

Effect of Corticosteroids on the Human Monocyte IgG and Complement Receptors

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ABSTRACT A quantitative in vitro assay was employed to directly assess the effect of corticosteroids on the IgG and complement receptor function of human mononuclear phagocytic cells. In this system corticosteroids were solubilized with cholesterol-phospholipid sonicated dispersions before exposure to mononuclear cells. Solubilized corticosteroids at concentrations between 10^{-4} and 10^{-3} M inhibited both IgG and complement receptor activity in a dose-response fashion. Inhibition was dependent upon the time of interaction of the mononuclear cells with corticosteroids and was half-maximal by 15 min. The inhibitory effect at all concentrations of hydrocortisone was partially overcome by increasing the number of IgG molecules per erythrocyte. Hydrocortisone also inhibited the binding of erythrocytes coated with both IgG and C3, despite the fact that when both were on the erythrocyte surface a synergistic effect on binding to mononuclear cells was observed. At the steroid concentrations employed, the capacity of mononuclear cells to exclude trypan blue and to take up latex particles and neutral red was unaffected. Mineralocorticoids also inhibited receptor activity, but the sex hormones were less effective. These studies demonstrate an effect of steroid hormones on cell membrane receptor function, and they suggest that an inhibition of the recognition system for IgG and C3 in vivo may explain, in part, the effect of corticosteroids in man.

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INTRODUCTION

The macrophage IgG and complement receptors are important in host defense (1, 2) and are responsible for the increased clearance of immunoglobulin- and complement-coated cells in patients with autoimmune hemolytic anemia and autoimmune thrombocytopenia. Corticosteroids are effective in the treatment of IgG-induced autoimmune hemolysis (3, 4). Existing data suggest that this effect results from a decrease in erythrocyte sensitization with antibody by decreasing antibody production (5, 6) or by decreasing the binding of antibody to the erythrocyte surface (6).

Previous studies have shown that corticosteroids decrease the clearance of antibody-coated red cells in man (3, 6) and rats (7) and IgG- and/or C3-coated red cells in guinea pigs (8). In the latter study Atkinson et al. demonstrated that IgG-sensitized erythrocytes were cleared in corticosteroid-treated guinea pigs as if they had fewer IgG molecules per red cell, suggesting a decreased recognition of immunoglobulin- or complement-coated cells by the macrophages of the reticulo-endothelial system of the steroid-treated animal. Indeed recent studies have shown that corticosteroids can affect macrophage (9) and monocyte (10) activity. However, none of these studies have shown an effect of corticosteroids on monocyte or macrophage receptor function. To assess this, we employed a quantitative in vitro model derived from that described by LoBuglio et al. (11), using human erythrocytes and homologous peripheral blood mononuclear phagocytic cells. In order to present poorly soluble steroids in a form which might interact more effectively with the mononuclear cell membrane, we first solubilized corticosteroids with cholesterol-phospholipid sonicated dispersions (12, 13). With these tools we examined the effect of corticosteroids on the IgG and C3 receptors of human mononuclear phagocytic cells.

METHODS

Isotonic Veronal-buffered saline, pH 7.4, containing 0.1% gelatin and 0.0015 M CaCl_2 and 0.0005 M MgCl_2 (VBS)¹ and Veronal-buffered saline containing 0.1% gelatin and 0.01 M ethylenedinitrilotetracetic acid (EDTA buffer) were prepared as previously described (14, 15). Rabbit monospecific antiserum to human IgM, IgG, and C3 (Behring Diagnostics, American Hoechst Corp., Somerville, N. J.); human anti-Rh D antiserum (Sera-Tec Biologicals, New Brunswick, N. J.); human C1 (Cordis Laboratories, Miami, Fla.); Hanks' balanced salt solution (HBSS) and Alsever's solution (Grand Island Biological Co., Grand Island, N. Y.); trypan blue (Allied Chemical Corp., Morristown, N. J.); neutral red (Fisher Scientific Co., King of Prussia, Pa.); and latex particles (The Dow Chemical Company, Indianapolis, Ind.) were obtained as indicated. Peroxidase stain was performed according to Kaplow (16).

Isolation of rabbit IgG anti-human IgG, anti-human C3, and complement components. 3 ml of rabbit anti-human IgG or rabbit anti-human C3 antiserum was applied to a 5×100 -cm Sephadex G-200 column equilibrated with 0.0035 M phosphate buffer, pH 7.4, containing 0.15 M NaCl and fractionated by upward flow at 15 ml per h. Each IgG fraction was pooled, concentrated to 20 ml, absorbed three times with D-positive human erythrocytes, and utilized in the quantitative IgG and C3 studies below.

Fresh frozen guinea pig serum was obtained from Rockland, Inc. (Gilbertsville, Pa.). Partially purified guinea pig C2 and human C1 were prepared by a modification of established methods (17, 18) as were cells coated with hemolytic antibody and the fourth component of complement (EAC4) (19).

Preparation of rabbit IgM antierythrocyte antiserum. Erythrocytes obtained from human donors were washed three times in EDTA buffer with removal of the buffy coat and two times with VBS. 1 ml of increasing concentrations of red blood cells (RBC) (1×10^8 – 3×10^9 RBC) in VBS was injected intravenously 3–4 times per wk over a 3-wk course into the ear vein of New Zealand white rabbits. Bleedings were done 5–10 days after the last injection and 3.5 ml of those antisera with the highest hemagglutinin activity were fractionated by Sephadex G-200 chromatography (5×100 cm as above) and the IgM-containing peak pooled so as to contain no detectable IgG by Ouchterlony analysis (20). The IgM antierythrocyte antibody was stored at 4°C .

Preparation of human erythrocytes sensitized with human anti-D antisera. Whole blood was obtained from D-positive donors and either diluted threefold with Alsever's solution and held at 4°C or diluted with sodium heparin (Riker Laboratories, Inc., Northridge, Calif.) (10 U of heparin per 10 ml whole blood) and used immediately. The diluted whole blood was washed twice with EDTA buffer with removal of the buffy coat, resuspended to 1×10^8 RBC per ml with EDTA buffer, sensitized at 37°C with

an equal volume of anti-D antiserum diluted in EDTA buffer, washed once with EDTA buffer and twice with HBSS, and resuspended with HBSS to a final concentration of 5×10^7 erythrocytes per ml. In all experiments utilizing sensitized red cells, control erythrocytes were prepared by exposing washed erythrocytes to EDTA buffer at 37°C in the absence of antiserum. For experiments examining mononuclear cell binding of IgG-sensitized erythrocytes, red cells were radiolabeled with [^{51}Cr]sodium dichromate (100 μCi per ml of whole blood) (New England Nuclear, Boston, Mass.) by standard techniques (21) before sensitization. Hemagglutination titers were performed by a microtiter technique. 2.5×10^6 washed, anti-D-sensitized human erythrocytes in a volume of 25 μl were added to 50 μl of serial threefold falling dilutions of monospecific rabbit anti-human IgG or anti-human IgM antiserum diluted in EDTA buffer. The suspensions were mixed and incubated at 37°C for 30 min and at room temperature for an additional 30 min and examined for agglutination. In all experiments there was no detectable agglutination of anti-D-sensitized erythrocytes incubated with EDTA buffer alone or with anti-IgM antisera, while marked agglutination was seen with greater than 1:1,000 dilution of anti-IgG antisera.

Preparation of human erythrocytes sensitized with IgM and C3 (EIgMC3). Whole human blood obtained and washed with removal of the buffy coat as above was resuspended to 1×10^8 RBC per ml with EDTA buffer and sensitized at 37°C with an equal volume of IgM antierythrocyte antibody diluted in EDTA buffer, washed once with EDTA buffer, and washed twice with HBSS. 5×10^7 of these RBC were incubated with 1:16 dilution of fresh autologous serum as a source of C3 at 37°C for 15 min, washed three times with HBSS, and resuspended to 5×10^7 RBC per ml with HBSS. The number of IgM and C3 molecules per EIgMC3 was determined by C1 fixation and transfer (see below).

In all experiments utilizing EIgMC3, control erythrocytes sensitized with IgM alone without exposure to serum, as well as erythrocytes exposed to serum in the absence of IgM antibody, were employed. For experiments examining mononuclear cell binding of EIgMC3, red cells were radiolabeled with [^{51}Cr]sodium dichromate (100 μCi per ml of whole blood) before sensitization. Erythrocytes sensitized with subagglutinating concentrations of IgM antierythrocyte antibody were not agglutinated when incubated with anti-IgG antiserum. 30 molecules of IgM per RBC, as determined by C1 fixation and transfer (see below), were sufficient to cause detectable direct agglutination.

Quantitation of the number of immunoglobulin or C3 molecules per erythrocyte. The C1 fixation and transfer method was used to determine the average number of IgG molecules per erythrocyte in the sensitized erythrocyte population (22, 15). This technique provides a minimum estimate of the number of immunoglobulin molecules per cell (22). D-positive erythrocytes suspended in EDTA buffer were sensitized with twofold falling dilutions of human anti-D antiserum diluted in EDTA buffer as indicated in the preparation of IgG-sensitized erythrocytes. The average number of IgG C1-fixing sites generated was determined after sensitization with rabbit IgG anti-human IgG and typical dose-response curves with a slope of 1.0 were obtained, directly relating the number of C1-fixing sites to the number of IgG molecules per erythrocyte (23, 24). Similar results were obtained with three different preparations of anti-IgG. In all experiments erythrocytes

¹Abbreviations used in this paper: EDTA buffer, Veronal-buffered saline containing 0.1% gelatin and 0.01 M ethylenedinitrilotetracetic acid; EIgMC3, human erythrocytes sensitized with IgM and C3; HBSS, Hanks' balanced salt solution; HC, cholesterol-phospholipid mixtures containing hydrocortisone; RBC, red blood cells; VBS, isotonic Veronal-buffered saline, pH 7.4, containing 0.1% gelatin and 0.0015 M CaCl_2 and 0.0005 M MgCl_2 .

were sensitized with approximately 900 IgG molecules per RBC. At all concentrations of IgG per red cell examined no C1-fixing sites were generated in the absence of anti-IgG.

The number of IgM molecules per RBC was determined by direct C1 fixation and transfer after sensitization of human erythrocytes suspended in EDTA buffer with two-fold falling dilutions of IgM antierythrocyte antibody diluted in EDTA buffer (22, 15). Typical dose-response curves were obtained with a slope of 1.0. The slope of 1.0 has been taken to indicate that one IgM molecule is capable of fixing one molecule of C1 (23). In all experiments using IgM antibody, erythrocytes were sensitized with approximately 120 molecules of IgM per RBC.

The number of C3 molecules per EIGMC3 was estimated by determining the number of C1-fixing sites generated after exposure to 2-mercaptoethanol and IgG anti-C3 (25). Erythrocytes sensitized with 120 molecules of IgM antibody were incubated with serial twofold falling dilutions of autologous serum as a source of C3 for 15 min at 37°C, washed once with VBS, incubated with 0.05 M 2-mercaptoethanol for 30 min at 37°C, washed twice in EDTA buffer, and incubated with an optimal concentration of anti-C3 (25, 26) before C1 fixation and transfer. Typical dose-response curves with a slope of 1.0 directly relating the number of C1-fixing sites generated by anti-C3 to the concentration of serum employed were obtained. The number of C1-fixing sites generated by anti-C3 relates to a minimum estimate of the absolute number of C3 molecules per RBC (25, 26).

In all experiments using EIGMC3, erythrocytes were sensitized with 120 molecules of IgM and a 1:16 concentration of serum. This concentration of serum generated approximately 2,500 C1-fixing sites with anti-C3. This method of analysis of cell-bound C3 detects both C3b and its major degradation product, C3d. IgM-sensitized erythrocytes were exposed to autologous serum for 15 min at 37°C; after this incubation period, one would expect most of the cell bound C3 to be in the form C3b (27). EIGMC3 were immune adherence positive.

Immune adherence assay. Immune adherence assays were performed as previously described (27). 5 μ l of 3.3×10^7 IgM-sensitized erythrocytes or EIGMC3 were placed in 20 μ l of VBS. 50 μ l of 5×10^7 washed human erythrocytes (possessing the immune adherence receptor) were added to each well. The preparations were mixed and incubated for 30 min at 37°C and immune adherence activity examined at 15 and 30 min.

Preparation of mononuclear cell monolayers. Mononuclear cell monolayers were prepared by the method of LoBuglio et al. (11) modified as below. Whole blood was obtained from normal human donors, anticoagulated with sodium heparin (10 U per ml of whole blood), and applied to a Ficoll-Hypaque density gradient (28). The mononuclear cell layer was harvested, diluted with HBSS to 1×10^6 mononuclear cells per ml, and 1 ml was placed in replicate 35-mm plastic tissue culture dishes (Falcon Plastics, Oxnard, Calif.) for 45 min at room temperature; non-adherent cells were decanted and the adherent cells were washed five times with HBSS. In all experiments monolayer plates contained greater than 97% mononuclear cells of which approximately 80% were peroxidase positive. Approximately 90% of cells morphologically appeared to be monocytes when stained with Wright's-Giemsa and examined by light microscopy. The number of mononuclear cells per monolayer was quantitated by light microscopy

with a Kpl-W 125 Zeiss microscopy eyepiece (Carl Zeiss, Inc., New York). The mean number of mononuclear cells per field (2.5×10^{-3} mm²) was determined by examining 100 consecutive fields. The number of mononuclear cells per monolayer (8.6×10^3 mm²) was then calculated. Trypan blue exclusion was performed by standard techniques.

Mononuclear cell binding of radiolabeled, IgG-sensitized erythrocytes was achieved by incubating 1 ml (5×10^7 per ml) of unsensitized or IgG-sensitized erythrocytes with each monolayer for 2 h at 37°C, decanting unbound erythrocytes and washing five times with HBSS; the monolayers were then lysed with H₂O and the lysate was assayed for radioactivity in a gamma counter (Nuclear Chicago, Searle Analytic, Inc., Des Plaines, Ill.) or air-dried and stained with Wright's-Giemsa for light microscopy. All experiments were performed in triplicate. Less than 1% of the mononuclear cells bound red cells when incubated with unsensitized erythrocytes, and the ratio of unsensitized to sensitized red cells bound per monolayer was 1:10 and usually 1:20 in all experiments. Inhibition of mononuclear monolayer cell binding of sensitized erythrocytes was determined in all experiments as follows: $\{1 - [\text{cpm of lysate (monolayer incubated with steroid)}/\text{cpm of lysate (monolayer incubated with buffer)}]\} \times 100$.

Mononuclear cell binding of EIGMC3 was assessed in a similar manner by incubated mononuclear monolayer cells at 37°C for 90 min with radiolabeled EIGMC3 (5×10^7 RBC). The number of bound erythrocytes was determined as in the IgG receptor assay above.

Solubilization of steroids. Steroids were solubilized in cholesterol-phospholipid mixtures by a modification of the method of Bruckdorfer et al. (12). 23 mg of unesterified cholesterol (Sigma Chemical Co., St. Louis, Mo.) and 40 mg of the phospholipid 1,2-dipalmitoyl lecithin (Grand Island Biological Co.) were added with 5 mg of steroid to 10 ml of 0.15 M NaCl. The mixture was placed in a fluted metal container surrounded by an ice slurry and subjected to 70 W for 60 min with a Branson sonifier (Heat Systems-Ultrasonics, Inc., Plainview, N. Y.) using a standard tip. All sonicated cholesterol-phospholipid mixtures were centrifuged at 21,800 g for 30 min to remove sedimented insoluble material. Cholesterol-phospholipid mixtures containing hydrocortisone are designated "HC." Such cholesterol-phospholipid mixtures have been previously shown to cause no change in red cell or platelet cholesterol:phospholipid ratio (29, 30).

Ingestion of neutral red or latex particles. Neutral red (0.1%) was diluted 1:10 with HBSS, and 1 ml was incubated at room temperature for 10 min with replicate monolayers. A cover slip was added and the percentage of cells demonstrating neutral red uptake determined by light microscopy. In each experiment 100 consecutive cells were examined. Phagocytosis of latex particles was determined by incubating 1 ml of 1.0- μ m latex particles (5×10^7 /ml) in HBSS with replicate monolayers for 1 h at 37°C and the percentage mononuclear cells containing > 4 latex particles per cell determined by counting 100 consecutive cells in each experiment under phase microscopy.

Steroids. Hydrocortisone, deoxycorticosterone, β -estradiol, testosterone, progesterone hydrocortisone phosphate, hydrocortisone acetate, hydrocortisone succinate, prednisone, dexamethasone, and aldosterone were obtained from Sigma Chemical Co.

Statistics. The results are expressed as mean \pm SEM. Comparisons were evaluated by using Student's *t* test for unpaired samples.

RESULTS

The effect of HC on mononuclear cell IgG receptor activity was first examined by incubating IgG (anti-D)-sensitized erythrocytes with mononuclear cells in the presence of 1 ml of 8×10^{-4} M HC or buffer (Fig. 1). HC caused a 66% inhibition of the binding of sensitized erythrocytes to the mononuclear cell monolayer. Preincubation of the monolayers with HC for 30 min at 37°C before the introduction of IgG-sensitized erythrocytes increased this inhibition to 97%. Washing the monolayers after 30 min of preincubation with HC did not diminish the capacity of HC to inhibit erythrocyte binding. In all subsequent experiments, monolayers were washed three times after a 30-min preincubation with HC. Although HC inhibited the attachment of IgG-coated red cells, it did not cause the release of mononuclear-cell-bound erythrocytes from their mononuclear cell attachment site when added for 30 min at 37°C (Fig. 1, D).

To further assess the effect of HC, monolayers were preincubated with 4×10^{-4} M and 8×10^{-4} M HC for varying time intervals (Fig. 2). No inhibition of IgG-coated erythrocyte binding was observed with up to 5 min of preincubation with 8×10^{-4} M or up to 15 min of preincubation with 4×10^{-4} M HC. Thereafter, a progressive inhibition of the capacity of the mononuclear cells to bind IgG-sensitized erythrocytes was observed.

The effect of hydrocortisone concentration on the inhibition of mononuclear cell IgG receptor activity was examined in two ways (Fig. 3). First, hydrocortisone was solubilized with cholesterol-phospholipid mixtures (HC) and the effect of varying dilutions of HC with buffer was studied. In addition, cholesterol-phospholipid

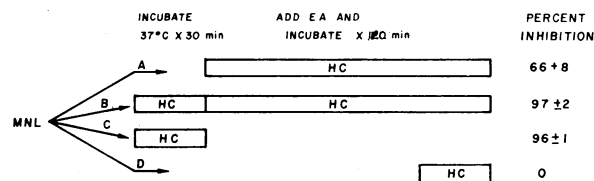


FIGURE 1 Inhibition of IgG receptor activity by 8×10^{-4} M hydrocortisone (HC). Four experiments (A-D) are shown. In each experiment all additions were to replicate mononuclear cell monolayers (MNL). In all experiments erythrocytes were sensitized with 900 IgG per RBC (EA). In exp. A, HC was added with EA for 120 min at 37°C . In exp. B, MNL were preincubated for 30 min at 37°C with HC before the addition of EA. In exp. C, MNL were preincubated with HC and washed three times before the introduction of EA for 120 min at 37°C . In exp. D, MNL were first incubated with EA for 90 min at 37°C , washed three times and then incubated with HC for 30 min at 37°C to assess displacement of EA from mononuclear cells by HC.

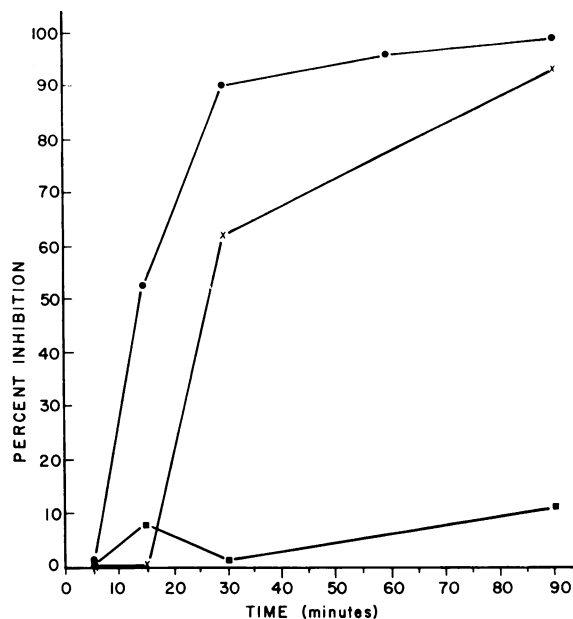


FIGURE 2 Kinetics of inhibition of IgG receptor activity by hydrocortisone. ● represents the effect of 8×10^{-4} M HC and × the effect of 4×10^{-4} M HC. ■ represents the effect of cholesterol-phospholipid mixtures without hydrocortisone.

mixtures were prepared with a constant amount of cholesterol-phospholipid and a varying amount of hydrocortisone. Little inhibition was seen after preincubation of the mononuclear cells with hydrocortisone at concentrations up to 2.5×10^{-5} M. Thereafter, inhibition was progressive, the half-maximal point being at approximately 2×10^{-4} M. Preincubation with solubilized cholesterol-phospholipid mixtures without hydrocortisone inhibited the binding of sensitized erythrocytes <10%.

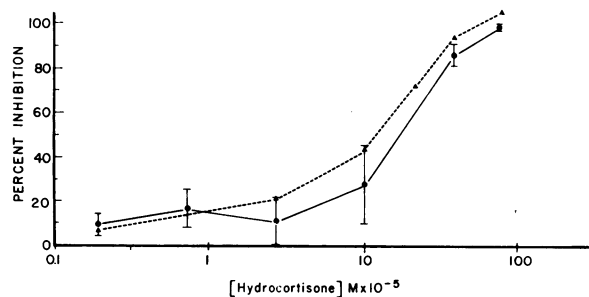


FIGURE 3 Inhibition of IgG receptor activity by hydrocortisone. ▲ represents cholesterol-phospholipid mixtures containing a constant amount of cholesterol and phospholipid but different concentrations of hydrocortisone. ● represents twofold falling dilutions of a mixture containing cholesterol (1.4 mg/ml), phospholipid (2.4 mg/ml), and HC (8×10^{-4} M).

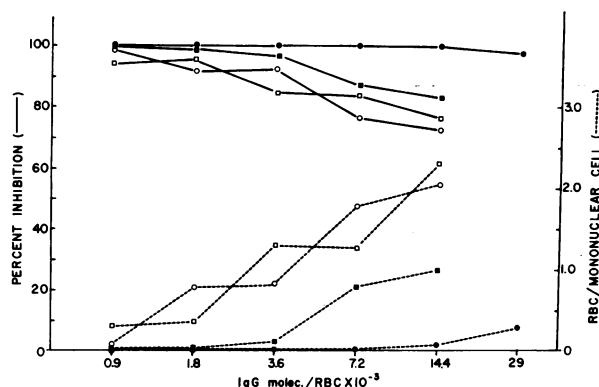


FIGURE 4 IgG receptor inhibition by hydrocortisone: effect of increasing IgG/RBC. Monolayer cells were preincubated with 8×10^{-4} M HC (●), 4×10^{-4} M HC (■), 3×10^{-4} M HC (□), and 2×10^{-4} M HC (○). With increased IgG per erythrocyte the inhibitory effect of low concentrations of HC was partially overcome.

There is evidence that erythrocytes more heavily sensitized with IgG may still be cleared *in vivo* in corticosteroid-treated animals (8). Therefore, the effect of the number of IgG molecules per red cell on the ability of HC-treated mononuclear cells to detect IgG-coated erythrocytes was examined. With increasing numbers of IgG molecules per red cell there was a progressive decrease in the inhibitory effect of HC (Fig. 4). At all concentrations of HC examined ($2-8 \times 10^{-4}$ M), inhibition was achieved at each level of IgG sensitization in a dose-dependent fashion.

The effect of HC on the mononuclear cell C3 receptor was examined in a similar fashion. When mononuclear cells preincubated with HC were incubated with red cells coated with C3, results similar to those obtained with IgG-coated cells were observed (Fig. 5). Inhibition was 90% at HC concentrations of 8×10^{-4} M and approximately half-maximal at 1×10^{-4} M HC. Neither IgM-sensitized erythrocytes not incubated with serum nor unsensitized erythrocytes incubated in serum were bound to the monolayers.

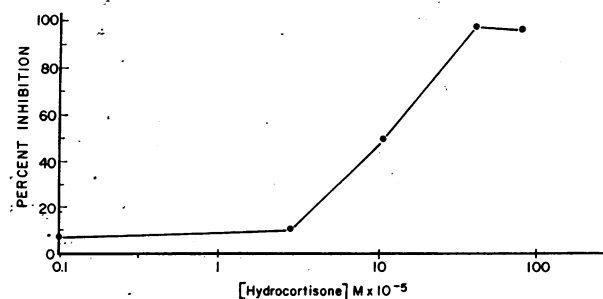


FIGURE 5 Inhibition of monocyte C3 receptor by hydrocortisone.

TABLE I
Inhibition of Mononuclear Monolayer Cell Binding of Erythrocytes Coated with IgG and/or Complement by HC

RBC coated with:	Red cells bound per mononuclear cell preincubated with:		
	HC		Buffer
	4×10^{-4} M	3×10^{-4} M	
IgG	0.1	0.3	2.4
C3	0.1	0.2	2.5
IgG + C3	0.4	1.5	7.2

The average number of red cells bound per mononuclear cell was calculated from the radioactivity (^{51}Cr) per monolayer (1×10^6 mononuclear cells).

The capacity of HC to inhibit erythrocytes sensitized with both IgG and C3 was also examined (Table I). IgG-sensitized erythrocytes were coated with C3 and the binding to monolayers compared to that of erythrocytes sensitized with either C3 or IgG alone. The number of IgG and C3 molecules per erythrocyte was the same whether erythrocytes were sensitized with both IgG and C3 or whether they were sensitized with IgG or C3 alone. In order to directly compare the number of IgG- with the number of C3-coated red cells bound per mononuclear cell, the results are expressed in terms of red cells per mononuclear cell. Concentrations of IgG (900 IgG C1-fixing sites) and C3 (1,250 C1-fixing sites) per erythrocyte were selected that caused equal numbers of erythrocytes to be bound per mononuclear cell. The effect of IgG plus C3 on the binding of erythrocytes to mononuclear cells appeared to be synergistic. HC (3 and 4×10^{-4} M) pretreatment inhibited mononuclear cell binding of erythrocytes sensitized with both IgG and C3; however, this inhibition was less marked than that seen with either IgG or C3 alone.

The effect of preincubation with HC (8×10^{-4} M) on mononuclear cell function is shown in Table II. HC did not diminish the number of mononuclear cells per monolayer, nor did it increase the percentage of mononuclear cells capable of excluding trypan blue. The capacity of mononuclear cells to take up neutral red or latex particles was also unaffected.

The capacity of other steroid hormones to influence the IgG receptor activity of mononuclear cells was also examined. Monolayers were preincubated with each steroid (300 $\mu\text{g}/\text{ml}$) solubilized in cholesterol-phospholipid mixtures (Table III). As with HC, the corticosteroid derivatives prednisone and dexamethasone markedly inhibited the binding of IgG-coated red cells. Mineralocorticoids also caused a marked inhibition of IgG receptor activity, but the sex hormones had a less profound

TABLE II
Effect of 8×10^{-4} M HC on the Mononuclear Monolayer

	Trypan blue uptake	Mononuclear cells per monolayer*	Neutral red uptake	Latex uptake (>4 particles/cell)
	%		%	%
CPM + hydrocortisone	8 ± 5.5	1.1×10^5	94 ± 1.8	78 ± 5.1
CPM	9 ± 1.0	1.1×10^5	95 ± 1.2	68 ± 4.6
Saline	6 ± 5.1	1.2×10^5	97 ± 1.3	82 ± 12.0

CPM, cholesterol-phospholipid mixtures without hydrocortisone; CPM + hydrocortisone, cholesterol phospholipid mixtures solubilized with 8×10^{-4} M hydrocortisone.

* Mean of quadruplicate monolayers for each experimental condition (representative experiment).

inhibitory effect. Water-soluble derivatives of hydrocortisone had a variable effect. Hydrocortisone hemisuccinate was equivalent to hydrocortisone, whereas hydrocortisone phosphate and hydrocortisone acetate had significantly less inhibitory activity.

DISCUSSION

These studies demonstrate an effect of steroid hormones on the IgG and C3 receptor function of human peripheral blood mononuclear phagocytic cells. Previous studies in steroid-treated animals suggested a decreased recognition of IgG- and complement-coated cells by macrophages (9, 10). To assess this in vitro we examined the human peripheral blood mononuclear phagocytic cell, a cell closely related to the tissue macrophage (31), and employed a homologous in vitro model which permits quantitative evaluation of IgG and C3 receptor function.

TABLE III
IgG Receptor Inhibition by Steroids (300 μ g/ml)

	Molarity	Percent inhibition
Hydrocortisone	8×10^{-4}	96 ± 1.2
Prednisone	8×10^{-4}	93 ± 7.5
Dexamethasone	8×10^{-4}	80 ± 16.0
Mineralocorticoids		
Aldosterone	8×10^{-4}	89 ± 3.5
Deoxycorticosterone	9×10^{-4}	72 ± 9.4
Sex hormones		
β -Estradiol	1×10^{-3}	29 ± 1.0
Testosterone	1×10^{-3}	46 ± 2.5
Progesterone	1×10^{-3}	43 ± 12.0
Hydrocortisone succinate	6×10^{-4}	100 ± 1.0
Hydrocortisone phosphate	6×10^{-4}	67 ± 13.8
Hydrocortisone acetate	7×10^{-4}	33 ± 15.9
Cholesterol	8×10^{-4}	9 ± 3.4

In vitro studies involving steroid hormones are limited by the low solubility of steroids in aqueous solutions. However, steroids may be solubilized by sonication with cholesterol-phospholipid mixtures (12). Mixtures with a cholesterol:phospholipid molar ratio of 1.0 have been used in our studies, since it is known that this proportion does not affect either the lipid composition or the function of other human peripheral blood cell membranes (29, 30).

Solubilized hydrocortisone (HC) inhibited mononuclear cell IgG and C3 receptor function in a dose-response fashion (Figs. 3 and 5). The effect of HC was directly on the mononuclear cell monolayer, as it was not reversed by washing (Fig. 1) and was dependent upon the time of interaction of HC with the mononuclear cells (Fig. 2). However, once IgG-coated erythrocytes were bound to their mononuclear cell attachment site, HC did not cause their removal (Fig. 1).

Mononuclear cells were viable as determined by trypan blue exclusion and at the concentrations of HC examined nonimmune phagocytosis of latex particles and neutral red was unaffected while IgG receptor activity was markedly inhibited (Table II). Whether higher concentrations of solubilized hydrocortisone affect nonimmune phagocytosis of monolayer cells as well, as has been demonstrated by others with steroids in animal models (32, 33, 7), remains to be determined.

Our data demonstrate that HC also inhibits the binding of erythrocytes coated with complement whether or not IgG is also present. Erythrocytes with C3b on their surface are immune adherence positive (34, 35, 27) and our complement-coated red cells were positive in the immune adherence reaction. C3b-coated erythrocytes bind to a macrophage receptor which is similar to and perhaps identical with (36) the immune adherence receptor. Recently, evidence has been presented for a receptor for the fourth component of complement (C4b) on human polymorphonuclear leukocytes (37) and

monocytes (38). In this study we have not distinguished between C3b and C4b, and thus the relative contribution of each in binding erythrocytes to the mononuclear monolayer cells is undetermined.

In our system, as has been previously described (15, 39, 40), complement is required for the binding of IgM-sensitized cells to macrophages, and at the concentration of IgM employed we have confirmed the absence of a receptor for IgM on human peripheral blood monocytes. The clearance of erythrocytes in IgM-induced hemolytic anemia in man (cold agglutinin disease) is dependent upon the macrophage complement receptors (26, 15, 27). Corticosteroid therapy is not as effective in cold agglutinin disease as in warm antibody (IgG) autoimmune hemolytic anemia (3, 4). Corticosteroids, however, have been demonstrated to decrease the clearance of IgM-sensitized cells in an animal model (8). This apparent discrepancy may relate to the large number of C3b molecules per erythrocyte in cold hemagglutinin disease or to species differences in the systems involved.

Our data are in keeping with the *in vivo* clinical (6) and experimental (8) evidence that the improved red blood cell survival with corticosteroids can be overcome when the concentration of IgG per erythrocyte is increased. Increasing the number of IgG molecules per erythrocyte resulted in increased numbers of erythrocytes bound per HC-treated mononuclear cell at all concentrations of HC (Fig. 4). Furthermore, with increased numbers of IgG per erythrocyte the percentage inhibition observed at each concentration of HC became less marked.

Erythrocytes coated with IgG and complement are cleared more rapidly *in vivo* than erythrocytes coated with IgG alone (15, 41, 42), and corticosteroid treatment is less effective in improving the clearance of cells sensitized with both IgG and C3 (8, 41). This is consistent with our observation that erythrocytes coated with both IgG and C3 bind more effectively to HC-treated mononuclear cells than red cells coated with either IgG or C3 (Table I).

Mononuclear cell binding of IgG- and C3-coated cells is greater than the additive binding observed with cells coated with either IgG or C3 (Table I). This suggests a synergistic interaction between the IgG and complement receptors. Mantovani et al. previously showed that complement markedly enhanced the binding of IgG-sensitized cells to mouse peritoneal macrophages (40). However, they did not examine macrophage binding of complement-coated cells alone. Huber et al. studied the human monocyte IgG and C3 receptors in a heterologous system which employed sheep red blood cells and rabbit IgG antibody. Their studies revealed a cooperative interaction between the monocyte IgG and C3 receptors in phagocytosis (39).

Although not assessed directly, it appears that the locus of action of steroid hormones in these studies is at the membrane level. While a direct effect of steroid hormones on membranes has been postulated, it has been difficult to establish (43). A wide range of steroids can be incorporated into natural and artificial lipid membranes (44, 45); however, there does not appear to be a constant relationship between the ability of a steroid to do so and the structural features of the steroid molecule. The effect of steroids on membranes has been observed most vividly in their ability to stabilize or labilize artificial lipid membrane vesicles (46, 47). In addition, some steroid hormones bind to the membrane proteins of red cells and this appears to underlie the effect of progesterone on the storage of human red cells (48). It is unclear whether the effect which we have observed results from a direct effect on membrane lipids, a direct effect on membrane proteins (e.g., the receptors themselves), or an indirect effect on the membrane exerted from within the cell.

Rinehardt et al. recently examined the effect of corticosteroids *in vitro* (10) and *in vivo* (49) on the human peripheral blood monocyte. They observed no inhibition of IgG receptor activity and the complement receptor was not studied. However, their study differs in that monocytes were not preincubated with steroid and they did not employ corticosteroids in a lipid, membrane-soluble form. Additionally, our studies indicate that inhibition of IgG receptor activity is dependent upon the concentration of hydrocortisone as well as the number of IgG molecules per erythrocyte. The concentration of hydrocortisone employed in our studies are similar to plasma steroid levels that have been achieved *in vivo* (50, 51). Nonetheless, whether hydrocortisone can directly affect IgG receptor function *in vivo* remains to be determined.

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