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Regulatory and effector T-cells are differentially modulated by Dexamethasone ☆

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Abstract It is assumed that the ratio between effector T cells (Teff) and regulatory T cells (Tregs) controls the immune reactivity within the T-cell compartment. The purpose of this study was to investigate if Dexamethasone (Dex) affects Teff and Tregs subsets. Dex induced on Tregs a dose and time-dependent apoptosis which resulted in a relative increase of Teff. After TCR activation, Dex induced a strong proliferative inhibition of Teff, but a weaker proliferative inhibition on Tregs. These effects were modulated by IL-2, which not only restored the proliferative response, but also prevented Dex-induced apoptosis. The highest dose of IL-2 prevented apoptosis on all FOXP3 + CD4+ T cells. Meanwhile, the lowest dose only rescued activated Tregs (aTregs), probably related to their CD25 higher expression. Because Dex did not affect the suppressor capacity of aTregs either, our results support the notion that under Dex treatment, the regulatory T-cell compartment maintains its homeostasis.
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1. Introduction

Regulatory T cells (Tregs) have been characterized as CD4+ T cells expressing CD25, FOXP3 and very low amounts of CD127. This low expression of CD127 allows differentiation of Tregs from naive and memory conventional T cells.

As recently reported, FOXP3 + CD4+ T cells include three phenotypic and functionally distinct cellular subpopulations. Two of them having *in vitro* suppressive activity, were

Abbreviations: Tregs, regulatory T cells; rTregs, resting Tregs; aTregs, activated Tregs; Teff, effector T cells; GC, glucocorticoids; Dex, Dexamethasone; GR, glucocorticoid receptor; APCs, antigen presenting cells; geoMFI, geometric mean fluorescence intensity.

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characterized as FOXP3^{low}CD45RA + resting Treg cells (rTregs) and FOXP3^{high}CD45RA- activated Tregs (aTregs). A third subset of FOXP3^{low}CD45RA- cells was found to be a cytokine-secreting cell population without suppressor activity, and was identified as FOXP3+ non-Tregs [1,2].

Under physiological conditions, the magnitude of the immune reactivity within the T-cell compartment is largely proportional to the ratio between effector T cells (Teff) and Tregs. In this context, an impaired function and/or homeostasis of Tregs have implications in the development of several common autoimmune or inflammatory diseases.

However, Tregs not always operate under physiological conditions, and the immune state might be altered by drugs frequently used in the medical practice, some of them having cytotoxic activity.

Glucocorticoids (GC) are potent anti-inflammatory and immunosuppressive agents used in the treatment of numerous autoimmune and inflammatory diseases. GC mediate their biological effects through the binding to an intracellular receptor that translocates to the nucleus and targets specific DNA sequences resulting in the blockage of several inflammatory pathways [3,4] and the induction of apoptosis [5]. These events lead to the inhibition of T cells [6,7] and/or altering the function of dendritic cells [8,9].

The sensitivity of Tregs to steroid drugs such as Prednisone and Dexamethasone (Dex) has been the subject of conflicting data. In the murine system, some studies reported that Dex increased the proportion of Tregs, both in peripheral blood and secondary lymphoid organs [10,11]. However, additional studies in mice models of asthma [12] or multiple sclerosis [13] indicated that GC induced a decrease in the number of Tregs. In humans, a first report [14] claimed that the treatment of patients with bronchial asthma with GC (either systemic or inhaled) induced the increase of circulating Tregs. Later on, several small *in vivo* studies also pointed out towards there being a positive correlation between the administration of GC and the frequency of Tregs in patients with different autoimmune diseases [15–17]. By contrast, two recently larger studies performed in patients with bronchial asthma or autoimmune connective tissue diseases, arrived at the opposite conclusion [18,19]. These contradictory results may have at least two possible explanations. Firstly, there is a large heterogeneity in the characterization of Tregs. Several studies only defined Tregs as being CD4+ CD25^{high}, but it is well known that many of these cells represent activated T cells instead of Tregs [20–22]. Secondly, most of these studies were performed in patients with an autoimmune background and it is likely that these individuals already had an impairment of Tregs frequency and/or function.

Tregs differ in many aspects from cytotoxic Teff. The sensitivity of Tregs to apoptosis is of outmost importance to sustain the equilibrium between effector and suppressor forces. Tregs have a variable sensitivity to apoptosis which is influenced by factors such as the cytokines secreted within the inflammatory environment, the type of antigenic stimulation and the proliferation rates [23].

Our study provides evidence that during the course of an immune response, GC exert differential effects on both, effector and regulatory T cells by inducing a strong inhibition of the proliferation of Teff and a differential apoptosis of Tregs. Finally GC effects can be modulated by IL-2, which even in very low amounts could differentially impact on Tregs survival and function.

2. Material and methods

2.1. Subjects

Buffy coat was obtained from 1 unit of blood collected from 15 male and 15 non-pregnant healthy female donors (average 34 years, range 27–42 years), and processed immediately after volunteer's donations. Infectious and endocrine disorders were cleared in all patients, who were not undergoing any kind of treatment. This study has been approved by the Investigation and Ethics Committee at the Hospital de Clínicas "José de San Martín" and informed consent was obtained from all donors.

2.2. Peripheral blood mononuclear cell (PBMCs) isolation

PBMCs were obtained from buffy coats through a Ficoll–Hypaque (GE Biosciences) density gradient centrifugation.

2.3. Cell sorting

CD4+ T cells were purified by negative selection by using CD4+ T cell MACS beads (Miltenyi Biotec), following manufacturer's instructions. Different subsets of FOXP3 + CD4+ T cells and Teff were isolated by staining purified CD4+ T cells with anti-CD4 PerCP, anti-CD25 PE and anti-CD45RA FITC antibodies (all from BD Biosciences) and sorting with a FACSAria II Flow cytometer (Becton Dickinson), yielding four populations: CD25^{low}CD45RA + (rTregs), CD25^{high}CD45RA− (aTregs), CD25^{low}CD45RA− (FOXP3+ non-Tregs), CD25− (Teff). Cells were collected into RPMI 1640 medium (Hyclone) plus 50% heat-inactivated fetal calf serum and washed once for further studies. The expression of FOXP3 in sorted cells determined by Flow cytometry was detected in >90% of aTregs, >80% of rTregs and FOXP3+ non-Tregs, and in less than 0.5% of Teff.

2.4. Cell lines

HeLa human cervix adenocarcinoma cells were maintained in complete culture medium following the recommendations from the American Type Culture Collection.

2.5. Flow cytometry

Freshly isolated or *in vitro*-cultured cells were stained with anti-CD4 (PerCP or APC), anti-CD25 (PE or APC-Cy7), anti-CD45RA (PE-Cy7, APC or FITC), and anti-CD127 (PE), all from BD Biosciences. Intracellular detection of FOXP3 with anti-FOXP3 (PE or Alexa Fluor 488), Ki-67 antigen with anti-Ki-67 (FITC) and BCL-2 with anti-BCL-2 (FITC) antibodies was performed on fixed and permeabilized cells following the manufacturer's instructions. Negative control samples were incubated with an isotype-matched mAb. Data was acquired using a FACSAria II (Becton Dickinson) and was analyzed with FlowJo software. Statistical analyses are based on at least 100,000 events gated on the population of interest.

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