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A subset of CD8$^+$ T cells acquiring selective suppressive properties may play a role in GvHD management

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ABSTRACT

Difficulty in segregating graft-versus-tumor effect (GvT) from graft-versus-host disease (GvHD) remains a major limitation of allogeneic stem cell transplantation (Allo SCT). Naturally occurring regulatory T cells have been suggested to suppress alloreactive T cells involved in GvHD; however, their non-selective suppressive effect raises concern regarding probable attenuation of the GvT effect. Recent studies suggested inducible CD8 (iCD8) cells to be useful in suppressing autoimmune reactions, although their function in the Allo SCT setting has not been fully explored. The current study assessed in-vitro the properties of iCD8 T cells, generated in response to allogeneic dendritic cells (DCs), imitating the Allo SCT conditions. CD25$^-$ peripheral blood mononuclear cells (PBMCs) were stimulated with allogeneic DCs in mixed lymphocyte culture (MLC). The resultant iCD8$^+$CD25$^+$ population was isolated and assessed for phenotypic markers, cytokine expression profile, cell proliferation, inhibitory capacity and anti-viral response. The generated CD8$^+$CD25$^+$FOXP3$^+$ T cells selectively inhibited the primary allogeneic response, without attenuating T cell response against other stimuli, such as mitogens or a cytomegalovirus (CMV) recall antigen. In conclusion, iCD8$^+$CD25$^+$ cells suppress allogeneic stimulation, while maintaining the capacity to respond to infectious pathogens. These cells could be potentially efficient in the Allo SCT setting, where GvHD prevention is required.

1. Introduction

The anti-tumor effect obtained with Allo SCT is often offset by a high mortality rate, caused by a simultaneous GvHD. Generation of an immunosuppressive cell population that selectively inhibits GvHD without adversely affecting post-transplant immune reconstitution and anti-tumor immunity remains a major challenge for improving patient outcome.

Naturally occurring CD4 regulatory T cells (nTregs) have been suggested to oppose GvHD [1,2]. However, their non-selective inhibitory effect [3] raises the question whether their administration would also inhibit GvT activity, mediated through the induction of response targeted against antigens shared by tumor and healthy cells, tumor-specific antigens, or over-expressed self-antigens (proteinase-3, WT-1) [4].

Regulatory CD8$^+$ T cells, both expanded and inducible, possessing suppressive function, were mainly explored in the cancerous [5] and chronic-infection settings [6]. The abundance of these cells in involved tissues proved to be associated with a worse prognosis, given their ability to promote tumor progression and infection spread by suppressing an effective immune response [5,6].

Conversely, the employment of suppressive capacities of regulatory CD8 cells for treating T cell-dependent autoimmune disorders such as multiple sclerosis (or its non-humanized version; experimental autoimmune encephalomyelitis/EAE) and insulin dependent diabetes mellitus (IDDM) appears promising, leading to inhibition of T cell-related autoimmune inflammatory responses [7,8].

Characteristics and potential applicability of CD8 regulatory T cells for preventing GvHD, a life-threatening complication mediated through donor's T cell activation by host's DCs, have not been fully elucidated [9–11].

2. Objective

The purpose of the current study was to explore in-vitro the profile and significance of induced CD8$^+$ T cells, generated in response to allogeneic DCs, aiming to mimic the allogeneic stem cell transplant setting, where selective inhibition of GvHD without attenuating the GVT response is required.

Abbreviations: Allo SCT, allogeneic stem cell transplantation; CMV, cytomegalovirus; DCs, dendritic cells; EAE, experimental autoimmune encephalomyelitis; GvHD, graft-versus-host disease; GvT, graft-versus-tumor; iCD8, inducible CD8; IDDM, insulin dependent diabetes mellitus; IRB, Institutional Review Board; mAb, monoclonal antibodies; MLC, mixed lymphocyte culture; nTregs, naturally occurring CD4 regulatory T cells; PBMCs, peripheral blood mononuclear cells.

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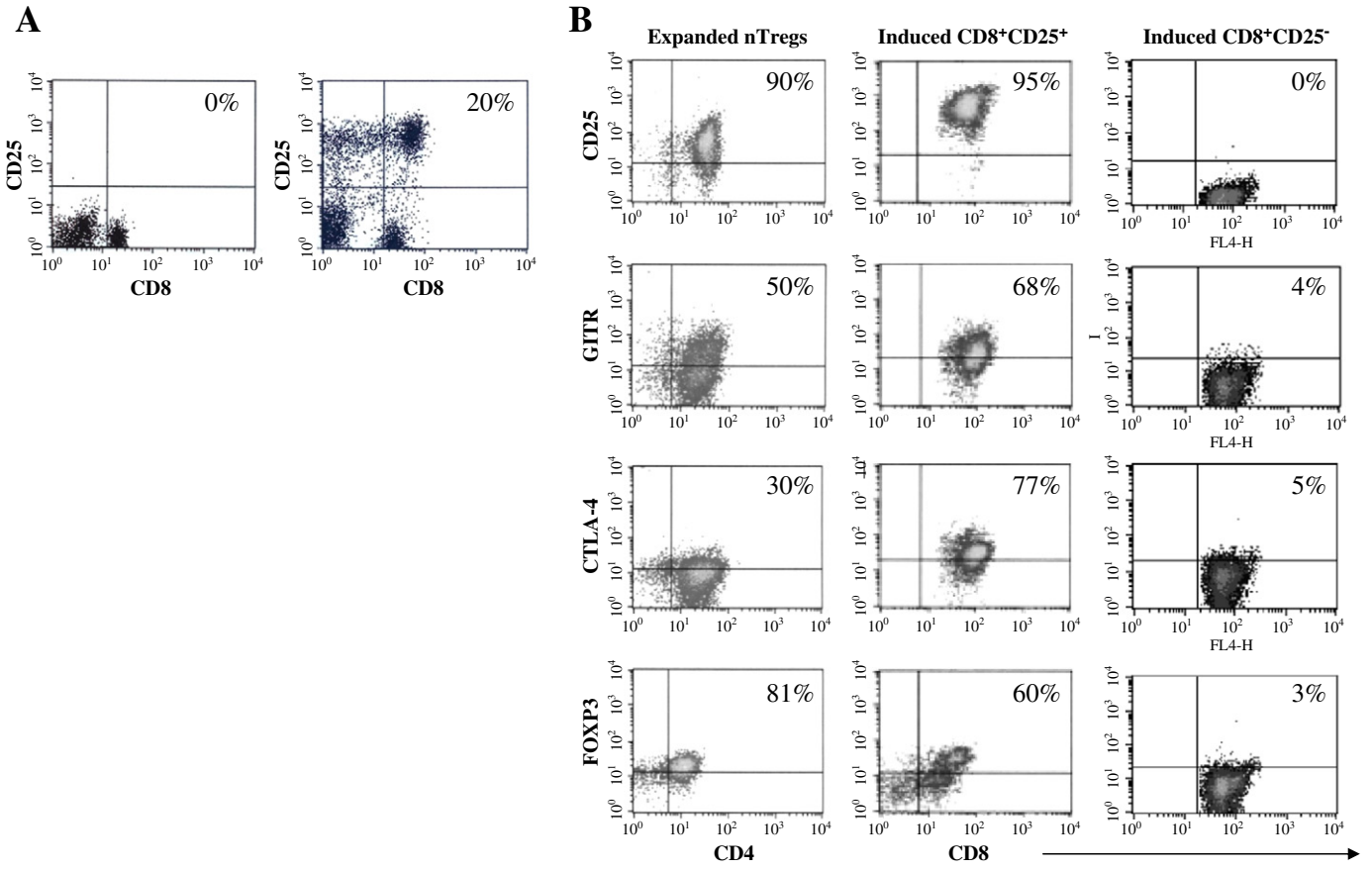

Fig. 1. Induced $CD8^+CD25^+$ T cells express regulatory markers. CD25 depleted PBMCs were stimulated with irradiated DCs for 6 days. (A) Induction of $CD8^+CD25^+$ T cells was assessed with flow cytometry using PE-anti-CD25 and FITC-anti-CD8 monoclonal antibodies. The dot plot represents one of the 7 experiments prior to (left panel) and following (right panel) induction. (B) $ICD8^+CD25^+$ and $CD8^+CD25^-$ T cells were isolated and stained with FITC-anti-GITR, FITC-anti-CTLA-4 or intracellular FITC-anti-FOXP3 as shown in the representative FACS dot plot (n = 5). Expanded nTregs were induced and analyzed in the same experiment.

3. Material and methods

3.1. Human samples

Peripheral blood mononuclear cells (PBMCs) obtained from healthy donors (IRB approval number 2419) were isolated by centrifugation over Ficoll–Hypaque gradients (Sigma-Aldrich, St Louis, MO, USA). All experiments were performed using PBMCs obtained from different unrelated donors.

3.2. Flow cytometery and intracellular staining

Cells were stained with the following monoclonal antibodies (mAb): CD25, CD4, CD8, CD3, and CD45 (BD Biosciences, San Jose, CA); GITR and CTLA-4 (R&D systems, Minneapolis, Minnesota, USA). The antibodies were conjugated to FITC, PE, PerCP or APC.

For intracellular staining, cells were activated with 40 ng/ml PMA and 1 μg/ml ionomycin (Sigma-Aldrich) and treated with 2 μM/ml GolgiStop (BD Bioscience). Upon staining for surface markers, cells were permeabilized (cytofix-cytoperm kit, BD Bioscience) and incubated with FITC anti-FoxP3 (clone PCH101, eBioscience) and PE conjugated anti-cytokine mAb: IL-2, IFN-γ, IL-10 (BD Pharmigen) or TGF-β1 (clone TB21, IQ products Groningen, The Netherlands). Necrotic cells were detected by the addition of 0.1 mg/ml propidium iodide (PI) (Sigma-Aldrich). Apoptotic cells were detected by Annexin V (APC) apoptosis detection kit (eBioscience).

Flow cytometry analysis was done using the CellQeust software on a fluorescence-activated cell sorting (FACS) — Calibur instrument (BD Bioscience).

3.3. Dendritic cell generation

Monocyte-derived DCs were generated from adherent PBMCs cultured with 1% AB plasma (Tel Hashomer Blood Bank, Tel-Aviv, Israel), 1000 U/ml granulocyte-macrophage colony stimulating factor (GM-CSF) and 500 U/ml interleukin-4 (IL-4) (R&D systems, Minneapolis, Minnesota, USA) for 5 days. Maturation was induced by addition of 1000 U/ml tumor necrosis factor α (TNF-α), 300 U/ml IL-1β, 1000 U/ml IL-6 (R&D systems), and 1 μg/ml prostaglandin E2 (PGE-2) (Sigma-Aldrich) for 48 h.

3.4. Allo-stimulation and purification of cell populations

$CD25^-$ PBMCs generated by negative selection using anti-CD25 magnetic beads (Miltenenyi Biotech) were stimulated with mature irradiated DCs (7500 cGy, Gammacell 300) in the mixed lymphocyte culture (MLC) containing 10% AB plasma for 5 days. After stimulation, $CD8^+$ T cells were purified by negative selection using the untouched $CD8^+$ T cell isolation kit (Miltenyi Biotech).

$CD8^+CD25^+$ cells were isolated by positive selection, using anti-CD25 magnetic beads. Naturally occurring $CD4^+CD25^+$ T (nTregs) cells were expanded in parallel with DCs. For functional analysis, expanded nTregs