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Research Article

The present study was undertaken to determine the effect of in vivo hydrocortisone on the kinetics of subpopulations of normal human peripheral blood (PB) thymus-derived (T) cells. Normal volunteers received a single i.v. dose of hydrocortisone, and blood was taken just before, as well as 4, 24, and 48 h after hydrocortisone administration. T cells were purified from each specimen, and proportions and absolute numbers of T lymphocytes bearing receptors for the Fc portion of IgG (T_G) and for the Fc portion of IgM (T_M) were enumerated by rosetting T cells with bovine erythrocytes which had been coated with either antiovine erythrocyte IgG or IgM. 4 h after i.v. administration of hydrocortisone, T_M cells decreased from 52 ($\pm 5\%$) to 23 ($\pm 6\%$) of PB T cells ($P < 0.01$) and the absolute number of T_M cells decreased from 1,028 (± 171) per mm^3 to 103 (± 23) per mm^3 ($P < 0.001$). In contrast, relative proportion of T_G cells increased from 22 ($\pm 4\%$) to 66 ($\pm 7\%$), while the absolute numbers of T_G cells were essentially unchanged ($P > 0.2$). In vitro studies involving preincubation of T cells with hydrocortisone before rosette determination of T_G or T_M cells demonstrated that the decrease in absolute numbers of T_M cells did not represent hydrocortisone interference with T_M rosette formation, nor did it represent a switch of [...]

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The Differential Effect of In Vivo Hydrocortisone on the Kinetics of Subpopulations of Human Peripheral Blood Thymus-Derived Lymphocytes

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ABSTRACT The present study was undertaken to determine the effect of in vivo hydrocortisone on the kinetics of subpopulations of normal human peripheral blood (PB) thymus-derived (T) cells. Normal volunteers received a single i.v. dose of hydrocortisone, and blood was taken just before, as well as 4, 24, and 48 h after hydrocortisone administration. T cells were purified from each specimen, and proportions and absolute numbers of T lymphocytes bearing receptors for the Fc portion of IgG (T_G) and for the Fc portion of IgM (T_M) were enumerated by rosetting T cells with bovine erythrocytes which had been coated with either antiovine erythrocyte IgG or IgM. 4 h after i.v. administration of hydrocortisone, T_M cells decreased from 52 ($\pm 5\%$) to 23 ($\pm 6\%$) of PB T cells ($P < 0.01$) and the absolute number of T_M cells decreased from 1,028 (± 171) per mm^3 to 103 (± 23) per mm^3 ($P < 0.001$). In contrast, relative proportion of T_G cells increased from 22 ($\pm 4\%$) to 66 ($\pm 7\%$), while the absolute numbers of T_G cells were essentially unchanged ($P > 0.2$). In vitro studies involving preincubation of T cells with hydrocortisone before rosette determination of T_G or T_M cells demonstrated that the decrease in absolute numbers of T_M cells did not represent hydrocortisone interference with T_M rosette formation, nor did it represent a switch of T_M cells to T_G cells. Thus, administration of hydrocortisone to normal subjects produces a selective depletion from the circulation of T lymphocytes which possess receptors for the Fc portion of IgM (T_M cells) and of T cells which possess no detectable Fc receptor ($T_{\text{non-M, non-G}}$ cells). T_G cells are relatively resistant to the lymphopenic effect of hydrocortisone. These data clearly demonstrate that in vivo corticosteroids have a differential effect on the kinetics

of identifiable and distinct subsets of cells in the human T-cell class.

INTRODUCTION

Corticosteroids are important chemotherapeutic agents in clinical medicine and are used in a variety of diseases which are believed to be caused by inflammatory or immunological phenomena (1). Studies from our laboratory (2, 3) and others (4) have shown that in vivo corticosteroid administration to normal subjects causes a selective depletion of thymus-derived (T) lymphocytes from the peripheral circulation, with the absolute number of bone marrow-derived (B) cells decreasing to a lesser extent. Kinetic studies with ^{51}Cr -labeled autologous peripheral blood lymphocytes in guinea pigs (5) and in humans (6) have shown that hydrocortisone-induced lymphopenia represented not a destruction of cells, but a redirection of traffic of predominantly recirculating lymphocytes from the peripheral blood pool to other lymphocyte pools, particularly to the bone marrow.

We have also shown that in vivo corticosteroids selectively deplete functional subpopulations of lymphocytes as measured by proliferation to mitogens (2, 7). We suggested at that time that in addition to selectively affecting T cells more than B cells, corticosteroids probably also had a selective effect on subsets within the T-cell class, since we found that 4 h after in vivo administration of 400 mg of hydrocortisone, the blastogenic response of peripheral blood lymphocytes to phytohemagglutinin was unchanged, while the response to concanavalin A was markedly reduced (2).

Recently, Moretta and his colleagues have delineated distinct subpopulations of human T lymphocytes based on the presence or absence of cell surface receptors for the Fc portion of IgG (T_G cells) or

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IgM (T_M cells) (8–10). They have further shown in a system employing pokeweed mitogen-stimulated B-cell production of intracytoplasmic antibody that the T_M cells are the T “helper” cells and the T_G cells are the T “suppressor” cells (11).

The present study was undertaken to determine the differential effects of in vivo administration hydrocortisone on the kinetics of these distinct subpopulations of T cells in normal subjects.

METHODS

Subjects. The methods for i.v. hydrocortisone administration have been previously described in detail (2, 3). Briefly, after informed consent was obtained, six normal adult volunteers of either sex (age range 20–26 yr) received a single dose of 400 mg of hydrocortisone sodium succinate (The Upjohn Co., Kalamazoo, Mich.). Heparinized venous blood was drawn just before (0 hour) and 4, 24, and in some subjects, 48 h after hydrocortisone administration.

Cell suspensions. Purified mononuclear cell suspensions were obtained by standard Ficoll-Hypaque (Ficoll, Pharmacia Fine Chemicals, Inc., Piscataway, N. J.; Hypaque, Winthrop Labs, New York) density centrifugation of peripheral blood, and T-cell-enriched mononuclear cell suspensions were obtained by sheep erythrocyte rosetting of lymphocytes followed by centrifugation of rosetted cells over Ficoll-Hypaque gradients as previously described in detail (11, 12).

Surface markers on purified T cells. T cells bearing receptors for the Fc portion of IgG (T_G) and IgM (T_M) were determined by rosetting T cells with bovine erythrocytes which had been coated with either antbovine erythrocyte IgG or IgM as previously described by Moretta and colleagues (8–11).

Surface immunoglobulin-positive (sIg+) cells were identified and enumerated by staining cell suspensions with fluorescein-conjugated purified goat F(ab)₂ fragments of antibodies to human Ig.

In order to rule out the possibility that hydrocortisone binding to T lymphocytes might interfere with T_M or T_G rosette formation, control experiments were performed in which T cells were exposed to hydrocortisone in vitro before rosetting for T_M or T_G markers. Purified T cells were incubated for 1 h at 37°C in media or with either 0.1 mM, 10 μ M, or 1 μ M hydrocortisone and washed three times in TC 199 media; and rosette determinations for T_G or T_M were performed as described above.

RESULTS

Base-line values for lymphocyte subpopulations in normal subjects. In the present study, 86 \pm 3% of peripheral blood lymphocytes were found to be sheep erythrocyte-rosette positive (T cells) and 14 \pm 3% were found to be non-T lymphocytes (B cells plus other non-T lymphocytes). Of the purified T cells (95 \pm 1% T cells), 52 \pm 5% possessed a receptor for the Fc portion of IgM (T_M cells) and 22 \pm 4% were found to have a receptor for IgG (T_G cells). The remaining percentage of T cells needed to make up 100% are hereafter designated as T cells with neither IgM nor IgG Fc receptors ($T_{non-M, non-G}$ cells), and made up of 26 \pm 6% of T cells. The normal values for T_M and T_G

in our laboratory from studies in 20 normal subjects are 56 \pm 4% and 17 \pm 2%, respectively, and are virtually identical with the values in the present study as well as to those originally reported by Moretta and his colleagues (8, 9).

Effect of in vivo hydrocortisone on the kinetics of T-cell subpopulations. As shown previously, in vivo hydrocortisone administration to normal subjects produced a profound lymphopenia (from 2,206 \pm 147 to 845 \pm 136 lymphocytes/mm³) maximal at 4 h after drug administration which selectively affected T cells as compared to non-T cells (2, 3). 24 h after hydrocortisone administration there was a slight rebound lymphocytosis which returned to normal at 48 h (2). In contrast to the T-cell fraction of lymphocytes, the absolute number of non-T cells (B cells plus other non-T cells) did not decrease but remained essentially the same ($P > 0.2$). At 4 h after hydrocortisone administration, the percent of T_G cells in the total T-cell population rose significantly ($P < 0.001$), while the percent of T_M cells fell ($P < 0.01$). The percent of $T_{non-M, non-G}$ cells followed the pattern of the T_M cells and also decreased ($P < 0.05$) (Fig. 1). At 24 h, there was a slight rebound increase in T_M cells which was not significantly different from the 0-h value, and a slight but nonsignificant decrease in T_G . By 48 h after hydrocortisone, all values had returned to base line.

When absolute numbers of lymphocytes in the T-cell subpopulations were calculated (Fig. 2), it was found that at 4 h after hydrocortisone administration, the numbers of T_M cells markedly decreased ($P < 0.001$). In addition, the number of $T_{non-M, non-G}$ cells significantly decreased ($P < 0.02$), while the absolute number of T_G cells were essentially unchanged ($P > 0.2$). At 24 h, there was a rebound increase in the numbers of T_M

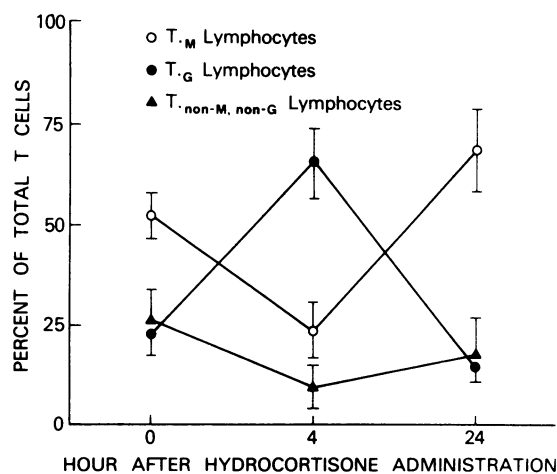


FIGURE 1 The changes of percentages in T-cell subpopulations after in vivo hydrocortisone administration. Each point represents the mean \pm SEM of six experiments.

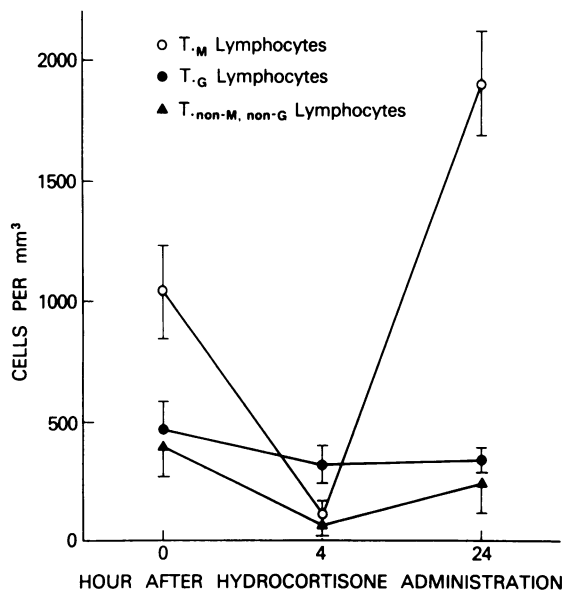


FIGURE 2 The selective decrease of absolute numbers of T_M and T_{non-M, non-G} cells after in vivo administration of hydrocortisone. Each point represents the mean \pm SEM of five experiments.

cells ($P < 0.02$), which returned to base line at 48 h. At 24 h, T_G cells were still within the range of base line. The number of T_{non-M, non-G} cells did not rebound as did the T_M.

Thus, the data show that 4 h after in vivo administration of hydrocortisone, there was a selective elimination of T_M and T_{non-M, non-G} cells from the peripheral circulation. In addition, the relative proportion of T_G cells increased, while the absolute numbers of T_G remained unchanged.

To determine whether hydrocortisone administration reversibly caused IgG Fc receptor-negative T cells to transiently bind IgG, and thus appear as IgG Fc receptor positive cells, T cells from 4 h after hydrocortisone administration were incubated overnight and rosette determinations were repeated for T_G cells. We found the percent of T_G cells to be stable. Before and after overnight incubation the proportions of T_G cells were the same ($61 \pm 6\%$ and $61 \pm 8\%$, respectively; data represent mean \pm SEM of five experiments).

In addition to hydrocortisone effecting a selective depletion of T_M cells from the circulation, other possibilities exist which could explain the observed changes in numbers of T_M cells: (a) Hydrocortisone may decrease the binding of the T_M IgM receptor to IgM. (b) Hydrocortisone may cause T_M or T_{non-M, non-G} cells to switch their receptors and become T_G cells.

Effect of in vitro preincubation of T cells with hydrocortisone. In order to rule out the possibility

that in vivo hydrocortisone binding to the lymphocyte surface might have interfered with the in vitro binding of IgM-coated bovine erythrocytes to the IgM Fc receptor, T cells were incubated in vitro with 0.1 mM, 10 μ M, and 1 μ M hydrocortisone at 37°C for 1 h and then washed in media before placing them in overnight cultures to generate the IgM Fc receptor. We found that preincubation of T cells with as much as 0.1 mM hydrocortisone (suprapharmacologic amounts) did not inhibit the ability of T_M cells to express their IgM receptors or to bind bovine erythrocytes coated with IgM.

To evaluate the possibility that hydrocortisone might promote the switch of T_M cells to T_G cells, purified T cells were incubated with varying concentrations of hydrocortisone for 1 h at 37°C and then washed before T_G rosette formation. In these experiments, we found that preincubation of T cells in varying concentrations of hydrocortisone did not significantly change the percent of T_G cells in purified T-cell suspensions.

DISCUSSION

The present study has demonstrated that hydrocortisone administered intravenously to normal subjects in a single 400-mg dose caused a selective and transient depletion of T_M and T_{non-M, non-G} cells from the peripheral circulation, while T_G cells were relatively refractory to this lymphopenic effect. Furthermore, we have demonstrated that these changes in proportions and absolute numbers of T_M cells represents an actual depletion of these cells from the circulation and does not represent a decreased ability of T_M to bind IgM or an increased ability to T_G to bind IgG. Similarly, in vitro controls support the concept that T_M cells are being depleted from the circulation and are not switching to T_G cells.

In an elegant series of papers, Moretta and his colleagues have described the T_M subset of human T lymphocytes which comprise approximately 55–75% of human T cells and which bind the Fc portion of the pentameric form of IgM (8, 13). Other laboratories have now confirmed these findings in human T cells (14) and, as well, have demonstrated T_M cells in mouse T-cell suspensions (15).

T cells bearing receptors for IgG have been previously shown to exist in humans by several laboratories (9, 16, 17). Moretta and others showed that T_M and T_G cells are separate subsets of human T cells and that in T-cell suspensions from normal human peripheral blood, the receptors do not seem to exist on the same cell simultaneously (8). In addition, Moretta and his colleagues have demonstrated that in the system of human B cells stimulated to produce intracytoplasmic Ig by pokeweed mitogen, the T_M cells are the "helper" T lymphocytes and the T_G cells, after

activation with immune complexes, are the "suppressor" T lymphocytes (11). Furthermore, T_M and T_G cells are also functionally separable on the basis of differential blast transformation to phytohemagglutinin, in that T_M cells respond well to phytohemagglutinin while T_G cells do not (10). Thus, the initial observations of Moretta et al. suggest that T_M and T_G are distinct subsets of human T cells and may in some ways be analogous to the Ly 1 (helper) and Ly 2,3 (suppressor) T-cell subsets defined in the mouse (18).

The selective effect of *in vivo* hydrocortisone on T- versus B-cell kinetics is now well established (2-4). Our previous findings (5, 6) using labeled autologous lymphocytes which showed that hydrocortisone causes a redistribution of predominately recirculating T lymphocytes out of the circulation (most likely to the bone marrow compartment and possibly to the spleen) can now be further refined. On the basis of response to hydrocortisone, T_M and T_{non-M, non-G} cells belong to the recirculating pool of T cells, while T_G cells are predominately nonrecirculating lymphocytes.

Yu et al. (4) did note a relative increase in percentage of Fc receptor-positive mononuclear cells remaining in the circulation after oral administration of prednisone and also that the absolute number of Fc receptor-positive cells decreased less than did the absolute number of T cells. This relative increase in Fc receptor-positive cells after *i.v.* hydrocortisone administration to normal subjects was also noted by us together with a concomitant increase in antibody-dependent cellular cytotoxicity 4 h after hydrocortisone administration (19). However, these observations (4, 19) were made on unfractionated mononuclear cell suspensions in which IgG Fc receptors are found on monocytes, B cells, null cells, and as just recently recognized, on a small fraction of T cells (9).

In contradistinction, the present study describes the selective and differential effects of corticosteroids on subpopulations of purified T cells recognized by surface markers which until recently were not described (8-10). Our data suggest that T_G cells, after *in vivo* hydrocortisone administration, have a kinetic pattern similar to Fc receptor-positive B cells. Although only speculative, this nonrecirculatory characteristic of B cells and T_G cells may relate to the mutual presence of a similar or identical Fc receptor for IgG on each cell type.

It has been suggested that patients with autoimmune disease associated with hypergammaglobulinemia such as systemic lupus erythematosus have decreased numbers of functioning suppressor cells and that this lack of suppression may be responsible for the production of autoantibodies seen in this type of disease (20, 21). Because of their anti-inflammatory and immunosuppressive properties and their efficacy in con-

trolling the inflammatory manifestations of disease (1), corticosteroids have long been used as the primary mode of therapy in autoimmune disease. It is possible that the selective depletion of a helper T-cell subpopulation by *in vivo* corticosteroids with the resulting relative increase in proportion of the suppressor T-cell subpopulation contributes to the therapeutic effect of this drug in diseases associated with lack of suppressive immunoregulatory control. It is obvious that much more information is needed to completely define the full range of functional capabilities of these various lymphocyte subpopulations before a full understanding of the mechanisms of corticosteroid-mediated immune regulation is to be achieved. Hopefully, the present observation of the selective and differential lymphopenic effect of *in vivo* hydrocortisone on identifiable and distinct T-cell subsets will help provide insight into the mechanisms of immunoregulation by corticosteroids. In this regard, it may be possible to define a degree of specificity in these agents which for so long have been characterized by the nonspecificity of their therapeutic effect.

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