
<td>9.2 ± 0.7</td>
</tr>
<tr>
<td>Range</td>
<td>1.4–17.5</td>
</tr>
<tr>
<td>No. (%) of patients with AIDS</td>
<td>21 (50)</td>
</tr>
<tr>
<td>Lymphocyte subsets, %</td>
<td></td>
</tr>
<tr>
<td>CD4⁺ Mean ± SEM</td>
<td>25.3 ± 2.2</td>
</tr>
<tr>
<td>Range</td>
<td>1–55.5</td>
</tr>
<tr>
<td>CD8⁺ Mean ± SEM</td>
<td>44.9 ± 2.2</td>
</tr>
<tr>
<td>Range</td>
<td>12.5–79</td>
</tr>
<tr>
<td>Virus load for entire cohort, mean copies/mL</td>
<td>400</td>
</tr>
<tr>
<td>Antiretroviral therapy received, no. (%) of patients</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>8 (19)</td>
</tr>
<tr>
<td>HAART</td>
<td>34 (81)</td>
</tr>
</tbody>
</table>

NOTE. CT, combination therapy.

Table 2. Characteristics of HIV-1–infected children, according to antiretroviral treatment (ART).

<table>
<thead>
<tr>
<th>ART status</th>
<th>No. (%) of patients</th>
<th>Duration of ART, monthsᵃ</th>
<th>Duration of uVL, monthsᵇ</th>
<th>CD8⁺CD38⁺ cell percentageᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>11 (26.2)</td>
<td>...</td>
<td>11.9 ± 4.4 (2–35)</td>
<td>61.3 ± 5.8 (36.6–85.3)</td>
</tr>
<tr>
<td>Monotherapy</td>
<td>3 (7.1)</td>
<td>20.5 ± 10.2 (0.7–35)</td>
<td>5.2 ± 3.4 (1.8–8.6)</td>
<td>65.8 ± 9.2 (47.4–75.4)</td>
</tr>
<tr>
<td>Combination</td>
<td>14 (33.3)</td>
<td>43.3 ± 5.7 (18.1–97)</td>
<td>17.4 ± 3.8 (0.2–39)</td>
<td>70.1 ± 3.0 (54.8–88.5)</td>
</tr>
<tr>
<td>HAART</td>
<td>14 (33.3)</td>
<td>55.9 ± 8.9 (0.9–109)</td>
<td>14.4 ± 4.4 (1.1–47.5)</td>
<td>66.0 ± 4.0 (40.1–90.2)</td>
</tr>
</tbody>
</table>

NOTE. uVL, undetectable virus load.

DISCUSSION

In recent years, HAART has been successfully used for the treatment of HIV-1–infected subjects, with a great reduction in HIV-1–related morbidity and mortality [21, 22]. The efficacy of HAART has meant that many patients achieve suppression of VL to less than the limits of detection (i.e., a uVL) along with an increase in the CD4⁺ T lymphocyte count [23–25], with a generally good outcome [26, 27]. However, most therapeutic regimens have failed to achieve a complete suppression of HIV-1 replication in children [11]. The early identification of HIV-1–infected patients who are likely to experience therapeutic failure represents a major challenge for their management, because early identification would allow the initiation of alternative successful therapies. In the present study, we confirmed that CD8⁺CD38⁺ T cell count is a marker of immune activation and that this expression decreases with the control of HIV-1 replication in HIV-1–infected children receiving HAART. Most probably, CD8⁺ T cell activation was driven by HIV-1 replication; once the level of viral replication was reduced, CD8⁺ T cell activation normalized. This finding further emphasizes why long-lasting viral suppression is required to normalize the immune system. In this study, we report the reduction in the activation stage of CD8⁺ T cells in the peripheral blood, although we lack data on the effect of viral suppression on CD8⁺ T cells in lymphoid tissue.

The increase in the CD8⁺CD38⁺ T cell count has been associated with elevated VLs in HIV-1–infected children [2] and adults [28]. Tilling et al. [13] monitored the changes in the CD8⁺CD38⁺ T cell count in peripheral blood samples obtained from patients receiving HAART. They found that CD8⁺CD38⁺ T cell counts decreased in patients who maintained a VL of ≤70.6% did not have a rebound in the VL of >400 copies/mL and/or >5000 copies/mL. All HIV-1–infected children with CD8⁺CD38⁺ levels of >70.6% had a rebound in the VL of >400 copies/mL and/or >5000 copies/mL.
<50 copies/mL, indicating that CD8<sup>+</sup>CD38<sup>+</sup> T cell count may represent a marker of residual viral replication when the VL decreases to less than detectable levels after HAART intervention. In our study, we observed a progressive decrease in the CD8<sup>+</sup>CD38<sup>+</sup> T cell count with duration of uVL, implying that, in the absence of further viral stimulation, CD38 expression in CD8<sup>+</sup> T cells must have differentiated to another cellular subset or down-regulated their high CD38<sup>+</sup> cell phenotype. These findings support the utility of assessing the level of CD38 expression to monitor the response to HAART.

The initiation of HAART induces a noteworthy decrease in both the percentage and absolute number of CD8<sup>+</sup>CD38<sup>+</sup> T cells in peripheral blood and lymphoid tissues [29, 30]. In addition, the lack of response to HAART has been shown to be associated with the lack of reduction in the VL, the lack of improvement in the CD4 count, or both [31, 32]. In a similar way, persistence of increased CD8<sup>+</sup>CD38<sup>+</sup> T cell counts predicts the maintenance of high VL in HAART-treated HIV-1–infected children [14]. In our experience, there is a dramatic rebound in the VL parallel with the median CD8<sup>+</sup>CD38<sup>+</sup> percentage or CD8<sup>+</sup>HLA-DR<sup>+</sup>CD38<sup>+</sup> percentage. In the analysis of the data, we controlled for the association of HLA-DR with CD38 in activated T cells. We analyzed CD8<sup>+</sup>HLA-DR<sup>+</sup>CD38<sup>+</sup> and CD8<sup>+</sup>HLA-DR<sup>+</sup>CD38<sup>+</sup> separately to control the interaction of HLA-DR<sup>+</sup> and CD38<sup>+</sup> markers. We did not find an association between them and the duration of uVL and virological failure (data not shown). We later performed a multivariate Cox regression analysis with HLA-DR and CD38 values solely. We found that CD38 level was still valid as predictive marker of virological failure, whereas HLA-DR level was not. Finally, we performed a multivariate Cox regression analysis considering the CD8<sup>+</sup>CD38<sup>+</sup> T cell percentage and previous antiretroviral therapy protocols to assess virological failure. We found that

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**Table 3. Results of Cox regression analysis of the increase in the virus load (VL) according to median T cell subset value at baseline.**

<table>
<thead>
<tr>
<th>T cell subset</th>
<th>Risk of increase in the VL of &gt;400 copies/mL</th>
<th>Risk of increase in the VL of &gt;5000 copies/mL</th>
<th>P</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell percentage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD8&lt;sup&gt;+&lt;/sup&gt;CD38&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4.5 (1.3–15.3)</td>
<td>2.9 (0.9–9.2)</td>
<td>.015&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.050&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD8&lt;sup&gt;+&lt;/sup&gt;HLA-DR&lt;sup&gt;+&lt;/sup&gt;</td>
<td>2.2 (0.7–6.4)</td>
<td>2.2 (0.7–6.8)</td>
<td>.179</td>
<td>.146</td>
</tr>
<tr>
<td>CD8&lt;sup&gt;+&lt;/sup&gt;HLA-DR&lt;sup&gt;+&lt;/sup&gt;CD38&lt;sup&gt;+&lt;/sup&gt;</td>
<td>2.9 (0.9–9.1)</td>
<td>3.7 (1.1–12.7)</td>
<td>.065</td>
<td>.032&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MFI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD8&lt;sup&gt;+&lt;/sup&gt;HLA-DR&lt;sup&gt;+&lt;/sup&gt;</td>
<td>2 (0.7–5.8)</td>
<td>2 (0.7–6.1)</td>
<td>.199</td>
<td>.181</td>
</tr>
<tr>
<td>CD8&lt;sup&gt;+&lt;/sup&gt;CD38&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1.8 (0.6–5.3)</td>
<td>1.9 (0.6–5.5)</td>
<td>.273</td>
<td>.236</td>
</tr>
</tbody>
</table>

**NOTE.** MFI, median CD38-relative fluorescence intensity; RR, relative risk.

<sup>a</sup> Statistically significant
prior antiretroviral therapy protocol did not have any significant influence on CD8⁺CD38⁺ T cell percentage. Similarly, the duration of virological suppression did not have any significant influence (data not shown).

However, increased CD38 expression in CD8⁺ T cells obtained from patients with Epstein-Barr virus infection and other infections has been reported [33, 34]. The CD8⁺CD38⁺ T cell count may represent a sensitive marker for the early prediction of occurrence of infectious diseases in HIV-1–infected patients treated with HAART. Thus, high CD38 values might not be specific for the reactivation of HIV-1 infection. Therefore, in the absence of other infections, the CD38 value might be a useful prognostic factor for HAART-treated HIV-1–infected patients [29]. Moreover, the CD8⁺CD38⁺ T cell count was higher in children with AIDS, but the incidence of virological failure incidence was not greater among these children (data not shown). This highlights the importance of CD8⁺CD38⁺ T cell count as a marker of virological failure.

In conclusion, in this study, we demonstrate—to our knowledge, for the first time—that CD8⁺CD38⁺ T cell count is a good long-term prognostic marker of therapeutic failure in HIV-1–infected children. However, there are certain limitations to our current results—in particular, the small sample size and the strict inclusion criteria—that somewhat restrict our conclusions and their external validity. Early signs of immune activation might warn the clinician about the patient’s noncompliance with therapy or about viral rebound during HAART. Further prospective studies, including a more systematic follow-up, are needed to assess the sensitivity of the CD8⁺CD38⁺ T cell count as a marker of the control of viral replication in HIV-1–infected children.

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