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Clinical Research

Patient HLA-DP–Specific CD4$^+$ T Cells from HLA-DPB1–Mismatched Donor Lymphocyte Infusion Can Induce Graft-versus-Leukemia Reactivity in the Presence or Absence of Graft-versus-Host Disease

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A B S T R A C T

Clinical studies have demonstrated that HLA-DPB1–mismatched allogeneic stem cell transplantation (allo-SCT) is associated with a decreased risk of disease relapse and an increased risk of graft-versus-host disease (GVHD) compared with HLA-DPB1–matched SCT. In T cell–depleted allo-SCT, mismatching of HLA-DPB1 was not associated with an increased risk of severe GVHD, but a significant decreased risk of disease relapse was still observed. To investigate whether patient HLA-DP–specific CD4$^+$ T cell responses were frequently induced after T cell–depleted HLA-DPB1–mismatched allo-SCT and donor lymphocyte infusion (DLI), we developed a method to screen for the presence of HLA-DP–specific CD4$^+$ T cells using CD137 as an activation marker and analyzed 24 patient–donor combinations. The patients suffered from various B cell malignancies, multiple myeloma, and myeloid leukemias. Patient HLA-DP–specific CD4$^+$ T cells were detected after DLI in 13 of 18 patients who exhibited a clinical response to DLI, compared with only 1 of 6 patients without a clinical response to DLI. Eight patients developed significant GVHD. These data show that patient HLA-DP–specific CD4$^+$ T cells frequently occur after HLA-DPB1–mismatched T cell–depleted allo-SCT and DLI, and are associated with graft-versus-leukemia reactivity both in the presence and absence of GVHD.

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INTRODUCTION

After allogeneic hematopoietic stem cell transplantation (allo-SCT), donor-derived T cells recognizing mismatched antigens on residual malignant cells can induce strong graft-versus-leukemia (GVL) reactions. Treatment of patients with relapsed leukemia, lymphoma, or multiple myeloma with allo-SCT followed by donor lymphocyte infusion (DLI) can result in long-lasting complete remission [1-6]. Unfortunately, the beneficial GVL effects of DLI are often accompanied by graft-versus-host disease (GVHD). To reduce the risk of GVHD, patients and donors are preferably matched for HLA-A, -B, -C, -DRB1, and -DQB1 (8/8 or 10/10 match) [7-9].

HLA-DPB1 is often not taken into consideration in donor selection, since the overall mortality of patients who underwent HLA-DPB1–matched or –mismatched allo-SCT did not statistically differ. However, HLA-DPB1 matching status did have an impact on GVL reactivity and GVHD [8,10-13]. In T cell–depleted allo-SCT, mismatching for HLA-DPB1 has been associated with a significant decreased risk of disease relapse with no increased risk of severe GVHD [11]. The role of HLA-DP as a transplantation antigen was confirmed by the isolation of polyclonal HLA-DP–specific CD4$^+$ T cells from skin biopsies of patients with GVHD after HLA-DPB1–mismatched allo-SCT [14,15]. We previously demonstrated a profound GVL effect with only minimal skin GVHD caused by polyclonal HLA-DP–specific CD4$^+$ T cells in a patient responding to HLA-DPB1–mismatched DLI for refractory chronic B cell leukemia, suggesting that HLA-DP–specific CD4$^+$ T cells can be involved in GVHD, as well as in selective GVL reactivity [5].

The beneficial effect of mismatching for HLA-DPB1 in T cell–depleted allo-SCT on disease relapse has been reported to be more pronounced in patients with acute lymphoblastic leukemia compared with those with myeloid leukemias [16]. High expression of HLA class II molecules, including HLA-DP, is found in most B acute lymphoblastic leukemia and B chronic lymphocytic leukemia cells, whereas myeloid leukemic cells show more variable expression of HLA-DP. However, HLA-DP–expressing myeloid leukemic cells have been demonstrated to be recognized and lysed by HLA-DP–specific CD4$^+$ T cells [5,17]. Thus, both B cell and myeloid hematologic malignancies with sufficient HLA-DP expression may be susceptible to an HLA-DP–mediated GVL effect.

The aim of the present study was to investigate whether HLA-DP–specific CD4$^+$ T cell responses frequently occur after HLA-DPB1–mismatched allo-SCT and DLI, and whether the development of patient HLA-DP–specific CD4$^+$ T cell responses was associated with beneficial clinical responses (ie, GVL effect) or GVHD. We therefore analyzed HLA-DP–specific immune responses and clinical responses in 24 patients treated with DLI after 10/10-matched, HLA-DPB1–mismatched T cell–depleted allo-SCT.

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To analyze the emergence of an allogeneic HLA-DP–specific immune response after DLI administration, we developed an assay to screen for allogeneic HLA-DP–specific $CD4^+$ T cells. In this assay, we used HLA class II–negative HeLa cells transduced with all molecules relevant for HLA class II processing as stimulator cells and transduced patient- or donor-specific HLA-DP molecules into these cells to measure the emergence of patient HLA-DP–specific $CD4^+$ T cells in peripheral blood (PB). Using these HLA-DP– transduced HeLa cells, we introduced patient and donor HLA-DPB1 molecules as single variables to detect HLA-DP–specific immune responses. Patient HLA-DP–specific $CD4^+$ T cells were found in 72% of the patients who demonstrated a clinical response after DLI, but only in 1 of 6 patients (17%) without a clinical response to DLI. Patient HLA-DP–specific $CD4^+$ T cells were found in patients with GVHD and in patients with selective GVL reactivity without GVHD. HLA-DP–specific $CD4^+$ T cell responses were observed in patients suffering from B cell malignancies, multiple myeloma, and myeloid leukemias.

MATERIALS AND METHODS

Cell Collection and Preparation

PB samples were obtained from patients and healthy stem cell donors after approval by Leiden University Medical Center's Institutional Review Board and provision of informed consent in accordance with the Declaration of Helsinki. Samples were collected during standard follow-up after allo-SCT and DLI (ie, 6 weeks, 3 months and 6 months). In some individuals, additional samples were available. PB mononuclear cells (PBMNCs) were isolated by Ficoll-Isopaque separation and cryopreserved. Stable Epstein-Barr virus (EBV)-transformed B cell lines were generated using standard procedures. EBV lymphoblastic cell lines (LCLs) and HeLa cells were cultured in Iscove's modified Dulbecco's Medium (IMDM; BioWhittaker, Verviers, Belgium) supplemented with 10% FBS (BioWhittaker).

Patient Selection and Characteristics

The study included a total of 24 patients who underwent a 10/10-matched, HLA-DPB1–mismatched T cell–depleted allo-SCT followed by DLI between 2000 and 2008 (Table 1). The patients suffered from multiple myeloma (n = 8), B cell malignancies (n = 7), and myeloid leukemias (n = 9). Fifteen patients received a single DLI, and 9 patients received 2 or more DLIs. Indications for DLI included mixed chimerism, persistent or progressive disease, and EBV posttransplantation lymphoproliferative disorder. Leukocyte chimerism in bone marrow was determined by short tandem repeat analysis. In the absence of relapse or GVHD, the level of mixed chimerism was stable in the first months after allo-SCT. DLI was administered on an intention-to-treat basis in accordance with standard protocols. The first DLI was given between 4 months and 26 months after allo-SCT. At 4 months, 0.15*10e6 $CD3^+$ T cells were administered. At 6 months, 1.5*10e6 $CD3^+$ T cells were given for mixed chimerism or persistent disease, and 2.5*10e6 $CD3^+$ T cells were given for progressive disease. At 9 months, 2.5*10e6 $CD3^+$ T cells were administered. Dose escalation to a maximum of 5*10e7 $CD3^+$ T cells was provided to patients receiving subsequent DLIs. The first DLI was freshly harvested, and cryopreserved material was used for subsequent DLIs.

Flow Cytometry

The monoclonal antibodies (mAbs) anti–CD3 fluorescein isothiocyanate (FITC), anti–nerve growth factor receptor phycoerythrin (PE), anti–CD4 peridinin chlorophyll protein (PerCP), anti-CD137 allophycocyanin (APC), and anti–IFN-γ APC were obtained from BD Biosciences (San Jose, CA). Anti-CD154-PE was obtained from Beckman Coulter (Fullerton, CA). Anti–HLA-DP-PE mAbs were purchased from Leinco Technologies (St. Louis, MO). Flow cytometry analysis was performed on a BD flow cytometer, and cell sorting was done with a BD FACSAria cell-sorting system.

Transduction with Different HLA-DP Constructs

HeLa cells and EBV-LCLs were transduced with different HLA-DPA1 and HLA-DPB1 molecules as described previously [18]. In brief, HeLa cells were transduced with CD80, HLA-DM, and invariant chain (HeLa-II cells) to allow appropriate co-stimulation and processing of HLA-DP molecules. Purified HeLa-II cells were then transduced with different combinations of HLA-DPA1 and HLA-DPB1 molecules and selected based on positive staining with anti–HLA-DP-PE antibodies. HLA-DP–transduced EBV-LCLs were selected based on marker gene expression.

Characterization of HLA-DP–Specific $CD4^+$ T Cells in PBMNCs

To validate the use of HLA-DP–transduced HeLa-II cells as stimulator cells to detect HLA-DP–specific $CD4^+$ T cells in PB, we used PBMNCs from a patient in whom we previously demonstrated a profound HLA-DPB1*03:01–specific immune response. To determine the optimal method for detecting HLA-DP–specific $CD4^+$ T cells, we compared percentages of activated $CD4^+$ T cells using different activation markers after various incubation periods. Cryopreserved PBMNCs were thawed, and $CD4^+$ T cells were positively selected using magnetic CD4 beads (Miltenyi Biotec, Bergisch Gladbach, Germany) in accordance with the manufacturer's instructions. Then 0.1 × 10e6 purified $CD4^+$ T cells were stimulated with 0.03 × 10e6 control HeLa-II cells or HeLa-II cells transduced with patient HLA-DPB1*03:01 or donor HLA-DPB1*04:02 for 6-44 hours in 150 μL of culture medium supplemented with 10% human serum and 10 IU/mL of IL-2 (Chiron, Amsterdam, the Netherlands).

For intracellular IFN-γ and CD154 staining, cells were incubated in the presence of 10 μg/mL of Brefeldin-A (Sigma-Aldrich, St. Louis, MO); harvested after 6, 10, or 18 hours of incubation; surface-stained with CD4-FITC– and CD3-PerCP–labeled mAbs, fixed with 1% paraformaldehyde; and permeabilized using 0.1% Saponin (Sigma-Aldrich). Then cells were stained intracellularly with CD154-PE– and IFN-γ APC–labeled mAbs and analyzed by flow cytometry.

To determine the expression of surface CD154 and CD137, cells were harvested after 16, 22, or 44 hours of incubation; washed; surface-stained with CD4-FITC–, CD3-PerCP–, CD154-PE–, and CD137-APC–labeled mAbs; and analyzed.

To confirm specificity of CD137-expressing $CD4^+$ T cells, 0.75 × 10e6 purified $CD4^+$ T cells were stimulated with 0.15 × 10e6 HLA-DPB1*03:01–transduced HeLa-II cells, and after 44 hours of incubation, CD137-expressing $CD4^+$ T cells were sorted single cell per well into U-bottom microtiter plates. Proliferating $CD4^+$ T cell clones were expanded using nonspecific stimulation and third-party feeder cells. IFN-γ production in response to HLA-DPB1*03:01– or HLA-DPB1*04:02–transduced HeLa-II cells, donor EBV-LCLs, or HLA-DPB1*03:01–expressing EBV-LCLs was tested. To determine IFN-γ production, 5000 $CD4^+$ T cells were co-cultured with 30,000 stimulator cells in 150 μL of medium. After overnight incubation, supernatants were harvested, and IFN-γ production was measured by ELISA (CLB, Amsterdam, the Netherlands).

Analysis of HLA-DP–Specific $CD4^+$ T Cells after Allo-SCT and DLI in 24 Patients

To quantify the presence of HLA-DP–specific $CD4^+$ T cells in PBMNC, we analyzed $CD4^+$ T cells, derived from donors and from patients after allo-SCT both before and after DLI, for CD137 expression in response to stimulation with patient or donor HLA-DPB1 molecules. Samples obtained between 6 weeks and 6 months after DLI were analyzed according to availability and moment of clinical response. $CD4^+$ T cells were positively selected from PBMNCs using magnetic CD4 beads (Miltenyi Biotec) in accordance with the manufacturer's instructions. A total of 0.15 × 10e6 $CD4^+$ T cells were stimulated with 0.03 × 10e6 HeLa-II cells, HeLa-II cells transduced with donor or shared HLA-DPB1 molecules, or HeLa-II cells transduced with patient-specific HLA-DPB1 molecules in 150 μL of IMDM supplemented with 10% human serum and 10 IU/mL of IL-2. After 44 hours of incubation, CD137 expression on $CD3^+/CD4^+$ T cells was analyzed by flow cytometry.

Clinical Responses after HLA-DPB1–Mismatched T Cell–Depleted Allo-SCT and DLI

Clinical responses to DLI were defined as beneficial clinical responses or GVHD. Beneficial clinical responses were defined as a sustained increase in donor chimerism to ≥99% donor cells, or a decrease in malignant cells or disease markers resulting in either complete disappearance or a reduction of >90%. Clinically important GVHD was considered present in case of acute GVHD (aGVHD) grade II or greater or extensive chronic GVHD (cGVHD). aGVHD was graded according to the Glucksberg criteria. cGVHD was graded according to the Shulman criteria. Lesions of the mouth matching criteria for cGVHD in the absence of other signs of cGVHD were classified as limited cGVHD. Clinical data were analyzed by a hematologist who was not informed of the presence or absence of HLA-DP–specific $CD4^+$ T cells.

Statistical Analysis

The association between the presence of patient HLA-DP–specific $CD4^+$ T cells and clinical responses was assessed using the 2-tailed Fisher exact test. The Student *t* test after logarithmic transformation was used to evaluate for a statistically significant difference in magnitude between T cell responses directed against permissive mismatches and those directed against nonpermissive mismatches.

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