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*

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

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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

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

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/m^2). 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, 1.11-6 × 10^8/kg). The median CD34$^+$ cell count was 11 × 10^6/kg (range, 3-18 × 10^6/kg), and the median CD3$^+$ cell count was 8.95 × 10^7/kg (range, 3.87-14.8 × 10^7/kg). The CD34$^+$ fraction obtained after CD34$^+$ positive selection contained a median count of 10.4 × 10^6 CD34$^+$ cells/kg (range, 2.92-12 × 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, 1.58-9.2 × 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, 0.6-12.2 × 10^5/kg) and CD14$^+$ monocytes (median, 1.21 × 10^6/kg; range, 1.07-1.6 × 10^6/kg). CD19$^+$ B cells were almost completely eliminated (median, 0 × 10^4/kg; range, 0-2 × 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 × 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