The Effect of Hydrocortisone on the Kinetics of Normal Human Lymphocytes

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Lymphocyte kinetic studies employing $^{51}$chromium-labeled autologous lymphocytes were performed in nine normal volunteers in order to determine the effects of hydrocortisone administration on the recirculating versus the nonrecirculating intravascular lymphocyte pools. Following infusion of labeled cells, the recirculating portion of the labeled cells rapidly equilibrated with the total intravascular lymphocyte pool and the vastly larger total-body recirculating lymphocyte pool, so that by 1 hr following infusion 21.8% ± 3.2% of the labeled lymphocytes were left in the circulation. Four hundred milligrams of intravenous hydrocortisone administered 24 hr after infusion of labeled cells caused a profound but transient lymphocytopenia which was maximal at 4 hr with return of lymphocyte counts to normal by 24 hr after injection. Concomitant with the lymphocytopenia there was a dramatic increase in lymphocyte specific activity (cpm per $10^6$ lymphocytes), while the total lymphocyte-associated radioactivity remaining in the circulation was unchanged, indicating that corticosteroid administration depleted the unlabeled recirculating cells. As the lymphocyte counts returned to normal following hydrocortisone, the specific activity also returned to normal. These studies indicated that hydrocortisone administration caused a transient lymphocytopenia by a preferential depletion of the recirculating portion of the intravascular lymphocyte pool.

Corticosteroid administration in normal humans has been demonstrated to cause a transient depletion of lymphocytes, predominantly of the thymus-derived (T) cell population. It has been proposed that this corticosteroid-induced lymphocytopenia results not from a destruction of cells but from a redistribution of lymphocytes out of the circulation into other body compartments, much like that which has been observed in certain animal species. The phenomenon of the recirculation of lymphocytes has been well established both in animals and in man. Since recirculating lymphocytes can freely migrate into and out of the circulation and since they are composed predominantly of T lymphocytes, it is reasonable to assume that the corticosteroid-induced depletion of predominantly T lymphocytes is due to a redistribution of these intravascular recirculating lymphocytes. In order to test this hypothesis, radioactive chromium ($^{51}$Cr)-labeled lymphocyte kinetic studies have been employed to determine the effects of acutely administered hydrocortisone on the recirculating lymphocyte pool in normal human volunteers.

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MATERIALS AND METHODS

Subjects

Nine normal volunteers (six females, three males; ages 19-25 yr) were used in the study. Informed consent was obtained, and the subjects were taking no medications during the study. Five subjects received a single injection of 400 mg of hydrocortisone sodium succinate (OHC) intravenously (i.v.) at the time designated below. Four subjects served as controls and did not receive OHC.

Separation and 51Cr-labeling of Leukocytes

At 8 a.m. on the first day of the study, 450 ml of citrate dextrose anticoagulated blood were withdrawn from the antecubital vein via a 16-gauge needle and collected in a sealed sterile plastic bag to which two satellite bags were connected (Fenwal Laboratories, Division of Travenol Laboratories, Morton Grove, Ill.). Separation and labeling of leukocytes were performed by a slight modification of a previously described method. One hundred milliliters of 6% dextran in 0.9% saline (Abbott Laboratories, North Chicago, Ill.) were added to the bag of whole blood, and the cells were sedimented at room temperature at 1 g for 45 min. The leukocyte-rich supernate was expressed into a satellite bag using a plasma extractor (Fenwal Laboratories), and platelets were removed by differential centrifugation (150 g for 3 min at 20°C). The white blood cells (WBC) were incubated in 20 ml of plasma dextran with 500 sCi of 51Cr (sodium radiochromate, E. R. Squibb & Sons, Inc., Radiopharmaceutical Dept., New Brunswick, N.J.) for 30 min at 37°C. Fifty milligrams of ascorbic acid (Eli Lilly & Co., Indianapolis, Ind.) were then added to reduce the remaining unbound chromate. The labeled cells were washed twice with and resuspended in 50 ml of autologous plasma-dextran and reinfused over a 1-3-min period. The total time from withdrawal of blood to reinfusion of labeled cells was approximately 2½-3 hr, and the viability of the reinfused labeled cells was always greater than 95% (trypan blue dye exclusion test). The employment of these sealed plastic bags with attached satellite bags allowed the separation, labeling, washing, and reinfusion of cells to be accomplished without breaking sterile conditions or exposing the blood components to air. All cell types in the leukocyte-rich fraction obtained from dextran sedimentation were labeled and reinfused. The lymphocyte-associated radioactivity was determined from Hypaque-Ficoll-separated cells from an aliquot of preinfusion cells and from blood samples taken throughout the study. The reasons for this approach will be discussed further on.

Hypaque-Ficoll separation of mononuclear cells was performed on an 8-ml aliquot of labeled cells taken from the bag before reinfusion and from 20-ml samples of blood drawn at the designated time intervals after reinfusion. These Hypaque-Ficoll-separated mononuclear cells contained approximately 85% lymphocytes, 15% monocytes, and less than 1% granulocytes. During the study, several Hypaque-Ficoll-separated cell samples drawn 24 hr or more after the reinfusion of labeled cells were plated on glass petri dishes to remove monocytes by glass adherence and so determine precisely what per cent of the radioactivity of these separated cells was in the lymphocyte fraction. It was found that greater than 95% of the radioactivity was associated with the lymphocytes. This lack of monocyte-associated radioactivity in the cell suspensions is best explained by the fact that most of the reinfused labeled monocytes had been cleared from the circulation by this time. To rule out the possibility that any of the radioactivity of the Hypaque-Ficoll-separated cells was associated with labeled platelets (since variable numbers of platelets are often seen in smears of Hypaque-Ficoll-separated cell fractions), platelet counts and radioactivity determinations were performed on the cell fractions before and after differential centrifugation to remove platelets. In calculations throughout the study, we determined the lymphocyte-associated radioactivity as well as the specific activity of lymphocytes (cpm per 106 lymphocytes) from these Hypaque-Ficoll-separated cell samples. Cells were counted in a Coulter Counter (Model Fn, Coulter Electronics, Inc.), and radioactivity was measured in an automatic gamma counter (Series 1185, Nuclear-Chicago, Des Plaines, Ill.). The total lymphocyte-associated radioactivity contained in the circulation at each individual time point was determined by converting the lymphocyte-associated radioactivity in the 20-ml sample of blood to the total amount in the intravascular compartment, assuming blood volume as 7% of body weight.

Blood samples were drawn at 15 min and 1, 2, 3, 6, 12, and 24 hr after the reinfusion of labeled cells. The five subjects who received the single dose of 400 mg OHC intravenously got their dose
immediately after the 24-hr sample. Blood samples were drawn 4 and 8 hr after OHC administration (28 and 32 hr after infusion of cells), and further samples were drawn daily for 6 days. Subjects who did not receive OHC had blood samples drawn at the same time intervals. In addition to the blood samples taken for determination of lymphocyte radioactivity, samples for WBC counts and differentials were taken at each time interval.

There are several reasons why we chose to label and reinfuse dextran-sedimented WBC and perform our more involved Hypaque-Ficoli separation of lymphocytes and determination of lymphocyte-associated radioactivity on the blood samples drawn after reinfusion of labeled cells. First, it was learned from preliminary experiments that as minimal manipulation of cells as possible prior to reinfusion lessened the chance of altering or damaging cells and appreciably increased the proportion and time of survival after reinfusion. Second, Hypaque-Ficoll separation and labeling of purified lymphocytes before reinfusion would entail manipulating cells outside the sterile, sealed bags. This procedure would increase the small but nonetheless definite chance of microbial contamination. Third, there is no real advantage in this particular study in removing granulocytes before labeling the cells, since the half-life of granulocytes is sufficiently short that most of the labeled granulocytes would no longer be present by the time the OHC was administered at 24 hr to determine the effect on lymphocyte kinetics. Fourth, removal of granulocytes and monocytes by nylon or glass-bead column purification prior to labeling was avoided, since this procedure has been shown to remove selectively certain lymphocyte populations.

At the time each blood sample was drawn, body surface counts were measured over the spleen, sternal bone marrow, liver, and lung using a Tri-Carb scintillation spectrometer with a shielded 2-inch thallium-activated, sodium iodide crystal (Model 3002, Packard Instrument Co., Downers Grove, Ill.).

RESULTS

Since the initial ⁵¹Cr labeling of cells was done on the leukocyte-rich supernate from dextran-sedimented whole blood, it was necessary to rule out the possibility that the radioactivity which was assayed in the samples drawn following reinfusion of labeled cells was due to contaminating platelets, granulocytes, or red blood cells (RBC). As mentioned above, determinations of lymphocyte-associated radioactivity and specific activity of lymphocytes were done on Hypaque-Ficoll-separated cells. These suspensions were macroscopically free of RBC. This fact was confirmed by examination of cytocentrifuged smears stained with Wright-Giemsa stain. It was found that only rare RBC per high-power field were seen in these smears. However, in order to rule out totally the possibility that these few contaminating RBC were significantly contributing to the activity of the samples, Hypaque-Ficoll suspensions of specimens from four subjects at different time periods in the study were examined for activity prior to and following hypotonic lysis of RBC. It was found that this maneuver decreased the activity of the specimens by only 5.5% ± 1.2%. This small decrease was due most likely to the minimal damage to the mononuclear cells themselves by the hypotonic exposure as well as to the small amount of elution of label consistently seen with washing alone.

As mentioned previously, granulocytes make up only about 1% of the Hypaque-Ficoll mononuclear cell layer, and so any contribution by granulocytes to the total activity of the cell suspension would be negligible. In addition, the half-life of granulocytes is sufficiently short that most of the labeled granulocytes would have been cleared from the circulation by 24 hr after reinfusion.

The most significant potential problem with contamination is with labeled platelets. It is clear that Hypaque-Ficoll-separated mononuclear cells may contain substantial numbers of platelets. Despite the fact that platelets were re-
moved by differential centrifugation prior to $^{51}$Cr-labeling, it was not certain that all platelets were removed, and thus some were labeled and reinfused with the labeled WBC. In order to determine what contribution, if any, labeled platelets made to the activity of the Hypaque-Ficoll suspensions which were assayed for lymphocyte activity, the effect of removal of platelets from the suspensions by differential centrifugation (150 g for 3 min at 20°C) upon the specific activity of lymphocytes was determined in two subjects. Table 1 shows that removal of all detectable platelets from suspension A and greater than 90% of the platelets from suspension B resulted in only minimal decreases in the lymphocyte specific activity. These data indicate that neither contaminating RBC, granulocytes, nor platelets significantly influenced the activity of the mononuclear cell suspensions.

Following reinfusion of cells, rapid equilibration of labeled lymphocytes with the total intravascular as well as extravascular compartments occurred. This equilibration was similar to that described in previous lymphocyte kinetic studies. By 1 hr following reinfusion of labeled cells, the mean per cent of total injected lymphocyte-associated radioactivity remaining in the circulation for all nine subjects was 21.8% ± 3.2%, with a range of 12.0% - 36.2%. In order to determine more accurately the kinetics of labeled versus unlabeled lymphocytes remaining in the circulation after equilibrium had been reached, together with the effect of OHC administration on these kinetics, the amount of lymphocyte-associated radioactivity left in the circulation at any given time, as well as the specific activity of lymphocytes, was expressed as the per cent of the 1-hr value.

Figure 1 illustrates the effect of a single dose of 400 mg OHC on lymphocyte kinetics. Prior to the administration of OHC (at 24 hr), the total circulating lymphocyte counts showed very little variability and were the same in the treated and control groups. Likewise, the per cent of the 1-hr lymphocyte specific activity, as well as the per cent of the 1-hr-lymphocyte-associated radioactivity remaining in the circulation, were the same for the treated and control groups, and both showed a gradual linear decay. Following administration of a single 400 mg dose of OHC intravenously, there was a dramatic decrease in total circulating lymphocyte counts which was maximal at 4 hr. Counts returned to normal by 24 hr following OHC administration. Thereafter, the lymphocyte
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Counts in both groups were the same for the remainder of the study (6 days). At the same time as the decrease in lymphocyte counts in the OHC-treated group was seen, there was a marked increase in lymphocyte specific activity which returned to normal as the lymphocyte counts returned to normal and subsequently resumed the gradual linear decay pattern identical to that of the untreated group. However, during the period of corticosteroid-induced lymphocytopenia, the amount of lymphocyte-associated radioactivity was unchanged and continued to follow a gradual linear decay pattern identical to that of the control group. Hence, the depletion of circulating lymphocytes caused by corticosteroid administration was predominantly from the unlabeled lymphocytes in the circulation. This conclusion was determined by the fact that the amount of lymphocyte-associated radioactivity in the circulation remained unchanged while lymphocyte specific activity markedly increased during the decrease in total circulating lymphocyte counts.

In addition to the fact that there was no increase in egress of labeled lymphocytes from the circulation following OHC administration, neither was there any redistribution of labeled cells already present in the spleen, bone marrow,
liver, and lung. Figure 2 illustrates that once the labeled cells entered these tissues there was no change in the distribution of labeled cells in the treated group following administration of OHC.

DISCUSSION

The phenomenon of the recirculation of lymphocytes has been clearly demonstrated in certain animal species\textsuperscript{9-11} as well as in man.\textsuperscript{12} Two distinct populations of lymphocytes have been shown to exist in certain animal species based on circulation properties.\textsuperscript{9-11} The recirculating portion of the intravascular lymphocyte pool, comprised of approximately two-thirds of lymphocytes in the circulation, belongs to a vastly larger total-body recirculating lymphocyte pool.\textsuperscript{10} These intravascular recirculating cells can freely migrate into and out of the circulation. They are in constant equilibrium with the extravascular portion of this total-body recirculating pool contained in the thoracic duct lymph and certain areas of the spleen, lymph nodes, and bone marrow.\textsuperscript{9-11,21} The major proportion of these recirculating lymphocytes has been shown to be thymus derived.\textsuperscript{10,13} The smaller fraction of intravascular lymphocytes belongs to the
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The nonrecirculating pool which is not capable of freely migrating from the circulation, and these cells probably live out their life span within the intravascular compartment. In the present study, despite the fact that the reinfused cells were minimally manipulated and were greater than 95% viable, there was a rapid initial clearance of approximately 78% of labeled lymphocytes within the first hour after reinfusion. Thereafter there was a gradual and steady decay of lymphocyte-associated radioactivity remaining in the circulation. This observation is very similar to the findings in previous human lymphocyte kinetic studies. The explanation has been put forth that the recirculating fraction of the reinfused lymphocytes which is capable of freely migrating out of the circulation, rapidly equilibrates with the vastly larger total-body recirculating pool. The lymphocyte-associated radioactivity remaining within the intravascular circulation is due then, for the most part, to the nonrecirculating fraction of the reinfused labeled lymphocytes which cannot migrate out of the circulation. Since the recirculating labeled lymphocytes have become so diluted in the much larger total-body pool, one would expect them to contribute very little to the radioactivity in the circulation as they continue to recirculate between the intravascular and extravascular compartments.

The present study shows that the transient lymphocytopenia caused by a single dose of 400 mg of OHC is due to a depletion of recirculating lymphocytes from the intravascular compartment. Precisely at the point of OHC-induced maximal lymphocytopenia, there was a dramatic increase in lymphocyte specific activity, while the lymphocyte-associated radioactivity remaining in the circulation was no different from that of the control group (Fig. 1). These data clearly demonstrate that the lymphocytes which had been depleted from the circulation by OHC administration were unlabeled recirculating cells, and the labeled non-recirculating lymphocytes in the circulation were not noticeably affected. Of interest is the fact that, as the total lymphocyte count returned to normal 24 hr following OHC administration, the lymphocyte specific activity returned to and followed the identical decay curve as that of the untreated control group, indicating that the depleted cells had been replaced in the circulation by unlabeled, recirculating lymphocytes which were capable of reentering the intravascular compartment and restoring the total lymphocyte count to normal. This finding is quite consistent with previous findings that administration of 400 mg of OHC to normal subjects results in a preferentially greater depletion of the T-lymphocyte population of circulating lymphocytes defined functionally and by surface markers. This predominantly T-lymphocytopenia was maximal at 4 hr following OHC, with a return to normal total numbers and proportions of lymphocyte populations by 24 hr. The present observation of return to the normal linear regression of specific activity following transient depletion of cells together with previous evidence that suprapharmacologic doses of corticosteroids in vitro do not cause a lysis or death of normal human lymphocytes argues strongly for a redistribution phenomenon as the explanation of the corticosteroid-induced lymphocytopenia noted here. Studies in the mouse, guinea pig, and rat have shown that corticosteroids cause a circulating lymphocytopenia by effecting a redistribution of T-lymphocytes out of the circulation into other body compartments, particularly the bone marrow. In addition, studies in the mouse, guinea pig, and rat have shown that corticosteroids cause a circulating lymphocytopenia by effecting a redistribution of T-lymphocytes out of the circulation into other body compartments, particularly the bone marrow. In addition, studies in the mouse, guinea pig, and rat have shown that corticosteroids cause a circulating lymphocytopenia by effecting a redistribution of T-lymphocytes out of the circulation into other body compartments, particularly the bone marrow.
this phenomenon of corticosteroid-induced redistribution of lymphocytes can actually cause a lymphocytosis in special circumstances. Shaw et al. demonstrated that corticosteroid administration caused a temporary lymphocytosis in patients with chronic lymphocytic leukemia which was explained, at least in part, by the redistribution of lymphocytes from peripheral organs such as the spleen into the circulation.

Surface organ radioactivity counting demonstrated that, after equilibrium had been reached following reinfusion, and after labeled cells had distributed to different tissues, there was little redistribution of label in tissues following OHC administration. This finding was not surprising, since the labeled recirculating lymphocytes had already equilibrated and left the circulation by that time, and we have seen (Fig. 1) that OHC administration caused no appreciable depletion of labeled lymphocytes from the circulation. Surface body counting of organ radioactivity is at best a very crude measurement. At the time of OHC administration, equilibrium had already been reached, and the lymphocytes which were depleted from the circulation were nonlabeled recirculating cells which had replaced the recirculating lymphocytes during equilibration. It is unlikely that any further shifts of labeled cells between organs at the time of OHC administration would be appreciated by surface counting in experiments of this sort because of the tremendous dilution factor in the total-body recirculating lymphocyte pool and the necessarily small amount of radioactive label allowable in normal volunteer studies.

The present study demonstrates that in humans the lymphocytopenia induced by acutely administered corticosteroids is due to a preferential depletion from the circulation of recirculating lymphocytes. These cells under normal circumstances are capable of migrating into and out of the intravascular compartment and are in equilibrium with the total-body recirculating lymphocyte pool. The mechanisms whereby corticosteroid administration causes this selective redistribution of the intravascular portion of the recirculating lymphocyte pool remain unknown. However, the fact that enzymatic alteration of molecular configurations on the surface of lymphocytes profoundly affects their recirculating capacities suggests that the present alterations in circulation may be directly associated with pharmacologically induced changes in the lymphocyte surface.

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