Immunoglobulin Production Induced In Vitro by Glucocorticoid Hormones

T CELL-DEPENDENT STIMULATION OF IMMUNOGLOBULIN PRODUCTION WITHOUT B CELL PROLIFERATION IN CULTURES OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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ABSTRACT The direct effects of steroid hormones on the production of immunoglobulins and DNA synthesis by human T and B lymphocytes was evaluated in cultures of peripheral blood mononuclear cells. As detected by a reverse hemolytic plaque assay, the addition of 0.1 mM to 10 nM hydrocortisone to lymphocytes in culture in the absence of other stimulants or mitogens, resulted in the dramatic induction of immunoglobulin production with responses comparable to those seen in similar cultures stimulated with pokeweed mitogen. Steroid-stimulated immunoglobulin production was first seen after 48 h and peaked at 8–10 d of culture. The production of IgG, IgA, and IgM was induced following incubation with steroid. Glucocorticoids, but not estrogens or androgens, were capable of mediating this effect, and only compounds with affinity for the glucocorticoid receptor were active. The induction of immunoglobulin production was dependent on both T cells and monocytes; cultures depleted of either cell type did not produce immunoglobulin when stimulated with glucocorticoid hormones. Proliferation of B cells or T cells could not be detected by [^{3}H]thymidine incorporation or total cell recovery from steroid-stimulated cultures, even though such cultures demonstrated marked increases in immunoglobulin production. The mechanism responsible for this functional maturation of B cells to become high rate immunoglobulin producing cells is as yet undefined, although it appears to involve more than merely steroid mediated inactivation of suppressor T cells.

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INTRODUCTION

Corticosteroid modulation of immune responses has been the focus of a great deal of research in both human and animal systems. Although steroids are generally regarded as immunosuppressive agents, all components of the immune system are not equally affected by these compounds, and at times steroid actions on different components appear to be antagonistic. For example, conflicting data have been published regarding effects of steroids on B cells and antibody production. In vivo, steroids are known to cause transient lymphopenia in the peripheral blood of man with a much more pronounced depletion of T cells than B cells resulting in a relative increase in the percentage of circulating B cells compared with T cells (1, 2). However, the B cells obtained from peripheral blood following in vivo treatment with glucocorticoids have been reported to produce less than normal amounts of immunoglobulin in culture (3).

Early studies evaluating in vivo effects of relatively low doses of steroids reported no significant effect on serum immunoglobulin levels in man (4). More recent findings suggest that high dose administration of steroid results in modest decreases in serum immunoglobulins, particularly IgG and IgA, which may persist for periods of several months after treatment is stopped (5, 6). Specific antibody synthesis in man, however, has not been shown to be suppressed by corticosteroids, and in one study, enhancement was demonstrated (7). In young chickens, treatment with cortisone resulted in the mobilization of B lymphocytes from the Bursa and enhanced the differentiation into mature immunoglobulin secreting plasma cells in the peripheral lymphoid tissues (8).

Several in vitro studies of immunoglobulin production by human lymphocytes have also revealed a stimulatory effect of steroids (9, 10). In some instances where suppressor cells are known to be present, steroids appear to enhance in vitro immunoglobulin synthesis, and this response has been thought to reflect inactivation or inhibition of suppressor cell function (11, 12). While evaluating in vitro steroid action on suppressor cells, we noted the ability of glucocorticoids, in the absence of any known mitogen, to markedly enhance immunoglobulin production. The further definition of this observation is the subject of this report.

METHODS

Preparation of mononuclear cells. Heparinized blood was obtained from healthy adult volunteers and the mononuclear cell population prepared by standard density gradient centrifugation on a mixture of sodium diatrizoate and Ficoll (LSM, Bionetics, Kensington, Md.).

Preparation of B and T cell populations. The mononuclear cell population was mixed with 2-aminoethylisothiouronium bromide (AET)[1]-treated sheep erythrocytes (SRBC) (13) at a ratio of 1:150 and incubated for 2 h at 4°C. The erythrocyte-lymphocyte mixture was then gently resuspended and the rosetting cells were separated from those failing to form rosettes on another LSM gradient. The SRBC were removed by lysis with an NH_4Cl-lysing buffer. The rosetting population was demonstrated to contain ~97% rosette-positive cells, and will be referred to as the "T cell population." The interface, nonrosetting cells, contained B lymphocytes, monocytes, "null" cells and <3% E rosette-positive cells and will be referred to as the "B cell population."

Lymphocyte culture. The cell populations were suspended at 1×10^6 cells/ml in RPMI 1640 medium supplemented with 2 mM glutamine, 100 IU penicillin, 100 μg streptomycin, and 10% heat-inactivated fetal calf serum, and cultured in 15-ml round-bottomed tubes (Falcon 3033, Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, Calif.) at 37°C in a humidified 5% carbon dioxide incubator. Pokeweed mitogen (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.), Epstein-Barr virus (supernatant from cell line B95-8), hydrocortisone 21-sodium succinate (Upjohn Co., Kalamazoo, Mich., Solucortef), 6-alpha methylprednisolone 21-sodium succinate (Solumedrol), prednisolone 21-sodium succinate, 17-ethinyl estradiol, D-aldosterone, dehydroandrosterone (all from Sigma Chemical Co., St. Louis, Mo.), testosterone, diethylstilbesterol, 11-α cortisol, 11-β cortisol (Steriloids, Pauling, N. Y.), or R5020, a synthetic progestin, (14) (kindly supplied by Dr. Marc E. Lippman) were added to appropriate cultures. At termination of culture, the cells were washed four times with 15 ml media and the number of immunoglobulin-secreting cells was determined with a reverse hemolytic plaque assay as previously described (15). Plaques were counted on a video-based automatic plaque counter (Optomax, Hollis, N. H.).

Proliferation as determined by [^{3}H]thymidine incorporation. Cells were cultured at 1×10^6 cells/ml in a total volume of 0.2 ml in 96-well U-bottom tissue culture plates (Linbro Scientific Co., Hamden, Conn.). [^{3}H]thymidine (NET 027, New England Nuclear, Boston, Mass., sp act 6.7 Ci/mmol, final concentration 1 Ci/culture) was added during the final 4 h of culture and the plates then harvested. Results of liquid scintillation counting are expressed as mean counts per minute of triplicate cultures.

Preparation of monocyte-depleted population. Mononuclear cells at a concentration of 10^7 cells/ml in balanced salt solution containing 20% fetal calf serum were mixed with an equal volume of Lymphocyte Separator Reagent (Technicon Instruments Corp., Tarrytown, N. Y.) and incubated at 37°C with continuous rocking for 45 min. A magnet was then used to remove the iron filings and iron-containing phagocytic cells and the residual lymphoid cells were then subjected to another LSM density gradient centrifugation. The interface cells contained <2% cells staining for alpha napthyl butyrate esterase (16).

RESULTS

Stimulation of immunoglobulin production by hydrocortisone. Peripheral blood lymphocytes cultured with pokeweed mitogen (PWM) or 10 μM hydrocortisone were harvested on day 7 and examined for immunoglobulin-secreting cells (IgSC) with a reverse hemolytic plaque assay. In 20 normal subjects, PWM induced an average increase in IgSC of 5.9-fold over that found in cultures maintained in medium alone [5331 (1.22) vs. 911 (1.22)IgSC/culture]. Strikingly, in parallel cultures stimulated with 10 μM hydrocortisone without the addition of any other known B cell activator an average 8.8-fold increase in IgSC was found [8027 (1.14)IgSC/culture]. Cultures from each of the 20 normal subjects responded to steroid stimulation with a range of response from 2- to 60-fold increase over control cultures. To demonstrate that this response required *de novo* protein synthesis, cycloheximide (0.05 mM), or puromycin (0.05 mM) were added to the cultures during the final hour. These inhibitors of protein synthesis blocked by 82–99% plaque formation induced by glucocorticoids, as well as blocking immunoglobulin synthesis in response to PWM and the Epstein-Barr virus (EBV).

The results presented here have been expressed as the number of IgSC obtained per culture to eliminate potential differential effects of cell survival or proliferation caused by the various stimulants tested. In fact, with 10 μM hydrocortisone as the stimulant, the average cell recovery from 8-d cultures initially consisting of 10^6 cells was only 0.23×10^6. Cell recovery in unstimulated cultures averaged 0.35×10^6, and 1.1×10^6 cells were recovered from PWM-stimulated cultures. Thus, for example, when the data from Table I are expressed as IgSC per recovered cell, the response in steroid stimulated cultures is even more striking. With PWM stimulation, an average of 4,840 IgSC were present in 10^6 recovered cells, while in

[1] *Abbreviations used in this paper*: AET, 2-aminoethylisothiouronium bromide; EBV, Epstein-Barr virus; FCS, fetal calf serum; IgSC, immunoglobulin-secreting cells; NHS, normal human serum; PBMC, peripheral blood mononuclear cells; PWM, pokeweed mitogen; RHPA, reverse hemolytic plaque assay; SRBC, sheep erythrocytes.

TABLE I
Class of IgSC Induced by Hydrocortisone Induced by Hydrocortisone

	IgSC/culture*		
	IgG	IgA	IgM
Unstimulated	84 (1.71)	28 (1.50)	217 (1.94)
Hydrocortisone	1,521 (1.16)	1,078 (1.14)	3,309 (1.20)

* IgSC on day 8 of cultures of 1×10^6 PBMC.

hydrocortisone-stimulated cultures, 34,900 IgSC were found in 10^6 recovered cells.

Kinetics of glucocorticoid-induced immunoglobulin secretion. The kinetics of the development of IgSC in cultures of human peripheral blood mononuclear cells (PBMC) stimulated with PWM or 10 μM hydrocortisone as measured by the reverse hemolytic plaque assay (RHPA) are depicted in Fig. 1. Cultures stimulated with PWM had the expected marked enhancement in polyvalent IgSC production with maximum response occurring between 4 and 6 d of culture. Addition of 10 μM hydrocortisone to cultures of PBMC without the addition of other mitogens resulted in enhanced IgSC production comparable to that seen with PWM. Similar to PWM, hydrocortisone-stimulated immunoglobulin production was not detected until after 2 d in culture, but its peak effect was delayed until 8–10 d of culture.

Assessment of cellular proliferation by [^{3}H]thymidine incorporation. Unfractionated mononuclear cells were simultaneously evaluated for the incorporation of tritiated thymidine and production of IgSC. PWM added to cultures resulted in the expected potent

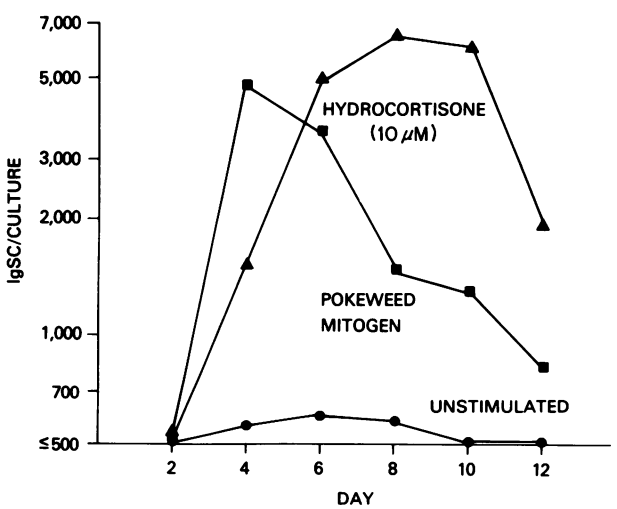

FIGURE 1 Kinetics of appearance IgSC in cultures of human mononuclear cells cultured with pokeweed mitogen or 10 μM hydrocortisone (geometric mean of seven experiments).

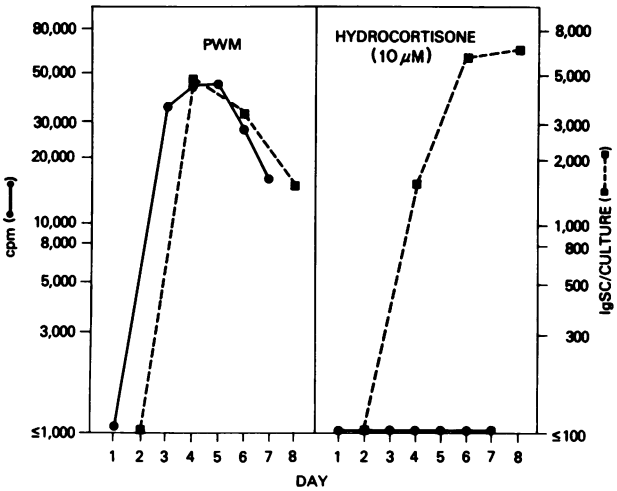

FIGURE 2 Comparison of IgSC production and [^{3}H]thymidine incorporation in cultures stimulated with PWM or 10 μM hydrocortisone (geometric mean of five experiments).

stimulatory effect on thymidine incorporation between 3 and 8 d coinciding with the appearance of IgSC (Fig. 2). In marked contrast, hydrocortisone did not induce thymidine incorporation greater than control cultures at any time during the culture period. IgSC production was again most marked at 8–10 d. This lack of proliferation above background occurred in hydrocortisone-stimulated PBMC cultured in media supplemented with either fetal calf serum or normal human serum. Significant proliferation was also not detected in cultures of isolated T or B cell populations, or mixtures of B cells with either irradiated or unirradiated T cells (data not shown).

Comparison of glucocorticoids and nonglucocorticoids on the induction of IgSC production. Evaluation of the dose effects of hydrocortisone yielded the curve seen in Fig. 3. Enhancement of IgSC production as measured on day 8 of culture was present with as low a concentration as 0.1 nM hydrocortisone. Marked stimulation was evident with hydrocortisone in the 10 nM to 0.1 mM range. 1 mM hydrocortisone, not shown, was inhibitory to cultures with few IgSC present at day 8 of culture.

To determine if the stimulatory action was unique to glucocorticoids, we compared the effects of a variety of steroid hormones on immunoglobulin production. As shown in Fig. 3, the glucocorticoids evaluated, hydrocortisone, dexamethasone, prednisolone, and methylprednisolone, all had a marked stimulatory effect. The reason for the variation in potency compared with the relative in vivo activities of these compounds is unknown, but is perhaps related to the particular chemical derivatives (succinate, acetate, etc.) of the natural compounds chosen for study. Testosterone, diethylstilbesterol, and R5020, a synthetic progestin, as

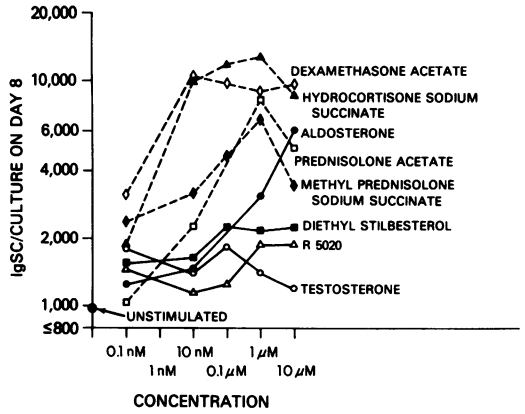

FIGURE 3 Comparison of various concentrations of steroid compounds on IgSC activity (geometric mean of five experiments).

demonstrated, as well as dehydroandrosterone and ethinyl estradiol (data not shown), all had minimal effect on immunoglobulin production. Perhaps the small amount of cross-reactivity of these agents with glucocorticoids was able to mediate this modest stimulation over base line. Interestingly, aldosterone, primarily a mineralocorticoid, did show a stimulatory response at the higher concentrations. This presumably relates to its known glucocorticoid activity and capacity to bind to the glucocorticoid receptor (17). It thus appeared that glucocorticoids specifically mediated this stimulatory response. This issue was more directly addressed by evaluating the necessity for binding to the glucocorticoid receptor. 11-β cortisol is a potent glucocorticoid. In contrast, 11-α cortisol, a stereoisomer differing only at the 11 position does not bind to the glucocorticoid receptor and is not biologically active. The 11-β isomer induced marked enhancement in IgSC production [Δ-8659 (1.25)IgSC/culture] comparable to other glucocorticoids, while the 11-α compound was inactive [Δ-224 (1.62)IgSC/culture]. Thus it appears that glucocorticoids via binding to their cellular receptor mediate the stimulatory effect on immunoglobulin production.

Class of immunoglobulin produced in glucocorticoid stimulated cultures. To determine whether the glucocorticoid-induced response was restricted or polyclonal in nature, we evaluated the isotypes of immunoglobulins being secreted. As depicted in Table I, hydrocortisone enhanced the production of IgG, IgA, and IgM significantly over control values. Small numbers of IgM anti-SRBC plaques were also detected. In addition, IgE production as determined by double antibody radioimmunoassay was enhanced in cultures from ~75% of normal and atopic individuals.[2] Thus the stimulation was polyclonal and not restricted to a single class of immunoglobulin.

T cell and monocyte dependence of the glucocorticoid effect. In order to assess the question of cellular cooperation necessary for the glucocorticoid effect, we examined the response of B and T cell populations to hydrocortisone. As shown in Fig. 4, PWM is a T cell-dependent polyclonal B cell activator, having little effect on a purified population of B cells. In contrast, EBV is able to activate B cells directly to become IgSC, thus functioning as a helper T cell-independent activator. Hydrocortisone enhancement of IgSC production proved to be T cell dependent, the B cell fraction alone being unresponsive to hydrocortisone. With the addition of T cells, marked immunoglobulin production was again noted.

Because T cell-dependent polyclonal activation appears to be correlated with monocyte dependence, we examined the role of monocytes in glucocorticoid stimulation. Removal of phagocytic cells with iron filings results in populations of lymphocytes containing <2% monocytes. The addition of hydrocortisone to phagocyte-depleted cultures resulted in no enhancement in immunoglobulin production contrasted with the marked increases seen in unseparated populations (Fig. 5). Such monocyte-depleted cells, however, are still extremely sensitive to stimulation by a monocyte independent B cell activator such as EBV.

Effect of T cell irradiation or steroid pulsing on steroid-induced Ig production. Since both steroids

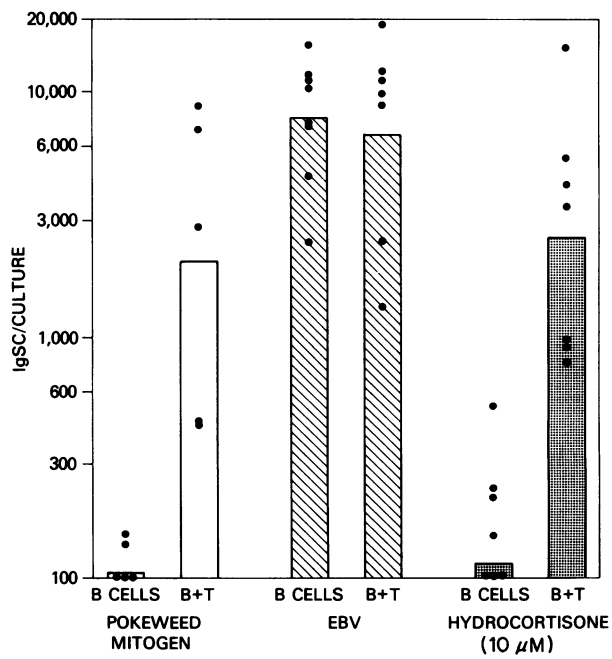

FIGURE 4 T cell requirement for the production of IgSC in cultures stimulated with PWM, 10 μM hydrocortisone or EBV.

[2] Grayson, J., Blaese, R. M., and Waldmann, T. A. Unpublished data.

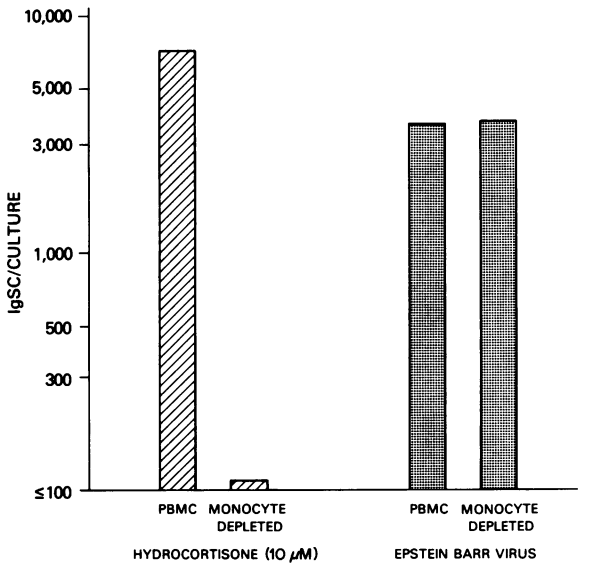

FIGURE 5 Monocyte requirement for the development of IgSC in cultures stimulated with 10 μM hydrocortisone or EBV.

and irradiation are known to inactivate suppressor cells in certain circumstances, we attempted to evaluate whether hydrocortisone in our system was merely inactivating naturally occurring suppressor cells and thus permitting spontaneous B cell activation. From Fig. 6, it is apparent that isolated B cells in culture without exogenous stimulation produce only low numbers of IgSC, as do isolated B cells treated with hydrocortisone (Fig. 4). The addition of T cells or irradiated T cells without any additional stimulant results in essentially no increase in immunoglobulin-producing cells. However, the combination of B cells and irradiated T cells is still extremely sensitive to stimulation with hydrocortisone, showing a marked enhancement of IgSC production. These data would suggest that glucocorticoid stimulation of immunoglobulin production in vitro is not the result of simple inactivation of a radiosensitive suppressor T cell population that ordinarily prevents spontaneous B cell maturation. However, this experiment does not exclude the possibility that a radioresistant T suppressor cell population could be inactivated by glucocorticoids, thus allowing spontaneous B cell maturation to active immunoglobulin production.

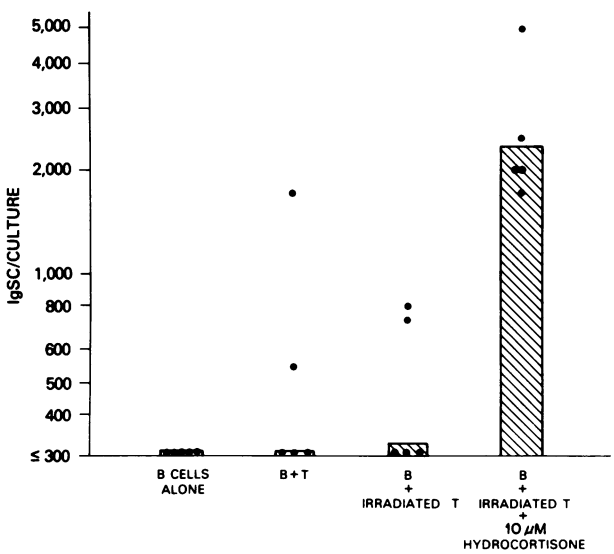

FIGURE 6 Effect of irradiation of T cells on immunoglobulin production.

In addition, to test the possibility that steroids might be lysing a population of suppressor cells, we incubated cultures for 24 h in the presence of 0.1 μM hydrocortisone, washed the cells, and examined the cultures for immunoglobulin production at 7 d. Hydrocortisone-pulsed cells did not demonstrate increased IgSC compared with controls [95 (1.60) vs. 47 (1.53)] in contrast with cultures exposed to hydrocortisone for 7 d [8586 (1.16)].

Effect of the serum source on steroid-induced immunoglobulin production. To determine whether the stimulation of immunoglobulin production by steroids was related to the particular serum source used to supplement the culture medium, six different lots of fetal calf serum were tested. Each of these FCS lots, as well as horse serum, was able to support steroid-induced immunoglobulin production. Table II shows data from two additional experiments demonstrating that this response can also be obtained when certain normal human serum samples are used to supplement the culture medium.

DISCUSSION

The polyclonal induction of immunoglobulin production in cultured human lymphocytes by glucocorticoids

TABLE II
Enhanced IgSC Production in the Presence of Human Serum

	IgSC/culture*
Experiment 1	
Media	110
PWM	9,280
Hydrocortisone 10 μM	1,040
Experiment 2	
Media	260
PWM	1,080
Hydrocortisone 10 μM	740

* 1×10^6 PBMC cultured in RPMI 1640 and 10% human serum and harvested on day 7. IgSC determined by RHPA.

in the absence of identifiable mitogenic or antigenic stimulation and in the absence of cellular proliferation is not easily explained within the context of most current theories of B cell differentiation and maturation. Therefore, it is essential to rule out potential trivial explanations for these findings.

The possibility that these observations represent artifacts of the RHPA such as plaques being produced by dead or dying immunoglobulin containing cells preferentially lysed by the steroids seems very remote. First, the IgSC detected in steroid-stimulated cultures appear late in culture and are T cell dependent. B cell cultures which should be enriched for immunoglobulin-containing cells do not respond to steroid stimulation with increased plaque formation unless T cell "help" is provided to the system. Furthermore, culture supernatants assayed by a double antibody radioimmunoassay demonstrate increased immunoglobulin accumulation in cultures similar to that seen with the reverse hemolytic plaque assay and the amount recovered after 12 d in culture far exceeds the amount recovered by lysis of the initial cell population and determination of intracellular immunoglobulin concentration.[3] Finally, incubation of the steroid-stimulated cultures for the final hour of culture with cycloheximide or puromycin, inhibitors of protein synthesis, almost completely abolished IgSC detectable in the reverse hemolytic plaque assay. Therefore, glucocorticoids are capable of inducing *de novo* synthesis of immunoglobulins of all classes in cultures of human peripheral blood lymphocytes.

A critical question is whether the culture conditions might be contributing some unidentified stimulus which synergizes with the steroids to promote immunoglobulin production. It is very difficult to exclude this possibility with certainty. FCS has stimulatory effects in many culture situations and contains several growth factors and probably endotoxin. Certainly "background proliferation" in cultures of human peripheral blood lymphocytes supplemented with FCS is often considerably higher than such "background proliferation" in cultures supplemented with normal human serum (NHS). For this reason, we tested whether corticosteroids would induce immunoglobulin production in cultures of human lymphocytes supplemented with NHS rather than FCS. As shown (Table II), certain cultures containing NHS could also respond with immunoglobulin production when hydrocortisone was added, suggesting that these observations were not the result of hydrocortisone providing a "permissive environment" for some cryptic mitogen present in FCS to induce B cell maturation. However, it must be noted that ~90% of human sera tested failed to support steroid-induced immunoglobulin production. Interestingly, mixtures of these nonsupportive human sera with FCS actually suppressed the expected steroid-induced response suggesting that these human sera are inhibitory rather than lacking the "cryptic mitogen." This inhibitory activity of human serum presumably explains the failure to demonstrate steroid-induced immunoglobulin production in systems employing media supplemented with pooled human serum (10).

The observation that steroids induce a marked increase in immunoglobulin production in culture without inducing significant cellular proliferation is also striking. Considerable evidence has been presented demonstrating that B cells may be induced to proliferate extensively, as with anti-immunoglobulin stimulation, without differentiating to produce immunoglobulin (18, 19). More recently, the differentiation or maturation of B cells to immunoglobulin production without concomitant proliferation has also been reported in both murine and human systems (20–23). In the mouse, evidence suggests that the differentiating cell population is separate from the proliferating one or that maturation occurs in two stages or requires two signals, with one stage proceeding in the absence of DNA synthesis (24–26). Studies with human lymphocytes evaluating this issue have yielded conflicting data. It appears that the activation of antigen specific B cells may result in immunoglobulin synthesis without a detectable proliferative response. Geha (27) demonstrated antibody synthesis without proliferation in human B cells in the presence of T cell helper factors stimulated with ragweed antigen. In addition, certain leukemic B cells appear to be able to mature to plasma cells in the presence of PWM and autologous T cells without concomitant cell division (28). Of course, one cannot totally rule out the possibility that proliferation could be occurring below the level of detection in these as well as in our system.

The experiments with a variety of steroid hormones of different structure clearly demonstrate that appropriate stimulation via the glucocorticoid receptor is essential for the immunoglobulin production response to occur. Androgens and estrogens are ineffective, while all of the steroids tested which had significant binding affinity for the glucocorticoid receptor were capable of inducing immunoglobulin production in these cultures. Such receptors have been demonstrated to be present on B cells, T cells, and monocytes (29, 30).

Glucocorticoids exert a variety of effects on cellular metabolism (31–33). However, the mechanisms by which steroids act are still not completely defined, although the model most consistent with much of the current data suggests that glucocorticoids freely enter the cell and bind to their specific steroid-

[3] Grayson, J., Blaese, R. M., and Waldmann, T. A. Unpublished data.

binding receptor in the cytoplasm. This complex is then transported to the nucleus and signals RNA synthesis. In humans, the number of glucocorticoid receptors of a given cell does not appear to correlate with its sensitivity to the effects of steroids (29, 34). Thus, although B cells, T cells, and monocytes all contain large numbers of glucocorticoid receptors, they do not appear to be equally affected by glucocorticoids. Studies are currently in progress in our laboratory to determine whether B cells, T cells, or monocytes are the primary targets mediating glucocorticoid stimulation of immunoglobulin production.

It has been repeatedly observed that glucocorticoids may exert profound effects on certain T cell functions while leaving other T cell functions totally intact. In steroid sensitive species such as the mouse, corticosteroids lyse the majority of thymocytes leaving only a small population of functionally active cells that behave quite differently from the entire thymocyte population in many respects. In steroid-resistant species such as man, corticosteroids have also been shown to have striking differential effects on functionally distinct lymphocyte subpopulations. Waldmann et al. (11) demonstrated that suppressor T cells from patients with common variable hypogammaglobulinemia could be inactivated in vitro by the addition of hydrocortisone to the cultures, and that helper T cell activity was preserved in these cultures. Similar steroid-sensitive T cells have been demonstrated in a variety of pathologic and physiologic situations in man, including studies of patients with infectious mononucleosis (12), in vitro immunoglobulin production by cord blood lymphocytes (35), and in mixtures of normal lymphocytes at high T:B ratios where suppression is commonly seen. We have previously shown that low dose hydrocortisone (1 μM) blocks proliferation in the autologous mixed lymphocyte reaction (MLR) while not affecting the allogeneic MLR (36) and the proliferating cell in the autologous MLR has been demonstrated to be a suppressor cell in some systems (37). Thus, since suppressor T cells appear to be particularly sensitive to inactivation by glucocorticoids, the possibility must be considered that the steroids in our experiments are inactivating a natural suppressor T cell which is important in preventing the spontaneous maturation of B cells to immunoglobulin production. There is scant evidence supporting "spontaneous maturation" but no entirely satisfactory system has been used to study this issue. In our experiments, isolated B cells cultured with or without steroids did not spontaneously mature into IgSC. In most systems in which T suppressor cells are inactivated by steroids, they are also preferentially inactivated by irradiation (e.g. common variable hypogammaglobulinemia, infectious mononucleosis, newborns). However, purified B cells mixed with irradiated T cells as a source of suppressor-depleted T cell help also did not spontaneously mature to secrete immunoglobulins. If glucocorticoids were added to the mixture of B cells and irradiated T cells, augmented maturation of the B cells to immunoglobulin secretion was seen. This does not rule out the possibility that a radioresistant, steroid-sensitive suppressor T cell could be responsible for preventing spontaneous B cell maturation, but such a postulated suppressor cell must have properties quite different from those of suppressor cells defined to date. Perhaps experiments employing T helper factors in place of intact T cell populations mixed with steroid-stimulated B cells will resolve this issue.

Another potential explanation for these observations is that glucocorticoids are important for the normal maturation process of B cells to become IgSC. In this case, the cells which mature to immunoglobulin production in vitro with steroid stimulation were primed "in vivo" and are merely completing their normal sequence of maturation in a (now) more appropriate in vitro environment (i.e., one containing glucocorticoids). If this were the case, glucocorticoids might be expected to promote a consistent early increase in immunoglobulin production since B cells stimulated in vivo several days earlier would be closer to active immunoglobulin synthesis when put into culture than cells activated just before being placed in culture. However, the kinetics of in vitro steroid-induced immunoglobulin production do not match such a prediction and immunoglobulin synthesis actually lags behind the response elicited by mitogens such as PWM. Furthermore, the continued T cell dependence of this response in vitro makes "in vivo" activation of these cells less likely since in many systems, helper T cell influence is required for only a relatively brief period early in the course of B cell activation.

If glucocorticoids do not play an essential role in normal B cell maturation, it remains a possibility that these hormones are able to induce the maturation of certain cellular metabolic pathways in B cells as they do in other types of cells, and that this altered state of cellular metabolism results in the facilitated immunoglobulin production observed. It is becoming increasingly apparent that large numbers of B cells may be stimulated to differentiate morphologically into immunoglobulin-containing cells detectable by immunofluorescence without a concomitant increase in IgSC detectable by the reverse hemolytic plaque assay. From our experience and that of others (38), the number of high rate IgSC may represent only 10–25% of cells identifiable as containing intracytoplasmic immunoglobulin in cultures of human lymphocytes stimulated with PWM. In addition, the number of lymphocytes with intracytoplasmic immunoglobulin in control cultures (i.e., no mitogen added) is also often much higher than the "background" level of IgSC and

more nearly approaches the number of IgSC seen in steroid containing cultures. These cytoplasmic immunoglobulin-containing cells which do not secrete enough immunoglobulin to be detected in the reverse hemolytic plaque assay may represent senescent cells or perhaps abortively differentiated B cells or even a type of B memory cell. Experiments are in progress to determine if steroids alter the ratio of high rate IgSC to cells containing intracytoplasmic immunoglobulin as detected by immunofluorescence. If steroids were to induce a higher ratio of immunoglobulin secretion by this type of B cell, then the lack of proliferation might be more easily understood. As potential support for the concept that steroids are inducing immunoglobulin secretion by a more mature B cell is the observation that human cord blood lymphocytes do not respond to steroid stimulation with increased IgSC in culture (39). Cord blood B cells are immature in that they respond less well than adult B cells to in vitro stimulation with PWM or EBV, they are restricted to production of IgM, and of course, they should have few memory B cells. Furthermore, the steroid-induced response in adult lymphocytes tends to parallel expected memory B cell frequency. For example, normal subjects produced little IgE in cultures stimulated with PWM, EBV, or hydrocortisone. However, glucocorticoids induce a high level of *de novo* IgE synthesis in lymphocytes from some atopic patients, even though stimulation with PWM or EBV fails to induce such synthesis.[3]

These observations of the capacity of steroid hormones at physiologic concentrations in vitro to induce the maturation of certain B cells to actively secrete immunoglobulin obviously raises a great many questions regarding the potential role of these hormones in the normal function of the immune system and in particular, the maturation and differentiation of B cells and the cellular cooperative events associated with these B cell changes. Although there are few answers to these questions as yet, recognition of the presence of this hormone effect may be essential for a clearer understanding of many experimental systems addressing basic questions of differentiation and maturation of immunologic functions.

REFERENCES

1. Yu, D. T. Y., P. J. Clements, H. E. Paulus, J. B. Peter, J. Levy, and E. V. Barnett. 1974. Human lymphocyte subpopulations. Effect of corticosteroids. *J. Clin. Invest.* **53:** 565–571.
2. Fauci, A. S., and D. C. Dale. 1974. The effect of in vivo hydrocortisone on subpopulations of human lymphocytes. *J. Clin. Invest.* **53:** 240–246.
3. Saxon, A., R. H. Stevens, S. J. Ramer, P. J. Clements, and D. T. Y. Yu. 1978. Glucocorticoids administered in vivo inhibit human suppressor T lymphocyte function and diminish B lymphocyte responsiveness in in vitro immunoglobulin synthesis. *J. Clin. Invest.* **61:** 922–930.
4. Schwartz, R. S. 1968. Immunosuppressive drug therapy. *In* Human Transplantation. F. T. Rapaport and J. Dausett, editors. Grune & Stratton, Inc., New York. **28:** 440–471.
5. Butler, W. T., and R. D. Rossen. 1973. Effects of corticosteroids on immunity in man. Decreased serum IgG concentration caused by 3 or 5 days of high doses of methylprednisolone. *J. Clin. Invest.* **52:** 2629–2640.
6. Settipane, G. A., R. K. Pudupakkam, and J. H. McGowan. 1978. Corticosteroid effect on immunoglobulin. *J. Allergy Clin. Immunol.* **62:** 162–166.
7. Tuchinda, M., R. W. Newcomb, and B. L. DeVald. 1972. Effect of prednisone treatment on the human immune response to keyhole limpet hemocyanin. *Int. Arch. Allergy.* **42:** 533–544.
8. Rusu, U. M., and M. D. Cooper. 1975. *In vivo* effects of cortisone on the B cell line in chickens. *J. Immunol.* **115:** 1370–1374.
9. Smith, R. S., N. A. Sherrman, and E. Middleton. 1972. Effect of hydrocortisone on immunoglobulin synthesis and secretion by human peripheral lymphocytes *in vitro*. *Int. Arch. Allergy.* **43:** 859–870.
10. Fauci, A. S., K. R. Pratt, and G. Whalen. 1977. Activation of human B lymphocytes. IV. Regulatory effects of corticosteroids on the triggering signal in the plaque-forming cell response of human peripheral blood B lymphocytes to polyclonal activation. *J. Immunol.* **119:** 598–603.
11. Waldmann, T. A., S. Broder, R. Krakauer, R. P. MacDermott, M. Durm, C. Goldman, and B. Meade. 1976. The role of suppressor cells in the pathogenesis of common variable hypogammaglobulinemia and the immunodeficiency associated with myeloma. *Fed. Proc.* **35:** 2067–2072.
12. Tosato, G., I. Magrath, I. Koski, N. Dooley, and R. M. Blaese. 1979. Activation of suppressor T cells during Epstein-Barr virus induced infectious mononucleosis. *N. Engl. J. Med.* **301:** 1133–1137.
13. Pellegrino, M. A., S. Ferrone, M. P. Dietrich, and R. A. Reisfeld. 1975. Enhancement of sheep red blood cell human lymphocyte rosette formation by the sulfhydryl compound 2-amino-ethylisothiouronium. *Clin. Immunol. Immunopathol.* **3:** 324–327.
14. Raynaud, J. P. 1977. R5020, a tag for the progestin receptor. in Progress in Cancer Research and Therapy. Vol. 4. McGuire, W. L., J. P. Raynaud, and E. E. Baulieu, editors. Raven Press, New York. 9–21.
15. Lawrence, E. C., R. M. Blaese, R. R. Martin, and P. M. Stevens. 1978. Immunoglobulin secreting cells in normal human bronchial lavage fluids. *J. Clin. Invest.* **62:** 832–835.
16. Koski, I. R., D. G. Poplack, and R. M. Blaese. 1976. A nonspecific esterase stain for the identification of monocytes and macrophages. in In vitro method in cell-mediated and tumor immunity. B. R. Bloom, and J. R. David editors. Academic Press, Inc., New York 358–362.
17. Samuels, H. H., and G. M. Tomkins. 1970. Relation of steroid structure to enzyme induction in hepatoma tissue cultured cells. *J. Mol. Biol.* **52:** 57–74.
18. Vitetta, E., E. Pure, P. Isakson, L. Buck, and J. Uhr. 1980. The activation of murine B cells: the role of surface immunoglobulins. *Immunol. Rev.* **52:** 211–230.
19. Finkelman, F., and P. Lipsky. 1979. The role of cell membrance immunoglobulin in primate B lymphocyte differentiation. *Immunol. Rev.* **45:** 117–139.
20. Andersson, J., and F. Melchers. 1974. Maturation of mitogen-activated bone marrow-derived lymphocytes in the absence of proliferation. *Eur. J. Immunol.* **4:** 533–539.

21. Attallah, A. M., T. J. Yeatman, P. D. Noguchi, and J. C. Petriciani. 1979. Is DNA synthesis a requisite for the differentiation of B lymphocytes into immunoglobulin-secreting plasma cells. *Int. Arch. All. Appl. Immunol.* **60:** 132–139.
22. Mudawwar, F., E. Yunis, and R. S. Geha. 1978. Antigen-specific helper factor in man. *J. Exp. Med.* **148:** 1032–1043.
23. Chen, W. Y., J. Munoz, H. H. Fudenberg, E. Tung, and G. Virella. 1981. Polyclonal activation of human peripheral blood B lymphocytes by formaldehyde-fixed Salmonella paratyphi B. I. Immunoglobulin production without DNA synthesis. *J. Exp. Med.* **153:** 365–374.
24. Vitetta, E. S., J. Forman, and J. R. Kettman. 1976. Cell surface immunoglobulin. VIII Functional differences of B lymphocytes bearing different surface immunoglobulin isotypes. *J. Exp. Med.* **143:** 1055–1066.
25. Gronowicz E., and A. Coutinho. 1975. Selective triggering of B cell subpopulations by mitogens. *Eur. J. Immunol.* **4:** 771–776.
26. Andersson, J., A. Coutinho, and F. Melchers. 1979. Mitogen-activated B cell blasts reactive to more than one mitogen. *J. Exp. Med.* **149:** 553–564.
27. Geha, R. S., F. Mudawwar, and E. Schneeberger. 1977. The specificity of T cell helper factor in man. *J. Exp. Med.* **145:** 1436–1448.
28. Fu., S. M., N. Chiorazzi, H. G. Kunkel, J. P. Halper, and S. R. Harris. 1978. Induction of *in vitro* differentiation and immunoglobulin synthesis of human leukemia B lymphocytes. *J. Exp. Med.* **148:** 1570–1578.
29. Lippman, M. E., and R. Barr. 1977. Glucocorticoid receptors in purified subpopulations of human peripheral blood lymphocytes. *J. Immunol.* **118:** 1977–1981.
30. Werb, Z., R. Foley, and A. Munck. 1978. Interaction of glucocorticoids with macrophages. Identification of glucocorticoid receptors in monocytes and macrophages. *J. Exp. Med.* **147:** 1684–1694.
31. Thompson, E. B. and M. E. Lippman. 1974. Mechanism of action of glucocorticoids. *Metab. Clin. Exp.* **23:** 159–203.
32. Werthamer, S., C. Hicks, and L. Amaral. 1969. Protein synthesis in human leukocytes and lymphocytes: 1. Effect of steroids and sterols. *Blood.* **34:** 348–356.
33. Tormey, D. C., H. H. Fudenberg, and R. M. Kamin. 1967. Effect of prednisolone on synthesis of DNA and RNA by human lymphocytes *in vitro*. *Nature (Lond.).* **213:** 281–282.
34. Fauci, A. S., T. Murakami, D. Brandon, D. L. Loriaux, and M. Lipsett. 1980. Mechanisms of corticosteroid action on lymphocyte subpopulations. Lack of correlation between glucocorticoid receptors and the differential effects of glucocorticoids on T cell subpopulations. *Cell. Immunol.* **49:** 43–50.
35. Tosato, G., I. T. Magrath, I. R. Koski, N. J. Dooley, and R. M. Blaese. 1980. B cell differentiation and immunoregulatory T cell function in human cord blood lymphocytes. *J. Clin. Invest.* **66:** 383–388.
36. Ilfeld, D. N., R. S. Krakauer, and R. M. Blaese. 1977. Suppression of the human autologous mixed lymphocyte reaction by physiologic concentrations of hydrocortisone. *J. Immunol.* **119:** 428–434.
37. Sakane, T., A. D. Steinberg, and I. Green. 1978. Failure of autologous mixed lymphocyte reactions between T and non-T cells in patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. U. S. A.* **75:** 3464–3468.
38. Ginsburg, W., F. D. Finkelman, and P. E. Lipsky. 1978. Circulating and mitogen-induced immunoglobulin-secreting cells in human peripheral blood: Evaluation by a modified reverse hemolytic plaque assay. *J. Immunol.* **120:** 33–39.
39. Grayson, J., N. J. Dooley, I. R. Koski, and R. M. Blaese. 1981. Effects of steroid hormones on immunoglobulin production in adult and cord blood lymphocytes. *Pediatr. Res.* **15:** 596.