

CD45RA depletion in HLA-mismatched allogeneic hematopoietic stem cell transplantation for primary combined immunodeficiency: A preliminary study

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Background: Combined immunodeficiencies (CIDs) form a heterogeneous group of inherited conditions that affect the development, function, or both of T cells. The treatment of CIDs with allogeneic hematopoietic stem cell transplantation (HSCT) is complicated by a high incidence of life-threatening infections and an increased risk of graft-versus-host disease (GVHD).

Objective: In view of the growing evidence that alloreactivity is mainly derived from human naive T cells, the selective depletion of naive T cells from allografts might constitute a way of reducing alloreactivity while maintaining memory T-cell responsiveness to pathogens.

Methods: Five consecutive patients with CIDs and chronic viral infections underwent an allogeneic, HLA-mismatched HSCT. Given the patients' infection status and the potential risk of severe GVHD in the mismatched setting, the CD34⁺ fraction of the allograft was depleted of naive T cells by using magnetic CD45RA beads.

Results: Engraftment occurred in 4 of the 5 patients. No severe GVHD occurred. In the 4 engrafted patients viral infections were cleared within 2 months of the HSCT, and both cellular and humoral immunity were re-established within a year of the HSCT. An early T-cell response against viral pathogens was documented in 2 patients.

Conclusion: The present pilot study shows that clinical-grade depletion of naive T cells from an allograft through the use of magnetic CD45RA beads seems to be a feasible and efficacious option for the treatment of patients with CIDs at high risk of GVHD, infection, or both in an HLA-mismatched setting. (*J Allergy Clin Immunol* 2014;■■■:■■■-■■■.)

Key words: Combined primary immunodeficiency, hematopoietic stem cell transplantation, graft-versus-host disease, viral infection, immunomagnetic CD45RA depletion, naive T cell

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Supported by Assistance Publique Hôpitaux de Paris.

Disclosure of potential conflict of interest: M. Cavazzana's institution has received or has grants pending from the European Research Council and CELL-PID, as does that of F. Touzot, A. Fischer, J.-M. Luby, C. Picard, B. Terniaux, and J. Magalon. The rest of the authors declare that they have no other relevant conflicts of interest.

Received for publication May 22, 2014; revised July 21, 2014; accepted for publication August 12, 2014.

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0091-6749/\$36.00

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<http://dx.doi.org/10.1016/j.jaci.2014.08.019>

Abbreviations used

ATG:	Anti-thymocyte globulin
BM:	Bone marrow
CID:	Combined immunodeficiency
CMV:	Cytomegalovirus
DLI:	Donor lymphocyte infusion
GVHD:	Graft-versus-host disease
HSCT:	Hematopoietic stem cell transplantation
MMUD:	Mismatched unrelated donor
MUD:	Matched unrelated donor
TCR:	T-cell receptor
TEMRA:	Terminally differentiated effector memory re-expressing CD45RA

Primary combined immunodeficiencies (CIDs) represent a heterogeneous group of genetic disorders that affect the development, function, or both of T cells. In addition to increased susceptibility to infections, patients affected by CIDs present with a wide range of clinical symptoms, including autoimmunity, granulomatous inflammation, lymphoproliferation, and an increased risk of malignancy.^{1,2} CIDs can lead to death in infancy or childhood. Allogeneic hematopoietic stem cell transplantation (HSCT) is the treatment of choice for CIDs because it enables the replacement of dysfunctional hematopoietic lineages with normal cells.³ The results of HSCT in patients with CIDs lacking an HLA-matched sibling remain unsatisfactory because of the high risk of graft rejection (caused by residual immunity), a high mortality rate (caused by exacerbation of viral infections present before transplantation), and a high incidence of graft-versus-host disease (GVHD). For all these reasons, donor choice has a major effect on the likelihood of a successful outcome.

In the European Stem Cell Transplant for primary Immune Deficiencies in Europe registry of patients with CIDs, the overall survival rate is around 47%.⁴ We found similar results for 24 consecutive patients with CIDs undergoing transplantation in the Necker Children's Hospital (Paris, France) between 2006 and 2013 with a matched unrelated donor (MUD) or a mismatched unrelated donor (MMUD). Overall survival was around 50%, with severe (grades III-IV) GVHD being the primary cause of death (our unpublished data).

Given that donor T cells are key mediators of both post-transplantation immunity and GVHD, researchers have developed a variety of approaches for removing the potentially harmful effects of donor T cells while retaining their beneficial effects.⁵⁻⁷ Studies in mice have shown that alloreactivity is

mainly derived from the naive T-cell repertoire.^{8,9} There is also a growing body of evidence to suggest that alloreactivity in human subjects is mainly associated with the naive T-cell compartment. Hence depletion of naive T cells from the allograft is an appealing approach for patients at high risk of GVHD or infection during the posttransplantation period.¹⁰⁻¹² In the present pilot study 5 patients with CIDs presenting with chronic infections underwent allogeneic, HLA-mismatched HSCT with a CD45RA-depleted allograft.

METHODS

Patients

Five patients given CID diagnoses (MHC class II expression deficiency [n = 2], CD25 deficiency [n = 2], and ORAI1 deficiency [n = 1]) underwent transplantation with CD45RA-depleted allografts between September 2011 and September 2012 at Necker Children's Hospital. All patients presented with severe chronic infection with 1 or more viruses. Three patients also had various immune dysfunctions (Table I). Patient P5 had previously undergone HSCT with a 10/10 MUD allograft that had failed to engraft. In the absence of an available HLA-identical donor or a MUD, HSCT was performed with a 5/6 mismatched sibling donor (patient P1) or a 9/10 MMUD (patients P2-P5). Both donor and recipient HLA status were determined by using high-resolution class I and II molecular DNA typing. The patients were offered allogeneic transplantation with CD45RA depletion in accordance with French regulatory requirements. All patients provided informed consent. The patients underwent myeloablative conditioning with busulfan (with a target cumulative area under the curve of 85-90 mg/L·h) and fludarabine (160 mg/m²). Four patients (patients P2, P3, P4, and P5) received a reinforced regimen, with the addition of thiotepa (10 mg/kg). All patients, with the exception of patient P2, who had a high burden of viral infections, received 4 infusions of 2.5 mg/kg anti-thymocyte globulin (ATG; Thymoglobulin; Genzyme, Framingham, Mass). ATG was administered as an immunotherapeutic treatment to increase the chance of engraftment. Except for patient P1, who received it on day -4, ATG was administered before chemotherapy (on day -20) so that it could be eliminated before infusion of the graft (Table I). The change over time in the active fraction of circulating ATG was measured, as described previously.¹³ All patients received GVHD prophylaxis with cyclosporine for 6 months and mycophenolate mofetil for 3 months. Acute GVHD was assessed and graded according to established standardized criteria.¹⁴

Preparation of the CD45RA-depleted allograft

The harvested bone marrow (BM) preparation underwent a 2-fold selection process to maintain the subpopulation of CD45RA-expressing hematopoietic stem cells.¹⁵ First, the BM preparation was T lymphocyte depleted with a CD34⁺ immunomagnetic CliniMACS system (Miltenyi Biotec, Bergisch Gladbach, Germany). The positive fraction from this selection contained CD34⁺ hematopoietic stem cells and fewer than 5000 CD3⁺ cells/kg of the recipient's body weight.

Second, the negative fraction was then CD45RA depleted with the CD45RA CliniMACS kit (Miltenyi Biotec). The CD34⁺CD45RA⁻ fraction from the second selection step was infused into the patient, along with the CD34⁺ fraction (Fig 1).

Monitoring of immune reconstitution

Flow cytometric analysis was performed on a BD FACSCanto II system running BD FACS DIVA software (BD Biosciences, Heidelberg, Germany). All mAbs were obtained from BD Biosciences or Miltenyi Biotec. Pathogen-specific CD4⁺ and CD8⁺ T cells were detected by using intracellular IFN-γ or pentamer staining. Briefly, PBMCs were stimulated *in vitro* for 6 hours with Peptivator cytomegalovirus (CMV) pp65 (Miltenyi Biotec). Brefeldin A was added 2 hours after the start of incubation. Cells were stained for CD3, CD8, CD4 (BD Biosciences), and intracellular IFN-γ. For HLA-A1 and HLA-A2 patients, specific staining with a Pro5 MHC class I pentamer

(Pro-immune, Oxford, United Kingdom) was used for detection of CMV-specific T cells. The proliferation of PBMCs was assayed, as described previously.¹⁶

RESULTS

Validation of the CD45RA depletion process

The donors' BM collections yielded a median mononuclear cell count of 3.2×10^8 /kg of the recipient's body weight (range, $1.11\text{--}6 \times 10^8$ /kg). The median CD34⁺ cell count was 11×10^6 /kg (range, $3\text{--}18 \times 10^6$ /kg), and the median CD3⁺ cell count was 8.95×10^7 /kg (range, $3.87\text{--}14.8 \times 10^7$ /kg). The CD34⁺ fraction obtained after CD34⁺ positive selection contained a median count of 10.4×10^6 CD34⁺ cells/kg (range, $2.92\text{--}12 \times 10^6$ /kg) and a median CD3⁺ cell count of 1700/kg (range, 1300-5000/kg; Table II). A median dose of 2.5×10^6 CD3⁺ cells/kg (range, $1.58\text{--}9.2 \times 10^6$ CD3⁺ cells/kg) was infused into the patient. The median recovery of CD45RO⁺CD3⁺ T cells from the CD34⁺ fraction was 24.7% (range, 9.9% to 39.6%; Table II and see Table E1 in this article's Online Repository at www.jacionline.org).

The procedure eliminated more than 99.9% of CD45RA⁺ cells, and the median log depletion was 4.2 (range, 3.9-4.3). It is noteworthy that CD8⁺ T cells specific for pathogens of interest (mainly CMV) were retained by the process, despite the loss of terminally differentiated effector memory cells re-expressing CD45RA (TEMRA) (see Fig E1 in this article's Online Repository at www.jacionline.org). The CD45RA-depleted fraction also included CD16⁺CD56⁺CD3⁻ natural killer cells (median, 1.6×10^5 /kg; range, $0.6\text{--}12.2 \times 10^5$ /kg) and CD14⁺ monocytes (median, 1.21×10^6 /kg; range, $1.07\text{--}1.6 \times 10^6$ /kg). CD19⁺ B cells were almost completely eliminated (median, 0×10^4 /kg; range, $0\text{--}2 \times 10^4$ /kg).

Hematologic reconstitution

The median post-HSCT follow-up period was 22 months (range, 9-32 months). Graft failure was observed in patient P4, who received the lowest dose of CD34⁺ cells (2.9×10^6 /kg). Nine months after HSCT, patient P4 died of infectious complications after prolonged aplasia. All other patients showed engraftment after a median of 15 days (range, 13-40 days), as confirmed by an analysis of PBMC chimerism (Table III). It is noteworthy that patients P1 and P3 transiently displayed mixed chimerism (in the CD3⁺ and CD3⁻ compartments for patient P1 and in the CD3⁺ compartment for patient P3). In view of the mixed chimerism, we performed donor lymphocyte infusions (DLIs) at days +36 and +42 (with $10^6 \times$ CD3⁺ cells per kilogram each time) for patient P1. Patient P3 did not receive DLI because of the unavailability of the donor. Over the first year, the mixed chimerism observed in both patients converted progressively to full donor chimerism. Because patient P5 remained dependent on platelet transfusion 3 months after HSCT, a booster transplant (with a CD34⁺ selected allograft) from the same donor enabled rapid myeloid reconstitution.

Clinical outcomes

Patient P1 had grade I GVHD at day +48 (ie, 12 days after the first DLI), which was treated with steroids. No GVHD was

TABLE I. Patients' characteristics

	Patients				
	P1	P2	P3	P4	P5
Diagnosis	ORAI1 deficiency	MHC class II deficiency	CD25 deficiency	CD25 deficiency	MHC II deficiency
Age	7 mo	23 mo	34 mo	6 mo	35 mo
Viral infection	CMV	Enterovirus, norovirus	CMV, adenovirus	CMV	EBV, enterovirus, rhinovirus
Immunopathologic manifestations	None	Colitis, hepatitis	Eczema/enteropathy	Eczema/enteropathy AIHA	None
Donor	Sibling 8/10 (HLA-DR mismatched)	MMUD 9/10 (HLA-A mismatched)	MMUD 9/10 (HLA-C mismatched)	MMUD 9/10 (HLA-C mismatched)	MMUD 9/10 (HLA-A mismatched)
Conditioning regimen	ATG, 10 mg/kg Days -4 to -1 Busulfan Fludarabine	Busulfan Fludarabine Thiotepa	ATG, 10 mg/kg Days -20 to -17 Busulfan Fludarabine Thiotepa	ATG, 10 mg/kg Days -20 to -17 Busulfan Fludarabine Thiotepa	ATG, 10 mg/kg Days -20 to -17 Busulfan Fludarabine Thiotepa
GVHD prophylaxis	Cyclosporine MMF	Cyclosporine MMF	Cyclosporine MMF	Cyclosporine MMF	Cyclosporine MMF

AIHA, Autoimmune hemolytic anemia; MMF, mycophenolate mofetil.

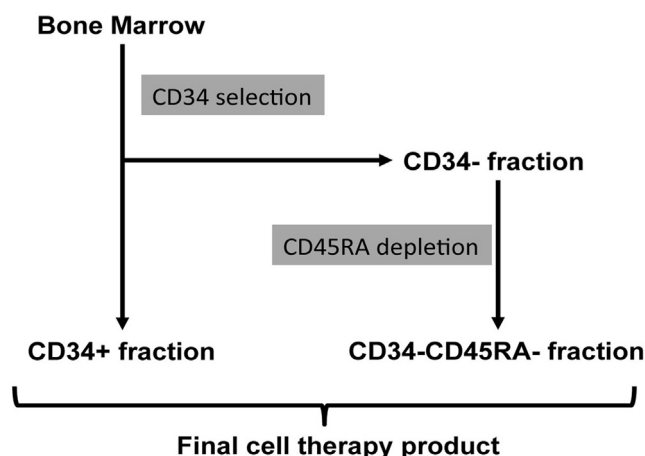


FIG 1. Scheme of the CD45RA depletion process. The BM sample undergoes 2 selection steps. First, *in vitro* T-lymphocyte depletion of BM was performed with a CD34⁺ immunomagnetic CliniMACS system (Miltyen Biotec). The negative fraction was then CD45RA⁺ depleted with a CD45RA CliniMACS kit. The final cell therapy product consists of the CD34⁺ and CD34⁻CD45RA⁻ negative fractions.

observed in the 3 other evaluable patients. Immunosuppressive therapy was withdrawn in all cases 6 months after HSCT. None of the patients had clinically relevant viral manifestations after HSCT. Patient P1 had persistent CMV viremia before transplantation; a serum CMV viral load could not be detected after day +50 (coinciding with the development of a CMV-specific antiviral response, see below).

Patient P2 had protracted diarrhea (associated with enterovirus and norovirus carriage) and required parenteral nutrition before HSCT. He rapidly improved after transplantation; by day +20, gastrointestinal transit had normalized, and enterovirus shedding had ceased. The patient was weaned off parenteral nutrition at day +60.

Patient P3 presented with chronic CMV viremia before transplantation. A post-HSCT reactivation of CMV replication (in the absence of CMV disease) was successfully treated with a course of ganciclovir between days +30 and +40. The CMV viral load became negative 3 months after transplantation.

TABLE II. Graft characteristics

	Patients				
	P1	P2	P3	P4	P5
Source	BM	BM	BM	BM	BM
CD34 ⁺ fraction					
CD34 ⁺ cells ($\times 10^6$ /kg)	8.8	12.3	12	2.9	6.8
CD3 ⁺ cells/kg	5000	2150	1300	1600	1700
CD45RA ⁺ cells ($\times 10^7$ /kg)	7.26	2.0	2.04	7.8	12.6
CD3 ⁺ CD45RA ⁻ cells ($\times 10^6$ /kg)	1.58	9.2	2.5	2.6	1.7
CD4 ⁺ CD45RA ⁻ cells ($\times 10^6$ /kg)	1.34	8.2	1.4	2.3	1.4
CD8 ⁺ CD45RA ⁻ cells ($\times 10^6$ /kg)	0.12	0.99	0.47	0.15	0.1
Natural killer cells ($\times 10^5$ /kg)	1.22	2	0.8	0.6	1.6
CD3 ⁺ CD45RA ⁺ cells/kg	799	9230	2530	0	1750

We observed clearance of enterovirus shedding in patient P5 at 2 months after HSCT. Signs of immune dysfunction resolved in all patients. Patient P3 initially had a severe erythroderma before HSCT that gradually resolved within 4 months after transplantation. No immune reconstitution syndrome was experienced because viral clearance was achieved.

Immune reconstitution

Patient P1 received 2 nonmanipulated DLIs on days +36 and +42 after transplantation and thus was not further considered in our analysis of the time course of immune reconstitution. The 3 other patients displayed a steady immune reconstitution, with lymphocyte subset counts reaching normal values within a year of HSCT (Figs 2 and 3). Patient P2 had an especially fast immune recovery, with significant thymic production of T cells at 6 months after HSCT (as shown by the fact that 27% of the CD4⁺ T cells were CD31⁺CD45RA⁺ and 61% of the CD8⁺ subsets were CCR7⁺CD45RA⁺; Fig 2, D and E). The 3 evaluable patients displayed mitogen- and antigen-induced proliferation at 6 and 12 months after HSCT, respectively (see Table E2 in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org). Intravenous

TABLE III. Clinical outcomes

	Patients				
	P1	P2	P3	P4	P5
Engraftment (d)	Yes (13)	Yes (15)	Yes (10)	No	Yes (34)
Chimerism at engraftment	CD3 ⁺ 19% Dnr CD3 ⁻ 56% Dnr	100% Dnr	CD3 ⁺ 100% R CD3 ⁻ 97 % Dnr	100% R	100% Dnr
Chimerism d100	CD3 ⁺ 80% Dnr CD3 ⁻ 100% Dnr	100% Dnr	CD3 ⁺ : R>>Dnr CD3 ⁻ : 100% Dnr	100% R	100% Dnr
Chimerism d360	ND	100% Dnr	CD3 ⁺ : 70% Dnr CD3 ⁻ : 100% Dnr	ND	100% Dnr
DLI	Yes, days +36 and +44	No	No	No	No
Booster transplant	No	No	No	No	Yes, M3
GVHD	Grade 1 (skin), day +48, resolved	No	No	No	No
Viral disease	No	No	No	No	No
Outcome	AW (M40)	AW (M24)	AW (M21)	Death (M9)	AW (M18)

AW, Alive and well; Dnr, donor; ND, not done; R, recipient.

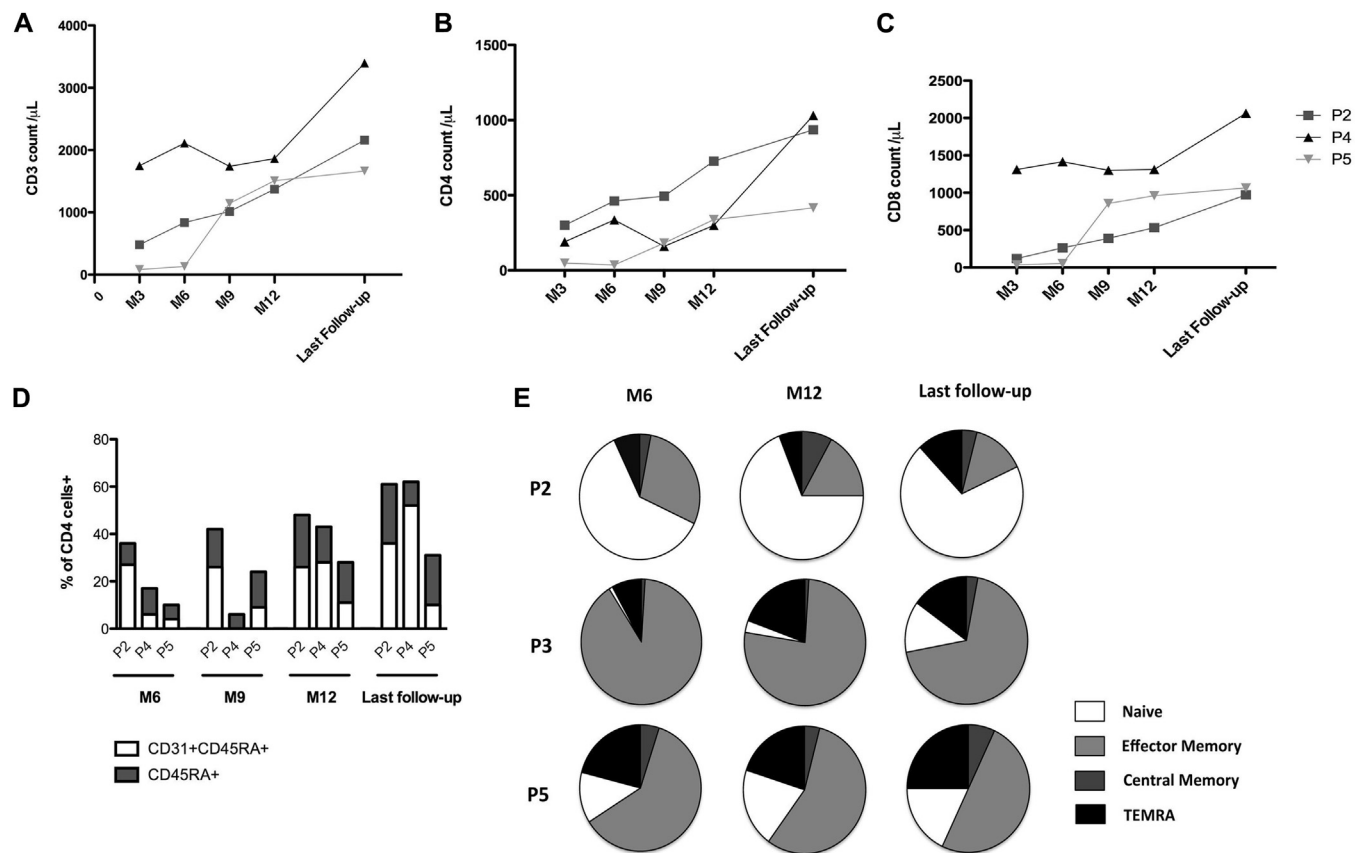


FIG 2. T-cell reconstitution after HSCT with a CD45RA-depleted allograft (patients P2, P3, and P5). **A**, Absolute CD3⁺ lymphocyte counts in peripheral blood of patients at 3, 6, 9, and 12 months and at last follow-up. **B**, Absolute CD3⁺CD4⁺ lymphocyte counts in peripheral blood of patients at 3, 6, 9, and 12 months and at last follow-up. **C**, Absolute CD3⁺CD8⁺ lymphocyte counts in peripheral blood of patients at 3, 6, 9, and 12 months and at last follow-up. **D**, Percentage of CD3⁺CD4⁺ lymphocytes expressing CD45RA and the CD31 markers in peripheral blood of patients treated with a CD45RA-depleted allografts at 6, 9, and 12 months and at last follow-up. **E**, Percentage of CD3⁺CD8⁺ lymphocyte subsets in patients at 6, 9, and 12 months and at last follow-up. Subsets were determined according to their expression of CCR7 and CD45RA, as follows: naive cells (CCR7⁺CD45RA⁺), effector memory cells (CCR7⁺CD45RA⁻), central memory cells (CCR7⁻CD45RA⁻), and TEMRA cells (CCR7⁻CD45RA⁺).

immunoglobulin replacement therapy was withdrawn in patients P2, P3, and P5 at 9, 16, and 17 months after HSCT, respectively. The patients maintained normal immunoglobulin levels thereafter (Table IV) and had normal antibody responses on immunization against tetanus toxoid, poliomyelitis, and diphtheria.

The development of early specific antiviral response after HSCT was confirmed in 2 patients. Before HSCT, patient P1 had chronic CMV viremia with no detectable CMV-specific CD8⁺ T cells. At day +38 (ie, only 1 day after the first DLI), CMV-specific T cells were detected by using pentamers restricted to

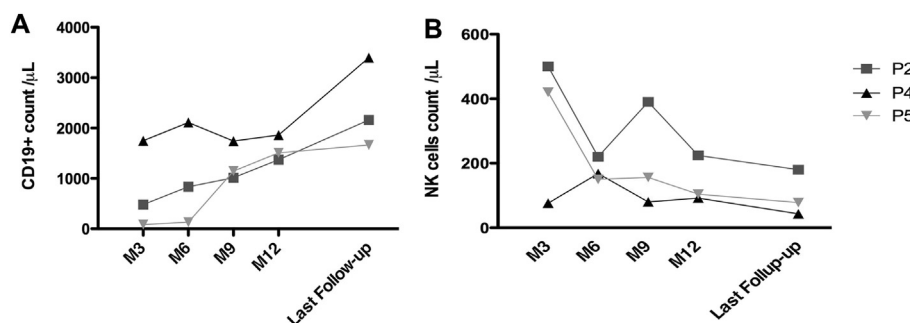


FIG 3. B and natural killer (NK) cell reconstitution after HSCT with CD45RA depletion. **A**, Absolute CD19⁺ lymphocyte counts in peripheral blood of patients at 3, 6, 9, and 12 months and at last follow-up. **B**, Absolute CD3⁺CD16⁺CD56⁺ lymphocyte counts in peripheral blood of patients at 3, 6, 9 and 12 months and at last follow-up.

TABLE IV. Serum immunoglobulin levels in patients P2, P3, and P5 at last follow-up (in grams per liter)

Patient ID	IgM	IgA	IgG
P2	1.52	0.89	6.34
P3	0.4	1.78	8.21
P5	0.33	0.88	10.08
Normal range	0.54-1.53	0.88-10.08	5.49-10.19

MHC HLA-A2 and the CMV pp65 antigen (Fig 4, A). Similarly, *in vitro* stimulation of lymphocytes with the pp65 antigen induced significant activation of CD8⁺ T cells, as shown by the detection of intracytoplasmic IFN- γ by using flow cytometry. In patient P3 reactivation of CMV replication occurred during the posttransplantation period. Before HSCT, patient P3 had CMV-specific CD8⁺ T cells that were detected by using pentamers restricted to MHC HLA-A1 and the CMV p50 antigen (Fig 4, B). After HSCT, CMV-specific CD8⁺ T cells with an effector memory phenotype (CD62L⁻CD45RA⁻) were detected at day +39 (Fig 4, B).

DISCUSSION

The present preliminary results show that CD45RA depletion by using immunomagnetic beads is feasible and might be a useful treatment approach in patients with a high risk of infectious complications and GVHD in a mismatched setting.

We opted for a double-selection strategy: we first isolated the CD34⁺ cells and then depleted the CD45RA cells in the CD34⁺ fraction. Given that CD45RA is expressed on hematopoietic stem cells, we feared that 1-step depletion would alter the hematopoietic reconstitution by depleting part of the CD34⁺ subset.¹⁵

The CD45RA depletion resulted in a greater than 4-log reduction in the number of CD45RA⁺ T cells. In line with the stronger expression of CD45RA on CD8⁺ T cells, we observed an increase in the CD4/CD8 ratio.¹⁷ The final product was almost completely devoid of naive T cells and contained predominantly memory T cells expressing the CD45RO marker. Given that *in vitro* T-cell depletion of allografts increases the risk of graft rejection (especially in the context of HLA incompatibility),¹⁸ we chose to reinforce the conditioning regimen (by adding thiotepa) in patients due to receive a graft from an MMUD. Graft failure was observed in only 1 patient affected by CD25

deficiency, who received a CD34⁺ cell dose of less than the recommended threshold in the genotypical setting.¹⁹ The most remarkable feature in the 4 engrafted patients was the seemingly uneventful post-HSCT period, with no clinically relevant viral infections and the rapid clearance of viruses detected in the pre-HSCT periods (ie, enterovirus, norovirus, and CMV) within 2 months of the transplantation. Importantly, no episodes of severe GVHD (ie, greater than grade II) occurred. The patients in whom engraftment occurred displayed rapid immune reconstitution, with restoration of cellular and humoral immunity within a year of HSCT. Interestingly, 2 patients (patients P1 and P3) exhibited an early myeloid engraftment with a mixed chimerism in the CD3⁺ compartment (with a high proportion of recipient cells). Patient P3's 2 HLA-mismatched populations coexisted until 1 year after HSCT. The mixed chimerism observed in patient P1 could result from T-cell depletion of the CD45RA⁻ fraction caused by ATG. However, we observed a similar transient mixed chimerism of the CD3⁺ compartment in patient P3 that progressively converted to full donor chimerism without any therapeutic intervention. This indicates the initial persistence of autologous T cells that are secondarily overcome by the expansion of the donor's memory T cells and thymic reconstitution from the donor's origin.

The CD45RA-depleted fraction contained CD8⁺ T cells with *in vivo* functional activity against pathogens, as shown by the rapid development of an immune response against CMV in 2 of our patients within the first 2 months after HSCT. This immune response was associated with clearance of CMV replication in both patients. Most of the cytotoxic cells detected in patient P3 displayed an effector memory phenotype, which agrees with the fact that TEMRA cells were removed by means of CD45RA depletion.²⁰ As shown recently by 2 research groups, CD45RA-depleted lymphapheresis products also contain CD4⁺ and CD8⁺ T cells with intact *in vitro* functional activity against CMV, adenovirus, EBV, and *Aspergillus* and *Candida* species antigens.^{21,22} We have also confirmed these data in preclinical experiments (data not shown).

Several studies in mice have suggested that naive T cells have a greater role in alloreactivity than memory T cells.^{8,9} There is also a growing body of evidence to suggest that in human subjects alloreactivity mainly derives from the naive T-cell compartment and not the memory T-cell compartment.¹⁰⁻¹² It has been shown that CD45RA depletion effectively reduces the number of alloreactive CD8⁺ T cells *in vitro*.²¹ No severe

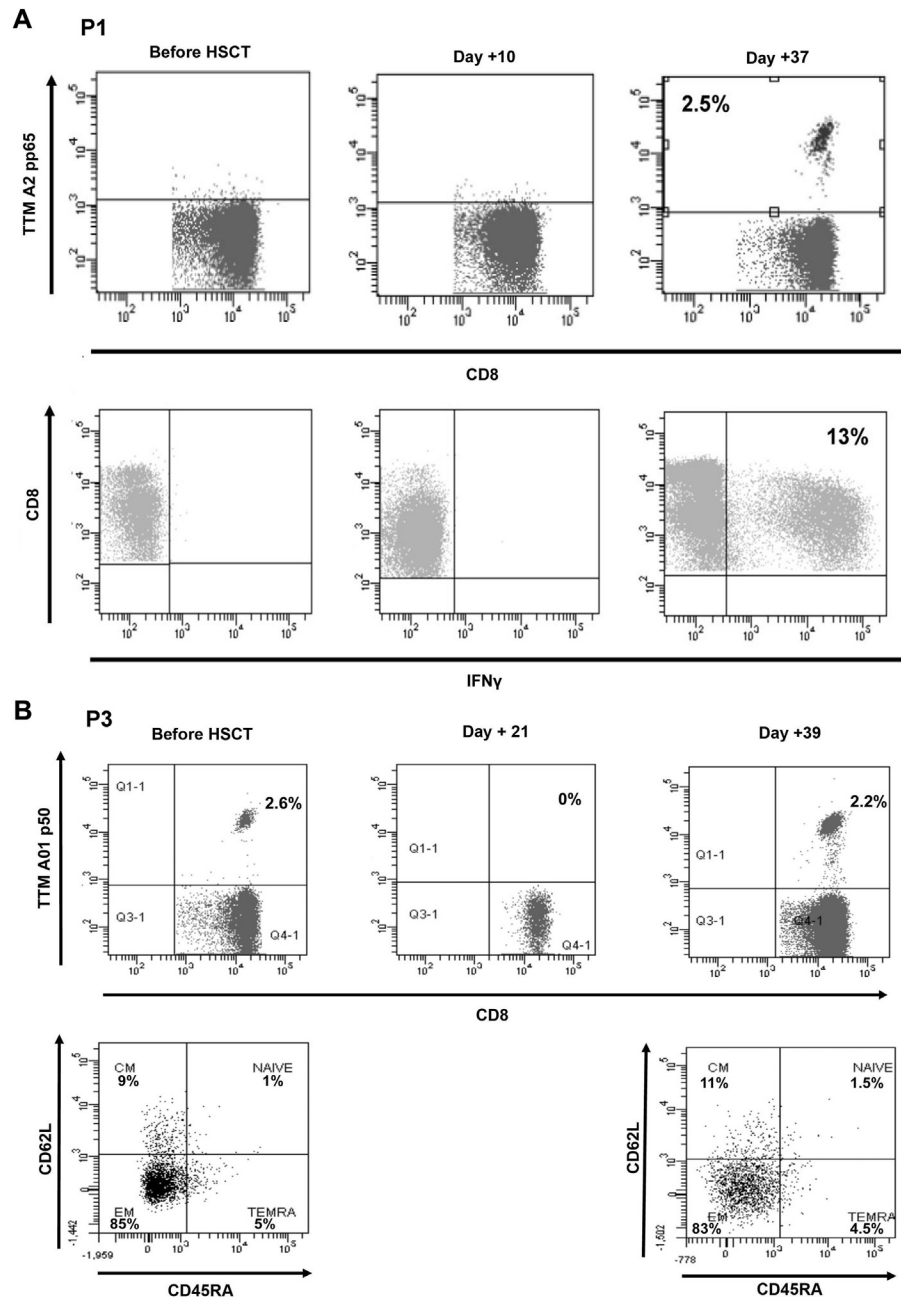


FIG 4. CMV-specific T-cell responses in patients treated with CD45RA-depleted HSCT. **A**, Detection of CMV-specific CD8⁺ T cells in patient P1 by using pentamers restricted to MHC HLA-A2 and the pp65 antigen before and after HSCT (days +10 and +37, upper panel). Detection of CMV-specific CD8⁺ T cells in patient P1 by using IFN- γ staining of CD8⁺ lymphocytes incubated *in vitro* with the Peptivator pp65 antigen before and after HSCT (days +10 and +37, lower panel) is also shown. Patient P1 received a DLI at day +36. **B**, Detection of CMV-specific CD8⁺ T cells in patient P3 by using pentamers restricted to MHC HLA-A1 and the p50 antigen before and after HSCT (days +21 and +39). Phenotypic analysis of CMV-specific CD8⁺ T cells according to CD62 ligand (CD62L) and CD45RA before and after HSCT (day +39) is also shown.

GVHD was observed in our patients, despite the fact that they were at a greater risk of this complication because of their underlying disease (MHC class II expression deficiency and CD25 deficiency), their health status (chronic infection and inflammation), and the mismatched setting.²³⁻²⁵ Only patient P1 had grade I GVHD 12 days after a nonmanipulated DLI infusion, suggesting a causative role of this DLI in the onset of GVHD.

Bertaina et al²⁶ have recently reported the efficiency of T-cell receptor (TCR) $\alpha\beta$ T-cell and CD19 cell depletion for preventing GVHD in children undergoing haploidentical HSCT for nonmalignant disorders. As new cell therapy processes are emerging, we need to precisely define the indications for each type of process. Compared with TCR $\alpha\beta$ /CD19 depletion, the CD45RA depletion process has the advantage of providing

TCR $\alpha\beta$ T cells with limited alloreactivity that are immediately active toward pathogens, thus permitting the rapid resolution of infection after HSCT. However, the ability of this process to sufficiently reduce the alloreactivity of T cells in a haploidentical setting remains to be demonstrated.

In conclusion, our preliminary data show that clinical-grade depletion of naive T cells from an allograft (by using magnetic CD45RA beads) is feasible and might be an effective option for HSCT in patients with CIDs at high risk of GVHD, infection, or both in a mismatched setting. The procedure could perhaps be improved by the use of peripheral stem cells as the initial product because this would enable the retrieval of a higher dose of CD34⁺ and CD3⁺CD45RA[−] cells. Further studies are now needed to (1) validate the safety of CD45RA depletion, (2) optimize the number of CD45RA[−] T cells infused, and (3) further assess the procedure's efficacy. Lastly, one could consider extending this treatment to patients undergoing HSCT for other PIDs or nonmalignant conditions.

Clinical implications: Clinical-grade depletion of CD45RA naive T cells from an allograft might be a treatment option for patients with CIDs at high risk of GVHD, infection, or both in an HLA-mismatched setting.

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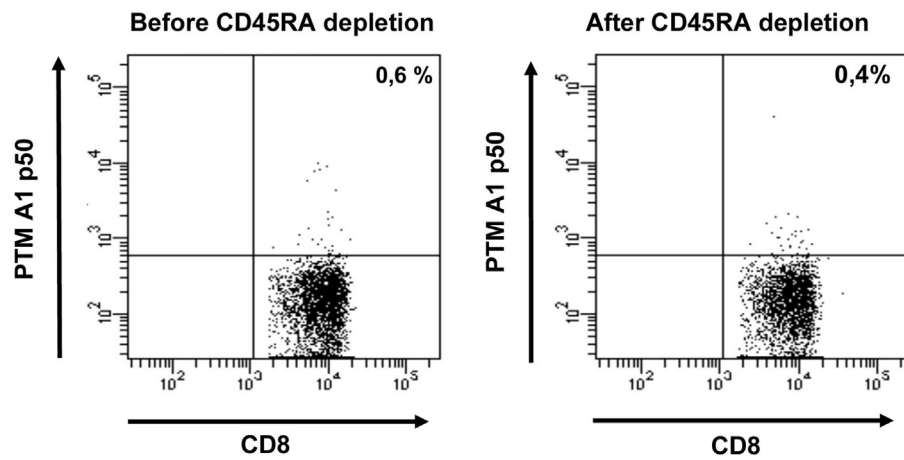


FIG E1. CD45RA depletion of the allograft maintains the presence of CMV-specific CD8⁺ T cells. CMV-specific CD8⁺ T cells are detected in the CD34⁺ fraction and the CD45RA-depleted allograft at a similar frequency by using pentamers specific for HLA-A1 and the CMV p50 antigen (patient P1).

TABLE E1. Cell counts per kilogram of recipient for the initial BM sample, the CD34⁺ fraction, and the CD45RA-depleted fraction

	Patients				
	P1	P2	P3	P4	P5
BM					
CD3 ⁺ cells	8.95×10^7	1.48×10^8	1.38×10^8	5.57×10^7	3.87×10^7
CD3 ⁺ CD4 ⁺ cells	6.29×10^7	5.42×10^7	8.61×10^7	3.30×10^7	1.95×10^7
CD3 ⁺ CD8 ⁺ cells	2.66×10^7	6.44×10^7	4.30×10^7	1.63×10^7	1.65×10^7
CD19 ⁺ cells	NA	NA	NA	NA	NA
CD14 ⁺ cells	NA	NA	NA	NA	NA
CD16 ⁺ CD56 ⁺ cells	NA	NA	NA	NA	NA
CD34⁺ fraction					
CD3 ⁺ CD45RO ⁺ cells	1.02×10^7	2.33×10^7	2.56×10^7	1.04×10^7	6.87×10^6
CD3 ⁺ CD4 ⁺ CD45RO ⁺ cells	4.70×10^6	1.99×10^7	1.62×10^7	9.63×10^6	5.88×10^6
CD3 ⁺ CD8 ⁺ CD45RO ⁺ cells	2.01×10^6	5.30×10^6	7.56×10^6	1.17×10^6	1.05×10^6
CD3 ⁺ CD45RA ⁺ cells	3.47×10^7	7.45×10^7	5.05×10^7	2.84×10^7	1.97×10^7
CD3 ⁺ CD4 ⁺ CD45RA ⁺ cells	1.54×10^7	2.84×10^7	2.50×10^7	1.74×10^7	1.53×10^7
CD3 ⁺ CD8 ⁺ CD45RA ⁺ cells	1.03×10^7	4.53×10^7	2.45×10^7	1.06×10^7	1.12×10^7
CD19 ⁺ cells	2.40×10^7	4.16×10^7	2.14×10^7	1.12×10^7	2.06×10^7
CD14 ⁺ cells	NA	1.33×10^7	NA	3.34×10^7	1.28×10^7
CD16 ⁺ CD56 ⁺ cells	8.12×10^6	3.85×10^7	6.34×10^6	4.68×10^6	4.30×10^6
CD34⁺CD45RA⁺ fraction					
CD3 ⁺ CD45RO ⁺ cells	1.58×10^6	9.23×10^6	2.53×10^6	2.57×10^6	1.73×10^6
CD3 ⁺ CD4 ⁺ CD45RO ⁺ cells	1.34×10^6	8.24×10^6	1.41×10^6	2.26×10^6	1.44×10^6
CD3 ⁺ CD8 ⁺ CD45RO ⁺ cells	1.22×10^5	9.98×10^5	4.69×10^5	1.56×10^5	1.03×10^5
CD3 ⁺ CD45RA ⁺ cells	7.99×10^2	9.23×10^3	2.53×10^3	0	1.75×10^3
CD3 ⁺ CD4 ⁺ CD45RA ⁺ cells	0	0	1.69×10^3	0	0
CD3 ⁺ CD8 ⁺ CD45RA ⁺ cells	0	3.99×10^3	4.69×10^2	0	0
CD19 ⁺ cells	0	0	2.04×10^4	0	1.29×10^5
CD14 ⁺ cells	1.60×10^6	1.07×10^6	NA	1.17×10^6	1.25×10^6
CD3 ⁺ CD16 ⁺ CD56 ⁺ cells	1.22×10^6	2.07×10^5	8.15×10^4	6.00×10^4	1.60×10^5

NA, Not applicable.

TABLE E2. Lymphocyte proliferation in response to PHA and tetanus toxoid in patients P2, P3, and P5 at 6 and 12 months after HSCT (cpm $\times 10^3$)

Patient ID	6 mo		12 mo	
	PHA	Tetanus toxoid	PHA	Tetanus toxoid
P2	129	ND	145	65
P3	62	ND	63	12
P5	48	ND	55	32
Normal values	>50	>10	>50	>10

ND, Not determined.