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Cellular Immune Reconstitution after Haploidentical Transplantation in Children

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ABSTRACT

Delayed immune reconstitution is 1 of the major contributions to the morbidity and mortality after haploidentical transplantation. Patients with a slow recovery of the innate and especially of the adaptive immune system are at high risk for severe and often lethal infections. The reason for delayed immune reconstitution after haploidentical transplantation include the T cell depletion (TCD) of the graft, the thymic dysfunction induced by pretransplant chemotherapies and by the conditioning regimens, and the occurrence of graft-versus-host disease (GVHD) and its treatment. The detailed analysis, understanding, and manipulation of the reconstitution of the cellular immune system will be of utmost importance to overcome the posttransplant immunodefcient status, and should result in a reduced risk of severe and overwhelming infections and hopefully also to a reduced risk of relapse through better immunological control of residual malignant cells.

KEY WORDS

Haploidentical stem cell transplantation • T cell depletion • Immune reconstitution • Alloreactive NK cells

RECONSTITUTION OF T CELLS

Various methods of T cell depletion (TCD) have been used in allogeneic stem transplantation [1-3]. Although the degree of TCD varied among these methods, they were all associated with a delayed reconstitution of the T-lymphocytes, resulting in severe and often lethal infectious complications in the posttransplant course.

Other methods use combined in vitro and in vivo TCD stategies without or with subsequent adoptive transfer of nonalloreactive donor cells [4,5].

More recently developed methods, such as $CD34^+$ positive selection, resulted in a high degree of TCD (5 log) and enabled the transplantation and tolerance induction across the HLA barrier [6,7], but were also associated with a high rate of infectious complications [8].

In our initial work, we have widely used the positive selection of haploidentical granulocyte–colony-stimulating factor (G-CSF)-mobilized peripheral $CD34^+$ stem cells as an indirect method of TCD in pediatric patients [9]. Using this approach, we were able to demonstrate in clinical trials that

(1)

1. Haploidentical transplantation with $CD34^+$ positively selected stem cells is possible without significant graft-versus-host disease (GVHD) [9] and is a reasonable alternative for children with malignant or nonmalignant diseases who do not have an HLA-matched related or unrelated donor [10].
2. The immune reconstitution after transplantation with $CD34^+$ selected stem cells is very delayed and associated with a high risk of severe and often lethal viral or fungal infections [11].

(2)

3. Alloreactive NK cells play a major role in the protection from posttransplant relapse [12,13] and might support the engraftment and protect from GVHD [14].

Initially, we have used myeloablative preparative regimen with the $CD34^+$ positive selection to ensure engraftment [9]. However, we have experienced a high treatment-related mortality (TRM) in association with myelablative conditioning regimens in the

haploidentical setting because of toxicity and delayed immune reconstitution. From these initial experiences with $CD34^+$ positive selection, we have concluded that an ideal graft should not only be composed of highly purified $CD34^+$ stem cells, but should also contain NK cells from a preferentially NK-alloreactive donor. Therefore, we have established a TCD [15] and a TCD and B cell depletion method [16] for haploidentical transplantations based on the Magnetic-activated cell sorter that retains large numbers of NK cells and other cells. The additional B cell depletion was introduced to avoid donor-derived posttransplant EBV-associated lymphoproliferative disease (Figure 1).

Based on experimental data in animal models that NK cells might facilitate engraftment [14], we hypothesized that NK-cell enriched CD3-depleted stem cells should support the engraftment of haploidentical stem cells and initiated clinical trials with CD3-depleted stem cells at St. Jude Children's Research Hospital Memphis using either a myeloablative total body irradiation (TBI)-containing preparative regimen or reduced intensity conditioning (RIC) approach. For in vivo B cell depletion, patients received a single dose of the anti-CD20 antibody Rituxan prior to stem cell infusion.

To assess the immune reconstitution, lymphocyte subsets were analyzed by flow cytometry. In addition, the thymic function was assessed by measuring T cell receptor excision circles (TRECs) as a measure for the de novo generation of thymocytes [17] and the T cell receptor beta repertoire was assayed by V-beta spectratyping.

The comparative analyis of the immune reconstitution after a myeloablative TBI-containing regimen (TBI 12 Gy, Thiotepa 10 mg/kg, Cytoxan 120 mg/kg, ATG, and anti-CD3) [18] and an RIC regimen (Fludarabine 200 mg/m^2, Thiotepa 10 mg/kg, Melphalan 120 mg/m^2, and anti-CD3) [19] showed an accelerated immune reconstitution of the $CD4^+$ T-helper lymphocytes and a rapid increase of $CD56^+$

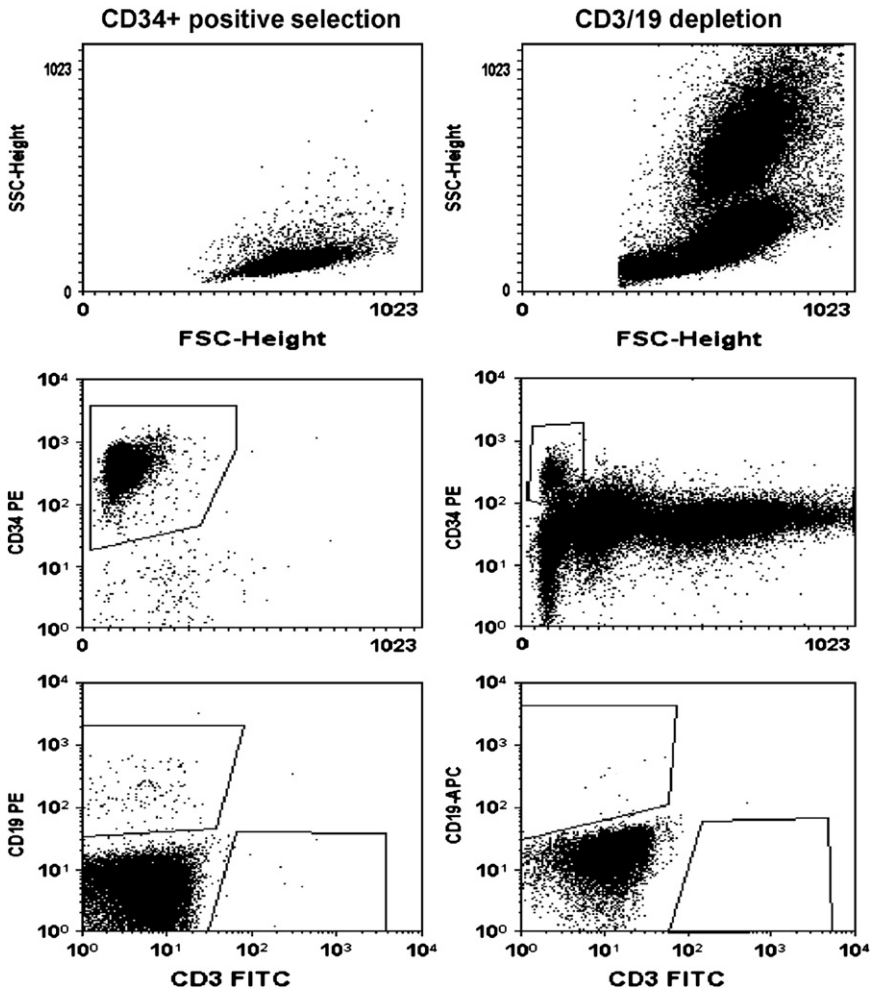

Figure 1. The fundamental difference between $CD34^+$ positive selected (left panel) and CD3/C19-depleted (right panel) mobilized peripheral stem cells from haploidentical donors is shown. Although $CD34^+$ positive selection results in a homogenous population of highly purified $CD34^+$ stem cells, CD3/C19 depletion results only in a low enrichment of $CD34^+$ cells, but retains large numbers of $CD56^+$ NK cells and other myeloid cells.

NK cells in the patients who received the RIC regimen. In addition, patients after RIC conditioning had a much faster thymic recovery as documented by a rapid increase of TREC and a faster reconstitution of the TCR, as measured by the V-beta spectratyping scores. The rapid immune reconstitution of the TCR in the RIC patients was associated with less reactivation of CMV and adenovirus (ADV) and in case of reactivation with much lower copy numbers [20]. In Figure 2, this comparative analyis is shown.

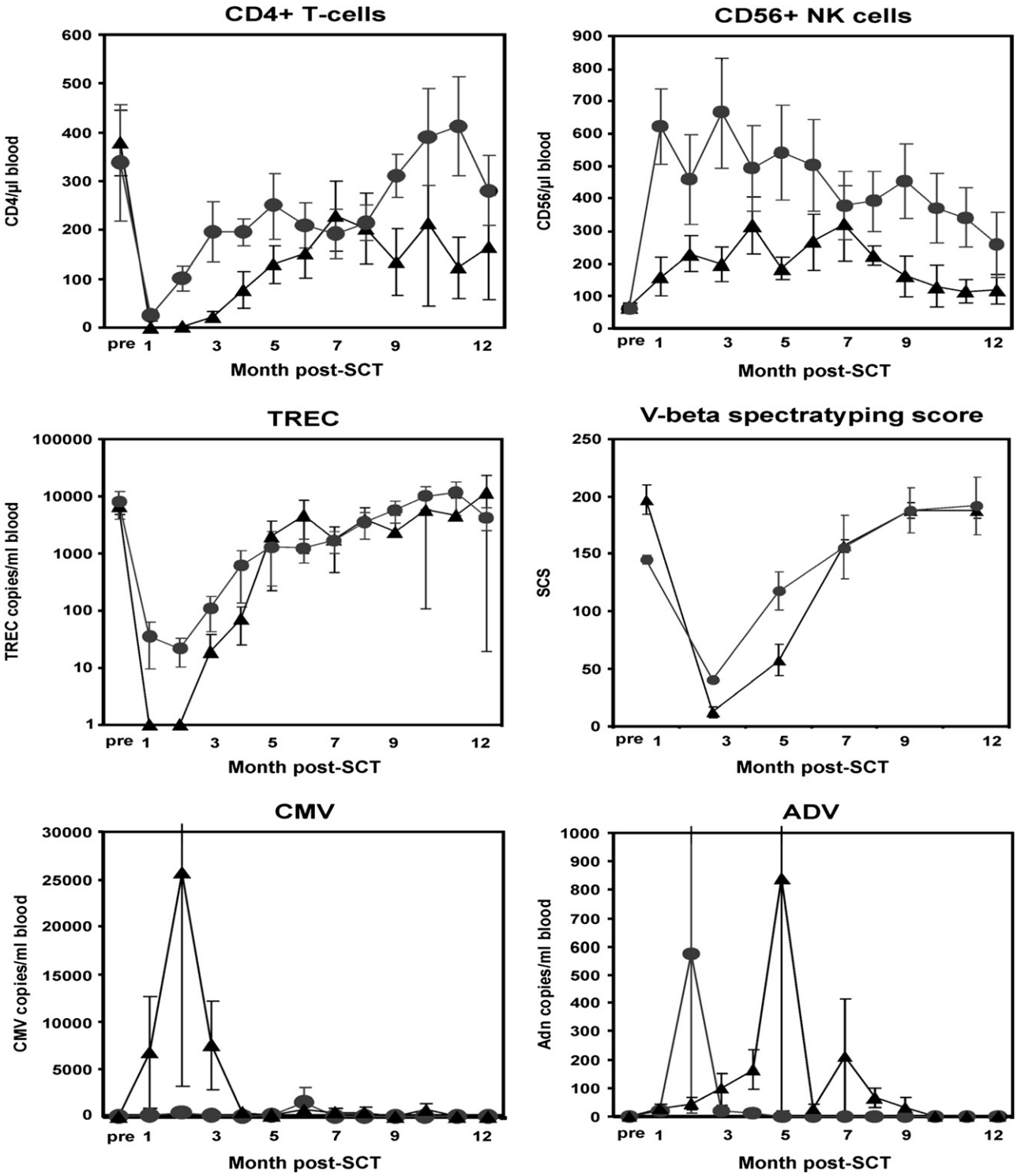

Figure 2. Comparative analyis of $CD4^+$ T-helper cells and $CD56^+$ NK cells after transplantion with haploidentical CD3-depleted PBSCs after TBI-containing myeloablative regimen (circle) or RIC regimens (triangle) (upper panel). In the middle panel, the recovery of the TRECs and the V-beta spectratyping score is depicted. In the lower panel, the copy numbers as determined by real-time PCR in patients with reactivation of cytomegalovirus (CMV) or adenovirus (ADV) is shown.

Based on these observations that a myeloablative approach is associated with a delayed de novo thymic generation of T-lymphocytes and a delayed establishment of the T cell receptor repertoire, we decided to abandon myeloablative conditioning regimens in patients undergoing haploidentical transplantations and initiated a clinical protocol using the IC approach for patients with malignant and nonmalignant diseases [21]. The conditioning regimen includes Fludarabine (160 mg/m^2), Thiotepa (10 mg/kg), and Melphalan (140 mg/m^2) and the anti-CD3 antibody OKT-3 (27 patients). In 10 patients with refractory malignant disease, we used clofarabine (200 mg/m^2) instead of fludarabine without any major toxicitiy (unpublished data).

ADOPTIVE TRANSFER OF VIRUS-SPECIFIC T CELLS

For patients who do not develop a specific T cell response to CMV or ADV and are therefore at high risk for severe infection, we have developed at the Children's University Hospital Tuebingen a rapid and GMP-conform and approved method for the in vitro isolation of CMV- or ADV-specific donor T cells for posttransplant adoptive transfer using the Interferon-gamma secretion method [22]. Briefly, donor mononuclear cells are incubated overnight with the viral antigen. The interferon-gamma secreting T cells, composed of CD4$^+$ and CD8$^+$ subsets, are then isolated using the interferon-gamma capture method. In Figure 3A, the purification steps are depicted. In Figure 3B, the in vivo expansion of ADV-specific T cells in patients with posttransplant ADV reactivation and the reduction of the ADV copy numbers or the clearance is show.

In a retrospective comparative analysis of the incidence of lethal viral infections after haploidentical transplantation after either CD34$^+$ selection and myeloablative regimens or CD3/CD19-depleted stem cells and RIC regimens in patients transplanted at the Children's University Hospital in Tuebingen, we have seen a major difference between the 2 approaches. Although almost 20% of the patients died from a viral infections after CD34$^+$ selection and myeloablation, none of the 37 patients who were transplanted to date (June 2007) with CD3/C19-depleted stem cells after RIC conditioning died from this complication. It is noteworthy that there was no death in this group from any infection. In Figure 4, the result of this comparative retrospective analysis is shown. In addition, the TRM after RIC conditioning was very low (1 out of 37), whereas the TRM rate was up to 35% in the patients who received myeloablation and CD34$^+$ positively selected stem cells [23].

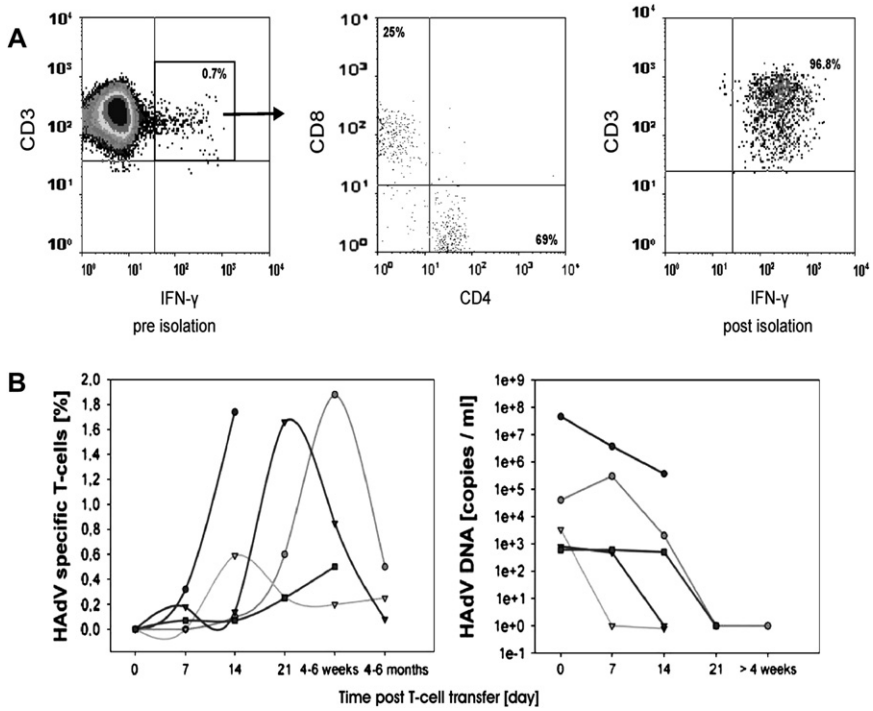

Figure 3. The in vitro purification steps of ADV-specific donor T cells are shown. Mononuclear cells from the haploidentical stem cell donor were isolated and incubated overnight with viral antigen. After stimulation, 0.7% of the CD3$^+$ T cells were found to secrete interferon-gamma and thus specific for ADV (A, left panel). These ADV-specific T cells are composed of CD4$^+$ and CD8$^+$ T cells (A, middle panel). After isolation using the interferon-gamma capture assay, the interferon-gamma secreting CD3$^+$ T cells are highly enriched and ready for infusion into the patient (C, right panel). In (B), the expansion of ADV-specific T cells in 5 patients with posttransplant ADV reactivation (left panel) and the concomitant decrease of the ADV copy numbers or the complete clearance (right panel) is shown.

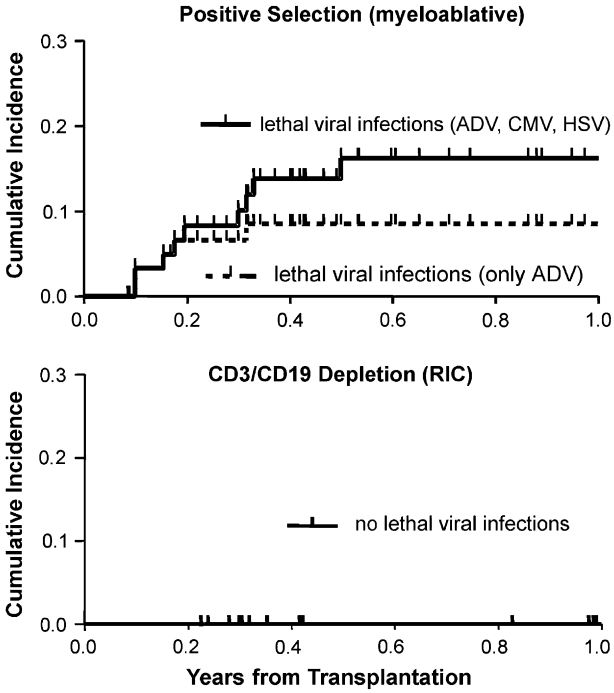

Figure 4. Comparative retrospective analysis of the cumulative incidence of lethal viral infections after myeloablative conditioning regimens and $CD34^+$ positive selection and after RIC regimen and CD3/CD19 depletion. In the myelablative group (upper panel), almost 20% of the patients died from a lethal infection. Half of these patients had a lethal ADV infection, whereas no death from ADV or any other viral, bacterial, or fungal infection was seen in the RIC group (lower panel).

RECONSTITUTION OF THE NK CELL REPERTOIRE

Alloreactive natural killer (NK) cells might play a major role not only in the prevention of infections, but also in the prevention of disease recurrence in patients with malignant diseases, especially in the context of RIC regimens. Therefore, a major focus of our work is directed on the analysis of the reconstitution of NK cells and especially of the killer inhibitory and activatory receptor (KIR and KAR) repertoire after haploidentical transplantation. We have shown previously that, in the context of $CD34^+$ positive selection and myeloablation, the alloreactive status between donor and recipient is predictive for the risk of relapse [12,13]. Using monoclonal antibodies against KIRs, the KIR repertoire of the donors and of the patients can be followed after transplantation by flow cytometry and can also be used to identify an NK-alloreactive donors [13]. In most of the patients, the KIR repertoire of the donor starts to reestablish after approximately 3 months posttransplant, whereas during the first months posttransplant, NK cells without KIR expression or with a skewed repertoire are the predominant cell population [11].

Besides the KIR repertoire, the number of inhibitory HLA class I molecules on leukemic targets and the functional activity of the NK cells (NK activity) also play a role in NK-mediated cytotoxicity against blasts [24]. We are therefore monitoring the NK activity of the all patients using the standard NK cytotoxicity assay against the standard target cell line K 562 and the number of surface inhibitory or activatory HLA-antigens on the patients' blasts.

ANALYSIS OF CHIMERISM AND MINIMAL RESIDUAL DISEASE

It has been shown that the establishment and maintenance of a complete donor chimerism is associatesd with a lower risk of relapse, and that decreasing donor chimerism might be a herald for relapse [25]. Although conventional assays are based on DNA analysis, the haploidentical situation between donor and recipients allows the analysis of chimerism via the different expression of HLA antigens on the cell surface of the hematopoietic cells. Therefore, we have established an easy and rapid method for the analysis of chimerism based on flow cytometry [26]. Because of the HLA mismatch, suitable anti-HLA antibodies can be identified in >80% of donor/recipients. Because most of the antibodies against lymphocyte surface antigen are available in conjugation with various fluorescent dyes, the method allows the exact analyis of donor- and recipient-derived cells and their subsets. With 1 mL of whole blood, an extensive analysis of all HLA-expressing cells and their subsets is possible. Because of the high sensitivity of the method, decreases of donor chimerism can be recognized very early.

In Figure 5A and B, a representative flowcytometric analysis of chimerism posttransplant is shown. In this example, the donor is HLA-A2/28 positive, whereas the recipient is HLA-A2/28 negative. As can be seen in the flowcytmetric analysis, all $CD56^+$ NK cells (A) and $CD3^+$ T-cells (B) are positive for HLA-A2/28, and thus are donor-derived.

In addition, the method can be used for the early detection of minimal residual disease (MRD) postransplant, similar to the already described flowcytometric MRD detection methods for residual leukemic blasts [27]. Because the malignant cells are recipient derived, a gate can be set on the cells expressing the patients HLA antigens, and the leukemic phenotype of these cells can be determined. This approach further increases the sensitivity of the method for early MRD detection. In Figure 5C and D, an example is shown. In this patient, peripheral recipient-derived $CD45dim^+$ positive cells were seen, whereas all donor-derived hematopoietic cells were $CD45bright^+$ (C). Additional staining showed that all these recipient derived $CD45dim^+$ cells had the leukemic phenotype ($CD34^+$) (D) of the patient's blasts prior to transplant.

Because the patient's blasts can easily be identified using HLA antibodies, the method will also allow the

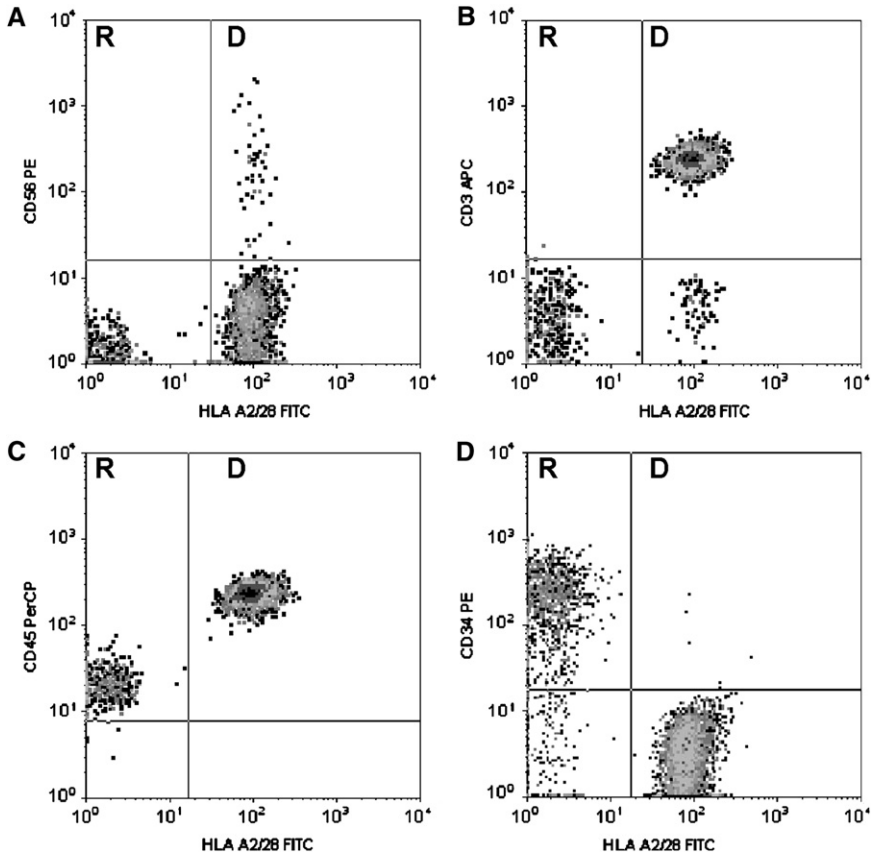

Figure 5. Flowcytometric analysis of peripheral blood chimerism posttransplant. In this example, the donor (D) is HLA-A2/28 positive, whereas the recipient (R) is HLA-A2/28 negative. At day +200 posttransplant, all NK cells (A) and T cells (B) are donor-derived. The HLA-A2/28 negative population stained positive for CD45dim, whereas all donor-derived hematopoietic cells were $CD45^+$ bright (C). Further analysis (D) showed that most of the HLA-A2/28 negative cells were $CD34^+$ and had the same leukemic phenotype as the patient's blasts prior to transplant.

analysis of the interaction of the donor-derived NK or T cells with the recipient-derived blasts by using flowcytometric approaches for assays of cellular cytotoxicity [28].

OUTLOOK

The very low rate of TRM and the absence of lethal infections after haploidentical transplantation using this RIC approach and CD3/CD19-depleted stem cells in pediatric patients offers a platform for further improving the outcome after transplantation. Especially in patients with malignant disease, further detailed analysis of the TCR and the repertoire of KIR and KAR receptors might help to better understand and manipulate the donor-derived immune reconstitution toward a powerfull graft-versus-malignancy effect. Newly described methods using oligonucleotide microarray technology for the detailed analysis of the TCR [29] or novel approaches for the quantitative analysis of the KIR and KAR expression of NK cells (X. Chen et al., submitted) in combination with flowcytometric analysis of recipient- and donor-derived effector cells will facilitate a better understanding of the interaction of reconstituting T and NK cells with the patients' malignant cells. The further availability of the donor after transplantation allows further intervention, such as the transfer of donor-derived virus-specific T cells [22] or purified NK cells in case of impending relapse [30]. Using this platform, further clinical studies using posttransplant strategies to further improve immune reconstition with cytokines [31,32] or to augment the antitumor effect with monoclonal antibodies [33,34] or other strategies are warranted.

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