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Anthony S. Fauci, David C. Dale

J Clin Invest. 1974;53(1):240-246. <https://doi.org/10.1172/JCI107544>.

Research Article

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The Effect of In Vivo Hydrocortisone on Subpopulations of Human Lymphocytes

ANTHONY S. FAUCI and DAVID C. DALE

*From the Laboratory of Clinical Investigation, National Institute of Allergy
and Infectious Diseases, National Institutes of Health,
Bethesda, Maryland 20014*

ABSTRACT This study was designed to determine the effect of in vivo hydrocortisone on subpopulations of lymphoid cells in normal humans. Subjects received a single intravenous dose of either 100 mg or 400 mg of hydrocortisone, and blood was drawn at hourly intervals for 6 h, and then again at 10 and 24 h after injection. Profound decreases in absolute numbers of circulating lymphocytes and monocytes occurred at 4–6 h after both 100 mg and 400 mg of hydrocortisone. Counts returned to normal by 24 h. The relative proportion of circulating thymus-derived lymphocytes as measured by the sheep red blood cell rosette assay decreased maximally by 4 h and returned to base line by 24 h after hydrocortisone. There was a selective depletion of functional subpopulations of lymphocytes as represented by differential effects on in vitro stimulation with various mitogens and antigens. Phytohaemagglutinin response was relatively unaffected, while responses to concanavalin A were significantly diminished. Responses to pokeweed mitogen were unaffected by 100 mg of hydrocortisone, but greatly diminished by 400 mg of hydrocortisone. In vitro responses to the antigens streptokinase-streptodornase and tetanus toxoid were markedly diminished by in vivo hydrocortisone. Reconstitution of monocyte-depleted cultures with autologous monocytes partially corrected the diminished response to antigens. This transient selective depletion of monocytes and subsets of human lymphocytes by a single dose of hydrocortisone is most compatible with a redistribution of these cells out of the circulation into other body compartments.

INTRODUCTION

Corticosteroids are widely used in clinical medicine as chemotherapeutic agents in the treatment of a diversity

Presented in part at the National Meeting of the American Federation for Clinical Research, 29 April 1973, Atlantic City, N. J.

Received for publication 14 June 1973 and in revised form 10 August 1973.

of diseases, especially those in which inflammatory or immunological phenomena are believed to play a predominant role (1). Despite this, little is known about the precise mechanisms of action of corticosteroids on the immune response in humans (1, 2). Several immunosuppressive effects of these agents have been observed to occur in man, including mild lymphocytopenia (3), decreased immunoglobulin production (4), and impaired expression of cutaneous delayed hypersensitivity responses (5). The striking heterogeneity of lymphoid cell populations within different species including man has been the subject of recent intense study (6). In some animal species, corticosteroids have been shown to have differential effects on subpopulations of lymphocytes (7, 8).

In man, different mitogens have been shown to differentially stimulate particular subpopulations of lymphocytes in vitro (9). Stimulation of thymus-derived lymphocytes by antigens in vitro is felt to require the interaction of macrophage and lymphocyte (10). The responsiveness of lymphocytes when cultured in vitro with various mitogens and antigens is generally agreed to reflect in vivo immunological function (11). Hence, in order to study the effects of in vivo hydrocortisone (OHC)¹ administration upon cellular immune responses in humans, normal volunteers were given intravenous OHC and the following parameters were measured: absolute numbers of circulating lymphocytes and monocytes, relative proportions of subpopulations of circulating lymphocytes, and the function of these subpopulations as determined by in vitro stimulation with various mitogens and antigens.

¹ *Abbreviations used in this paper:* B lymphocytes, bone marrow-derived lymphocytes; Con A, concanavalin A; HBSS, Hanks balanced salt solution; MEM-S, Eagle's minimum essential media modified for suspension culture; OHC, hydrocortisone; PHA, phytohemagglutinin; PWM, pokeweed mitogen; SK-SD, streptokinase-streptodornase; SRBC, sheep red blood cells; TCA, trichloroacetic acid; T lymphocytes, thymus-derived lymphocytes; TT, tetanus toxoid; WBC, white blood cell.

METHODS

Subjects. 10 normal adult volunteers of either sex, ages 20–27 yr each received a single dose of 100 mg and 400 mg of hydrocortisone sodium succinate (The Upjohn Co., Kalamazoo, Mich.) intravenously on separate occasions at least 1 wk apart. None of the subjects were taking any medications during the study period. The injections of OHC were given at 8 a.m., and subjects were required to remain in their rooms at minimal activity during the test period. Venous blood was obtained immediately before OHC injection (0 h), at hourly intervals for the first 6 h, and again at 10 and 24 h after administration. White blood cell (WBC) and differential counts, plasma cortisol levels, and Hypaque-Ficoll mononuclear cell separations were performed on the blood samples. Six other normal adult volunteers of similar sex and age range as the above subjects were used as normal controls. They underwent the same protocol studies except that they received 4 ml of normal saline intravenously instead of OHC.

Total leukocyte and differential counts. WBC counts were performed using a Coulter Counter (model F_n, Coulter Electronics, Inc., Hialeah, Fla.), and differential counts were performed on peripheral blood smears stained with Wright's stain. 200 cells were counted per smear by the same observer throughout the study.

Plasma cortisol levels. 10 ml of venous blood was obtained in a syringe moistened with heparin (Eli Lilly & Co., Indianapolis, Ind., 1,000 USP units per ml) at 0, 1, 4, and 24 h for determination of plasma cortisol levels. The determinations were kindly performed by Drs. M. B. Lipsett and L. D. Loriaux using a radioimmunoassay (12).

Preparation and culture of mononuclear cells. Mononuclear cells (lymphocytes and monocytes) were obtained by hypaque-ficoll separation (13) of heparinized venous blood obtained at 0, 1, 4, and 24 h. The mononuclear cells were washed three times in Eagle's minimum essential media modified for suspension culture (MEM-S) (Grand Island Biological Co., Grand Island, N. Y.). For culture, MEM-S was supplemented with 0.02 M L-glutamine, penicillin 100 U/ml, streptomycin sulfate 100 µg/ml (National Institutes of Health Media Supply Section), and 15% homologous AB serum (Antibodies, Inc., Washington, D. C.) in 5% CO₂. Triplicate cultures containing 2 ml of cells were incubated in air, at 100% humidity. The concentrations were adjusted so that each culture contained 0.5×10^6 lymphocytes per ml. The lymphocyte to monocyte ratio was consistently between 8 and 9 to 1 in the 0 h samples and between 20 and 30 to 1 in the 4-h samples. Mitogens or antigens were added to the cultures in previously determined optimal stimulatory doses. The mitogens used were: phytohemagglutinin (PHA) MR 68, lot K4402 (Wellcome Reagents Ltd., Beckenham, England), optimal dose 1 µg/ml of culture; concanavalin A (Con A), lot 2145 (Nutritional Biochemicals Corp., Cleveland, Ohio), optimal dose 10 µg/ml of culture; pokeweed mitogen (PWM), lot 180690 (Grand Island Biological Co., Grand Island, N. Y.), optimal dose 0.1 ml of 1:10 dilution in distilled water per 2 ml of culture. The antigens used were: streptokinase-streptodornase (SK-SD), lot 5-2201-66 (Lederle Laboratories Div., American Cyanamid Co., Pearl River, N. Y.), optimal dose 25 U streptokinase, 6.75 U streptodornase per ml of culture; tetanus toxoid (TT), lot L 01246 (Eli Lilly and Co., Indianapolis, Ind.), optimal dose 1.5 flocculation units per ml of culture. Cultures containing mitogens were incubated for 3 days and those containing antigens were incubated for 5 days. 4 h before harvesting, 2 µCi

of tritiated thymidine (6.7 Ci/mM, New England Nuclear, Boston, Mass.) were added to each culture tube. The cells were collected on Millipore filters using a 3025 Millipore sampling manifold (Millipore Corp., Bedford, Mass.). The filters were then washed with 10% trichloroacetic acid (TCA) and 95% ethanol (14), and placed in 10 ml of Aquasol (New England Nuclear). The TCA-precipitable activity was then counted in a liquid scintillation counter model LS-250 (Beckman Instruments, Inc., Fullerton, Calif.). The arithmetic mean of triplicate cultures was determined and the degree of stimulation was expressed as the difference in counts per minute per 10^6 lymphocytes between stimulated and unstimulated (control) cultures (Δ CPM).

Reconstitution of monocyte-depleted lymphocyte cultures. In order to determine to what extent, if any, depletion of circulating monocytes by hydrocortisone administration is responsible for decreased in vitro lymphocyte response to antigens, reconstitution experiments were performed. Six separate normal volunteers received 400 mg of OHC intravenously. As described above, Hypaque-Ficoll-separated mononuclear cells from 0 h and 4 h after OHC were cultured in the presence of SK-SD and TT. In addition, an aliquot of mononuclear cells from the pretreatment (0 h) sample was set aside and irradiated with 2000 R from a cobalt-60 gamma source (Gammator M, Kewaunee Scientific Equipment, Adrian, Mich.). This procedure renders lymphocytes unable to divide while mononuclear phagocytic cells can still function normally (15). This irradiated sample from 0 h was then added to cultures of post-OHC (4 h) cells in a concentration such that the number of monocytes added would equal the standard number of monocytes in the original 0 h culture. In this way, the 4-h cultures would have at least the same numbers of normal monocytes as the 0 h cultures. In order to control for any nonspecific effect of irradiated autologous lymphocytes on cultures, aliquots of 4-h cells (which are monocyte-depleted) were also irradiated and added back to standard 4-h cultures. Thus, we can determine the in vitro response to SK-SD and TT of cells before OHC administration, 4 h after OHC, 4 h after OHC plus monocyte-rich irradiated cells, and 4 h after OHC plus monocyte-depleted irradiated cells.

Proportion of circulating thymus-derived lymphocytes. The percentage of circulating lymphocytes which formed rosettes with sheep red blood cells (SRBC) was determined (16). In this assay thymus-derived (T) lymphocytes, but not bone marrow-derived (B) lymphocytes, bind SRBC to form rosettes. To 0.25 ml of the lymphocyte suspension (4×10^6 cells/ml) in Hanks balanced salt solution (HBSS) (National Institutes of Health Media Supply Section) was added 0.25 ml of 0.5% SRBC. The mixed cell suspension was incubated at 37°C for 5 min and centrifuged at 200 *g* for 5 min at 4°C, and the supernatant HBSS was then removed. 0.5 ml of fetal calf serum (Gray Industries, Inc., Fort Lauderdale, Fla.) which had been absorbed twice with SRBC was then added and the pellet was incubated at 4°C overnight. One-half the volume of supernate was then removed and the cells were gently resuspended by shaking. One drop of cell suspension was put on a glass slide and a cover slip was applied. 200 lymphocytes were counted by the same observer throughout the study using phase contrast optics at 400× magnification on a Zeiss microscope (Carl Zeiss, Oberkochen, West Germany). All lymphocytes binding more than three SRBC were considered positive. The total number of T cells per mm³ for

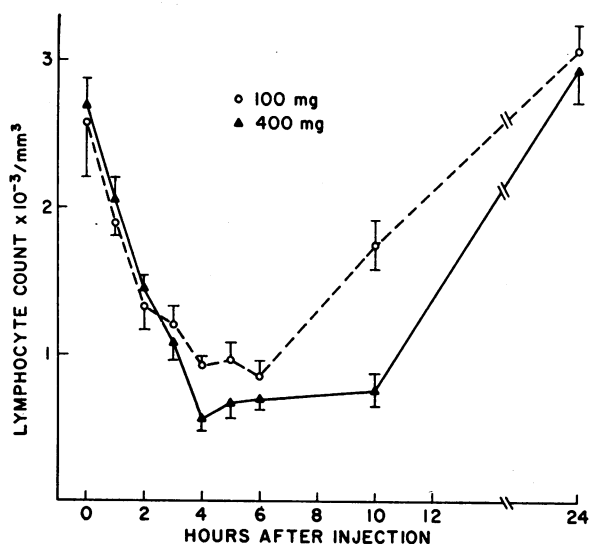


FIGURE 1 Absolute circulating lymphocyte counts after OHC administration. Mean levels (\pm SEM) for the various time intervals after injection of OHC are shown.

each individual was determined by multiplying the percent rosettes by the total lymphocyte count.

In order to rule out the possibility that corticosteroid binding to lymphocytes might interfere with SRBC rosette formation, a series of control experiments were performed. Hypaque-Ficoll-separated lymphocytes were collected from 10 normal adult laboratory personnel (six males, four females, ages 25–32 yr), one person per day for 10 separate days. The rosette assay was carried out exactly as described above except that the lymphocytes were first incubated for $\frac{1}{2}$ h at 37°C with varying concentrations (100 μ g/ml, 10 μ g/ml, 1 μ g/ml, and 0.1 μ g/ml) of OHC and then washed three times with HBSS before mixing with SRBC.

Analysis of results. The total circulating lymphocyte and monocyte counts as well as the percent rosettes and total rosettes per mm³ are expressed as the arithmetic mean with the standard error (SE) for the subjects at each time interval after OHC. For the stimulation with mitogens and antigens the results are expressed as the percent change in stimulation comparing 1, 4, and 24 h after OHC injection with 0 h (immediately before injection). The geometric mean of percent changes at each time interval for the experimental and control groups were compared applying Students' *t* test to the corresponding mean log values (17). The 70% confidence interval around each mean was derived by taking antilogs of the mean log \pm SE (18).

RESULTS

Plasma cortisol levels. Markedly elevated plasma cortisol levels of 131 (\pm 54) and 250 (\pm 60) μ g/100 ml at 1 h and 32 (\pm 4.3) and 137 (\pm 37) μ g/100 ml at 4 h after injection of 100 mg and 400 mg of OHC, respectively, were observed. By 24 h plasma cortisol levels had returned to base line in both groups.

Lymphocyte and monocyte counts. The effects of OHC on absolute circulating lymphocyte and monocyte counts are shown in Figs. 1 and 2. With both 100 mg and

400 mg of OHC, the lymphocyte counts show a sharp decrease reaching their lowest levels at 4–6 h after OHC, with a subsequent gradual increase so that by 24 h the counts have returned to base line. The curves for both doses of OHC are quite similar except for a slight lag in return to base line with 400 mg OHC as seen at the 10 h level.

The monocyte counts likewise fall abruptly reaching lowest levels at 4–6 h after OHC with a similar lag in return to base line seen with the 400 mg dose. At the 4–6-h levels, the monocyte counts approach zero, only to return to base line by 24 h.

T cell rosettes. The percent and absolute number per mm³ of rosette-forming T lymphocytes decreased maximally after both 100 mg ($P < 0.02$) and 400 mg ($P < 0.01$) of OHC (Table I). As with the absolute lymphocyte and monocyte counts, there was a return to base line by 24 h.

In vitro incubation of lymphocytes with OHC had no effect on the ability of these cells to form rosettes with SRBC. In the 10 separate subjects whose lymphocytes were incubated in HBSS as well as varying concentrations of OHC, there was no difference in the percent rosettes formed. In HBSS the mean percent of rosettes was 52.3 (\pm 3.5). When incubated with even the highest concentration of OHC (100 μ g/ml), the mean percent was 55.2 (\pm 2.8).

Response of cultured lymphocytes to mitogens and antigens. Table II lists the percent changes in lymphocyte stimulation with the different mitogens and antigens at various time intervals after OHC injection. The most dramatic reductions in stimulation are seen at 1 h and

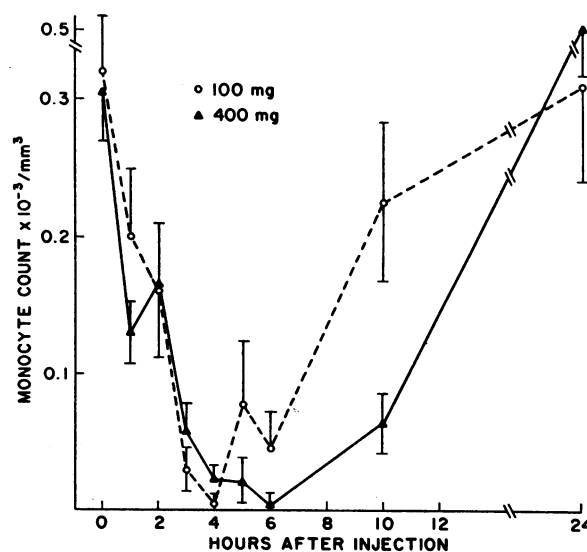


FIGURE 2 Absolute circulating monocyte counts after OHC administration. Mean levels (\pm SEM) for the various time intervals after injection of OHC are shown.

TABLE I
Effect of Hydrocortisone on Circulating T Lymphocytes

Time after OHC	Percent rosettes		Total rosettes (no./mm ³)	
	100 mg	400 mg	100 mg	400 mg
h				
0	48.5 (±3.5)*	49.8 (±4.2)	1252 (±191)	1325 (±129)
1	45.5 (±5.8)	35.8 (±5.4)	875 (±126)	726 (±122)
4	32.7 (±4.6)	30.9 (±4.1)	301 (± 41)	174 (± 33)
24	40.0 (±4.5)	52.3 (±2.9)	1189 (±186)	1411 (±108)

* Mean (±SE) for 10 subjects.

4 h after injection of 400 mg of OHC. A comparison of the differential effects of OHC on the in vitro lymphocyte responses to each stimulus at these time intervals and OHC dose is illustrated in Fig. 3.

At 1 h with 100 mg OHC (Table II) there is no significant change from base line with any of the mitogens and antigens tested. At 4 h with 100 mg OHC the response to PWM is essentially unchanged (+4%), the response to PHA is not significantly decreased (-21%, $P > 0.10$), while the response to Con A is markedly decreased (-59%, $P < 0.05$). The response to SK-SD is not significantly decreased (-26%, $P > 0.2$), while the response to TT is markedly decreased (-45%, $P < 0.02$) at this dose of OHC.

With 400 mg OHC several responses are reduced with a definite differential pattern (Fig. 3). At 1 and 4 h, the response to PHA is not significantly changed. The response to Con A is reduced slightly at 1 h, but still not significantly, while at 4 h the response to Con A is markedly decreased (-56%, $P < 0.01$) in sharp contrast to the response to PHA at 4 h. With 400 mg OHC, the response to PWM is not significantly changed at 1

h. At 4 h it is greatly decreased (-46%, $P < 0.05$) in contrast to its resistance to 100 mg OHC at 4 h (Table II). The responses to the antigens SK-SD and TT are highly sensitive to the effects of 400 mg of OHC both at 1 h and 4 h after injection (Fig. 3). The data given are those for optimal stimulatory concentrations of mitogens and antigens. In several experiments various concentrations of mitogens and antigens both greater and less than optimum were also tested. Similar differential sensitivities seen at optimal doses were also observed at these doses.

Reconstitution of monocyte-depleted lymphocyte cultures. The effects of adding monocyte-rich cells to monocyte-depleted cultures are shown in Table III. As in the previous experiments, 4 h after OHC administration there is a marked decrease in in vitro response to antigens SK-SD and TT. Adding irradiated, monocyte-rich cells from 0 h samples to monocyte-depleted 4-h cultures results in a partial, but not a complete reconstitution of the responses which is not seen when irradiated monocyte-depleted cells are added to the 4-h cultures ($P < 0.02$, paired sample *t* test). The lack of any

TABLE II
Effect of In Vivo Hydrocortisone on Lymphocyte Transformation

	1 h	4 h	24 h
Percent change in stimulation after 100 mg OHC*			
PHA	+5 (-15 to +29)	-21 (-36 to -3)	0 (-30 to +41)
Con A	-18 (-34 to +3)	-59 (-71 to -42)	+11 (-9 to +37)
PWM	+3 (-15 to +24)	+4 (-5 to +14)	+15 (-4 to +39)
SK-SD	+13 (-8 to +39)	-26 (-50 to +10)	+8 (-11 to +30)
TT	-8 (-12 to -4)	-45 (-59 to -25)	+3 (-18 to +29)
Percent change in stimulation after 400 mg OHC			
PHA	-8 (-17 to +1)	-17 (-26 to -6)	-11 (-21 to 0)
Con A	-20 (-29 to -10)	-56 (-63 to -48)	+2 (-20 to +30)
PWM	-16 (-22 to -9)	-46 (-57 to -33)	+2 (-16 to +26)
SK-SD	-32 (-37 to -25)	-60 (-73 to -39)	0 (-21 to +26)
TT	-23 (-28 to -16)	-66 (-71 to -60)	+16 (+3 to +31)

* Data given as geometric means of the percent changes in the groups studied; numbers in parentheses are 70% confidence intervals (18).

TABLE III
Effect of Monocyte Reconstitution on In Vitro Lymphocyte Response to Antigens After OHC Administration

Subject	0 h culture		4 h culture		4 h culture plus monocyte-rich cells		4 h culture plus monocyte-depleted cells	
	SK-SD	TT	SK-SD	TT	SK-SD	TT	SK-SD	TT
B. B.	27,493*	42,685	8,578	15,915	15,682	30,795	8,043	19,485
M. H.	103,845	53,766	73,908	21,338	89,270	20,868	62,275	21,250
C. C.	77,498	66,361	20,715	18,538	67,988	39,760	25,790	25,845
S. R.	54,226	74,929	25,325	16,160	35,485	26,630	24,650	19,668
P. P.	98,436	71,413	58,594	31,367	63,528	36,308	45,135	27,743
K. D.	50,368	13,656	13,013	1,405	20,705	5,353	13,333	1,443
Mean (\pm SE)	68,644 (\pm 12,166)	53,802 (\pm 9,398)	33,356 (\pm 10,853)	17,454 (\pm 3,964)	48,776 (\pm 11,953)	26,619 (\pm 5,064)	29,871 (\pm 8,320)	19,239 (\pm 3,816)

* cpm/10⁵ lymphocytes in stimulated cultures minus cpm/10⁶ lymphocytes in unstimulated cultures (Δ cpm).

effect of adding irradiated, monocyte-depleted cells to the 4 h culture also serves as a control demonstrating that adding irradiated autologous lymphocytes to standard cultures does not have a nonspecific adjuvant or suppressant effect on the response to antigens.

DISCUSSION

It has been firmly established that in man there exist heterogeneous subpopulations of lymphocytes. Two broad categories, bone marrow-derived and thymus-derived cells, can be identified by surface markers (16, 19). Functionally, these broad categories, as well as various

subsets are defined by their differential in vitro responsiveness to various mitogens (9). In addition, optimal in vitro responsiveness to antigenic stimuli requires the interaction of a critical proportion of phagocytic cells with the responding lymphocytes (10).

The present studies demonstrate that a single intravenous dose of OHC has a profound effect upon circulating lymphoid cells that is both rapid in onset and transient in duration. The absolute numbers of circulating lymphocytes and monocytes are decreased, with a definite pattern of decline and recovery after both a 100 and 400 mg dose of OHC (Figs. 1 and 2).

Of interest is the fact that the depletion of lymphocytes from the circulation is selective, in that there is a proportionately greater decrease in the number of T cells as measured by the SRBC rosette assay. This observation could indicate either a true selective depletion of circulating rosette forming T cells, or alternatively, an interference with the ability of these cells to form rosettes. The finding that high concentrations of OHC added to the rosette assay does not interfere with the ability of T cells to form rosettes is evidence against the latter point and strengthens the argument that there is a true selective removal of T cells from the circulation with OHC administration.

The heterogeneity of lymphocytes in lower animals has been well-defined, both by surface markers (20, 21) and functional properties (22-24). Corticosteroids have been shown to have a distinctly differential effect upon these subpopulations in that they cause selective depletion of cells from tissues (7, 25) and changes in their circulation patterns (8, 26) and in their functional capacities as determined by in vitro stimulation (27, 28). In addition, the interaction of antigen sensitive lymphocyte and nonimmune macrophage is affected by corticosteroids (29, 30).

In humans, it is possible to fractionate lymphocytes into functionally distinct subpopulations by glass wool

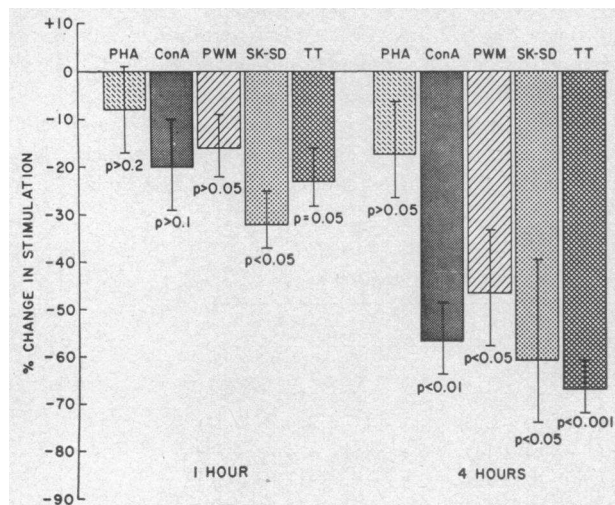


FIGURE 3 Differential effects of 400 mg of in vivo OHC on the in vitro lymphocyte response to various mitogens (PHA, Con A, PWM) and antigens (SK-SD, TT). Data shown are the geometric mean values together with 1 SE of the percent changes in lymphocyte stimulation at 1 h and 4 h after OHC administration. The P values represent the significance of these changes in response using a 2 sample *t* test comparing the subjects who received OHC with six controls who did not receive OHC but had similar lymphocyte studies done.

column separation (9). Recently, two subpopulations of lymphocytes were separated by passage through a nylon column (31). The subpopulations were distinguished by their differential responsiveness to *in vitro* stimulation by Con A. The column-adherent lymphocytes were highly responsive to Con A, while the nonadherent lymphocytes were relatively unresponsive to Con A. Both column-filtered and unfiltered cells responded nearly equally to both PHA and PWM. This mechanical depletion of a functional subpopulation of lymphocytes described above is analogous to the pharmacological depletion of subpopulations of lymphocytes by OHC administration demonstrated in the present study. That OHC removes lymphocytes from the circulation is clear from the profound decrease in absolute lymphocyte counts (Fig. 1). The cells which remained in the circulation after OHC administration were no different in response to PHA than circulating cells before OHC administration. In contrast, cells remaining in the circulation after OHC administration were markedly depleted of Con A-responsive lymphocytes as compared to circulating cells before OHC administration (Table II). The response to PWM was unchanged after 100 mg OHC, yet markedly diminished after 400 mg OHC. Hence, administration of OHC not only causes an absolute lymphopenia, but selectively depletes certain functional subpopulations of lymphocytes (Con A- and PWM-responsive cells).

The relative proportions of subpopulations of lymphocytes have been studied in several diseases (32, 33). In this regard, evidence presented here that corticosteroid administration can selectively deplete rosette-forming T cells as well as certain functional subpopulations of lymphocytes has potential clinical significance. As knowledge accumulates of the precise role of identifiable subpopulations of lymphocytes in the pathogenesis of certain immunologically mediated diseases, familiarity with the differential sensitivities of these subpopulations to corticosteroids will allow for greater therapeutic specificity in the use of these agents.

The effect of OHC administration on the *in vitro* response to antigens is quite dramatic (Fig. 3). This diminution of *in vitro* antigen responsiveness after OHC correlates well with the suppression of cutaneous delayed hypersensitivity to antigens during corticosteroid administration (5). The finding of differentially greater OHC sensitivity of *in vitro* antigen stimulation of lymphocytes as compared to PHA stimulation (Fig. 3) is in accord with others (34) who found that a group of renal transplantation patients who were receiving prednisone had suppression of lymphocyte stimulation by *Candida* antigen without significant inhibition of PHA response.

The mechanisms of this selectively greater depression by corticosteroids of antigen response is unclear. Mono-

cytes play a critical role in the *in vitro* response to antigens (10), and monocytes are established components of the cutaneous delayed hypersensitivity reaction (35). The dramatic fall in absolute monocyte counts at 4-6 h after OHC (Fig. 2) may be the crucial factor in the reduction of antigen responsiveness. The addition of irradiated, autologous monocyte-rich cell suspensions to the cultures of cells depleted of monocytes by OHC administration results in a partial, but not a complete reconstitution of response to antigens (Table III). This is in accord with studies (10) in which cell suspensions depleted of monocytes by glass bead column treatment had diminished *in vitro* responses to antigens. The deficient response was likewise partially corrected by the addition of autologous monocyte-rich cell suspensions. Hence, monocyte depletion caused by OHC administration clearly is responsible at least in part for the decreased *in vitro* response to antigenic stimulation seen in the present study. In addition, deficiency of one or more of the functional lymphocyte subpopulations which were shown to be selectively depleted may also contribute to the decreased *in vitro* response to antigens.

The precise mechanisms whereby the absolute lymphocyte counts decline rapidly and return to base line within 24 h are unclear. The mechanism for this decrease in cell numbers could be explained either by direct destruction or redistribution of lymphocytes out of the circulation to other body compartments. In man, there is no convincing evidence that corticosteroids even in pharmacologic doses cause significant destruction of normal lymphocytes *in vivo* (2). In addition, corticosteroids added *in vitro* to human peripheral blood lymphocyte (36) and thymocyte (37) suspensions in concentrations far exceeding attainable *in vivo* pharmacological levels had no cytolytic effects. It seems highly likely, therefore, that the dramatic depletion of circulating monocytes and subpopulations of lymphocytes following OHC administration is due to a redistribution of cells out of the circulation into other body compartments as has been described in certain animals (8, 26, 38). Experiments tracing the fate of labeled lymphocytes and monocytes after corticosteroid administration may clarify this hypothesis.

ACKNOWLEDGMENTS

The authors thank Ms. Kathy A. O'Meara, Ms. Sandra Crosby, and Mr. Stanley B. Ward for expert technical assistance.

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