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Alloreactivity of *ex vivo*-expanded T cells is correlated with expansion and CD4/CD8 ratio

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Background

We have demonstrated previously that retroviral-mediated transfer of a suicide gene into bone marrow (BM) donor T cells allows an efficient control of graft-versus-host disease (GvHD) after allogeneic BM transplantation. However, the 12 days of ex vivo *culture required for the production of gene-modified cells (GMC), including soluble CD3 monoclonal antibody (MAb)-mediated activation and expansion with interleukin (IL)-2, induced a decrease of GMC alloreactivity and a reversal of their CD4/CD8 ratio. Improving the culture protocol in order to maintain the highest alloreactivity is of critical importance in obtaining an optimal graft-versus-leukemia (GvL) effect.*

Methods

Peripheral blood mononuclear cells were activated with soluble CD3 MAb or CD3 and CD28 MAb co-immobilized on beads and expanded for 12 days in the presence of IL-2, IL-7 or IL-15 before analysis of alloreactivity and phenotype.

Results

Replacing the CD3 MAb by CD3/CD28 beads led to similar in vitro *alloreactivity but improved the expansion and* in vivo *alloreactivity of GMC. Replacing the IL-2 with IL-7, but not IL-15, or decreasing IL-2 or IL-7 concentrations, improved the* in vitro *alloreactivity of expanded cells but was associated with lower expansion. Indeed, the alloreactivity of expanded cells was negatively correlated with cell expansion and positively correlated with CD4/CD8 ratio and CD8 expression level.*

Discussion

Quantitative (i.e. low CD4/CD8 ratio) and qualitative (e.g. low CD8 expression) defects may account for the decreased alloreactivity of GMC. Using CD3/CD28 beads and/or IL-7 is more beneficial than CD3 MAb and IL-2 for preventing perturbations of the alloreactivity and phenotype of GMC.

Keywords

Alloreactivity, gene transfer, interleukin-2, interleukin-7, interleukin-15, T cell.

Introduction

T cells present in an allogeneic hematopoietic stem cell (HSC) graft are responsible for beneficial effects, such as anti-leukemic properties (graft-versus-leukemia effect; GvL), antiviral potential and engraftment facilitation. However, T cells also induce a major complication of HSC transplantation, graft-versus-host disease (GvHD), that results from the recognition of host antigens (Ag) by donor alloreactive T cells. We have demonstrated previously, in a phase I/II clinical trial, that donor T cells modified by retroviral-mediated *ex vivo* transfer of the herpes-simplex thymidine kinase (HS-tk) gene could be

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infused together with a T-cell-depleted HSC graft, in order to provide the beneficial effects of T cells [1]. If GvHD was to occur, ganciclovir (GCV) treatment was efficient at specifically depleting alloreactive HS-tk-expressing gene-modified cells (GMC), leading to GvHD resolution while preserving the other immune cells, such as non-alloreactive GMC and non-gene-modified immune cells.

During this clinical trial, the GMC preparation required a 12-day *ex vivo* culture period, including T-cell activation with a soluble CD3 monoclonal antibody (MAb) in the presence of interleukin-2 (IL-2), retrovirus-mediated gene transfer from day 3 to day 4, and a selection of the GMC from day 5 to day 12 [2]. However, we and other groups have observed, both *in vivo* in animal models and *in vitro* with human cells, that the CD3 MAb- or mitogen-induced cell activation required to transduce T cells and expand GMC leads to an impairment of alloreactivity in terms of GvHD induction, cytotoxic activity and proliferative response to allogeneic stimulation [3–9], as well as a decrease in anti-Epstein–Barr virus (EBV) reactivity [10–12] and skewed T-cell receptor β-chain variable region gene usage (TCRBV repertoire) [13,14], compared with non-cultured, non-transduced resting T cells obtained from murine spleen cells or human peripheral blood mononuclear cells (PBMC). We have reported that replacing the soluble CD3 MAb stimulation by CD3/CD28 MAb co-immobilized on beads was efficient at limiting, in part, the decrease of anti-EBV reactivity [10,12] and TCRBV repertoire skewing [14]. During our clinical trial, the *ex vivo* expansion of GMC was performed in the continuous presence of high concentrations of IL-2 [1]. Other cytokines of the same family, such as IL-7 or IL-15, have been shown to increase alloreactivity *in vitro* [15–18] and/or *in vivo* in murine models of GvHD [19–21]. As deleterious effects of donor T-cell-mediated alloreactivity can be controlled by GCV, our goal was to maintain the highest possible alloreactivity within GMC, in order to provide the most powerful GvL effect possible. For this purpose, we examined the effect of replacing the clinical-grade CD3 MAb by CD3 and CD28 MAb co-immobilized on clinical-grade beads and/or replacing IL-2 with IL-7 or IL-15 on the alloreactivity, phenotype and expansion rate of *ex vivo*-expanded T cells.

Methods

Activation and transduction of primary T cells

After isolation by whole blood centrifugation over Histopaque-1077 (Sigma, St Louis, MO, USA) and washing, PBMC from healthy volunteer adults were resuspended in a culture medium consisting of RPMI-1640 medium (Cambrex BioSciences, Clermont-Ferrand, France) supplemented with 10% human serum (EFS Bourgogne/Franche-Comté, Besançon, France). The cells (10^6 cells/mL) were activated in the presence of soluble CD3 MAb (10 ng/mL; OKT3, Jansen-Cilag, Levallois Perret, France) or CD3 and CD28 MAb-coated beads (Dynabeads® ClinExVivo™ CD3/CD28, formerly Xcyte™ Dynabeads®, 1×10^6 beads/10^6 cells; Invitrogen, Cergy Pontoise, France) in the presence of recombinant human IL-2 (Proleukin™, Novartis Pharma Canada, Dorval, Canada), IL-7 (R&D Systems, Lille, France) or IL-15 (R&D Systems), and incubated at 37°C, 5% CO_2 in a humidified incubator. Unless otherwise specified, cytokines were used at a final concentration of 500 IU/mL (IL-2) or 20 ng/mL (IL-7 and IL-15). The IL-2 concentration corresponded to the one used previously for the production of clinical-grade GMC [1,2]. The IL-7 and IL-15 concentrations were determined as being optimal in preliminary dose–response experiments because they were the lowest cytokine concentrations inducing the maximal thymidine incorporation by 4-day-activated phytohemagglutinin (PHA)-blast cells (data not shown). Cells were split every 2–3 days in culture medium supplemented with cytokines until day 12. When indicated, cells were spin-transduced at day 3 by centrifuging the cells for 2 h at 32°C at 1×10^6 cells/mL in the ΔLNGFR-encoding LNSN retroviral supernatant [22] kindly provided by Dr Bordignon (San Raffaele Hospital, Milano, Italy) in the presence of 5 μg/mL protamine sulfate (Sanofi-Synthelabo: Paris, France). ΔLNGFR-positive cells were immunomagnetically selected at day 5 as described previously [10] and expanded in culture medium supplemented with cytokines until day 12. Non-transduced cells expanded after CD3 or CD3/CD28 activation are referred as Co_{CD3} and $Co_{CD3/CD28}$ cells, respectively. ΔLNGFR-transduced and selected cells expanded after CD3 or CD3/CD28 activation are referred as GMC_{CD3} and $GMC_{CD3/CD28}$ cells, respectively. Cell expansions, expressed as relative cell number per input cell, were calculated from trypan blue numerations and