Glucocorticoids Activate a Suicide Program in Mature T Lymphocytes:

Protective Action of Interleukin-2

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INTRODUCTION

It is an established phenomenon that glucocorticoid hormones have an inhibitory action on the induction of T cell proliferation by antigens or mitogens.[1,2] These steroids also reduce the production of certain lymphokines like IL-2 and γ-Ifn[3,4] by susceptible cells. Furthermore, it is also well known that glucocorticoids have a lytic effect on immature cells of the immune system such as cortical thymocytes[5] and a T cell line.[6]

Lysis of glucocorticoid-sensitive cells is preceded by a set of morphological changes such as fragmentation of DNA and condensation of chromatin, blebbing of the cell surface and transient increase in buoyant density.[7] This cell death program is also involved in the elimination of self-recognizing lymphocytes in the thymus,[8] in the lysis of cells after treatment with anti-APO monoclonal antibody[9] or exposure to γ-irradiation.[10] Finally, growth factor deprivation will also lead to development of apoptosis in certain hemopoietic cells.[11-13]

In our laboratory we are studying the effects of glucocorticoid hormones on the growth of mature T lymphocytes in culture. In the present study we report that glucocorticoids induce apoptosis in concanavalin A-induced IL-2-dependent blasts of mouse spleen cells, through the activation of an endonuclease that cleaves chromatin in the linker region between nucleosomes. The death program does not take place when a saturating concentration of IL-2 is present in the culture medium.

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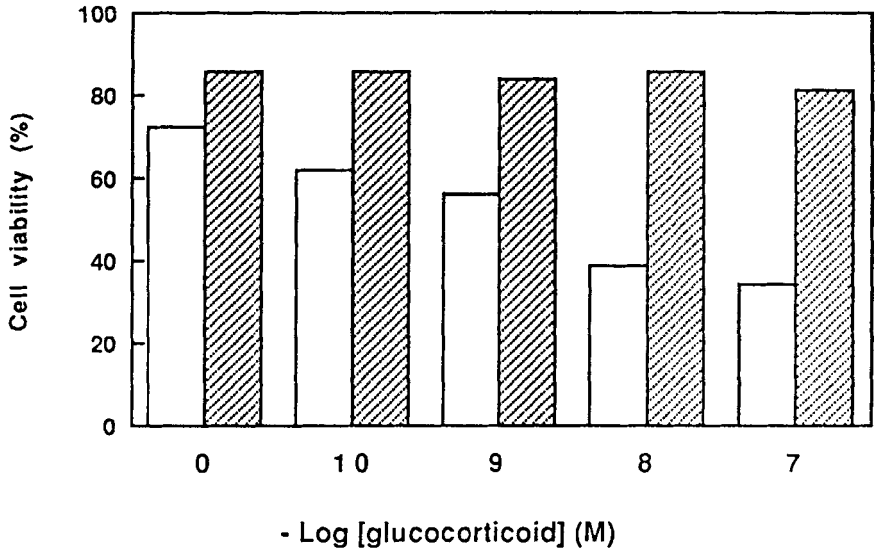

FIGURE 1. Con A blasts were treated for 24 h with various concentrations of fluocinolone acetonide in the absence (□) or in the presence (▨) of IL-2-containing conditioned medium (50 U/ml). Viability was determined by the trypan blue exclusion method.

MATERIALS AND METHODS

Materials

Steroid hormones and cycloheximide were purchased from Sigma (St. Louis, MO). Fetal bovine serum (FBS) was from Gibco Europe (Scotland). Concanavalin A was from Polysciences (Warrington, PA). All other chemicals were of the purest grade commercially available.

Cell Culture

IL-2-dependent blasts were obtained after stimulation of mouse spleen cells with concanavalin A (Con A) (3 μg/ml) for 3 days and were maintained in RPMI 1640 medium with 5% FBS, 2 mM L-glutamine, 50 μM 2-ME and IL-2-containing supernatant of Con A-induced mouse spleen cells.

Cell Viability

Viable cell numbers were determined by the trypan blue dye exclusion method.

Analysis of DNA Fragmentation in Agarose Gels

The DNA was obtained by phenol extraction followed by treatment with RNAse and proteinase K and analyzed by horizontal electrophoresis as previously described.[6]

RESULTS AND DISCUSSION

When IL-2-dependent blasts are incubated with the glucocorticoid analog fluocinolone acetonide (FA) in the absence of IL-2, a decrease in cell viability could be observed (FIG. 1). Sensitivity of Con A blasts to lysis by glucocorticoid hormone treatment is dependent on the dose of FA used and some lytic effect is already observed with 10^{-10} M of the steroid. At the highest concentration of FA (10^{-7} M) the number of viable cells in the culture has decreased to 45% of that in control cultures. Similar results have been obtained when Con A blasts are treated with dexamethasone although cell lysis is observed at concentrations ten times higher than those required with FA (results not shown). In contrast to these observations, when the equipment is carried out in the presence of a high concentration of IL-2 (>10 U/ml), viability of glucocorticoid-treated cultures is similar to that in control cultures (FIG. 1) suggesting that IL-2 interferes in the lytic pathway induced by glucocorticoids.

In order to get further insight into the mechanism involved in the lysis of IL-2-dependent T cell blasts by glucocorticoids we have analyzed the DNA of treated cells, as it is known that in immature T cells and in a T cell line these steroids induce a cell death program characterized by an early degradation of chromatin into oligonucleosome-length fragments.[5,6] FIGURE 2 shows that in both fluocinolone acetonide and dexamethasone-treated cultures the DNA is degraded with the typical "ladder" pattern of DNA fragments representative of apoptosis.[7] In contrast, the DNA of cells treated with either progesterone or testosterone, two

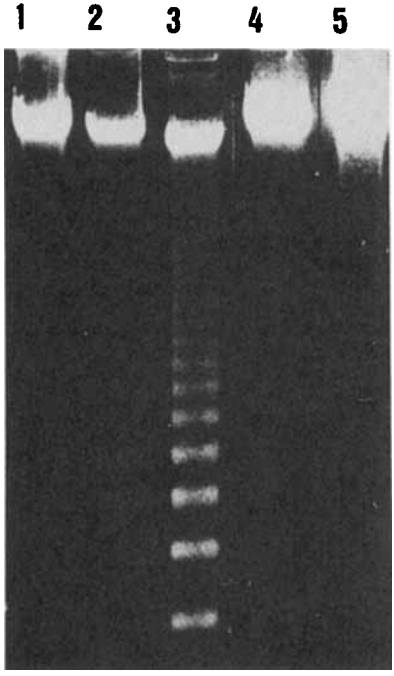

FIGURE 2. Cells were incubated for 8 h in the absence of IL-2 with: medium (1), dexamethasone 10^{-6} M (2), fluocinolone acetonide 10^{-6} M (3), progesterone 10^{-5} M (4) or testosterone 10^{-5} M (5). After the incubation DNA was extracted and analyzed as described under Materials and Methods.

steroid hormones that are inactive in eliciting glucocorticoid receptor-mediated actions,[14-17] is maintained in a high-molecular weight form. These findings suggest that the action of glucocorticoids on DNA fragmentation and cell viability in mature T lymphocytes is very likely mediated through the interaction with a specific glucocorticoid receptor inasmuch as the structure-activity relationship of these effects is similar to other receptor-mediated actions of these hormones.

It has been suggested that activation of apoptosis in thymocytes by glucocorticoids is a process requiring protein synthesis.[18] We were interested to know whether the DNA fragmentation induced by glucocorticoids in IL-2-dependent Con A blasts was also protein synthesis-dependent. Results in FIGURE 3 indicate that at least during 8 hours of treatment, inhibiting protein synthesis prevented dex-induced DNA cleavage. However, we have found that a prolonged incubation with cycloheximide (24 h) results in loss of cell viability indistinguishable from that caused by steroid treatment.

Very importantly, when cultures are treated with glucocorticoids in the presence of a saturating concentration of IL-2, activation of the endonuclease does not take place (FIG. 4), further indicating that a relationship must exist between degradation of chromatin (FIG. 4) and loss of viability (FIG. 1). These results are in agreement with the previously reported lack of effect of glucocorticoids on the viability and proliferation of CTLL-2 cells in the presence of IL-2.[6,17]

FIGURE 3. Con A blasts were incubated for 8 h in the absence of IL-2 with the following additions: medium (1), dexamethasone 10^{-6} M (2), cycloheximide 10 μg/ml (3) or dexamethasone 10^{-6} M and cycloheximide 10 g/ml (4). At this time DNA was extracted and fragmentation determined by horizontal electrophoresis as described under Materials and Methods.

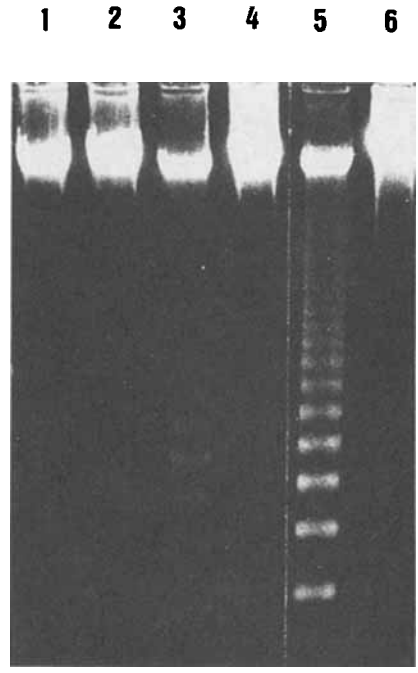

FIGURE 4. IL-2-dependent Con A blasts were incubated for 8 h with the following additions: (1) medium, (2) 50 U/ml IL-2, (3) 10^{-6} M dex, (4) 10^{-6} M dex and 50 U/ml IL-2, (5) 10^{-6} M FA or (6) 10^{-6} M FA and 50 U/ml IL-2. DNA fragmentation was analyzed as described in Materials and Methods.

All these results may indicate that the T cell response to a given antigen could be controlled not only by the availability of IL-2 but also through the action of hormones on endocrine origin that will switch on a programmed suicide mechanism and in that way terminate the immune response.

SUMMARY

Viability of T cell blasts obtained by concanavalin A stimulation of mouse spleen cells markedly decreases when these cells are exposed to glucocorticoid hormones in the absence of interleukin-2. The mechanism underlying the lysis of the mature lymphocytes seems to correspond to the apoptotic type of cell death inasmuch as an early degradation of DNA into oligonucleosome-length fragments is observed. Moreover, glucocorticoid-induced DNA fragmentation is delayed in the presence of an inhibitor of protein synthesis. Induction of the cell death program by glucocorticoids is most likely mediated through the interaction with a specific glucocorticoid receptor as suggested by the structure-activity relationship of the various steroids tested. Interestingly, the presence of a saturating dose of IL-2 during the treatment of concanavalin A blasts with glucocorticoids totally abolished DNA fragmentation and cell lysis.

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