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Transfer of PR1-specific T-cell clones from donor to recipient by stem cell transplantation and association with GvL activity

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Background

The curative effects of GvL following transfer of donor-derived T cells during allogeneic stem cell transplantation (SCT) are well established. However, little is known about the nature, origin and kinetics of the anti-leukemic T-cell responses involved.

Methods

*We used quantitative real-time PCR (qRT-PCR) for interferon-gamma mRNA production (IFN-γ) and PR1/HLA-A*0201 tetramer staining to detect PR1-specific CD8+ T-cell activity in a donor and a patient with CML. Unbiased strand switch anchored RT-PCR was used to further characterize specific clones in PR1 sorted CD8+ T-cell populations.*

Results

We identified PR1-specific $CD8^+$ T-cell clones from a donor pre-transplant, and demonstrated their transfer in the recipient's blood post-SCT using molecular tracking of Ag-specific T-cell receptors. PR1-specific $CD8^+$ T-cell populations were polyclonal, with a range of functional avidities for cognate Ag, and displayed predominantly effector memory phenotype early post-SCT, suggesting active stimulation in vivo. *Expansion of these PR1-specific $CD8^+$ T-cell clones in the recipient was followed by complete remission of CML.*

Discussion

This report represents the first direct demonstration that PR1-specific $CD8^+$ T-cell clones can be transferred during SCT, and supports the feasibility of pre-transplant vaccination strategies that aim to boost the number of anti-leukemic T cells in the graft.

Keywords

PR1, GVL, CML.

Introduction

Allogeneic hematopoietic stem cell transplantation (SCT) was initially performed for hematologic malignancies to replace diseased BM with marrow from a healthy donor. Today, more than three decades of clinical experience have shown that SCT not only reconstitutes the hematopoietic system in the recipient but also mediates a powerful and potentially curative anti-malignancy activity referred to as GvL [1–4]. However, little is known about the nature, origin and kinetics of the anti-leukemic T-cell responses that mediate GvL activity. Previously, we have demonstrated that memory $CD8^+$ T-cell responses to the leukemia-associated self-Ag PR1, a nine-amino acid HLA-A*0201-restricted peptide derived from proteinase 3 [5,6], are detectable at low frequencies in healthy donors [7]. These observations raise the possibility that the GvL effect

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is mediated by $CD8^+$ T cells present in the donor memory lymphocyte pool that are transferred by SCT and expand in the recipient.

In order to substantiate this hypothesis, we studied the donor of a patient with CML undergoing SCT. By isolating PR1-specific $CD8^+$ T cells in the donor directly *ex vivo*, we defined the individual clones comprising these Ag-specific responses. This knowledge allowed us to track the fate of these individual PR1-specific clones in the recipient after SCT and subsequent donor lymphocyte infusion (DLI). Further, we studied the kinetics, phenotype and functional avidity of the PR1-specific $CD8^+$ T-cell population, and correlated these parameters longitudinally with leukemia burden, as measured by molecular techniques, to detect the BCR-ABL fusion gene.

Methods

Case report

We treated a 25-year-old man with chronic phase CML with TBI (1200 cGy), fludarabine 125 mg/m^2 and CY 120 mg/kg, followed by transfusion of mobilized PBSC from his HLA-identical sister. The $CD34^+$ cell dose was 4.37×10^6/kg and the graft was T-depleted to a $CD3^+$ cell dose of 2×10^4/kg. No overt GvHD occurred post-SCT and complete donor chimerism was achieved after 12 months. Peripheral blood samples were obtained with written informed consent from both individuals before transplant and from the recipient periodically afterwards. The protocol was approved by the NIH Institutional Review Board.

qRT-PCR for BCR-ABL

Real-time quantitative RT-PCR (qRT-PCR) was used to monitor BCR-ABL mRNA expression in patient peripheral blood leukocytes, as described previously [8].

Peptide–HLA class I tetrameric complexes

PE-conjugated PR1/HLA-A*0201 and CMV/HLA*0201 tetramers were produced as described previously [7,9].

Determination of T-cell phenotype

Phenotypic analysis was performed by staining with PR1/HLA-A*0201 tetramer at 37°C, followed by counterstaining at 4°C with pre-titred MAb specific for CD4, CD8, CD14, CD16, CD19, CD27 and CD57 (PharMingen, San Jose, CA, USA), CD45RO (Dako, Carpinteria, CA, USA) and CD3 (Coulter, Miami, FL, USA). Alexa 430, FITC, Texas Red-PE (TRPE), PE, Cy5PE, Cy7PE and allophycocyanin (APC) were used as directly conjugated fluorophores. Data were acquired with a FACS DIVA (Becton Dickinson, San Diego, CA, USA). A minimum of 1.5×10^6 gated cells was acquired. Data analysis was performed using FlowJo software (TreeStar, San Carlos, CA, USA).

Detection of peptide-specific $CD8^+$ T-cell reactivity using qRT-PCR

To screen for peptide-specific $CD8^+$ T cells, we measured IFN-γ mRNA production by $CD8^+$ T cells stimulated with PR1 peptide (VLQELNVTV), as described previously [7]. IFN-γ mRNA expression was normalized to copies of CD8 mRNA from the same sample.

Chimerism analysis

The degree of lineage-specific donor/recipient chimerism was assessed using PCR-based analysis of short tandem repeats [10].

Flow cytometric cell sorting

All sorts were performed using a modified FACS DIVA (Becton Dickinson). Post-sort purity was consistently $> 99\%$.

Clonotypic analysis of specific $CD8^+$ T-cell populations

Unbiased identification of specific clones in sorted T-cell populations was performed using an unbiased strand-switch anchored RT-PCR, as described previously [11].

Results

Kinetics of the GvL response

The post-SCT course is outlined in Figure 1. Using qRT-PCR for peptide-specific IFN-γ mRNA production, we identified PR1-specific $CD8^+$ T cells in the donor but not in the patient prior to transplant; this was confirmed with PR1/HLA-A*0201 tetramer staining, which showed that 0.12% of all peripheral blood $CD8^+$ T cells in the donor recognized PR1 (Figure 2A). Seven weeks post-SCT, the patient received a pre-emptive DLI of 10^7 cells/kg. While present prior to DLI, the frequency of PR1-specific/total $CD8^+$ T cells identified by tetramer staining peaked 9 weeks post-SCT at 0.55% (Figure 2D); however, BCR-ABL transcripts remained detectable by qRT-PCR. To determine the functional avidity of PR1-specific $CD8^+$ T cells, the response to a range of PR1 peptide