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EFFECTIVE RNA LOADING OF CD34⁺ DERIVED DENDRITIC CELLS FOR ADOPTIVE IMMUNOTHERAPY

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A successful immunotherapeutic strategy for the treatment of relapsed Acute Lymphoblastic Leukemia (ALL) post stem cell transplantation requires the optimisation of multiple parameters. The aim of this project was to optimise RNA loading conditions into dendritic cells (DC) for eventual induction of leukemia specific cytotoxic T lymphocyte (CTL) responses. We hypothesized that electroporation would be an efficient technique with which to introduce IVT-RNA into cord blood CD34⁺ derived DC. The aims of this project were to: 1) to optimise variables affecting transfection efficiency (using IVT-enhanced green fluorescent protein (eGFP) mRNA as a model antigen), 2) optimise variables affecting cell viability, 3) determine the optimal day of RNA loading and 4) show that the electroporated CD34⁺ derived DC were functional. We designed experiments to investigate the effects of cuvette size, cell concentration, voltage, capacitance and electroporation medium on the viability and transfection efficiency of CD34⁺ derived DC. Additionally, we investigated factors pre and post electroporation including: incubation times and exposure of cells to different temperature environments that can influence viability and GFP uptake by CD34⁺ derived DC. Using our optimal conditions we achieved 91% GFP transfection efficiency with 90% cell viability, and 94% of Lin⁻CD11c⁺HLA-DR⁺ DC have taken up RNA. A crucial finding was the effect of temperature on cell membranes, significantly affecting transfection efficiency and cell survival. Cells will take up more RNA and survive better in a warm (37°C) environment compared to room temperature or ice. Transfection efficiency of CD34⁺ derived DC electroporated on day 9 of culture was consistently higher than that observed on day 7 of culture. Interestingly, electroporation did not further upregulate activation markers on CD34⁺ derived DC. CD34⁺ derived DC electroporated with Flu Matrix protein RNA under the optimised conditions are able to present antigen and be killed by flu CTL clones. In conclusion, we have developed an efficient and safe electroporation procedure that results in functional RNA loaded CD34⁺ derived DC that can now be used to stimulate CTL for adoptive immunotherapy.

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RAPAMYCIN-GENERATED CD4⁺, TH2 CELLS MODULATE GRAFT-VERSUS-HOST REACTION VIA AN IL-4 DEPENDENT MECHANISM

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Augmentation of T cell replete allografts with donor Th2 cells represents an approach to achieve a balance of type I and type II cytokine immunity post-BMT for mediation of a graft-versus-tumor (GVT) effect with regulation of graft-versus-host disease (GVHD). We have recently found that in vitro usage of high-dose rapamycin (10 μM) generates murine Th2 cells that potently inhibit allograft CD4⁺ and CD8⁺ T cell acquisition of IFN-based allospecificity, promote type II cytokine production in vivo, and reduce GVHD while preserving a component of GVT effect against allogeneic breast cancer cells. Because rapamycin-generated Th2 cells secreted reduced IL-4 and greatly reduced IL-5, IL-10, and IL-13 relative to control Th2 cells, we hypothesized that this population might regulate graft-versus-host reaction (GVHR) through a non-IL-4 mechanism. To address this possibility, CD4⁺ T cells from wild-type (wt) or IL-4 knock-out (IL-4 KO) donor C57Bl/6 mice were co-stimulated with anti-CD3, anti-CD28 coated beads in media supportive of Th2 differentiation (+IL-4, +IL-2, +IL-7) either without ("Th2") or with rapamycin (10 μM; "Th2.rapa"). Th2 or Th2.rapa cells generated from IL-4 KO donors were indeed

deficient in IL-4 secretion, but otherwise secreted a similar Th2-type cytokine pattern as Th2 or Th2.rapa cells expanded from wt donors. As shown (Table), relative to wt Th2 cells, wt Th2.rapa cells expanded greater in vivo after semi-allogeneic BMT (B6-into-CB6F1; 1050 cGy XRT; BMT supplemented with 20 x 10⁶ donor GVHR-inducing T cells) and more potently inhibited allograft CD4⁺ and CD8⁺ T cell acquisition of IFN-allospecificity; Th2.rapa cells also yielded a more dramatic shift toward type II cytokine secretion post-BMT (increased IL-4, IL-5, IL-10, and IL-13; each p < 0.05). In marked contrast, IL-4 deficient Th2 or Th2.rapa cells failed to modulate GVHR, as such recipients had levels CD4⁺ and CD8⁺ IFN-allospecificity similar to the GVHR control group. In conclusion, relative to control Th2 cells, rapamycin generated donor Th2 cells have an increased capacity to inhibit GVHR; although Th2.rapa cells secrete reduced IL-4 and greatly reduced IL-5, IL-10, and IL-13 in vitro, their ability to induce these cytokines in vivo post-BMT is significantly increased. Both Th2 and Th2.rapa cells are fully dependent upon IL-4 secretion for their inhibition of GVHR, and as such, these cells operate through a conventional Th2-type mechanism.

Table. Th2.rapa Cells Potently Inhibit GVHR Via an IL-4-dependent Mechanism

Trx Group ^a	Total # Donor T Cells ^b		# IFN-γ Allospecific Cells ^c	
	CD4	CD8	CD4	CD8
1 (no Th2)	5.2 ± 0.5	17.4 ± 1.9	1.5 ± 0.3	2.5 ± 0.6
2 (Th2 wt)	15.0 ± 2.1*	11.4 ± 1.2*	0.9 ± 0.2*	0.2 ± 0.1*
3 (Th2 IL-4 KO)	15.1 ± 1.5*	24.0 ± 1.6†	1.5 ± 0.2†	5.1 ± 0.9†
4 (Th2.rapa)	35.6 ± 2.5*	6.4 ± 0.2*	0.4 ± 0.1*	0.1 ± 0.1*
5 (Th2.rapa IL-4 KO)	25.6 ± 1.2*	10.1 ± 1.3†	1.9 ± 0.5†	2.1 ± 0.5†

^aCB6F1 mice were lethally irradiated (1050 cGy; n = 5 per trx group). Each mouse received B6 marrow (1 × 10⁶) and B6 T cells (20 × 10⁶). Groups 2-5 received in vitro-generated donor Th2 cells (10 × 10⁶ cells) that were generated from wild-type or IL-4 KO donors, and were generated either without or with rapamycin (10 μM). ^bAbsolute # of donor CD4 and CD8 splenic T cells was enumerated at day 7 post BMT using flow cytometry. ^cDay 7 splenic T cells were stimulated in vitro with CB6F1 dendritic cells, and the # of CD4 and CD8 cells producing IFN-γ was determined by cytokine capture flow cytometry. *Value is statistically different (P < 0.05) relative to the GVHR control group (group 1). †Value for the IL-4 KO group is statistically different from the result obtained with the respective wild type Th2 or Th2.rapa cohort (that is, group 5 vs. group 4 and group 3 vs. group 2).

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RISK FACTORS OF DEVELOPING ACUTE GRAFT-VERSUS-HOST DISEASE (AGVHD) AFTER HLA-MATCHED UNRELATED DONOR (MUD) TRANSPLANTATION -IMPORTANCE OF MAINTAINING THE BLOOD LEVEL OF TACROLIMUS EARLY AFTER TRANSPLANTATION

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The aim of this study was to identify risk factors for moderate to severe AGVHD in adults after MUD marrow transplantation and prophylaxis with tacrolimus and methotrexate (MTX). Seventy five adults with leukemia or marrow failure received a myeloablative preparative regimen and unmanipulated bone marrow graft from a HLA-serologically matched (50 allele match, 25 allele mismatch) unrelated donor. Tacrolimus (0.03 mg/kg/day) was given by continuous infusion from day -1 and the dose was adjusted to maintain whole blood steady state or trough levels between 10 and 20 ng/ml. MTX was administered on days 1,3,6, +/-11. 36 (48%) patients