Antagonistic and synergistic effects of glucocorticoids and IL-7 on CD4+ T cell activation

Igor Cima¹, Andrea Fuhrer², Thomas Brunner

Division of Immunopathology, Institute of Pathology, University of Bern, Martenstrasse 31, PO Box 62, 3010 Bern, Switzerland

Abstract

Glucocorticoids (GCs) are steroidal compounds widely used to treat chronic and acute inflammatory diseases. In particular, GCs at pharmacological doses induce apoptosis of activated and naïve T cells, inhibit their proliferation and block pro-inflammatory cytokine secretion. At physiological concentrations, the effect of these steroids on T cell immunity are not yet fully understood, and various studies reported paradoxical roles exerted by GCs on T cell immunity. Here, we show that GCs surprisingly induce proliferation of activated CD4+ T cells in the presence of IL-7, a cytokine secreted in the thymus and at mucosal sites. Increased proliferation is dependent on a GC-mediated survival of mitotic cells. Moreover, we observe a downmodulation of Th1 cytokine secretion in cells treated with GCs, an outcome which is not affected by the presence of IL-7. GCs exert thus a positive role in the presence of IL-7 by enhancing proliferation of CD4+ T cells and simultaneously a negative role by suppressing pro-inflammatory cytokine production.

Keywords: Apoptosis; Proliferation; Glucocorticoids; IL-7; T cells

1. Introduction

Proliferation, cell death and differentiation of lymphocytes are dynamic processes strongly influenced by various environmental signals. For example, GCs, particularly at pharmacological doses, are known for their anti-inflammatory properties, either by suppressing proliferation and the production of pro-inflammatory cytokines or by directly inducing apoptosis, in immature and mature T cells [1]. Thus, GCs are important therapeutic immunosuppressants commonly used in the treatment of acute and chronic inflammatory disorders. In contrast, cytokines binding to the common γ-chain receptor, such as IL-2, -7, -9, and -21, are typically mitogenic and induce survival signals [2]. Moreover, IL-7, opposite to GCs, was shown to induce the production of Th1 cytokines in activated T cells [3].

Cytokine and GC signaling are known to interact in resting and activated T cells, and the final outcome of these complex signaling networks defines differentiation, proliferation and cell death [4]. For instance, GCs were shown to upregulate the IL-7 receptor α-chain on human CD4+ T cells [5]. More recently, GCs were found to induce the expression of the pro-apoptotic Bcl-2 homolog Bim, providing a novel mechanism of GC-induced apoptosis [6]. Bim, on the other end was also found to be a central inducer of apoptosis in mature T cells upon IL-7 deprivation [7], thereby supporting the notion that IL-7 and GCs have clear opposing effects on T cell apoptosis. Interestingly, both IL-7 and GCs are synthesized and released by intestinal epithelial cells [8,9] and may regulate the fate of local T cells through prevention and induction of apoptosis, respectively.

In this study we have thus analyzed the putative opposing effects of IL-7 and GCs on T cell proliferation and apoptosis.
As expected, we found that IL-7 alone has a survival effect on T cells, whereas GCs alone have a suppressive effect on T cell proliferation. In marked contrast, we observed that combined treatment of T cells with IL-7 and GCs resulted in increased survival of mitotic T cells. At the same time, Th1 cytokine secretion was blunted in GC-treated cells, irrespective of the presence of IL-7.

2. Materials and methods

Pooled lymph node CD4+ T cells were purified from C57Bl/6 mice by antibody-coated magnetic beads (Miltenyi Biotech). The resulting T cell fraction was always >92% CD4+ and 99% CD8−. CD4+ T cells were stained with CFSE (carboxy-fluorescein diacetate succinimidyl ester) as described [10].

Fig. 1. GCs and IL-7 synergistically enhance proliferation and survival of CD4+ T cells. (A) GCs enhance IL-7 mediated proliferation of living CD4+ T cells. CD4+ T cells were incubated in the presence or absence of IL-7 and Cort for 4 days and Annexin V-negative cells were analyzed for their proliferation profile. A synergistic effect of IL-7 together with Cort was seen on the proliferation of living cells (n = 6 experiments, P = parent cell population). (B) Division index and % cells divided were quantified using the proliferation platform of the FlowJo software. Mean values of triplicates ± S.D. are shown. (C) Enhanced proliferation as the result of enhanced survival. Total proliferation rates were similar in IL-7-treated cells compared with IL-7 and Cort together (first panel, CSFE), whereas survival of divided cells was synergistically enhanced by adding Cort to IL-7-treated cells (second panel, Annexin V). A representative experiment out of three is shown. (D and E) The percentage of total divided cells (D) and Annexin V+ dead cells from the dividing population (E) was calculated as shown in (C). Mean values of triplicates ± S.D. of a typical experiment are shown. (F) GCs inhibit IL-7-induced cytokine secretion. Cells were stimulated for 3 days as indicated and cytokines in the supernatant were measured simultaneously using the Bio-Plex™ suspension array system. Mean values of triplicates ± S.D. are shown.
and activated with plate-bound anti-CD3 antibodies (αCD3, 10 μg/ml) in the presence or absence of murine IL-7 (5 ng/ml, Peprotech) and corticosterone (Cort, 500 nM, Acros Organics) for 3–4 days. Cell cultures were carried out in complete IMDM medium supplemented with 2% murine serum. Apoptosis was detected by Annexin V staining and proliferation by dilution of the CSFE labeling intensity. Flow cytometry data were analyzed using the FlowJo software (La Jolla, CA). In some experiments, proliferation data were compared using the division index. Division index refers to the average number of divisions that a cell has undergone, whereas the resting undivided population is not analyzed. The use of the division index parameter thus avoids a potential bias in the interpretation of the results due to variations in the number of cells in the resting population.

Cytokines in the supernatant were measured simultaneously using the Bio-Plex™ suspension array system (Biorad).

Data were analyzed in triplicates and differences were tested using the unpaired, two-tailed Student’s T-test with unequal variances.

3. Results and discussion

We were interested in analyzing the apparent antithetic effects of IL-7 and GCs on proliferation, survival and differentiation of activated CD4+ T cells. In an optimized in vitro model, purified murine CD4+ T cells were activated with plate-bound anti-CD3 in the presence of IL-7 and/or corticosterone, the major GC in mice. Proliferation and survival were monitored by flow cytometry, differentiation by the analysis of cytokines released into the supernatant. To our surprise, T cell receptor-activated CD4+ T cells proliferated better in the presence of both, corticosterone and IL-7, when compared with IL-7 or corticosterone alone, indicating that corticosterone- and IL-7-induced signals have a synergistic rather than antagonistic effect on T cell proliferation (Fig. 1A and B). In agreement with a previous report by Wiegens et al. this result also underlines that GCs, depending on the environmental conditions, can exert a positive role on T cell proliferation, which is completely antithetic to their classical anti-proliferative effect [11]. Interestingly, the synergistic effect of IL-7 and GCs on T cell proliferation was particularly evident at serum concentrations between 2% and 5%, and not observed anymore at higher concentrations (data not shown), indicating that additional serum factors may affect the qualitative outcome of T cell activation and proliferation. Moreover, this synergistic effect of GCs and IL-7 was not dependent on co-stimulatory signals as it was observed in the presence or absence of CD28 co-stimulation (data not shown).

T cell expansion is always the result of both, increased cell cycle progression and survival. We thus wanted to discriminate these two distinct events in the synergistic action of GCs and IL-7 on T cell proliferation. Cell death and proliferation were simultaneously assessed by flow cytometry, using Annexin V for the detection of phosphatidylserine exposure and CFSE detecting cell division. Interestingly, we observed that the increased corticosterone- and IL-7-mediated expansion of CD4+ T cells was mediated by increased survival rather than increased cell division (Fig. 1C–E). The combined treatment with GCs and IL-7 led to a better survival of dividing CD4+ T cells, whereas the total rate of cells, which had undergone cell division (dead and alive) did not change between the different conditions (i.e. IL-7 only, or IL-7 plus corticosterone) (Fig. 1D and E) indicating that cell cycle progression is not affected by the treatment of CD4+ T cells with IL-7 and/or corticosterone.

GCs are also known to inhibit the synthesis of Th1 cytokines, such as IFN-γ, IL-2 and GM-CSF, whereas IL-7 stimulates CD4+ T cells to produce these cytokines [3,12,13]. We were therefore interested whether GCs and IL-7 would have synergistic effects also in the regulation of Th1 cytokine synthesis. As expected, IL-7 alone led to an increase in production of IFN-γ, IL-2 and GM-CSF. Similarly, corticosterone alone inhibited the activation-induced synthesis of these cytokines. However, unlike their effect on T cell survival, no synergistic effect was observed between corticosterone and IL-7, and all cytokines were strongly down-regulated in the presence of both factors (Fig. 1F). Interestingly, the activation-induced expression of IL-4 was not strongly affected by GCs indicating that these steroids do not affect the expression of Th2 cytokines (data not shown). These results indicate that the synergistic effect of GCs on T cell survival in the presence of IL-7 may be distinct from the potent inhibitory activity of GCs on Th1 cytokine production.

In summary, we analyzed the interactions of GCs and IL-7 signaling on activated T cells. Provided separately, these signals are known to exert opposite effects on the proliferation, survival and differentiation of activated CD4+ T cells. The integration of the two signals, however, led to an unexpected synergistic effect on CD4+ T cells proliferation, due to an increased survival of mitotic cells. While the molecular mechanisms of these synergistic action of IL-7 and GCs remains to be elucidated, our findings describe a novel, surprising pro-survival activity of GCs on T cells with potential implication in the fine-tuning of immune homeostasis.

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References