ALTERATION OF CYTOKINE PRODUCTION AND FOXP3 - GR COLOCALIZATION IN DEXAMETHASONE TREATED REGULATORY T CELLS

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Introduction: Regulatory T cells (Tregs) play an indispensable role in maintaining immunological unresponsiveness to self-antigens and in suppressing excessive immune responses deleterious to the host. Tregs are produced in the thymus as a functionally mature subpopulation of T cells and can also be induced from naive T cells in the periphery. The fact that Tregs play a critical role in the immune system is evidenced by the severe autoimmune syndrome that results from a genetic deficiency in regulatory T cells (IPEX syndrome). Tregs are CD4+CD25high+, they express FoxP3 transcription factor (TF) while IL-2 and TGFβ cytokines are essential for their maturation. The goal of our work was to examine the typical cytokines of Tregs in the thymus and peripheral lymphoid organs and to determine the changes of the cytokine levels during glucocorticoid hormone (GC) treatment. We also investigated the changes of glucocorticoid receptor (GR) and FoxP3 localization in Tregs after GC analogue treatment.
Materials and methods: 4-6 week old Balb/c mice were treated for 2-4 days in vivo with high dose (20mg/kg) GC analogue Dexamethasone (DX, SIGMA). Cells from the thymus and spleen were isolated and stimulated with PMA/ionomycin O/N at 37°C. Cells were stained with cell surface (anti-CD4; anti-TGFβ) and intracellular (anti-FoxP3; anti-IL-10) antibodies. We determined the ratio of CD4+ T cells and Tregs and the changes in their cytokine production by flow cytometry. To investigate the GR and FoxP3 localization in DX treated and control thymic Tregs we first sorted CD4+/CD25high+ cells by FACS Aria and then stained them for intracellular GR and FoxP3.
Results: In the thymus in vivo GC treatment caused the elevation of Treg ratio from 0,5% to 8%, without changes of the absolute Treg cell number, which reflects the GC resistance of the these cells. In the spleen a slight decrease in Treg cell ratio (from 12% to 8%) was observed with a significantly diminished absolute cell number. We also measured the functional changes of the Treg cells after DX treatment. Both in the thymus and spleen of DX treated animals an elevation of TGFβ+ and IL-10+ Treg cell ratio was measured which further increased upon PMA/Ionomycin stimulation. Investigating the GR and FoxP3 localization by confocal microscopy we found 33% colocalization already in the untreated samples, which significantly increased after in vitro 30 min. of DX treatment. There was no significant difference in the FoxP3-GR colocalization between the control and the in vivo DX treated thymic Treg cells.
Discussion, conclusion: In thymic Tregs we observed GC resistance and low cytokine production, while in peripheral Tregs a higher rate of cytokine producing cells was detected, which were sensible to GC treatment. These suggest different signaling pathways of the two Treg cell populations. The functional consequence of FoxP3 and GR colocalization in thymic Tregs must be further investigated.

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