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Selective Immunosuppressive Activity of Steroids in Mice Inoculated with the Moloney Sarcoma Virus[1] (38503)

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The phenomena and reactions contributing to host cell-mediated immunological competence may be divided into at least two major phases. The first includes the inductive or sensitization phase, involving antigen-induced lymphocyte differentiation and proliferation. The second is the effector phase and comprises the expressions of the immune reactions, including elicitation of delayed hypersensitivity, lymphocyte-mediated injury to target cells, etc. The dissection of the diverse events reflecting cell-mediated immunity is, in part, dependent upon the type of assay employed and the phenomena underlying the response that is evaluated.

It has been reported recently (1, 2) that a synthetic steroid, 6-chloro-17α-hydroxypregna-1,4,6-triene-3,20-dione (CHP) is active in a series of models mediated by delayed hypersensitivity. Thus, although CHP had only moderate antiinflammatory activity in a number of standard assays for adrenal corticoid potency, e.g., thymolytic, granuloma and carrageenin edema, the steroid was essentially equipotent with cortisol in models of delayed hypersensitivity such as experimental allergic encephalomyelitis, adjuvant-induced arthritis, mouse skin delayed hypersensitivity and the survival of tail skin grafts in mice. This inhibition of delayed mediated mechanisms was not correlated with inhibition of 19 S and 7 S antibody formation, further distinguishing CHP from cortisol.

In view of the interesting apparent selectivity of CHP in the above models of immunological responsivity and its activity in prolonging the survival of skin allografts, we have examined the immunosuppressive activity of this steroid in a new model system that we have recently utilized (3, 4). Recognizing the broad nature of the term, immunosuppression, we wish to emphasize that it is used in this report to indicate the capacity of a host, in this case the CBA/Wh mouse, to prevent the progressive growth of the tumor induced by the murine sarcoma virus (Moloney) (MSV), and the influence of three steroids, CHP, cortisol and progesterone, on host resistance to tumor proliferation. The data obtained indicate that CHP, in contrast to the established immunosuppressive effects of a single large dose of cortisol or of progesterone, had no demonstrable immunosuppressive action in the experimental design described below.

Materials and Methods. The relative immunosuppressive activity of the steroids was assessed on the basis of the survival of mice with MSV-induced tumors. In newborn and adult mice (5), MSV causes a rapid induction of sarcomas at the site of virus inoculation. Peculiar to this tumor system is the high incidence of regression of autochthonous (primary) tumors (5). Immunological mediation of this tumor regression is suggested by the following observations. (a) Tumor regression depends on an intact immune system of the host. Tumors induced in normal adult mice regress spontaneously (6). In adult mice treated with cortisone (6) or exposed to sublethal X-irradiation (5), and in neonatally thymectomized mice (7), MSV-induced tumors do not regress but continue to grow, causing death of the host. This is also true of tumors induced in newborn mice (3–5). (b) MSV tumors possess tumor specific transplantation antigens (8). (c) A specific cellular and humoral response against the tumor and/or virus antigens, associated with progressive infiltration of the tumor by lymphocytes, precedes, accompanies and follows regression of the autochthonous tumor (9). (d) Large numbers of competent lymphoid cells given to immunosuppressed mice

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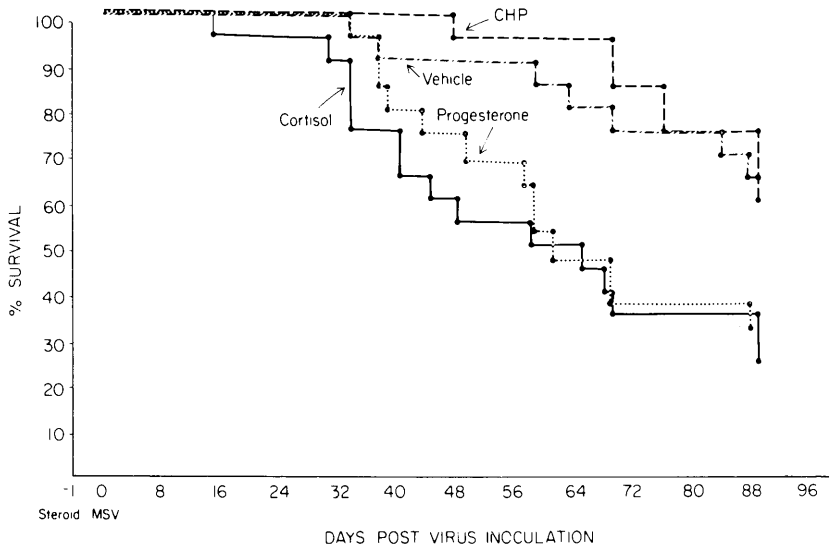

FIG. 1. Effect of cortisol, progesterone, CHP, and the steroid vehicle on the survival of mice with MSV induced tumors. The results are plotted as percentage of initial group surviving versus days postvirus inoculation.

bearing MSV-induced tumors increase survival and reduce tumor size (10).

The MSV used in the present study was kindly provided by Dr. J. B. Moloney of the National Cancer Institute and was diluted one part in 10 with phosphate buffered saline (pH 7.2) before use for inoculation. Eighty 5-week-old female CBA/Wh mice (average weight 18 g) from our own colony were divided into four groups of 20 animals each.[2] Mice in each group received a single intraperitoneal injection of 0.25 ml of CHP (25 mg/ml), progesterone (25 mg/ml), cortisol (25 mg/ml), or the suspending vehicle.[3] All steroids and vehicle were provided by Syntex Research. On the day following this injection, arbitrarily designated day 0, each mouse received 0.1 ml of the virus solution by intramuscular injection into the left hind limb.

Results. Figure 1 shows the survival curves over a 3-mo period for treated and untreated mice. Both the vehicle or CHP treated groups survived significantly longer than did the cortisol and progesterone treated group ($P < 0.05$).[4] There is no significant difference in mortality rates between the progesterone and cortisol-treated groups. The groups treated with CHP or the carrier vehicle also do not differ significantly from one another.

Only one animal (cortisol-treated) succumbed during the first 30 days. Fifty percent of the cortisol and progesterone treated animals died during the second month, at which time there was a 15% mortality in the control group and only a 5% mortality in the group receiving CHP.

During the third month, an additional 25% of the cortisol and progesterone treated mice died. This was one-half the number of those still alive at the end of 2 mo. At the end of the third month, only 40% of the control mice had died, as had 35% of the mice treated with CHP while 75% of the cortisol and progesterone treated animals had succumbed at this time.

Discussion. Two factors should be considered in evaluating the effects of the steroids used. First, the resistance of mice to

[2] One of the progesterone treated mice died on the first day of the experiment; this group therefore consisted of 19 animals.

[3] Composition: 0.9% NaCl, 0.5% Na carboxylmethycellulose, 0.4% polysorbate 80, 0.9% benzyl alcohol, 97.3% H_2O.

[4] Significance tested by Mann-Whitney *U* test, as described in Siegel, S., "Nonparametric Statistics for the Behavioral Sciences," pp. 116–127, 274–277, McGraw-Hill Book Co., New York (1956).

progressive growth of MSV-induced tumors is initially detectable in the second and third weeks of life and increases with age (11). The ability of 5-week-old mice to reject the tumor is not as great as that of older animals. This could contribute to the 40% mortality in the control group at 3 mo following virus inoculation. Second, relatively large doses of steroids were used.

Although progesterone was initially described as lacking significant immunosuppressive activity (12), this was subsequently attributed to the dose of steroid used (13). The data of the present study, supplemented by previous reports in the literature, particularly those of Munroe and Windle (14) and of Munroe (15), indicate that progestins, naturally occurring (progesterone) and synthetic (medroxy-progesterone) given in nonphysiological, large doses, are immunosuppressive agents and, like cortisol, may alter the capacity of animals to react against viral induced tumors.

A variable degree of host immunological nonresponsiveness has been reported in human pregnancy (16). However, it remains to be established whether or not a causal relationship obtains between the elevated blood levels of progesterone and the described immunological alterations in pregnancy.

The data reported here are of significance in demonstrating that in the experimental model employed, CHP was devoid of significant immunosuppressive activity. Since CHP does influence other parameters of host immunity (1, 2), our results add emphasis to the possible selective separation of specific parameters subsummed under the term, cell-mediated immunity.

Summary. A steroid, 6-chloro-17-hydroxypregna-1,4,6-triene-3,20-dione (CHP), that exhibits selective activity in several models of cellular immunity including an apparent inhibitory action on the elicitation of delayed hypersensitivity, was examined in a new, simple experimental model for assessing aspects of host cell-mediated immunological competence. This model is based upon the capacity of the adult mouse to prevent the progressive growth of tumors induced by the Moloney sarcoma virus. Two steroids reported to have immunosuppressive activity in other assay systems, namely, cortisol and progesterone, were also studied. Control mice and those injected with CHP maintained their capacity to reject the tumor. In contrast, significant numbers of mice receiving a single large injection of cortisol or progesterone succumbed to progressive tumor growth under the experimental conditions used. The data indicate that CHP, while influencing selected parameters of cellular immunity, e.g., the elicitation of delayed hypersensitivity, does not decrease the capacity of the host to mount a defense against the progressive growth of the Moloney virus-induced sarcoma. The results indicate that CHP may be useful in modulating specific aspects of cellular immunity without altering others. In addition, the experimental model described provides a simple method of assessing the possible immunosuppressive effects of naturally occurring and synthetic agents on viral-induced tumor growth.

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