Glucocorticoids Administered In Vivo Inhibit Human Suppressor T Lymphocyte Function and Diminish B Lymphocyte Responsiveness in In Vitro Immunoglobulin Synthesis

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ABSTRACT The effects of corticosteroid given in vivo on human lymphocyte subpopulation function were investigated using an in vitro system of pokeweek mitogen-stimulated immunoglobulin production. Peripheral blood lymphocytes were obtained from normal volunteers before and 4 h after the intravenous administration of methylprednisolone. Unfractioned peripheral blood lymphocytes showed a consistent decrease (mean ≅ 50%) in immunoglobulin and total protein synthesis after steroid administration. Utilizing separated thymus-derived (T) and bone marrow-derived (B) lymphocyte fractions, the pathophysiology of this alteration in immunoglobulin production was elucidated. B lymphocytes obtained after steroid treatment showed a markedly diminished immunoglobulin response (20% of normal) to normal T lymphocytes and to normal T cells that had been irradiated to remove suppressor T lymphocyte function. All major classes of immunoglobulin (IgG, IgM, and IgA) were affected. T lymphocytes procured after steroid administration were capable of providing normal amounts of T cell help for B cells in immunoglobulin production. However, suppressor T lymphocyte activity, observed with normal T lymphocytes at high T to B cell ratios, was absent from the poststeroid T lymphocytes. This loss of suppressor T lymphocyte function was not due to the presence of excess help as irradiated pre- and poststeroid T cells provided equal amounts of helper activity. On recombining the poststeroid treatment B cells, which are hyporesponsive in immunoglobulin synthesis, with the posttreatment T lymphocytes, which lack suppressor activity, diminished amounts of immunoglobulin were produced which correlate well with the effects observed with unseparated cells. Thus, corticosteroids have differential effects on the lymphocyte populations involved in immunoglobulin biosynthesis. B cell responsiveness is diminished, suppressor T lymphocyte activity is removed, and helper T lymphocyte function is unaffected.

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INTRODUCTION

Glucocorticoid hormones are the most important immunoregulatory agents available for use in humans and have been so for nearly 30 yr. Yet the mechanisms through which steroids exert their effects are obscure. Many reports have appeared demonstrating alterations of lymphocyte activity in vitro when incubated with steroids (1–6). However, these lymphocyte activities such as mitogen- or antigen-induced proliferation, lymphokine production, or development of cytotoxicity are now appreciated to be the end result of complex cell-cell interactions (macrophage-T, T-B, T-T, etc.) (7–9). Thus, the observed effect of steroids on such in vitro functions might result from the drugs' interaction with one or more of the participating cell types.

Even less is known about the cellular effects of steroids in vivo on components of the immune response. The continued use of these agents in man is associated with a loss of delayed hypersensitivity on skin testing (10) as well as decrease in immunoglobulin (Ig)[1] (11–13). Again the level of these functions (Ig

[1] *Abbreviations used in this paper*: Ig, immunoglobulin; PBL, mononuclear cell-rich peripheral blood leukocyte fraction; PWM, pokeweed mitogen; SDS, sodium dodecyl sulfate.

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production and delayed hypersensitivity) reflects not the sole activity of a single cell type but is the end result of immune system cell interactions. However, it is in diseases associated with alterations of these immune functions (i.e. decreased delayed hypersensitivity-sarcoidosis, excessive restricted antibody production-systemic lupus erythematosus and idiopathic thrombcytopenic purpura) that steroids have their most beneficial effect. To understand the actions of steroids in such conditions, it is necessary to evaluate the in vivo as well as the in vitro effect of glucocorticoids on the various immune cell populations. To this end we have examined the effect of glucocorticoids in vivo on the ability of human peripheral blood lymphocytes to synthesize Ig when stimulated in vitro with pokeweed mitogen (PWM). We have previously shown that by using separated B and T lymphocyte subpopulations in this system we can define B lymphocyte function, helper T lymphocyte function, and suppressor T lymphocyte activity (14). Furthermore, we have shown that the helper and suppressor T lymphocytes are distinct and separate populations.[2,3] Moretta et al. (15) have recently reported the physical separation of these T helper and T suppressor lymphocytes on the basis of their receptors for the Fc portion of IgM (helper) or IgG (suppressor). By analyzing the contribution of each of these lymphocyte subpopulations, we were able to define dual and opposing effects of glucocorticoid on Ig production. Methylprednisolone administered in vivo reduced the ability of B cells to respond in vitro to helper T lymphocytes. At the same time, this agent completely removed suppressor T lymphocyte activity. The modestly diminished Ig biosynthesis by unseparated mononuclear cell-rich peripheral blood leukocyte fraction (PBL) observed after in vivo steroid treatment can now be appreciated to be the result of the interplay between these two forces.

METHODS

Lymphocyte donors. Blood was drawn between 8–9 a.m. by venapuncture from healthy human volunteers whose ages ranged from 26- to 36-yr old. Methylprednisolone, 48.5 mg, (equivalent to 60 mg prednisone, kindly supplied by Upjohn Company, Kalamazoo, Mich.) was dissolved in reconstituting solution and then given i.v. over 1 min. 4 h later a further blood sample was obtained. In control experiments the exact same procedure was followed except that the subjects were injected with an equivalent volume of reconstituting solution free of methylprednisolone. Consent was obtained from all donors before entry into the tests. The protocol was approved by the Human Subject Protection Committee of the University of California, Los Angeles.

Cell characterization and quantitation. Leukocytes differential counts of the blood samples were assayed by standard techniques as described (16). T lymphocytes were recognized by their ability to form spontaneous sheep erythrocyte rosettes. Cells with receptors for the Fc portion of IgG were assayed by detection of binding aggregates of human Ig with subsequent indirect immunofluorescence. Complement receptor cells were enumerated by rosette formation with complement-coated zymosan particles. Cells with true membrane inserted Ig were detected by direct fluorescence after incubation at 37°C for 1 h with subsequent washing to remove cytophilic Ig from Fc receptors. These procedures are reported in detail elsewhere (16, 17). The absolute concentration of each population in the blood was calculated by multiplying the percent of the subpopulation by the absolute lymphocyte concentration.

Lymphocyte preparation. PBL was prepared by Ficoll-Hypaque (Ficoll, Pharmacia Fine Chemicals Inc., Piscataway, N. J.; Hypaque, Winthrop Laboratories, Evanston, Ill.) density sedimentation of heparinized peripheral blood. This fraction contained >95% mononuclear cells. The mononuclear fraction was subsequently separated into T and non-T cell populations by a modification of the sheep erythrocyte T lymphocyte rosette density separation technique. The sheep erythrocytes were pretreated with 2-aminoethylisothiuronium bromide hydrobromide before rosette formation (18). This technique yields a purified T lymphocyte preparation with insufficient B lymphocytes (1%) to demonstrate Ig production when up to 4×10^6 T cells were cultured (14). The B lymphocyte fraction contained between 1 and 5% T lymphocytes and 22–35% monocytes. Both the pre- and posttreatment PBL preparations yielded T and B fractions of equal purity as assessed by surface marker analysis. The presence of this low number of T lymphocytes permits a low level of B lymphocyte Ig production to occur; in the total absence of T lymphocytes (≤1%), the B cell fraction fails to synthesize any Ig (14). Irradiation (3,000 R) of lymphocytes was accomplished using a cobalt source in the Department of Radiologic Sciences at the Center for the Health Sciences, UCLA. Immediately after irradiation, the cells were washed twice in fresh medium.

Culture conditions. Either unfractionated, fractionated, or fractionated and recombined PBL were cultured in RPMI-1640 medium (Grand Island Biological Co., Grand Island, N. Y.) buffered with $NaHCO_2$ and supplemented with L-glutamine (10 mM), gentamicin (0.01%), and 15% heat-inactivated fetal calf serum. All cultures were done in a final volume of 1.5 ml in 13 × 100 mm plastic tubes (Falcon 2027 Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) and contained PWM (Grand Island Biological Co.) at a final dilution of 1/100 vol/vol. The tubes were incubated in a humidified atmosphere at 37°C with 5% CO_2 for 5 days. The cells were then centrifuged into a pellet, the medium was recovered, and the cells were resuspended in 0.5 ml of medium deficient in nonradioactive methionine and supplemented with ^{35}S-methionine (50 μCi/ml, 400 Ci/mM, New England Nuclear, Boston, Mass.). The cultures were incubated for a further 16 h.

Measurement of synthesized immunoglobulin. After the final incubation, duplicate cultures were centrifuged (500 *g* for 10 min), the culture medium removed, and the lymphocytes lysed with 1 ml lysis buffer (0.5% NP-40, 50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, 0.02% NaN_3, 5 mM KI, 2 mM methionine, 200 mM iodacetamide, 20 mM ε-amino caproic acid, pH 7.4). Nuclear and cellular debris

[2] Saxon, A., and R. Stevens. 1978. Suppression of immunoglobulin production in normal human blood: characterization of the cells responsible and mediation by a soluble T lymphocyte-derived factor. *Clin. Immunol. Immunopathol.* In press.

[3] Stevens, R. H., and A. Saxon. 1978. Anti-human helper T lymphocyte antiserum: generation and functional characterization. *Clin. Immunol. Immunopathol.* In press.

were removed by high speed centrifugation (20,000 *g* for 30 min). 10-ml aliquots of the resulting cytoplasmic supernate were precipitated with trichloroacetic acid (10%) to obtain total radioactive protein determinations. Remaining culture medium and cytoplasmic supernates were divided equally into tubes containing 5 μl of polyvalent anti-human Ig antisera and incubated for 20 min at room temperature. The resulting antigen-antibody complexes were removed by formalin-fixed *Staphylococcus aureus* bacteria (containing protein A which binds to the Fc portion of IgG) by the method of Kessler (19). After four washes the radioactive proteins were eluted with 100 μl of 4% sodium dodecyl sulfate (SDS)-6 M urea. 20 ml of the above radioactive protein solutions were precipitated with trichloroacetic acid and the radioactivity determined by liquid scintillation counting. The remainder of the radioactive samples were either directly analyzed by 10% SDS-polyacrylamide gels (acrylamide, 2,2-diallyltartaramide) (SDS-polyacrylamide gel electrophoresis) or reduced in dithiothreitol (0.05 M) and alkylated with iodoacetamide (0.1 M) before electrophoretic analysis. After electrophoresis, the radioactive Ig molecules were visualized by fluoroautoradiography (20). A marker sample containing radioiodinated (^{125}I) mu (μ), gamma (γ), and light chains was electrophoresed in parallel with each gel run for molecular weight determinations. Statistical analysis was performed using Student's *t* test.

RESULTS

The effect of methylprednisolone on the percent and number of lymphocytes in various subpopulations was measured (Table I). This information is critical to the understanding of levels of Ig synthesis observed in unfractioned PBL. We have previously shown that the Ig synthesis observed in unfractionated PBL reflects an interaction dependent on the B to T cell ratio (14). Thus, simple alterations in this B to T ratio without other effects would be expected to influence the amount of Ig synthesized per culture. 4 h after i.v. methylprednisolone administration, there was an absolute lymphopenia (average 50% decrease). The percent of T lymphocytes remaining was also consistently depressed ($P < 0.01$) leading to a marked fall in absolute T lymphocytes. The percent of B lymphocytes, Fc receptor cells, and complement receptor cells were increased after drug administration ($P < 0.025$, $P < 0.01$, and $P < 0.025$ respectively) but the absolute number of circulating cells in these populations was decreased due to the profound lymphopenia. The ratio of non-T to T cells after methylprednisolone administration was elevated from 0.14 to 0.27. Similar effects did not occur when subjects were injected with control dilutant only ($P > 0.05$ for all groups). These observations are in accord with observations we have reported (16).

Unfractionated PBL (2×10^6 cells), obtained at the same time as the studies reported above, were tested for their ability to synthesize Ig and cell protein. For each experiment the secreted Ig, cytoplasmic Ig, and total cell protein for PBL from the same donor before methylprednisolone administration were taken as 100%. The values for these same measurements from PBL obtained 4 h after steroid injection were then compared to the same individuals' base-line values (100%) (Fig. 1). The posttreatment PBL consistently demonstrated depressed Ig synthesis, both secreted (mean 50%, range 24–86%) and cytoplasmic (mean 56%, range 25–85%) as well as a similar decrease in total protein production (mean 54%, range 30–89%). PBL from con-

TABLE I

Effect of Methylprednisolone on the Circulating Lymphocyte Subpopulations

	Pretreatment				Posttreatment				Pretreatment				Posttreatment			
Subject	T*	Fc‡	C'§	MIg‖	T	Fc	C'	MIg	T	Fc	C'	MIg	T	Fc	C'	MIg
	%								*Absolute no./mm³*							
1	72.5	21.6	17.6	9.9	—	36.5	27.3	22.6	3,081	918	748	421	—	299	224	185
2	67.3	24.5	19.9	15.5	55.3	37.6	26.6	18.9	2,395	872	708	552	780	530	375	226
3	71.7	13.1	10.0	7.5	63.3	29.2	24.1	20.2	2,232	408	311	233	724	334	276	231
4	81.3	13.2	7.1	5.5	57.3	21.3	10.5	8.0	1,811	294	158	122	711	264	130	99
5	—	21.3	14.4	10.8	—	44.6	30.1	22.2	—	661	448	335	—	245	167	123
Mean	73.3	18.7	13.8	9.8	58.6	33.8	23.7	18.4	2,384	631	475	332	738	334	234	173
1 control	—	20.4	14.7	5.8	—	18.0	15.5	8.1	—	580	418	165	—	428	369	193
2 control	83.2	16.6	12.6	9.3	80.0	18.2	11.0	7.2	1,282	256	194	143	2,860	651	393	257
3 control	76.5	15.5	10.5	8.3	75.0	13.0	10.7	7.7	1,805	366	248	196	2,382	413	340	245
Mean	79.9	17.5	12.6	7.8	77.5	16.4	12.4	7.7	1,544	401	287	168	2,621	497	367	232

* T lymphocytes.

‡ Fc receptor cells.

§ Complement receptor cells.

‖ Membrane Ig-bearing lymphocytes.

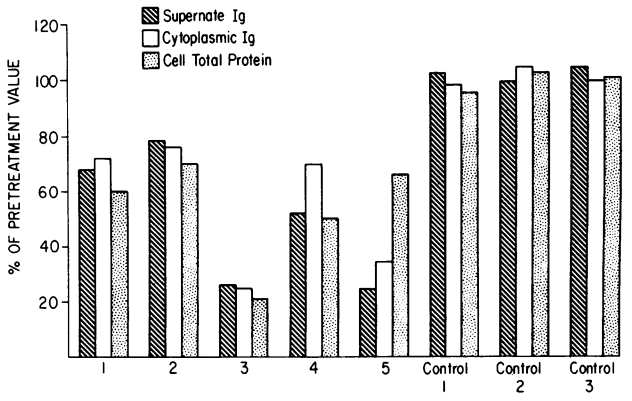

FIGURE 1 Effect in five donors of in vivo-administered steroid on the ability of 2×10^6 unfractionated PBL to produce secreted Ig (■), cytoplasmic Ig (□), and total protein (▨). The samples were obtained 4 h after methylprednisolone administration and compared to the values for these same measurements for each donor's PBL taken before drug administration (100% value). Controls (1–3) involved identical procedures except reconstituting fluid free of methylprednisolone was given.

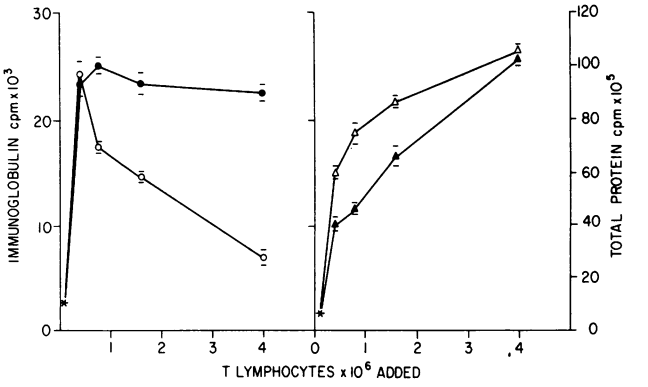

FIGURE 2 The effect of in vivo corticosteroid on circulating T lymphocytes' ability to regulate untreated B lymphocyte production of Ig. T lymphocytes, obtained either before (○, △) or 4 h after (●, ▲) steroid administration were added to a constant number (0.4×10^6) of pretreatment B fraction cells. The mean Ig (○, ●) and total protein (△, ▲) produced by these cultures are represented as well as the background levels for B fraction cells without T lymphocytes added (*). The actual values obtained with each of a pair of duplicate cultures is shown by the horizontal bars.

trol experiments did not show these alterations ($P < 0.025$ for cytoplasmic Ig, $P < 0.01$ for secreted Ig, and $P < 0.005$ for cell total protein).

The decreased Ig production could not be explained simply on the basis of the observed shift in B/T lymphocyte ratios (0.14–0.27) as this should yield cultures producing greater, not smaller, amounts of Ig (14). As we discussed in the Introduction, the depressed Ig production could have been due to steroids affecting any one or more of the interacting lymphocyte subpopulations.

To explore this question, PBL obtained from the same donor before and after (4 h) steroid injection were separated into T and B cell fractions and a series of crossover lymphocyte titrations were undertaken. The in vivo effect of methylprednisolone on peripheral blood T lymphocyte function is shown in Fig. 2. Pre- (normal) and posttreatment T lymphocytes were added in increasing numbers to a constant number of B fraction cells (0.4×10^6) obtained before steroid administration. The pretreatment T lymphocytes showed the normal dose response curve (14) consisting of a helper effect at low T lymphocyte numbers followed by a suppressor phase at higher T lymphocyte numbers. T lymphocytes obtained from the same individual after steroid treatment demonstrated an identical helper effect when added to the pretreatment B cells, but there was complete abrogation of suppressor T lymphocyte activity normally seen at high T cell numbers. Total protein synthesis by both sets of cultures were similar at high numbers but the posttreatment T cell cultures showed a somewhat diminished cell total protein at lower cell numbers (Fig. 2). Irradiation of normal T lymphocytes removes normal suppressor T lymphocyte activity (21).[2] When irradiated pre- and posttreatment T lymphocytes were added to normal B fraction cells, they gave identical levels of help with neither irradiated T population giving suppression (Table II). This demonstrated that removal of suppressor T lymphocyte activity from the pretreatment T cells by irradiation allowed them to now mimic the effect seen with irradiated T lymphocytes from the same donor 4 h after steroid treatment. Control values for unirradiated T populations are shown in Table III for reference.

To investigate the effect of the glucocorticoid treatment on the PBL B lymphocytes, B fraction cells obtained before and after injection of the drug were compared for their ability to produce Ig in collaboration

TABLE II
Effect of Irradiated T Lymphocytes Obtained Pre- and Poststeroid Injection on Pretreatment (Normal) B Cell (0.4×10^6) Ig Production

Time T lymphocytes obtained	T Lymphocytes	Ig	Total protein
	no.	*cpm × 10³*	*cpm × 10⁵*
0	0	2.4	9.7
Pre*	0.4	32.7	37.7
Post‡	0.4	33.6	30.7
Pre	1.6	51.6	75.1
Post	1.6	49.2	60.3
Pre	4.0	37.8	75.0
Post	4.0	36.5	73.7

* Before steroid administration.
‡ 4 h after steroid administration.

TABLE III

Effect of Untreated T Lymphocytes Obtained Pre- and Poststeroid Injection on Pretreatment (Normal) B Cell (0.4 × 10⁶) Ig Production

Time T lymphocytes obtained	T Lymphocytes	Ig	Total protein
	no.	*cpm × 10³*	*cpm × 10⁵*
0	0	2.4	9.7
Pre	0.4	36.1	66.0
Post	0.4	38.4	40.8
Pre	1.6	19.2	92.1
Post	1.6	41.7	80.4
Pre	4.0	4.9	130.6
Post	4.0	34.4	134.0

TABLE IV

Effect of Irradiated T Lymphocytes Obtained Presteroid Injection (Normal) on B Cells (0.4 × 10⁶) Obtained Pre- and Poststeroid Injection

Time B cells obtained	T Lymphocytes	Ig	Total protein
	no.	*cpm × 10³*	*cpm × 10⁵*
Pre*	0	2.4	9.7
Post‡	0	1.8	7.4
Pre	1.6	51.6	75.1
Post	1.6	7.4	29.6
Pre	4.0	37.8	75.0
Post	4.0	7.5	54.4

* Before steroid administration.

‡ 4 h after steroid administration.

with increasing numbers of untreated T lymphocytes (Fig. 3). The posttreatment B cells showed a reduced ability to respond with an Ig synthetic response, reaching only 25% of the Ig levels made by the B cells from the blood 4 h prior (Fig. 3). The reduced Ig synthesis could result from decreased ability of B lymphocytes to respond to T lymphocyte help or increased susceptibility to T lymphocyte suppression. To test these alternatives, the posttreatment B cells were mixed with irradiated untreated autologous T lymphocytes (which lack suppressor T lymphocyte activity) and were compared with the response of the pretreatment B cells (Table IV). Although the B cells procured after steroid infusion did increase their Ig production (approximately twofold) at both irradiated T lymphocyte levels, the levels remained 5- to 10-fold below that seen with B lymphocytes from the same individual before being given steroid.

The cumulative effect of these alterations in both circulating PBL B cell and T lymphocyte activity after treatment with methylprednisolone was investigated. Posttreatment T lymphocytes were titrated against a constant number of posttreatment B fraction cells and compared to the same titration using pretreatment T and B cells (Fig. 4). The posttreatment T lymphocytes again showed no suppressor effect when mixed with B

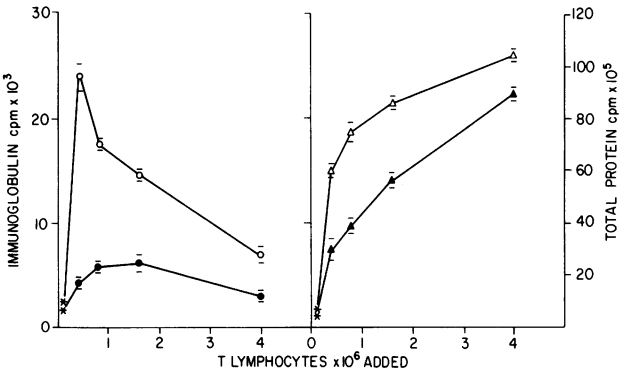

FIGURE 3 The effect of in vivo corticosteroid on circulating B lymphocytes' ability to produce Ig. An increasing number of untreated T lymphocytes was added to a constant number (0.4 × 10⁶) of B fraction cells obtained either before (○, △) or 4 h after (●, ▲) steroid administration. The mean Ig (○, ●) and total protein (△, ▲) produced by these cultures are shown. Background levels for Ig and total protein by the different B cell fractions alone are represented by asterisks (*). The actual values obtained with each of a pair of duplicate cultures is shown by the horizontal bars.

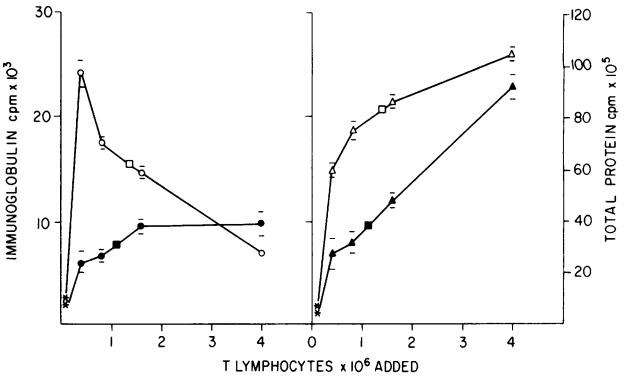

FIGURE 4 The cumulative effect on Ig production by in vivo corticosteroid treatment of B and T lymphocytes. Separated B and T cell populations, obtained 4 h after steroid treatment, were recombined (●, ▲, ■) and tested for their ability to produce Ig (●) and total protein (▲). Increasing numbers of T lymphocytes were added to a constant number (0.4 × 10⁶) of B cells. This was compared to an identical titration utilizing the same person's separated B and T lymphocyte populations obtained before steroid administration (○ = Ig, △ = total protein). Background levels for Ig and total protein are shown by asterisks (*). The predicted value for Ig and total protein production for unseparated PBL before (□) and after steroids (■) was also plotted by calculating untreated PBL to consist of 73% T lymphocytes and posttreatment PBL to contain 58% T lymphocytes (see Discussion). The symbols (●, ○, ▲, △) represent the mean values for the cultures while the actual experimental values obtained with each of a duplicate pair of cultures is shown by the horizontal bars.

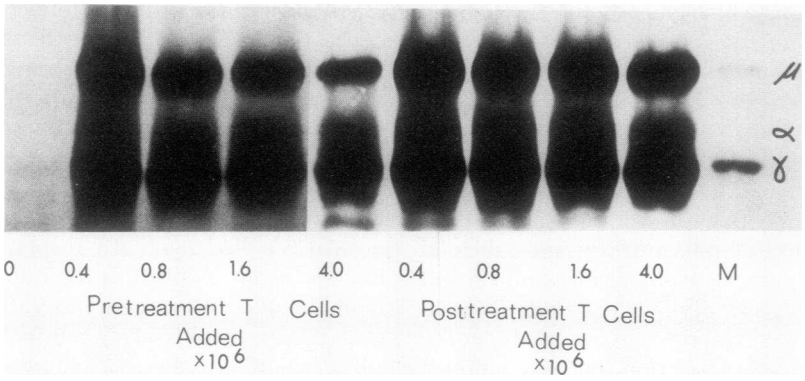

FIGURE 5 Ig heavy chains synthesized in vitro by a constant number (0.4×10^6) of untreated B fraction cells with increasing numbers of T lymphocytes obtained before (pretreatment) or 4 h after (posttreatment) methylprednisolone administration. The Ig molecules produced by B fraction cells alone is also shown (zero T cells added). The culture supernates were immune precipitated, reduced, alkylated, and run on 10% SDS polyacrylamide gels with subsequent fluoroautoradiography. Markers (M) consist of ^{125}I-labeled μ- and γ-chains. The position of α-chain is also shown.

cells obtained at the same time. The cumulative effect of mixing the hyporeactive B cells with the suppressor cell-free T cells was the production of a level of Ig and total protein higher than expected with normal (untreated) T lymphocytes but still lower than observed with B cells obtained before the subjects were given steroid.

Complete T and B lymphocyte titrations using pre- and posttreatment cells from the same person were performed on three individuals as well as on two separate occasions for the same donor. Each time the same effects were observed: loss of T suppressor cell function and inhibition of B cell responsiveness.

Subjects given steroids manifested changes in both B lymphocyte responsiveness and suppressor T lymphocyte activity. Whether these effects were manifest in alterations in all major Ig isotypes (IgM, IgG, and IgA) produced was analyzed. Radiolabeled Ig molecules produced from experiments were reduced, alkylated, and electrophoresed on 7.5% SDS polyacrylamide gels. The Ig molecules were then visualized by fluoroautoradiography. The poststeroid T lymphocytes which lacked suppressor activity demonstrated increased production of IgM, IgG, and IgA at high T cell numbers (Fig. 5). The poststeroid treatment B cells produced diminished amounts of all these isotypes (Fig. 6). Although allowing us to determine alterations in Ig isotypes between various points, this type of analysis is not quantitative enough to distinguish if there were differential effects on the amounts between the classes at any one point.

DISCUSSION

Previous reports have shown that after the administration of pharmacologic doses of corticosteroids, there is

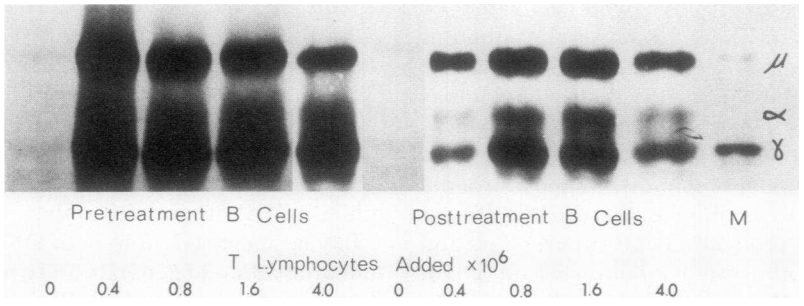

FIGURE 6 Ig heavy chains synthesized by a constant number (0.4×10^6) of B fraction cells obtained before (pretreatment) or 4 h after (posttreatment) methylprednisolone administration in the presence of increasing numbers of untreated (pretreatment) T lymphocytes. Ig molecules synthesized by B fraction cells in the absence of added T cells (0) are also shown. The culture supernates were immune precipitated, reduced and alkylated and run on 10% sodium dodecyl sulfate polyacrylamide gels with subsequent fluoroautoradiography. Markers (M) consist of ^{125}I-labeled μ- and γ-chains. The position of α-chain is also shown.

a profound lymphopenia which peaks between 3 and 6 h (16, 22). This lymphopenia is characterized by relative loss of circulating T cells leading to a downward shift in the observed PBL T/B cell ratio. From our previous work and others (8, 14, 23), such a shift in itself might be expected to lead to an increase in PWM-induced in vitro Ig production. However, PBL obtained after steroid injection showed a consistent decrease in secreted and cytoplasmic Ig and cell total protein. Theoretically, these observations could result from the loss of T helper lymphocyte activity, the enhancement of T suppressor lymphocyte activity, or the inhibition of B lymphocyte function. Because steroids are commonly thought of as immunosuppressive agents with greater effect on T cell functions (delayed hypersensitivity, cell-mediated reactions) than B cell function (Ig production), enhanced suppression or loss of help might be expected. However, through a series of separated T and B cell crossover experiments, these expectations were not confirmed. We demonstrated that T lymphocytes obtained 4 h after steroid administration had lost suppressor T cell activity present in the pretreatment (normal) T cells. The T helper lymphocyte activity in the pre- and poststeroid treatment samples was similar as shown by their ability (both unirradiated and irradiated) to help normal B cells reach equivalent maximums in Ig production.

The evidence showing a loss of T suppressor cells appears in contradiction to a recently reported study by Haynes and Fauci (24). They demonstrated that suppressor cells induced by concanavalin A were resistant to hydrocortisone in concentrations up to 0.1 mM. These suppressor cells were capable of inhibiting the plaque-forming cell response by human peripheral blood lymphocytes stimulated with PWM. There are several differences to be cognizant of between our studies. Most importantly their suppressor cells were induced by concanavalin A which appears to be a most potent stimulator of human suppressor cells (25–27) whereas our suppressor T cells were activated by PWM. The intensity of the concanavalin A stimulus toward suppression may be too great to be overcome by steroids whereas the weaker PWM stimulation may be more susceptible. However, both concanavalin A and PWM suppressor lymphocytes were sensitive to irradiation. Other differences include the exposure of the cells to steroid in vitro vs. in vivo, the use of different assays to measure Ig production and suppression, and the fact that our suppressor population was a T cell population whereas their suppressor cells could be found among multiple subpopulations of lymphoid cells. Possibly the concanavalin A-induced T suppressor lymphocyte is steroid sensitive as is the PWM-induced T suppressor but this effect was masked by other steroid-resistant suppressor cells activated by concanavalin A.

Steroids had a profound effect on the ability of B cells to produce Ig. The B cells obtained after steroid treatment only made 25% as much Ig as the pretreatment cells in response to normal T lymphocytes. This diminished Ig production was not due to an acquired hypersensitivity to normally present T suppressors. Irradiated T cells which lack T suppressors still could not stimulate the posttreatment B cells to approach a normal B cell Ig synthetic response. Thus, the B cells had an intrinsic defect in their ability to respond to T lymphocyte help with Ig biosynthesis. The combination of these dual effects seen in poststeroid treatment PBL, loss of T suppressor lymphocyte activity, and inhibition of B cell responsiveness, combine to give the result observed in unseparated PBL, a mean 50% decrease in Ig production. The experiments using combined posttreatment T and B lymphocytes showed a level of Ig and total protein synthesis in the poststeroid cells higher than expected if only the B cells were affected but lower than expected if only T suppressor activity were removed. These B/T titrations for pre- and posttreatment cells correlate well with the actual values obtained with unfractionated PBL. Unfractionated pretreatment PBL contained a mean of 73% T lymphocytes whereas posttreatment PBL had a mean of 58% T lymphocytes (Table I). With these percentages to calculate the number of T cells in 2×10^6 unfractionated PBL (for a standard culture), the predicted Ig and cell protein production by pre- or posttreatment unfractionated PBL can be plotted (Fig. 4). The predicted differences between the pre- and posttreatment values, 49% for Ig and 46% for cell protein, are in good agreement with the actual values obtained in unfractionated PBL experiments (Fig. 1).

The mechanism(s) through which corticosteroids bring about these differential effects on lymphocyte populations have not been directly investigated. However, steroids have been shown to inhibit the DNA synthetic response in lymphocytes (15). Studies by us as well as Seigal and Seigal (21) have shown that PWM-induced suppressor cells are dependent on DNA synthesis for their functional expression. T helper lymphocyte function, on the other hand, is DNA independent but depends on membrane activation (14). Thus, the differential need of T suppressors vs. T helpers for DNA synthesis may explain the observed steroid inhibition of T suppression only.

B lymphocytes require both DNA synthesis and protein production to generate an Ig response in the PWM driven in vitro system (14). B cells exposed to steroids in vitro have been reported elsewhere to show an inhibition of DNA response (16). We demonstrated that poststeroid treatment B cells also had a diminished total protein output as compared to pretreatment B cells. This evidence suggests that the mechanism of decreased DNA synthesis and subsequent diminished

B cell protein production on day 6 is operable in diminishing B cell responsiveness as it may be in inhibiting T suppressor cell function.

Another consideration is that corticosteroid receptors may not be uniformly represented on various T and (or) B cell subpopulations. Lippman and Barr (28) have recently reported that T and B lymphocytes have equal numbers of glucocorticoid receptors per cell and the receptor is uniform in its binding affinity. However, such populations consist of many subpopulations (i.e., T helper, T suppressor, T amplifier, B-T independent, and B-T dependent). Clearly steroid receptors may demonstrate differential representation on various functional subpopulations leading to differential susceptibility to glucocorticoid hormones.

The effects we observed with in vivo corticosteroid could be due to alterations in lymphocyte subpopulation activity in the blood as discussed above or could be due to a loss of reactive populations from the blood. Lymphocytes in man are "steroid-resistant" (29) and it is doubtful that steroids have caused a lysis of the affected cell populations. However, corticosteroids alter the representation of lymphocytes in the circulation by causing a redistribution of recirculating lymphocytes (30). Our results could be accounted for if suppressor T lymphocytes and a subpopulation of B cells more responsive in Ig production were selectively redistributed out of the circulation. A definitive answer to this awaits the establishment of an accepted marker for human T suppressor lymphocytes as well as a way of distinguishing the presence of possible B lymphocyte subpopulations. The evidence that glucocorticoids decrease in vivo Ig production and alter cell DNA suggests that the effects may not simply be due to redistribution effects.

Through whatever mechanisms, an in vivo pharmacologic dose of steroid has a profound effect on the immune function of Ig production. This results from the drug's interaction with and alteration of several of the immune cell populations involved in the final expression of this response; loss of T suppressor activity as well as inhibition of B cell responsiveness. In normal persons, the net effect was to decrease Ig production measured in vitro. However, in clinical situations, these effects may not be the same. In patients who already lack suppressor cell activity, as suggested in systemic lupus erythematosus (31), the main effect should be due to inhibition of B cell activity. The level of this effect might be magnified because T suppressor lymphocyte activity would already be lost. Indeed, Harrington (32) has presented evidence that pharmacologic doses of steroids (≥40 mg prednisolone/day) is associated with a decrease in antinuclear antibody titers to normal levels. On the other hand, in disease states such as sarcoidosis with anergy, where increased cellular suppression may play an important role in the hyporesponsive immune responses observed, steroids may increase immune responsiveness by interfering with this hypersuppression. Our data would give an explanation for the seemingly paradoxical result that has been observed in anergic sarcoid patients who do not react to intradermal tuberculin testing but will get a delayed hypersensitivity reaction if corticosteroids are injected locally with the tuberculin test (33). The locally injected steroids may inhibit T suppressor activity and thus allow for normal expression of delayed hypersensitivity. Similarly, in patients with common variable hypogammaglobulinemia solely on the basis of increased T suppressor lymphocyte activity, steroid inhibition of this suppression would be expected to overshadow their effect on B cell function and lead to increased Ig production. Indeed, Waldmann et al. (34) have reported increased Ig production in such a patient given steroids.

In clinical situations, steroids should be viewed as immunoregulatory agents, not immunosuppressive agents. Even considering only one immune function (Ig production), our report demonstrates how steroids, at one point in time, can have both enhancing and inhibitory actions. Studies as to the effects of chronically administered steroids in vivo on immune participating cells clearly need to be undertaken. The net result of steroid therapy, suppression or potentiation in any clinical setting will depend on the pathophysiology of the homeostasis achieved in that disease state before initiation of therapy. The immunopathophysiology of different disease states needs to be elucidated as well as the effects of steroids on components in various immune functions (as we have done for Ig production) to dissect the therapeutic role of steroids in human illnesses.

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