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Rapid monitoring of reconstituted Wilms' tumor suppressor gene-specific t lymphocytes after allogeneic stem cell transplantation using quantitative real time Polymerase chain reaction

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Background

Wilms' tumor suppressor gene (WT1) protein is over-expressed in some leukemias and various types of solid tumors, and it is considered to be an attractive target antigen for immunotherapy.

Methods

After ex vivo stimulation of peripheral blood mononuclear cells (PBMCs) from 12 patients with two HLA-A2 restricted WT1 peptides (Db126 and WH187), between 220 days and 32 months after allogeneic SCT, interferon-gamma (IFN-γ) quantitative real time polymerase chain reaction (qRT-PCR) and ELISPOT assays were used to evaluate WT1 responses in 22 samples.

Results

Both WT1 antigens induced significant quantities of IFN-γ mRNA production after 3 h in the qRT-PCR protocol. Among 18 samples of HLA-A2+, specific responses were detected in 27.8% (5/18) when Db126 peptide, compared to 16.7% (3/18) in the case of WH187 peptide. On the other hand, no detection (0/18) was obtained with ELISPOT, when both WT1 peptides were tested. In four HLA-A2- samples neither qRT-PCR nor ELISPOT detected any reconstituted WT1-reactive T cells in response to both peptides. As a result, the measurement of IFN-γ mRNA by qRT-PCR can be used to detect CTL responses 3 h after WT1 peptides stimulation of PBMCs.

Discussion

In conclusion, qRT-PCR allows rapid monitoring of WT1-reactive CTLs reconstitution after allogeneic SCT using patient's PBMCs.

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