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Effect of glucocorticoid deficiency after adrenalectomy on antitumor immunity

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Summary. We studied the effect of corticosterone after adrenalectomy on antitumor immunity in immunogenic tumors in mice. Antitumor immunity in the glucocorticoid deficient adrenalectomized mice (ADX mice) examined via comitant immunity and cytotoxic activity of spleen cells was compromised. Antitumor immunity was detected in ADX mice receiving sufficient supplementary doses of corticosterone. Loaded stress compromised the cytotoxic activity of the spleen cells in the ADX mice receiving adequate corticosterone, and the failure also contributed to the glucocorticoid deficiency because the activity was not affected by stress in the sham ADX mice. A matured effector cell activity was transferred to the glucocorticoid deficient ADX mice. We conclude that glucocorticoid deficiency compromises the antitumor immune response and that glucocorticoid might play an important role in the maturation of immunocompetent cells.

Introduction

Glucocorticoid administered exogenously suppresses the immune response by lympholysis or redistribution of effector cells from the circulation to other body compartments [5]. Besedovsky et al. [3] reported that serum corticosterone levels in rats were elevated during the immune response, but the role of the elevated corticosterone level in the immune response was not defined. Ablation of glucocorticoid by adrenalectomy enhanced graft rejection [4, 6] and suppressed tumor growth [2, 10]. Various stresses also suppress the immune response and the suppression contributes to elevated levels of serum glucocorticoid [12]. However, adrenalectomy did not prevent stress-induced immunosuppression [11] and a low concentration of glucocorticoid enhanced the mitogen response of lymphocytes, in vitro [13, 15].

We observed that adrenalectomy compromised the comitant immunity, antitumor immunity developed after tumor extirpation, and there was cytotoxic activity of the spleen cells against Meth A sarcoma in mice [9]. In the present study, we examined whether the failure of the antitumor immunity following adrenalectomy was a result of glucocorticoid deficiency and whether it could be restored by the administration of corticosterone.

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Materials and methods

Mice. Female BALB/c inbred mice 8 to 12 weeks old were purchased from The Shizuoka Agricultural Cooperative Association for Laboratory Animals (Shizuoka, Japan). These mice were housed in a specific pathogen-free room in the Laboratory of Animal Experiments, Kyushu University, with a room temperature of 22° C and light from 8:00 a.m. to 8:30 p.m.

Tumor. A methylcholanthrene-induced fibrosarcoma, Meth A sarcoma, of BALB/c origin was maintained in ascitic form by serial passages of 1×10^7 cells at weekly intervals.

Corticosteroid. Corticosterone (^{4}-pregnene-11 β, 21-diol-3,20-dione; Reichstein's substance H; Kendall's compound B, Sigma Chemical Co., St Louis, Mo.) was suspended at the required concentrations in sesame oil and 0.1 ml of the suspension was injected s.c.

Tumor transplantation and measurement of tumor size. Tumor cells were washed three times with Hanks' balanced salt solution (HBSS) and viable cells were adjusted to the desired concentration by trypan blue dye exclusion staining. Tumor cell suspension (0.1 ml) in HBSS was transplanted s.c. into the right flank of the mice. Tumor size was measured with a slide caliper and expressed in terms of area cubic millimeters, calculated by multiplying the longest and shortest diameters.

Spleen cells. The spleen was removed after femoral arterial exsanguination, crushed between two glass slides, and passed through two layers of gauze to remove the large fragments. After three washings of the filtrate with HBSS, viable cells were assessed by trypan blue dye exclusion staining.

Adrenalectomy. Adrenalectomy included removal of the surrounding fatty tissue and renal capsules for complete removal of accessory adrenocortical tissues. In addition, bilateral oophorectomy was performed. Sham adrenalectomized (ADX) mice underwent a 15 min exposure of the bilateral adrenal glands. The ADX mice were fed 0.85% saline ad libitum and corticosterone was given every evening. The minimum replacement dose, 0.3 mg was determined in preliminary experiments in which all ADX mice survived for over 14 days.

Irradiation. Radiation was delivered using a Shimadzu 250 kVp machine, operated at 200 kVp and 20 mA with a 0.3 mm copper and 1 mm aluminium filter. The mice were exposed to 400 rads whole body irradiation at a rate of 14.3 rads/min 24 h before use.

Stress. Restraint stress was applied, using a wire net, at room temperature for 2 h.

Comitant immunity. The antitumor immunity developed after tumor extirpation, was examined via the growth of retransplanted tumor: 5×10^6 viable tumor cells were tranplanted s.c. dorsally and removed on the 4th day. Then 10 days after the tumor resection, 1×10^6 viable tumor cells were retransplanted into the right flank of mice and the resulting tumor size was measured.

In vivo neutralization test. The cytotoxic activity of whole spleen cells was examined, using an in vivo neutralization test. Spleen cells were suspended at a concentration of 4×10^7 cells/ml and the tumor cells, 2×10^6 cells/ml, in HBSS. These cell suspensions were mixed in equal volumes (E/T = 20:1). This suspension (0.1 ml) was implanted s.c. into the right flank of mice irradiated 24 h before. The resulting tumor size was measured.

Statistics. Data were analyzed using Student's *t*-test. The differences were considered to be significant if the probability that the observed difference occurred by chance alone was less than 5%.

Results

Effect of corticosterone on comitant immunity

Adrenalectomy or sham adrenalectomy was performed at the same time as tumor resection. The ADX mice continued to receive 0.3 mg, 1 mg, or 2 mg of corticosterone every evening.

Growth of the retransplanted tumor in the ADX mice which continued to receive 0.3 mg was not suppressed but those of the mice continuing to receive 1 mg or 2 mg of corticosterone were suppressed as was tumor growth in the sham ADX mice (Fig. 1).

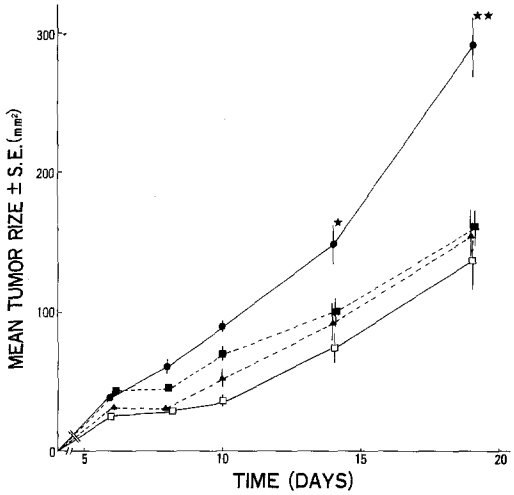

Fig. 1. Effect of corticosterone on comitant immunity. Tumor cells (5×10^6) were transplanted and adrenalectomy was performed at the same time as tumor resection 4 days after the transplantation. Then 1×10^6 tumor cells were retransplanted 10 days after the tumor resection to the adrenalectomized mice continuing to receive 0.3 mg (●——●), 1 mg (▲——▲), or 2 mg (■——■) of corticosterone daily and the sham adrenalectomized mice (□——□). * : Statistically significant ($P < 0.05$) compared to 1 mg and 2 mg. ** : Statistically significant ($P < 0.01$) compared to 1 mg and 2 mg. Vertical Bars: SE

Effect of corticosterone on cytotoxic activity of spleen cells

Adrenalectomy or sham adrenalectomy was performed with tumor resection on the 4th day after transplantation of 5×10^6 tumor cells. The ADX mice continued to receive 0.3 mg or 1 mg of corticosterone. At 14 days after tumor transplantation, spleen cells were obtained, and the cytotoxic activity examined.

Spleen cells from the ADX mice continuing to receive 1 mg of corticosterone suppressed the tumor growth compared to those of the ADX mice continuing to receive 0.3 mg (Fig. 2).

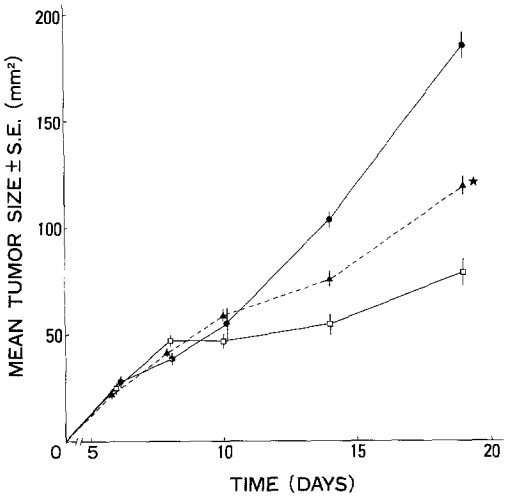

Fig. 2. Effect of corticosterone on cytotoxic activity of spleen cells. A mixture of 2×10^7 spleen cells with 1×10^6 tumor cells was transplanted into irradiated mice. The spleen cells were obtained 10 days after tumor resection of the adrenalectomized mice continuing to receive 0.3 mg (●——●) or 1 mg (▲——▲) of corticosterone daily and the sham adrenalectomized mice (□——□). * : Statistically significant ($P < 0.01$) compared to 0.3 mg. Vertical Bars: SE

Effect of loaded stress on ADX mice

The effect of loaded stress on the ADX mice was examined in terms of cytotoxic activity of the spleen cells. Adrenalectomy or sham adrenalectomy was performed with tumor resection. The ADX mice continued to receive 1 mg of corticosterone, which was sufficient for expression of the antitumor resistance. Restraint stress was applied for 2 h from the 4th day to the 9th day after tumor resection. The spleen cells were obtained the following day and cytotoxic activity examined.

The stress resulted in impairment of the cytotoxic activity of the spleen cells of the ADX mice, whereas in the sham ADX mice, the stress had no effect on the cytotoxic activity (Fig. 3).

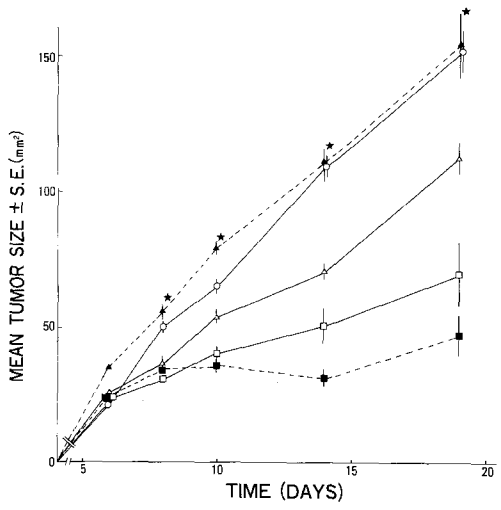

Fig. 3. Effect of loaded stress. A mixture of 2×10^7 spleen cells with 1×10^6 tumor cells was transplanted into irradiated mice. The spleen cells were obtained 10 days after tumor resection of adrenalectomized mice stressed (▲——▲) or nonstressed (△——△), sham adrenalectomized mice stressed (■——■), or nonstressed (□——□), and nonprimed mice (○——○). * : Statistically significant ($P < 0.01$) compared to nonstressed adrenalectomized mice. Vertical Bars: SE

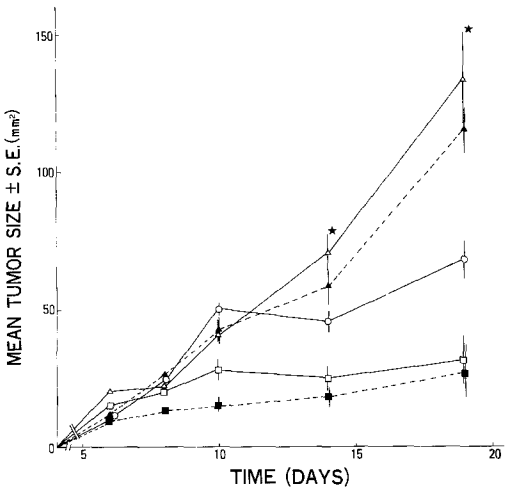

Fig. 4. Effect of glucocorticoid deficiency on the function of cytotoxic activity. A mixture of 2×10^7 spleen cells with 1×10^6 tumor cells was transplanted. Spleen cells were obtained 10 days after tumor resection. Spleen cells from adrenalectomized mice were transplanted into adrenalectomized mice (▲——▲) or sham adrenalectomized mice (△——△), those of sham adrenalectomized mice into adrenalectomized mice (■——■) or sham adrenalectomized mice (□——□), and of nonprimed mice into sham adrenalectomized mice (○——○). * : Statistically significant ($P < 0.01$) compared to sham adrenalectomized mice. Vertical Bars: SE

Effect of glucocorticoid deficiency on function of cytotoxic activity

Spleen cells were obtained by the same method as for the examination of the effect on cytotoxic activity from the ADX mice or sham ADX mice. The ADX mice continued to receive 0.3 mg of corticosterone. A mixture of 2×10^7 of these spleen cells was transferred with 1×10^6 tumor cells into the ADX mice or into the sham ADX mice and the resulting tumor size measured.

The spleen cells from the sham ADX mice suppressed the resulting tumor growth even when they were transferred into the ADX mice. However, those from the ADX mice did not affect growth of the tumor, while those from nonimmunized mice suppressed growth from the 14th day after the transfer (Fig. 4).

Discussion

We found that adrenalectomy compromised the antitumor immunity against Meth A sarcoma, as observed by the comitant immunity and cytotoxic activity of the spleen cells [9]. The failure of antitumor immunity was due to glucocorticoid deficiency, because by increasing the post-adrenalectomy dose of corticosterone, the above types of antitumor immunities were detected in the ADX mice.

Adrenalectomy has been reported to enhance allograft rejection [4, 6] but there have been few reports concerning the possibility that glucocorticoid deficiency could compromise the immune response. Antigen stimulation induces an elevation of serum corticosterone levels during the period of elevated immune response [3] and the glucocorticoid elevation could play some role in the immune response. The elevation of corticosterone may deplete the corticosteroid-sensitive immature cells of thymocytes which act as suppressor cells and enhance the immune response [1]. However, it was the effector activities that were affected in our experiments. Glucocorticoids play a permissive role in some hormone systems, especially in cyclic-AMP-dependent processes [8]. Cyclic-AMP plays a role in induction of the immune response [14, 15] and glucocorticoids may play a permissive role in the immune response, as is the case in erythropoiesis [7]. We speculate that an elevation of glucocorticoid levels may be required for an optimal immune response.

The tumor-bearing state was stress for the host and the demands for glucocorticoid may be relatively increased during periods of antitumor immunity, in comparison to that during allograft rejection. We examined whether the demands for glucocorticoid were increased for the immune response under conditions of stress. Daily injection of 1 mg glucocorticoid was sufficient to induce detectable antitumor immunity in the ADX mice and we loaded restraint stress on these mice. Such stress apparently compromised the antitumor immunity in the ADX mice but had no affect in the sham ADX mice.

Immunologically competent mature cells played an antitumor role, even when mixed with tumor cells and then transplanted into the glucocorticoid deficient ADX mice. Spleen cells from the glucocorticoid deficient ADX mice which were primed did not in any way affect the growth of tumor transplanted into the sham ADX mice. Nonprimed spleen cells from intact mice did have an antitumor effect 14 days after the transplantation. These results suggest that spleen cells from the glucocorticoid deficient ADX mice remained immature even after tumor challenge.

We conclude that glucocorticoid deficiency compromised the antitumor immune response and that glucocorticoid may play an important role in maturation of immunocompetent cells.

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