T-cell immune response controls the high incidence of adenovirus infection in adult allogenic hematopoietic transplantation recipients

Human adenovirus (HAdV) can produce disseminated disease with high mortality in pediatric allogenic hematopoietic stem cell transplant (allo-HSCT) recipients. In adult recipients, prospective studies addressing the incidence of HAdV infection and disease are lacking. The aim of this study was to characterize the kinetics of HAdV replication, the specific T-cell immune response, and the clinical impact of HAdV infection in adult allo-HSCT recipients. We found a high incidence of HAdV viremia, which was controlled by the T-cell immune response and without association with disseminated HAdV disease. Therefore, these data argue against the pre-emptive therapy for HAdV infection in adult allo-HSCT recipients.

The high variability on the reported rates of HAdV infection in HSCT recipients reflects the gap of knowledge regarding the natural history of the HAdV infection in these patients.[1-5] Although the overall mortality of HAdV infections is low in adults (1%), life-threatening disease has been associated with HAdV DNAemia.[5-7] However, this statement derives from studies made in pediatric allo-HSCT7[7] or retrospective evaluations of pediatric and adult patients selected after disseminated HAdV disease diagnosis.[6] In cases of disseminated disease, lethality rates reach up to 60-80% in children and adults.[5,7,8]

One hundred seventeen patients received an allo-HSCT from February 2016 to May 2018. Ninety-five of them (>18 years old) fulfilled the study requirements and were included (see the *Online Supplementary Materials and Methods*). Demographics and clinical characteristics of the patients are detailed in the *Online Supplementary Table S1*.

Fifty-eight (61.1%) patients showed HAdV viremia episodes, all cases belonging to the HAdV species C. Most episodes were transient viremia or blips, defined as HAdV detected in one isolated sample (59 of 80, 73.8%), with a median of one episode per patient. HAdV viremia occurred early (weeks -1 to +7) and blips earlier than persistent episodes (median 3 weeks [range: 1-5] *vs.* 7 weeks [range: 0-10], respectively). Viral loads showed wide variability, with lower levels observed in blips *versus* persistent episodes ($1.5x10^2$ [range: $8.1x10^1$-$5.4x10^2$] *versus* $9.8x10^2$ [range: $2.1x10^2$-$8.3x10^4$] copies/mL), and a median duration of 14 days in persistent episodes. Thirty-six (37.9%) patients had HAdV viremia <$5x10^2$ copies/mL and 22 (23.2%) ≥$5x10^2$ DNA copies/mL. The differences of viremia episodes between both groups are detailed in (Table 1). Six of 22 patients with ≥$5x10^2$ copies/mL had blips previously.

The incidence of HAdV viremia was similar to that reported in pediatric patients[5] but higher than those observed in adult allo-HSCT recipients (15-19.7%).[1,2] In agreement with other reports,[9,10] blips and persistent viremia episodes were detected early in the follow-up, suggesting that the HAdV viremia may arise from reactivation of the virus. This data could support the implementation of a HAdV pre-transplant screening, as has been already suggested.[10]

In adult allo-HSCT recipients, postulated risk factors for HAdV infection and disease include haploidentical or unrelated donor cord transplant, graft-*versus*-host disease (GvHD) of grade 3/4, and treatment with alemtuzumab or anti-timocytic globulin (ATG).[11] We did not find any association between HAdV viremia and these risk factors (*Online Supplementary Table S2*). Regarding the ATG use, it is important to point out that this result may be due to the low number of patients treated (10,5%). The use of rapamycin for GvHD prophylaxis in patients with a reduced intensity conditioning (20% of the patients treated with ATG and 60% of the whole series) may be another explanation for this lack of association. Results from our laboratory not yet published show an anti-HAdV activity of rapamycin, with an IC_{50} of 2.3 μM (selective index of 55), as reported for human cytomegalovirus (HCMV).[12] The lack of association with any demographic data, transplantation or clinical characteristics was independent of the level of HAdV viremia (*data not shown*).

Table 1. Characteristics of the human adenovirus (HAdV) viremia episodes depending on the level of the HAdV DNAemia.

	Low DNAemia* n (%)	High DNAemia** n (%)
Patients	36 (37.9)	22 (23.2)
Episodes per patient (median, IQR)	1 (1-2)	1 (1-1)
Blips episodes	59 (73.8)	18 (75.0)
Week of follow-up (median, IQR)	3 (1-5)	5 (2-8)
Maximum viral load (copies/mL; median, IQR)	1.3x102 ($7.9x10^1$-$2.1x10^2$)	3.2x103 ($1.1x103$-$2.1x10^4$)
Persistent episodes	15 (26.8)	6 (25.0)
Week of follow-up (median, IQR)	6 (-1-11)	7 (1-8)
Duration of viremia (days; median, IQR)	14 (7-14)	14 (14-14)
Maximum viral load (copies/mL; median, IQR)	$1.9x10^2$ ($8.1x10^1$-$2.6x10^2$)	$2.0x10^4$ ($1.8x103$-$7.3x10^5$)

*DNAemia < $5x10^2$ copies/mL; **DNAemia ≥ $5x10^2$ copies/mL; IQR: interquartile range.

In this study no patient developed symptomatic or disseminated HAdV disease nor received specific HAdV therapy during the follow-up. In addition, there was no chronologic association between HAdV viremia and the presence of other infections. Ten patients died due to different causes: five patients directly related to the transplantation; two patients of GvHD, two patients of thrombotic microangiopathy and one patient of veno-occlusive disease). Three patients had other infections: two patients with septic shock from pneumonia and *Clostridioides difficile* infection and one patient with *Aspegillus fumigatus* pneumonia). One patient relapsed with hematologic disease and one patient with multifactorial pulmonary fibrosis. There was not difference in the mortality between patients with (n=5, 8.6%) or without (n=5, 13.5%) HAdV viremia (*Online Supplementary Table S2*). The data of the present study does not support, in adult allo-HSCT patients, the threshold of ≥$5x10^2$ HAdV DNA copies/mL in blood as a marker of invasive HAdV infection,[9,13] since no patient developed disseminated HAdV disease, independently of the HAdV viremia level.

The HAdV-specific T-cell immune response was evaluated (*Online Supplementary Materials and Methods*) in 90 out of the 95 patients (in five patients there was loss of follow-up in specific time points). The number of patients with specific HAdV-specific T-cell immune response increased during the follow-up: 30 (33.3%), 65 (72.2%) and 78 (86.7%) of 90 patients at days +21, +56 and +100 after transplantation, respectively. Twelve

(13.3%) of the 90 patients did not develop HAdV-specific a T-cell immune response during the follow-up; three (25.0%) of them had one HAdV viremia blip episode.

The number of patients with $CD4^+$ T cells expressing IL-2 by day +56 ($P<0.05$) and $CD8^+$ T cells expressing IL-2 ($P<0.05$) and TNF-α ($P<0.05$) by day +21, was higher in those with *versus* without HAdV viremia (*Online Supplementary Table S3*). When the HAdV-specific T-cell immune response was compared between both groups higher expressions of IL-2 by $CD4^+$ T cells, and IL-2 and TNF-α by $CD8^+$ T cells were observed by days +56 and +21, respectively (*Online Supplemental Figure S4*). In contrast, when we compared the HAdV-specific T-cell immune response in patients with HAdV viremia higher *versus* lower than $5x10^2$ copies/mL the maximum cytokine expression was observed in $CD4^+$ T cells expressing TNF-α by day +100 (0.2% *vs.* 0.13%, $P<0.05$) and higher expressions of IL-2 by $CD4^+$ T cells and INF-γ by $CD8^+$ T-cells were observed by day +100 after the transplantation in patients with HAdV viremia ≥$5x10^2$ DNA copies/mL (Figure 1, *Online Supplementary Table S5*). Interestingly, patients with pre-transplantation (days -7 and 0) persistent HAdV viremia (n=4) had $CD4^+$ expression of INF-γ ($P=0.05$) and $CD8^+$ expression of IL-2, TNF-α and INF-γ ($P<0.05$) higher and/or earlier (at +21 and +56 days) than those with blips (n=12); this

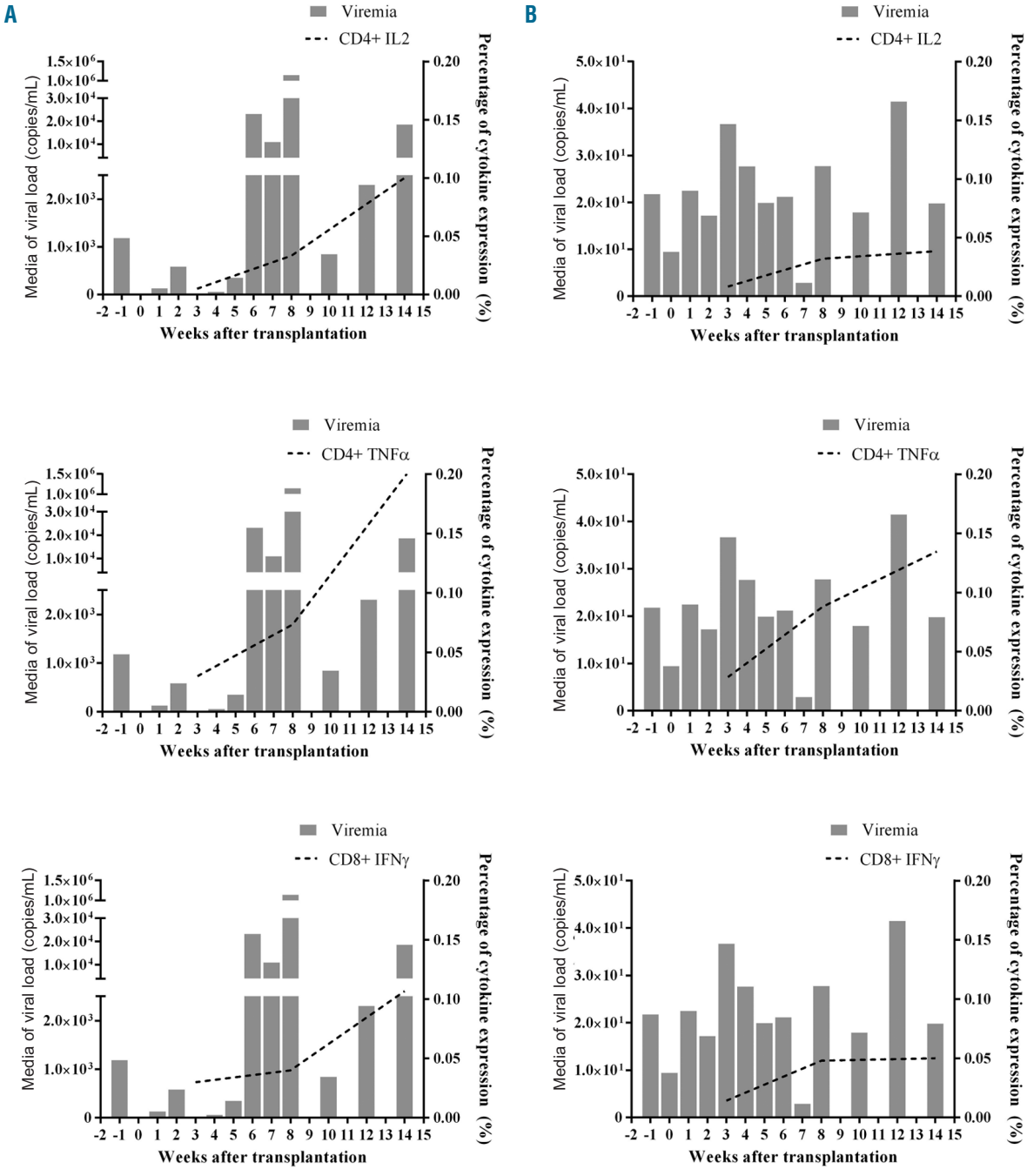

Figure 1. Human adenovirus-specific $CD4^+$ T cells expressing IL-2 and TNF-α and $CD8^+$ T cells expressing IFN-γ, on days +21, +56 and +100 after transplantation. (A) Patients with viremia ≥$5x10^2$ copies/mL. (B) Patients with viremia <$5x10^2$ copies/mL.

immune response paralleled with almost complete disappearance of viremia episodes (Figure 2). Finally, the percentage of $CD4^+$ and $CD8^+$ T cells and the $CD4^+/CD8^+$ T-cell ratio were similar in patients with and without HAdV viremia and independent of the level of viremia (*Online Supplementary Figure S1*).

In this study, we have characterized the HAdV-specific cellular immune response to identify the most appropriate immunologic marker to guide the management of HAdV infections in adult allo-HSCT recipients. Unlike Guérin-El Khourouj *et al.*,[14] who reported that HAdV-specific $CD4^+$ IFN-γ monitoring may be of help for the detection of high-risk allo-HSCT patients,[14] we found that the expression of IL-2 and TNF-α by $CD8^+$ T cells were the earlier markers in HAdV viremia patients. Moreover, specific $CD4^+$ and $CD8^+$ T-cell immune responses increased with a higher HAdV load in the blood. One interesting finding in the present study was the almost complete clearance of HAdV viremia, after 3 weeks of follow-up, in patients with persistent viremia before transplantation, which had higher expressions of INF-γ by $CD4^+$ and of IL-2, TNF-α and INF-γ by $CD8^+$ T cells, although the small number of these patients only permit to suggest this association. A limitation of our study is the lack of specific HAdV T-cell immune response evaluation in donors, which precludes to know if it has some impact on the kinetics and control of HAdV replication after the transplantation.

Currently, no definitive recommendations for the monitoring of HAdV viral loads and specific T-cell immune reconstitution as a basis for prophylaxis and therapy of HAdV infections in adult patients excist. In the pediatrics setting, the recommendations are limited to the threshold of HAdV DNAemia ≥$1x10^3$ copies/mL for the initiation of pre-emptive therapy, which is derived in most cases from retrospective and observational stu-dies.[3,15] A recent study suggests that the pretransplant detection of ≥$1x10^6$ HAdV DNA in stool is correlated with a higher risk of invasive HAdV infection.[13] In a European survey on the management of HAdV infection in allo-HSCT, physicians initiate preemptive therapy in adult patients with cidofovir in 29% and 14% of high and low-risk patients, respectively.[15] The high adverse effects observed with cidofovir represent a challenge for this decision in the absence of clinical data from prospective studies supporting the prediction value of viremia on disseminated HAdV disease in adult patients.

In summary, our results show that HAdV infection is a frequent event after allo-HSCT in adult patients, in contrast with the data from previous studies. The HAdV viremia may be persistent and with high viral loads. However, we found neither an association between the presence of HAdV viremia and the subsequent development of disseminated HAdV disease nor mortality. The HAdV-specific T-cell mediated immune response seems to control HAdV infections in adult recipients and to promote the early viremia clearance in adult patients with persistent viremia before transplantation. Therefore, the results from the present study do not support pre-emp-

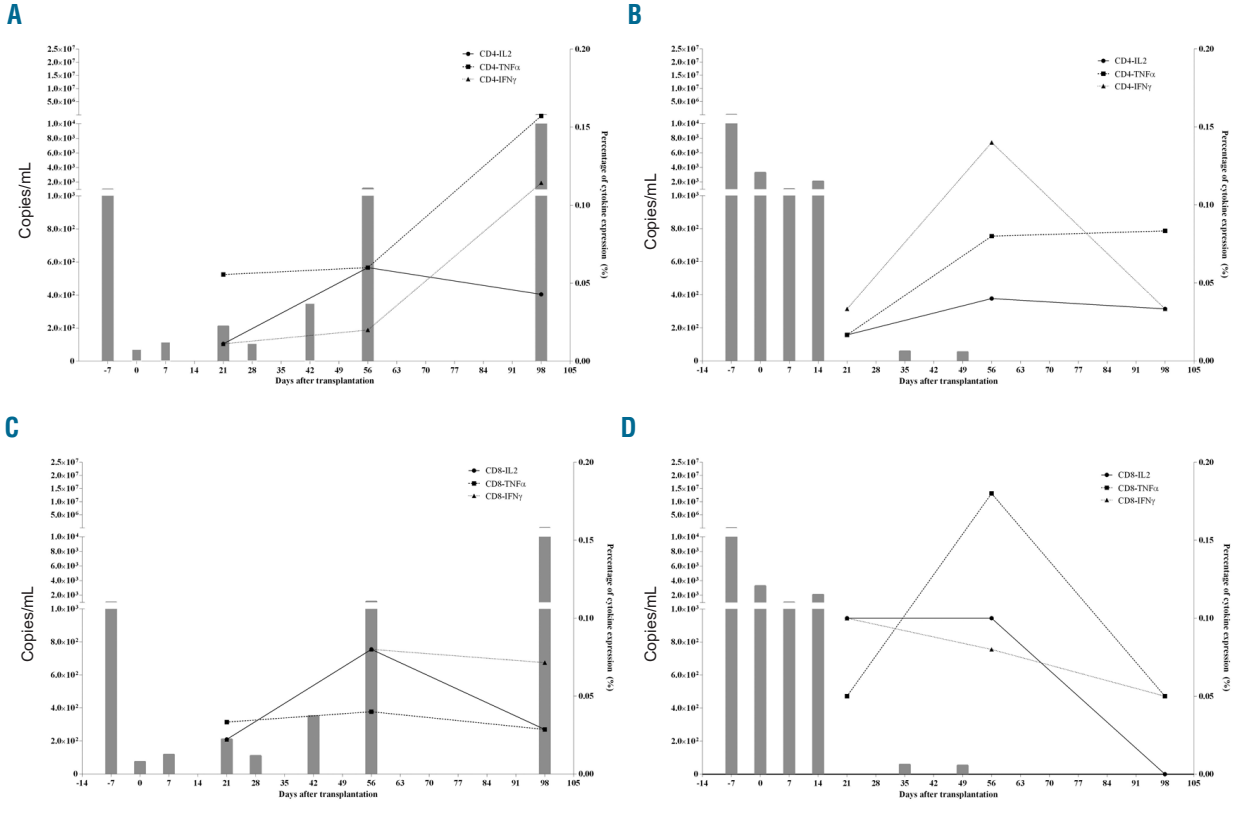

Figure 2 Human adenovirus-specific $CD4^+$ and $CD8^+$ T-cell immune responses in patients with blips and presistent viremia at days -7 and/or 0 pretransplantation. (A) and (C) $CD4^+$ and $CD8^+$ T cells, respectively, expressing IL-2, TNF-α and IFN-γ, on days +21, +56 and +100 after transplantation in patients with blips. (B) and (D) $CD4^+$ and $CD8^+$ T cells, respectively, expressing IL-2, TNF-α and IFN-γ, on days +21, +56 and +100 after transplantation in patients with persistent viremia.

tive therapy based only in the HAdV viremia in adult allo-HSCT recipients.

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