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Table S1: Induction of apoptosis following 2mM adenosine treatment of allostimulated, anti-CD3/CD28 bead stimulated, or unstimulated lymphocytes from 4 healthy donors measured by $\%PI^{+}AnnexinV^{+}$ cells in culture

	Allo-Stim[a]		**Bead-Stim**[b]		**Unstim**[c]	
Donor	NT[d]	+ADO[e]	NT	+ADO	NT	+ADO
1	9.39	11.1	13.7	14.7	34.4	34.5
2	6.44	7.12	10.2	11.4	16.6	16.5
3	4.53	5.59	7.7	9.1	15.6	15.5
4	7.27	7.67	6.95	7.34	20.8	21.2
	P=0.0426		P=0.0197		P=0.5707	

[a] Lymphocytes stimulated 5:1 with mix of mismatched DCs from 3 donors for 72 hours
[b] Lymphocytes stimulated 1:1 with anti-CD3/CD28 Beads for 72 hours
[c] Lymphocytes cultured unstimulated for 72 hours
[d] NT = No adenosine added
[e] +ADO = incubated with 2mM adenosine @ 37˚C for 5 minutes before PI, AnnexinV stain

Supplementary Materials and Methods

Generation of dendritic cells

Peripheral blood mononuclear cells (PBMC) were isolated from the LP products by ficoll separation method. Monocytes and lymphocytes were purified from the PBMC using the monocyte and lymphocyte negative isolation kits (Stemcell Technologies, Vancouver, BC, Canada). Dendritic cells (DCs) were generated from elutriated or magnetically purified monocytes monocytes by culturing them in 24 well plates for 4 days in media composed of a 50:50 mix of AIM V and RPMI 1640 media supplemented with 100 U/mL penicillin, and 100 µg/mL streptomycin (Invitrogen, Carlsbad, CA), 5% heat-inactivated normal AB serum (Gemini Bio-Product, Woodland, CA) and recombinant human (rh) granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4 (each 1,000 IU/mL, R&D Systems, Minneapolis, MN). DCs were matured with lipopolysachharide (LPS, 15ng/mL, Sigma, St. Louis, MO) for 24-48 hours before use.

CFSE labeling

1µM CFSE (5(6)-CFDA, SE; Life Technologies) solution was prepared in Phosphate buffered saline (PBS). Cryopreserved or cultured cells were washed in PBS and stained with 1mL of 1µM CFSE solution per 10^6 cells at room temperature (RT) in the dark for 10 minutes. An equal volume of filtered human serum was then added and incubated for 2 minutes at RT to absorb excess stain. The cells were then washed once with PBS and then once with media before culture.

Lentiviral vectors

The human immunodeficiency virus (HIV)-1-based gene transfer vectors used in this study are pRRLsin18.PPT.PGK.PRAME.Wpre (Lenti-PRAME) and pRRLsin18.PPT.MSCV.WT1.Wpre (Lenti-WT1). The vesicular stomatitis virus G protein (VSV-G) envelope encoding construct pMD.G, and the packaging construct pCMV ΔR8.91 were kindly provided by Prof. D. Trono, Department of Genetics and Microbiology, CMU, Geneva, Switzerland. Lentiviral vector particles were produced in 293T cells (ATCC, Manassas, VA) by three-plasmid transfection using a Calcium Phosphate transfection kit (Invitrogen) as previously described.[1] The viral

supernatants were concentrated to 50x by ultracentrifugation at 50,000g for 1.5 hours at 4°C. Viral pellets were resuspended in media and stored frozen at -80°C until use.

Lentiviral transduction of monocyte-derived DC

Day 4 immature DCs were transduced with WT1 or PRAME expressing lentiviral vector particles at a multiplicity of infection of approximately 30, in the presence of protamine sulfate (10μg/mL, Sigma), in a 24-well plate containing 1mL/well of DC culture medium supplemented with rhGM-CSF (1,000 IU/mL) and rhIL-4 (1,000 IU/mL). After 48 hours transduction, cells were washed and cultured in fresh DC culture medium containing rhGM-CSF and rhIL-4 in the presence of 15 ng/mL endotoxin-free LPS for 24 hours to induce maturation.

Flow cytometry

Cell viability was assessed by labeling cells with fixable VIVID (LIVE/DEAD® Fixable Violet Dead Cell Stain; Invitrogen) prior to surface stain. All the fluorescently labeled antibodies antibodies against various cell surface molecules used for phenotyping of various immune cell subsets as indicated in the results section and antibodies against the cytokines tumor necrosis factor (TNF)-α and interferon (IFN)-γ and transcription factor FOXP3 were purchased from BD Biosciences (San Jose, CA), Biolegend or eBioscience (SanDiego, CA) and were used according to the manufacturers recommendations. Intracellular staining for TNF-α and IFN-γ to assess T cell activity was performed using the Fixation and Permeabilization Solution Kit (BD Biosciences). Intracellular stain of transcription factor FOXP3 was performed with Foxp3 Staining Buffer Set (eBioscience). All flow cytometry data were obtained using the LSR Fortessa flow cytometer using BD FacsDiva software (both from BD Biosciences) and analyzed with Flow-Jo software (TreeStar, Ashland, OR).

Supplementary References

1. Chinnasamy D, Chinnasamy N, Enriquez MJ, Otsu M, Morgan RA, Candotti F. Lentiviral-mediated gene transfer into human lymphocytes: role of HIV-1 accessory proteins. *Blood*. 2000;96(4):1309-1316.