Reduced Number and Function of $CD4^{+}CD25^{high}FoxP3^{+}$ Regulatory T Cells in Patients with Systemic Lupus Erythematosus

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Abstract. CD4$^{+}$CD25$^{+}$ regulatory T cells (Tregs) play an important role in maintaining tolerance to self-antigens controlling occurrence of autoimmune diseases. Recently, it has been shown that the transcription factor forkhead box P3 (FoxP3) is specifically expressed on CD4$^{+}$CD25$^{+}$ T cells. FoxP3 has been described as the master control gene for the development and function of Tregs. We characterized CD4$^{+}$CD25$^{+}$CTLA-4$^{+}$FoxP3$^{+}$ T cells in 43 patients with systemic lupus erythematosus (SLE). Twenty of them comprised a group of newly admitted patients with the first manifestations of the disease, and the second group included patients that were treated with cytostatics and steroids. The results revealed a significant decrease in CD4$^{+}$CD25$^{+}$ and CD4$^{+}$CD25$^{high}$ T cells numbers in patients from group I compared with control and group II patients. Coexpression of FoxP3 on CD4$^{+}$CD25$^{+}$ T cells was significantly reduced in both groups regardless the therapy. The ability of Tregs to suppress proliferation of autologous CD8$^{+}$ and CD4$^{+}$ T cells was significantly reduced in both groups of patients compared to healthy donors. Our data revealed impaired production of Tregs in SLE patients that can be partly restored by conventional treatments.

1. Introduction

Regulatory CD4$^{+}$CD25$^{+}$ T cells (Tregs) represent a small subset of CD4$^{+}$ T cells that constitutively express the IL-2 receptor-α chain, CTL-associated antigen 4 (CTLA-4), glucocorticoid-induced TNF receptor (GITR), and class II MHC markers (Beacher-Allan et al. 2001). Unique lineage of immunoregulatory CD4$^{+}$CD25$^{+}$ T cells comprises ~5–10% of CD4$^{+}$ T cell population. Tregs do not proliferate in response to T cell antigens but can inhibit activation of other T cells by the contact-dependent or cytokine-mediated mechanisms (Shevach 2001). The Treg-specific gene, forkhead box protein P3 (FoxP3), encodes a transcription factor, which is explicitly expressed in Tregs (Hori et al. 2003). FoxP3 acts as a negative regulator of cytokine production by CD4 T cells and repress transcription of IL-2 and other cytokine genes including IL-4 and IFN-γ (Schubert et al. 2001).
FoxP3 can be induced upon TCR-mediated activation (Allan et al. 2005), and the function of FoxP3 is not restricted to Tregs (Chen et al. 2005). Small numbers of human CD4^+ and CD8^+ T cells transiently upregulated FoxP3 upon in vitro stimulation (Gavin et al. 2006). However, at present, high levels of expression of CD25 and FoxP3 are the most valued markers of CD4^+CD25^{high} Tregs.

In human, autoimmune diseases, including diabetes (Lindley et al. 2005), multiple sclerosis (Viglietta et al. 2004), rheumatoid arthritis (van Amelsfort et al. 2004), psoriasis (Sugiyama et al. 2005), and type II autoimmune polyendocrinopathy (Kriegel et al. 2004), are characterized by low numbers and/or defective function of CD4^+CD25^{high} T cells. However, reports on Tregs in patients with systemic lupus erythematosus (SLE) are controversial. Recent data indicate diminished numbers of CD4^+CD25^{+} T cells that mainly associated with the disease’s flares but not in remission (Crispin et al. 2003; Liu et al. 2004; Lee et al. 2006; Miyara et al. 2005). However, Alvarado-Sanchez et al. (2006) did not show any significant differences in the levels of regulatory T cells in SLE patients. The explanation of these differences might be in the variety in treatments and stages of the disease.

We aimed to estimate the total number and function of CD4^+CD25^{+}FoxP3^{+}CTLA-4^+ T cells and CD4^+CD25^{high}FoxP3^{+} cells in a group of newly admitted patients with early SLE manifestations prior to any therapy. Healthy volunteers and SLE patients after therapy served as controls.

2. Experimental Design

Forty-three patients with the diagnosis of active SLE according to the American College of Rheumatology criteria were enrolled in the study. The group included 35 women and 8 men, 14–49 (median 29.4) years old. Twenty newly admitted patients and 23 patients, who were recently treated with 3 g methylprednisolone/1 g cyclophosphamide pulse therapy, were studied prior to receiving corticosteroids. Seventeen matched healthy donors were included as controls.

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll gradient centrifugation. Antibodies used for flow cytometry were anti-CD3-FITC, anti-CD25-FITC, anti-CD4-PE, anti-CD8-PE, anti-CD19-PC5 (Beckman Coulter), anti-CD19-PC5, anti-CD152-PC5, anti-CD25-FITC (BD Pharmingen), and appropriate isotype controls. For the detection of intracellular markers, PBMCs were stained with surface membrane antibodies (anti-CD25-FITC and anti-CD4-PE), fixed, permeabilized with 0.1% saponin, and stained with anti-Foxp3-PC5 or isotype control antibody (eBioscience). Flow cytometry analysis was performed on FACSCalibur (Becton Dickinson) using CellQuest Pro software. The results are presented as the percentage of positive cells.

For CD4^+CD25^{+} cell isolation, PBMCs were labeled with Biotin-Antibody Cocktail and anti-Biotin MicroBeads (Miltenyi Biotec) followed by a negative selection of CD4^{+} T cells. Cells from the negative fraction were labeled with CD25 MicroBeads and positive CD4^{+}CD25^{+} cells were harvested. To increase the purity of cell populations, the positive fraction was isolated on magnetic