GLUCOCORTICOIDS AND CELLULAR IMMUNITY IN VITRO

Facilitation of the Sensitization Phase and Inhibition of the Effector Phase of a Lymphocyte Anti-fibroblast Reaction*

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Glucocorticoid hormones are widely used clinically to suppress the rejection of transplanted organs, a reaction in which lymphocyte-mediated cellular immunity, rather than antibodies, is thought to play an important role (1).

Cellular immune reactions may be divided into at least two major phases. The first phase is considered to be the inductive or sensitization phase which involves antigen-induced lymphocyte differentiation and proliferation. The second is the effector phase and comprises the expressions of the immune reaction, such as lymphocyte-mediated injury to target cells. Thus, glucocorticoid hormones could depress cellular immune reactions by inhibiting the induction of sensitization and/or by suppressing the effector mechanism of sensitized lymphocytes. Gabrielsen and Good (2) have recently reviewed the immunosuppressive properties of adrenal steroid hormones and other chemicals. They noted the difficulty of distinguishing between the establishment of sensitivity and its expression, in systems used to study cellular immunity reactions. Hence, it has been difficult to assign the immunosuppressive action of glucocorticoids to the inductive and/or the effector phases. Nevertheless, Gabrielsen and Good felt that, in addition to the remarkable effects of glucocorticoids in inhibiting the expression of sensitization, there was some evidence to indicate that cortisone administered around the time of sensitization probably modifies the sensitization process itself (2).

To analyze the effects of glucocorticoids on the induction of sensitization as well as on the effector phase of cellular immunity, we employed an in vitro lymphocyte antifibroblast reaction (3–5). Normal rat lymphocytes were sensitized against foreign rat or mouse embryonic fibroblasts in cell culture. Sensitized lymphocytes were then transferred to target fibroblast monolayers and fibroblast injury was measured. Prednisolone or hydrocortisone was added to the tissue culture medium during the sensitization phase and/or during the cytolytic effector phase of the lymphocyte-mediated reaction.

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It was found that these glucocorticoid hormones influenced each phase of the lymphocyte anti-fibroblast reaction. The presence of a glucocorticoid during the induction phase led to a marked reduction in the total number of lymphocytes surviving sensitization. However, the population of surviving lymphocytes contained a greater proportion of large transformed cells, and after the hormone was removed, was able to destroy several times more target fibroblasts than could an equal number of control-sensitized lymphocytes.

Glucocorticoid hormones influenced the effector phase by inhibiting lymphocyte-mediated damage to target fibroblasts. Inhibition occurred at concentrations of hormone that did not affect the viability of the previously sensitized lymphocytes. Thus, prednisolone and hydrocortisone appeared to suppress the expression of the cytolytic effects of sensitized lymphocytes, but did not interfere with the induction of sensitization, a process which they seemed to facilitate.

Materials and Methods

Glucocorticoids.—Stock solutions of sterile hydrocortisone sodium succinate (Organon, Inc., West Orange, N. J.) and prednisolone sodium tetrahydrophthalate (Ciba Corp., Summit, N. J.) were prepared in phosphate-buffered saline (PBS),[1] pH 7.2, and stored at concentrations of 0.1 and 1.0 mg/ml at 4°C.

Fibroblast Monolayers.—Primary cultures of fibroblasts were obtained from 16-day old inbred C3H/eb mouse embryos or from 17-day old inbred Lewis or inbred Wistar rat embryos by trypsinization, as described elsewhere (3–6). Explanted fibroblasts were seeded in plastic Petri dishes (Falcon Plastics, Los Angeles, Calif.) in Waymouth's medium supplemented with 5% calf serum. After 4 days of incubation (37°C, 10% CO_2 in moist air), the culture medium was replaced by lactalbumin hydrolysate in Earle's saline with 5% calf serum (LA) which was then changed every 4 days. Fibroblasts were transferred to sensitization cultures (3×10^6 fibroblasts, 60 mm plastic Petri dishes) and target cultures (0.7×10^6 fibroblasts, 35 mm plastic Petri dishes) after x-irradiation (2000 R). Target fibroblasts were labeled 4–5 days before use with radioactive chromium by incubation with ^{51}Cr as Na_2CrO_4 (Radiochemical Centre, Amersham, England) at a concentration of 2 μCi per target plate in 1 ml LA (5, 6). The radioactive medium was replaced by LA after 24 hr.

Lymphocyte Cultures.—Inbred Lewis rats were obtained from Microbiological Associates, Inc., Bethesda, Md. Inbred Wistar rats were supplied through the kindness of Mr. Shalom Joseph of the Department of Biodynamics of this Institute. Lymph nodes or spleens were removed from ether-anesthetized rats which weighed about 300 g. Cells were collected into cold PBS by gentle teasing (lymph nodes) or straining through a fine wire mesh (spleens) as previously described (3–6). Nucleated cells were counted and suspended in Dulbecco's modification of Eagle's medium with 20% horse serum (EM; Grand Island Biological Co., Berkeley, Calif.). Glucocorticoids were added to EM in the concentrations indicated in Results.

Sensitization was induced by incubating 4 ml of suspensions of lymphocytes with sensitizing fibroblast monolayers for 5 days. On the 3rd day of culture, 3 ml of EM was replaced by fresh EM which contained the added glucocorticoid. After sensitization, the lymphocytes were collected by repeated pipetting of the culture medium, resuspended in fresh EM, and trans-

[1] *Abbreviations used in this paper:* EM, Eagle's medium; LA, lactalbumin hydrolysate; PBS, phosphate-buffered saline.

ferred to target fibroblast cultures for assay of cytolytic activity. Large lymphocytes, small lymphocytes, and fibroblasts were distinguishable by their characteristic microscopic appearance.

Assay of Lymphocyte-Mediated Cytolysis.—Injury to fibroblasts was estimated by the per cent of the total ^{51}Cr which was released from target monolayers by the action of sensitized lymphocytes, as described by Berke and associates (5) with minor modifications (6). Briefly, ^{51}Cr-labeled target monolayers were washed with LA and incubated with 1.5 ml of EM containing the sensitized lymphocytes. A well-type sodium iodide crystal scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.), was used to measure the relative amount of ^{51}Cr released into the medium in each target culture. Each experimental value represents the mean of three target cultures. The standard error in these experiments was always less than 2%, similar to that described by Berke and associates (7). Spontaneous release of ^{51}Cr from

TABLE I

*Effects of Pretreating C3H Target Fibroblasts with Prednisolone**

Prednisolone pretreatment of C3H fibroblasts	Release of ^{51}Cr	
	Spontaneous	In presence of Lewis anti-C3H lymphocytes
μg/ml	%	%
0	12	36
0.5	13	36
5	12	37
50	12	37
500	11	35

* C3H target fibroblast monolayers were incubated for 24 hr with EM containing prednisolone. 4×10^6 Lewis lymphocytes which had been sensitized against C3H fibroblasts were then cultured with the target fibroblasts for 20 hr in the absence of prednisolone.

intact target fibroblasts was measured in triplicate in each experiment and the value was subtracted from each experimental result. The spontaneous release of ^{51}Cr usually was between 5 and 15%, and was not significantly influenced by incubation of the target monolayers with up to 10×10^6 unsensitized lymphocytes (6) or by the addition of hydrocortisone or prednisolone.

To learn whether lymphocyte-mediated cytolysis of fibroblasts might be influenced by treating the target fibroblasts with glucocorticoids, target fibroblasts were preincubated for 24 hr with EM containing concentrations of prednisolone from 0.5 to 500 μg/ml. The prednisolone was removed and sensitized lymphocytes were added to the target fibroblast cultures. The spontaneous and lymphocyte-mediated release of ^{51}Cr was measured after 24 hr of incubation. It was found (Table I) that pretreating the target fibroblasts with prednisolone had no significant effect on the release of ^{51}Cr.

RESULTS

The Effects of Glucocorticoids on the Induction of Sensitization.—The effects of prednisolone and hydrocortisone on the induction of small lymphocytes into sensitized effector cells were studied by adding glucocorticoids to the medium of the sensitization cultures and measuring the number of lymphocytes and their cytolytic capacity at the termination of the sensitization period.

Fig. 1 shows the results of an experiment in which 50 × 10^6 Wistar rat lymph node cells were sensitized against allogeneic Lewis rat fibroblasts in medium containing 0–100 μg/ml of prednisolone.

It was found that the number of lymphocytes recoverable from the sensitizing cultures was progressively reduced by the addition of increasing concentrations of prednisolone (Fig. 1). Cytolytic capacity was measured by incubating

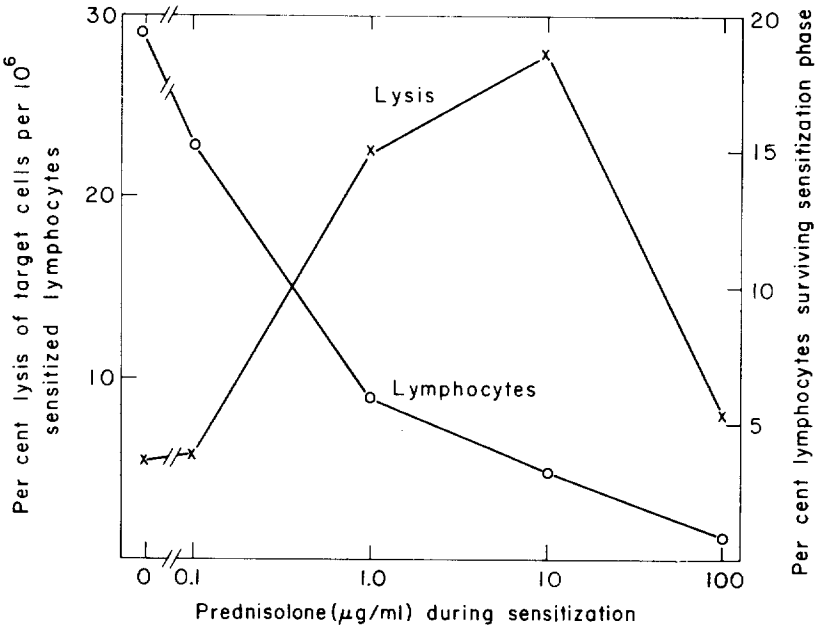

FIG. 1. The effects of prednisolone on the induction of sensitization. Cultures of 50 × 10^6 Wistar rat lymph node cells were sensitized against Lewis rat fibroblasts in the presence of prednisolone. The per cent of lymphocytes surviving sensitization was then counted. The per cent lysis of Lewis target fibroblasts by 1 × 10^6 of the sensitized lymphocytes was measured after 18 hr of incubation in the absence of prednisolone.

1 × 10^6 washed, sensitized lymphocytes with target fibroblasts for 18 hr. Lymphocytes which had been sensitized without added prednisolone or with a concentration of 0.1 μg/ml caused about 5% lysis of target fibroblasts (Fig. 1). Sensitization in the presence of 1.0 and 10 μg/ml was associated with target fibroblast lysis of 23 and 28%. Lymphocytes sensitized in the presence of 100 μg/ml of prednisolone showed no significant increase in cytolytic activity when compared with control cultures.

Thus, prednisolone, at concentrations of 1 and 10 μg/ml was associated with both a decrease in the number of recoverable lymphocytes and an increase in their cytolytic capacity.

It was possible that the augmented cytolytic capacity resulting from induction in the presence of glucocorticoids was not related to sensitization of lymphocytes against specific antigens, but rather to a nonspecific increase in cytotoxicity which could be manifested against fibroblasts antigenically unrelated to the sensitizing fibroblasts. To test this possibility (Fig. 2), Lewis rat spleen cells were sensitized against xenogeneic C3H mouse fibroblasts in the presence of concentrations of hydrocortisone from 0 to 10 μg/ml. The sensitized lymphocytes were collected and 1×10^6 were incubated with antigen-specific C3H or with syngeneic Lewis target fibroblasts. In this experiment,

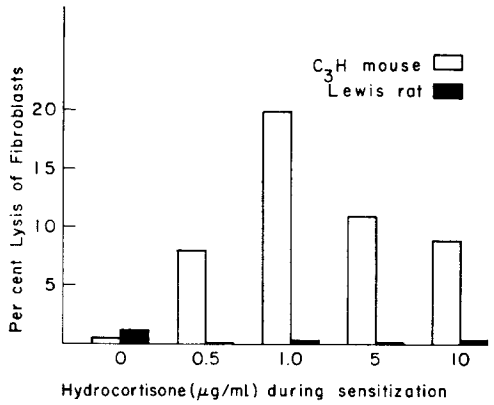

FIG. 2. Target fibroblasts. The immunospecificity of the effects of hydrocortisone on the induction of sensitization. Cultures of 27×10^6 Lewis rat spleen cells were sensitized against C3H mouse fibroblasts in the presence of hydrocortisone. Per cent lysis of antigen-specific C3H and syngeneic Lewis target fibroblasts by 1.2×10^6 of the sensitized lymphocytes was measured after 6 hr of incubation in the absence of hydrocortisone.

the release of ^{51}Cr from target fibroblasts was measured after 6 hr to learn if augmented cytolysis could be demonstrated within the time of one lymphocyte generation. It was found (Fig. 2) that Lewis spleen lymphocytes which were sensitized against C3H fibroblasts in the absence of hydrocortisone were unable to cause significant lysis to target fibroblasts during 6 hr of incubation. However, lymphocytes which were sensitized in the presence of 1 μg/ml of hydrocortisone caused 20% lysis of antigen-specific C3H target fibroblasts but no significant lysis of syngeneic Lewis target fibroblasts (Fig. 2).

These results indicated (*a*) that induction in the presence of hydrocortisone does not impair the immunospecificity of the effector phase, and (*b*) that augmented cytolysis can be expressed within one generation after incubation in hydrocortisone. This finding argues against the possibility that sensitization takes place in the target culture in the absence of hydrocortisone. It also sug-

gests that augmented cytolysis is not merely the result of increased lymphocyte proliferation.

The Effects of Glucocorticoids on Lymphocyte Kinetics During Sensitization.— To gain insight into the process by which glucocorticoids appear to promote sensitization we measured the kinetics of the effects of glucocorticoids on the number of lymphocytes and their degree of transformation during induction.

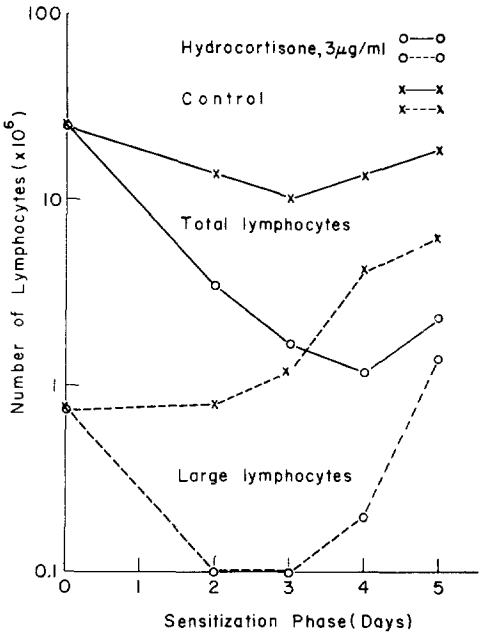

FIG. 3. The effects of hydrocortisone on the cell kinetics of the sensitization phase. Cultures of 25 × 10^6 Lewis rat lymph node cells were sensitized against C3H mouse fibroblasts in control medium and in the presence of hydrocortisone (3 μg/ml). On days 2, 3, 4, and 5, all the cells in two culture dishes were collected by using a trypsin solution and the lymphocytes, small and large, were counted. The indicated points represent the average number of cells counted in each pair of culture dishes. The numbers of cells did not differ by more than 12% in each of the pairs.

Comparable results were obtained in five experiments in which lymphocytes derived from lymph nodes or spleens were induced in medium containing 1–3 μg/ml of hydrocortisone or prednisolone. Figs. 3 and 4 show the results of an experiment in which Lewis rat lymph node cells were seeded on sensitizing monolayers of C3H fibroblasts with or without 3 μg/ml of hydrocortisone. All the cells from two cultues plates were collected on days 2, 3, 4, and 5 by using a trypsin solution (0.3%). The total number of viable lymphocytes and the number of large transformed lymphocytes, including those adherent to fibroblasts, were counted.

The kinetics of changes in the lymphocyte population are shown in Fig. 3. In control medium, the total number of lymphocytes fell from 25 × 10^6 to a low point on day 3 of 10 × 10^6 cells and then increased to 19 × 10^6 on day 5. Large transformed lymphocytes began to appear in increasing numbers from day 3 (1 × 10^6) to day 5 (6 × 10^6). In the presence of 3 μg/ml of hydrocortisone, the loss in total number of lymphocytes was more marked and there remained only about 1 × 10^6 lymphocytes on day 4. The total number of

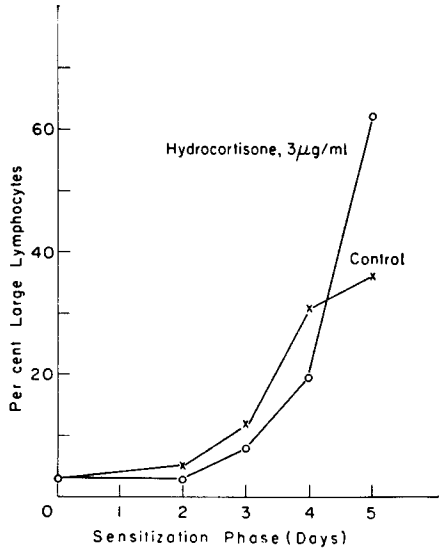

FIG. 4. The effects of hydrocortisone on the per cent of large lymphocytes during sensitization. The per cent of large lymphocytes found in the experiment shown in Fig. 3 was computed on days 2, 3, 4, and 5. Lysis of C3H target fibroblasts by 1 × 10^6 of these sensitized lymphocytes was measured after 18 hr. The lytic activity of the hydrocortisone-treated lymphocytes was fourfold greater than that of the control lymphocytes (20:5).

lymphocytes, however, increased to about 2 × 10^6 at the end of day 5. The number of large lymphocytes fell to about 0.1 × 10^6 on day 3 and then rose to about 1.4 × 10^6 on day 5 (Fig. 3).

Fig. 4 shows the per cent of large lymphocytes which appeared during the sensitization reaction presented in Fig. 3. The per cent of large lymphocytes was about the same in both hydrocortisone-treated and control cultures until day 3. By day 5, however, 63% of the hydrocortisone-treated lymphocytes and only 36% of the control lymphocytes appeared as large transformed lymphocytes.

At the termination of sensitization, 1 × 10^6 lymphocytes were transferred to target fibroblast monolayers. The lymphocytes which had been sensitized in

the presence of hydrocortisone produced fourfold more lysis than the sensitized control lymphocytes. These findings indicate that the presence of hydrocortisone or prednisolone is associated with a marked decrease in the number of lymphocytes during the initial stages of the induction of sensitization. However, the relative number of large lymphocytes among the total lymphocyte population rises sharply toward the end of the induction phase, producing cultures which are richer in large transformed lymphocytes than are control cultures. This suggests that glucocorticoids may promote the selection and proliferation of specifically reactive lymphocytes from among the initial population.

TABLE II

Augmented Cytolytic Effects of Equal Numbers of Large Lymphocytes Measured by Direct Cell Count

Sensitization phase*	Lytic phase‡				
	No. of lymphocytes × 10^6				Injured fibroblasts
Hydrocortisone	Prelysis		Postlysis		
	Total	Large	Total	Large	
μg/ml					%
0	1.9	0.5	1.2	0.4	4
2	0.6	0.5	2.4	2.1	96

* 28 × 10^6 Lewis spleen cells were sensitized against C3H fibroblasts.

‡ The indicated numbers of total and sensitized large lymphocytes were incubated with C3H target fibroblasts. After 18 hr the cultures were trypsinized and the numbers of viable lymphocytes and fibroblasts were counted.

Augmented Cytolytic Effect Measured by Direct Cell Count.—The above results raised the question of whether the increase in the per cent of large transformed cells could account for the increased cytolytic effect of lymphocytes induced in the presence of glucocorticoids. In order to study this, Lewis rat spleen cells were sensitized against C3H mouse fibroblasts with or without 2 μg/ml of hydrocortisone (Table II). The sensitized lymphocytes were collected and 1.9 × 10^6 control lymphocytes and 0.6 × 10^6 treated lymphocytes (each population containing the same number of large lymphocytes, 0.5 × 10^6) were transferred to C3H target fibroblasts. After 18 hr of incubation, surviving fibroblasts and lymphocytes were collected by use of a trypsin solution. Viable fibroblasts and lymphocytes were counted. It was found in this experiment that 96% of the target fibroblasts were either completely lysed or made permeable to eosine Y by the lymphocytes which had been sensitized in the presence of hydrocortisone. Only 4% of the target fibroblasts were injured by lymphocytes which had been sensitized in the absence of hydrocortisone. The

number of nontreated lymphocytes seemed to decrease slightly during the cytolytic reaction. On the other hand, the number of hydrocortisone-treated lymphocytes increased fourfold during the reaction (Table II).

Thus, the cytolytic capacity of hydrocortisone-treated lymphocytes was greater than that of the nontreated lymphocyte cultures, although each cell population initially contained an equal number of large transformed lymphocytes. Fibroblast injury was confirmed in this experiment by direct count of target fibroblasts and, therefore, detection of the augmented cytolytic effect was not limited to the ^{51}Cr method.

TABLE III

*Effects of Preincubating Spleen Cells with Hydrocortisone**

Sensitization phase of Lewis anti-C3H				Lytic phase‡
Hydrocortisone		Recoverable cells ($\times 10^6$)		
Preincubation 8 hr	5 day sensitization	Total cells	Large cells	Ratio of activity
μg/ml	*μg/ml*			
3	3	0.6	0.2	3
None	3	1.0	0.4	3
0	0	7.3	1.4	1
None	0	7.2	1.3	1

* 25×10^6 Lewis spleen cells were preincubated for 8 hr or seeded directly on C3H fibroblasts with or without hydrocortisone.

‡ 1×10^6 sensitized spleen cells were transferred to C3H target fibroblasts for 20 hr. The ratio of activity was computed by comparing the per cent lysis of each culture to the control cultures which were neither preincubated nor treated with hydrocortisone.

The Effects of Preincubating Lymphocytes with Glucocorticoids Before Exposure to Fibroblast Antigens.—Experiments were carried out to study the effect of preincubating lymphocytes with glucocorticoids before exposing them to the sensitizing fibroblasts. Table III shows the results of an experiment in which Lewis spleen cells were either preincubated for 8 hr or seeded directly on C3H fibroblast monolayers, with or without 3 μg/ml of hydrocortisone. It was found that preincubation with hydrocortisone appeared to reduce by about 50% the total number of lymphocytes as well as the number of large lymphocytes, compared to sensitization cultures which were exposed to hydrocortisone and sensitizing fibroblasts simultaneously (Table III). Nevertheless, the hydrocortisone-treated lymphocytes which survived preincubation demonstrated the same threefold increase in cytolytic activity as did treated lymphocytes which were not preincubated. Preincubation of lymphocytes in control medium had no effect on the number of recoverable cells. Three other similar experiments produced comparable results.

Thus, exposure to glucocorticoids before exposure to antigen appeared to

reduce the final numbers of sensitized lymphocytes but did not inhibit the degree of sensitization of the surviving lymphocytes.

The Influence of Glucocorticoids on the Effector Phase of the Lymphocyte Anti-fibroblast Reaction.—The influence of glucocorticoids on the effector phase was studied by measuring the cytolytic activity and the number of sensitized lymphocytes during the lytic reaction in the presence of various hormone concentrations. Table IV and Fig. 5 show the results of an experiment in which Lewis lymph node cells which had been sensitized against C3H fibroblasts in the absence of added glucocorticoids were incubated with target fibroblasts in medium which contained 0–1000 μg/ml of prednisolone. The presence of pred-

TABLE IV

*Effects of Prednisolone on Number and Cytolytic Activity of Sensitized Lymphocytes**

Prednisolone	No. of viable lymphocytes ($\times 10^6$) after lytic phase		Cytolysis of fibroblasts	Inhibition
	Total	Large cells		
μg/ml			%	%
0	2.8	1.1	45	—
1	3.1	1.0	29	36
10	2.1	0.7	18	60
100	1.5	0.4	17	62
1000	0.2	0.04	2	96

* Lewis lymph node cells were sensitized against C3H fibroblasts in the absence of prednisolone. The sensitized lymphocytes were collected and 2.7×10^6 lymphocytes, of which 0.8×10^6 were large, were transferred for 22 hr to C3H target fibroblasts in EM containing prednisolone.

nisolone at a concentration of 1 μg/ml was associated with a 36% inhibition of lymphocyte-mediated cytolysis but no impairment of lymphocyte viability. To confirm this result we studied the effects of prednisolone at concentrations of 0–20 μg/ml. Fig. 6 demonstrates that at concentrations of 0.05–5 μg/ml there was no decrease, and perhaps even a significant increase (33% at 0.5 μg/ml) in the number of sensitized lymphocytes at the termination of the cytolytic reaction. Nevertheless, prednisolone at concentrations of 0.5, 1.0, and 5.0 μg/ml inhibited cytolytic activity by 25%, 50%, and 67% respectively. Prednisolone at a concentration of 20 μg/ml produced a 36% decrease in lymphocyte number.

Thus, prednisolone at concentrations of about 1 μg/ml suppressed the effector phase of the lymphocyte anti-fibroblast reaction without demonstrating a significant lympholytic effect on previously sensitized lymphocytes.

The effects of hydrocortisone on the effector phase were also studied and found to be similar to those of prednisolone.

The Effects of Prednisolone During Sensitization and Lytic Reactions.—Experiments were done to test whether lymphocytes treated with glucocorticoids during induction would be resistant to their inhibitory effects on the cytolytic effector phase. Table V shows the results of an experiment in which Lewis lymph node lymphocytes were sensitized against C3H fibroblasts with or without 1 μg/ml of prednisolone. After the sensitization phase, 1.5×10^6

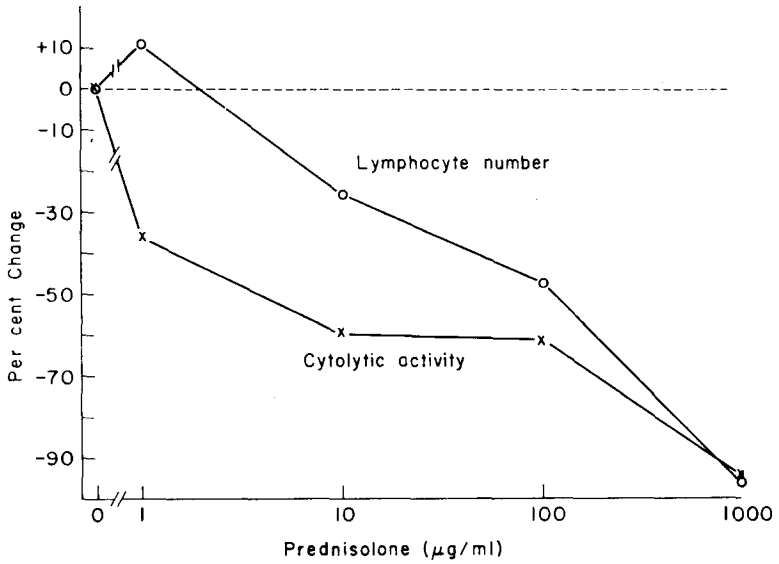

FIG. 5. The influence of prednisolone (0–1000 μg/ml) on the effector phase. Lewis rat lymph node cells were sensitized against C3H mouse fibroblasts and 2.7×10^6 of the sensitized lymphocytes were incubated with C3H target fibroblasts in the presence of 0–1000 μg/ml of prednisolone. Per cent lysis of fibroblasts and number of lymphocytes were measured after 22 hr. Per cent change in lymphocyte number and cytolytic activity were computed by comparison with control cultures which were incubated without prednisolone.

lymphocytes of both kinds were incubated for 20 hr with C3H target fibroblasts with or without 1 μg/ml of prednisolone. It was found that lymphocytes which had been sensitized in the presence of prednisolone demonstrated over twice the cytolytic activity of sensitized control lymphocytes. Nevertheless, the cytolytic effects of both control and treated lymphocytes were inhibited by about 50% by the presence of 1 μg/ml of prednisolone during the cytolytic reaction. Thus, the inhibitory effects of glucocorticoids on lymphocyte-mediated cell injury were not annulled by the presence of prednisolone during the induction phase.

DISCUSSION

The in vitro lymphocyte anti-fibroblast reaction used in these studies may be considered to be a model of cellular immunity. Small lymphocytes are in-

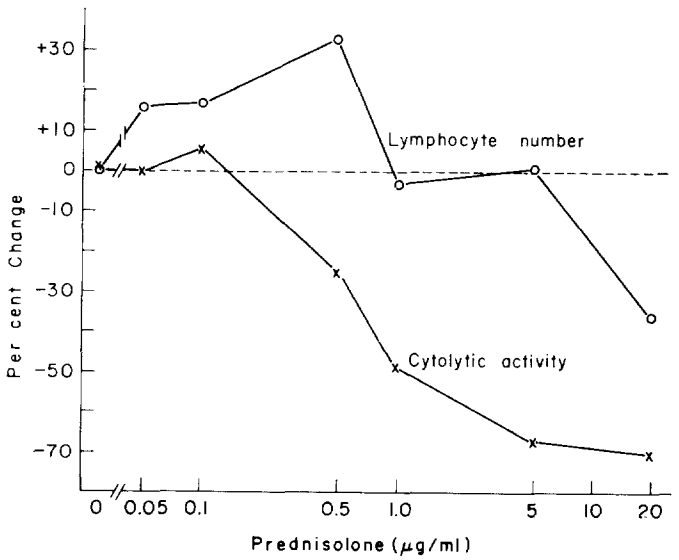

FIG. 6. The influence of prednisolone (0–20 μg/ml) on the effector phase. The conditions were the same as the experiment shown in Fig. 5, except that the prednisolone concentration was in the range of 0–20 μg/ml.

TABLE V

Effects of Prednisolone During Sensitization and Lytic Reactions

Sensitization phase*	Lytic phase‡		
Prednisolone	Lysis		Inhibition of lysis by prednisolone
	Without prednisolone	Prednisolone, 1 μg/ml	
μg/ml	%	%	%
0	19	9	53
1	42	20	52

* 20×10^6 Lewis rat lymph node cells were sensitized against monolayers of C3H mouse fibroblasts in control medium and in medium containing 1 μg/ml prednisolone.

‡ 1.5×10^6 sensitized lymphocytes were incubated for 20 hr with C3H target monolayers with or without 1 μg/ml of prednisolone.

duced to transform into large cells through contact with antigenically foreign fibroblasts. These sensitized lymphocytes can be activated by the sensitizing antigens to injure target fibroblasts. Extracellular antibody or complement have no demonstrable role in this reaction and close contact between living

lymphocytes and target fibroblasts appears to be required for injury to occur (3, 4, 6). The lymphocyte anti-fibroblast reaction, therefore, provided a unique tool for observing the influence of glucocorticoids on the induction phase as well as on the effector phase of a cellular immune response in vitro.

The presence of about 1 μg/ml of hydrocortisone or prednisolone during induction resulted in a decrease in the total number of lymphocytes, but caused a marked increase in the per cent of large transformed lymphocytes and in the cytolytic capacity of the sensitized lymphocytes. On the other hand, the presence of glucocorticoids during the effector phase was associated with an inhibition of lymphocyte-mediated lysis of target fibroblasts at concentrations of hormone which did not impair the viability of the previously sensitized lymphocytes.

The influence of glucocorticoids on the induction phase can be explained by a decreased susceptibility to the lymphotoxic effects of these hormones of transformed, sensitized lymphocytes compared to that of small lymphocytes. This is evidenced by the marked decrease in the number of small lymphocytes at the beginning of induction and the apparent proliferation of sensitized lymphocytes in the latter stage of induction which was accentuated in the presence of hydrocortisone (Fig. 3). Thus, contact with sensitizing fibroblasts might initiate changes in specifically reactive lymphocytes which lead to both transformation and resistance to glucocorticoid toxicity. Those small lymphocytes which are not capable of induction by contact with the sensitizing fibroblasts would remain vulnerable to the toxic effects of the hormones. It has been shown that glucocorticoids inhibit protein synthesis and DNA and RNA metabolism of small unsensitized rat lymphocytes (8). In preliminary studies we have found however that synthesis of protein and DNA by sensitized lymphocytes is unimpaired, at least during 24 hr of incubation, by comparable concentrations of hormones.[2] Thus, glucocorticoids may act to select antigen-reactive lymphocytes from among the initial lymphocyte population.

The effects on sensitization of nonglucocorticoid immunosuppressive agents such as azathioprine have been studied by employing the same in vitro cell culture system.[3] It was found that the presence of azathioprine also led to a profound decrease in the number of recoverable lymphocytes. However, in contrast to the selective action of glucocorticoids, azathioprine-treated lymphocytes were unable to injure target fibroblasts after the drug was removed.

In the absence of glucocorticoids a relatively larger number of lymphocytes which are not sensitized appear to survive the induction phase. Some of these unsensitized lymphocytes may be transformed into large cells by mitogenic substances released by sensitized lymphocytes (9). Hence, at the end of the induction phase a smaller fraction of the large lymphocytes in control cultures

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[2] Stavy, L., I. R. Cohen, and M. Feldman. Unpublished results.

[3] Treves, A., and L. Stavy. Unpublished results.

may be specifically active against target fibroblasts. This could explain the apparent increase in cytolytic activity per large cell observed after sensitization in the presence of hydrocortisone (Table II).

Preincubating small lymphocytes with hydrocortisone before exposing them to sensitizing fibroblasts led to a decrease in the yield of sensitized cells but did not impair their augmented cytolytic activity (Table III). It is possible that some of the potentially reactive lymphocyte population may have been irreversibly damaged by preincubation with hydrocortisone for 8 hr. Subsequent interaction of the surviving lymphocytes with sensitizing fibroblasts may have initiated cellular changes leading to the transformation of the remaining potentially reactive lymphocytes. These findings suggest that glucocorticoids may eliminate small lymphocytes randomly in the absence of sensitizing antigens.

It has been shown that the effector phase of the lymphocyte anti-fibroblast reaction involves at least two stages (6). The first stage comprises activation by antigen of the effector mechanism of the sensitized lymphocytes. Recognition of antigen by a receptor on the surface of the lymphocyte would appear to initiate this reaction. The second stage may be considered to be the cytolytic process itself. This stage is not antigen-specific since lymphocytes activated by antigen may inflict damage upon any fibroblast with which contact is made. Inhibition of target-cell injury by glucocorticoids might therefore result from interference with either or both stages of the effector phase. It is not likely, however, that glucocorticoids block the recognition of antigen by lymphocytes. Rosenau and Moon have demonstrated (10) that hydrocortisone prevents cytolysis of target cells without inhibiting antigen-specific clustering of lymphocytes about the target cells. In addition, our finding that sensitization occurs in the presence of glucocorticoids suggests that the ability of lymphocytes to interact with antigen persists. It is likely therefore that glucocorticoids inhibit the effector mechanism of transformed lymphocytes at a stage distal to the interaction of the receptor with antigen. The nature of the effector mechanism is unknown. However, glucocorticoids have been shown to stabilize lysosome membranes (11) and it is conceivable that lysosomal enzymes might have some role in target-cell injury as well as in lymphocyte activation (12, 13).

It is quite possible that our results pertain only to rat lymphocytes in an in vitro experimental system. Nevertheless, it might be useful to relate our in vitro findings to cellular immunity in vivo in view of the wide clinical use of glucocorticoid hormones as immunosuppressive agents.

Despite the fact that glucocorticoids may act to reduce the total number of small lymphocytes, the immune process of sensitization itself may evolve unhindered or may even be facilitated by glucocorticoids in vivo as it is in vitro. The development of specifically sensitized lymphocytes might not be recognized as long as glucocorticoids are present to inhibit damage to target cells.

Therefore, it appears questionable whether glucocorticoid hormones ought to be used routinely to suppress lymphocyte-mediated immune reactions during the period of primary sensitization. Our findings, however, support the use of glucocorticoids as agents to suppress tissue damage mediated by lymphocytes which already have been sensitized. Glucocorticoids could also be used to reduce the number of potentially reactive lymphocytes by treating graft recipients, for example, before the foreign graft is applied. Glucocorticoid treatment should be stopped, however, when the patient first receives the foreign graft.

Additional practical applications of the results of our studies involve the use of glucocorticoids to promote the development in vitro of populations of lymphocytes which exhibit a high degree of specific sensitization. We are currently studying aspects of transplantation and tumor immunity by sensitizing lymphocytes to tissue antigens with the aid of glucocorticoids in vitro (14).

SUMMARY

We studied the influence of glucocorticoids on the sensitization phase as well as on the cytolytic effector phase of an in vitro lymphocyte-mediated immune reaction. Lymphocytes obtained from the spleens or lymph nodes of unimmunized inbred rats were sensitized against foreign rat or mouse embryonic fibroblasts in cell culture. The capacity of the sensitized lymphocytes to produce a cytolytic effect was tested by transferring them to target fibroblast cultures. Injury to target fibroblasts was measured by release of radioactive ^{51}Cr from previously labeled fibroblasts or by direct count of viable fibroblasts after incubation with sensitized lymphocytes. Various concentrations of water-soluble hydrocortisone or prednisolone were added to cell cultures during the 5 day sensitization phase and/or during the subsequent cytolytic effector phase and the influence of these hormones on the number and cytolytic capacity of the lymphocytes was measured.

During the sensitization phase, the presence of glucocorticoid hormones, at concentrations of about 1 μg/ml, led to a profound decrease in the total number of recoverable lymphocytes. However, the per cent of large transformed lymphocytes was much greater in these treated cultures. The antigen-specific cytolytic capacity per cell of the glucocorticoid-treated lymphocytes, after the hormone was removed, was several times greater than that of lymphocytes sensitized in the absence of added hormones.

Glucocorticoids influenced the effector phase of the reaction by inhibiting lymphocyte-mediated injury to target fibroblasts. The hormones, at concentrations of about 1 μg/ml, inhibited the cytolytic effect by about 50% without reducing the viability of the sensitized lymphocytes. Dose-dependent toxicity to lymphocytes and increasing inhibition of cytolytic effect appeared at higher concentrations of hormones.

Thus, hydrocortisone and prednisolone, at concentrations of about 1 μg/ml, did not suppress the induction of sensitization, a process which they seem to facilitate in vitro. However, similar concentrations of these hormones appear to inhibit the cytolytic effector mechanism of sensitized lymphocytes. These findings may be relevant to the use of glucocorticoids as immunosuppressive agents in vivo.

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