Effect of Corticosteroids on Lymphocyte Activation

By David Tak Yan Yu

Human peripheral blood lymphocytes were stimulated by concanavalin A, sodium periodate, and neuraminidase plus galactose oxidase. Response to mitogens was measured by the amount of tritiated thymidine incorporated as well as the percent of "giant sheep red blood cell rosettes" generated. The thymidine incorporation was diminished by the absence of monocytes or the presence of corticosteroids. The percent of giant rosettes generated was not influenced by either change. This finding suggested that considerable lymphocyte activation could still take place in the presence of corticosteroids. When subjects received 60 mg of prednisone, they developed lymphopenia 5 hr later. The circulating lymphocytes at that time responded less well to mitogen stimulation when measured by both thymidine incorporation and percent giant rosettes, suggesting a selective sequestration of mitogen-responsive lymphocytes outside the circulatory compartment.

CORTICOSTEROIDS are potent pharmacologic agents.[1] Part of their efficacy may be related to their ability to suppress lymphocyte activation. Lymphocytes become activated when they are cultured in vitro with mitogens, allogeneic lymphocytes, and specific antigens. The rates of synthesis of DNA, RNA, and protein are increased, and lymphokines are released. Whereas the increases in synthesis are inhibited by corticosteroids,[2-7] lymphokine production may or may not be affected.[8-13] These data suggest that though corticosteroids can suppress lymphocyte activation, the suppression is incomplete.

The present investigator has recently reported that when human lymphocytes are activated by mitogens they acquire the ability to form rosettes in vitro with increased numbers of sheep red blood cells (SRBC). These are called "giant SRBC rosettes," and constitute a convenient marker of activated lymphocytes. In contrast to the conventional measurements of the gross rates of DNA, RNA, protein synthesis, or lymphokine production, this method of assessing lymphocyte "activation" allows direct enumeration of activated lymphocytes.[14,15] The term "activation" is used here to indicate that the lymphocytes have undergone some changes as a result of reaction with the mitogens.

The present paper is concerned with the application of this technique to examine the effect of corticosteroids.

MATERIALS AND METHODS

Cell Culture

Mononuclear cells were separated from heparinized blood samples of healthy human volunteers on Ficoll-Hypaque gradients. Whenever indicated, monocytes were removed by incubating these cells with carbonyl iron and then removing them with a magnet.[7] To stimulate cells with

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sodium periodate ($NaIO_4$) or neuraminidase plus galactose oxidase (NGAO), they were washed twice with phosphate-buffered saline (PBS) and incubated for 30 min at 4°C with 0.5 m*M* $NaIO_4$ (Sigma Chemical Co., St. Louis, Mo.), or at 37°C with 50 U/ml of neuraminidase (Behring, Somerville, N.J.) plus 0.5 U/ml of galactose-oxidase (Worthington, Freehold, N.J.). After one wash with Hank's balanced salt solution (HBSS) they were cultured for 48 hr in RPMI-10% human AB sera (Roswell Park Memorial Institute) at 1×10^6 cells/ml in a 37°C 5% CO_2 incubator.

To stimulate cells with concanavalin A (Con A), they were washed twice with HBSS and then cultured for 72 hr with 12 μg/ml of Con A (Calbiochem, San Diego, Calif.). To test the effect of corticosteroids, cultures were carried out with 10^{-4}-10^{-8} *M* hydrocortisone hemisuccinate (Sigma), methylprednisolone (Upjohn, Kalamazoo, Mich.), or prednisolone (Schering, Bloomfield, N.J.). Hydrocortisone hemisuccinate and methylprednisolone were dissolved in HBSS. Prednisolone was first dissolved at 5 mg/ml in ethanol and then diluted with HBSS. These reagents were added immediately after addition of Con A or treatment by NGAO or $NaIO_4$. The details of some of these methods have been reported previously.[14]

To assay DNA synthesis, lymphocytes were cultured in triplicate in 1 ml per tube as described. Four hours prior to termination, 2 μCi of tritiated thymidine were added to each tube (^{3}H-TdR, 5 Ci/mmole; Amersham/Searle, Arlington Heights, Va.). The cells were precipitated with 10% trichloroacetic acid and the amount of radioactivity assessed by scintillation counting.[14]

Rosette Assays

To assay SRBC rosettes, lymphocytes were washed twice with HBSS and resuspended at 5×10^6 cells/ml. SRBC were washed three times with saline and resuspended at 1% suspension in HBSS-10% fetal calf serum (FCS). The latter were first heat-inactivated and adsorbed with SRBC. Then 0.1 ml of each of the cell suspensions was mixed in 10 × 35 mm tubes, centrifuged at 200 *g* for 10 min, and kept at 4°C overnight before resuspension. Rosettes were assayed in hemocytometers.

To assess giant SRBC rosettes, a drop of each of the rosette suspensions was put on a glass slide under a cover slip and allowed to dry partially at room temperature for about 15 min. The SRBC surrounding the lymphocytes in each rosette spread out in a monolayer. Rosettes with ≥36 SRBC per rosette were defined as "giant SRBC rosettes." Nonviable cells were recognized by their failure to exclude trypan blue, and were not included in the assessment. The viability of all the samples exceeded 90%; 200-300 cells were counted in each sample. Results of SRBC rosettes were recorded as percent of lymphocytes forming SRBC rosettes. Results of giant rosettes were recorded as percent of SRBC rosettes forming giant rosettes (SRBC-RFC). The details of this method and the rationale have been previously reported.[14,15]

Effect of Prednisone Administration

Studies were also done to assess the effect of administration of prednisone in vivo on the circulating lymphocytes of human subjects. Consent was obtained from the subjects prior to the test. The protocol was approved by the Human Subject Protection Committee of the University of California, Los Angeles. Five healthy subjects, three female and two male, 28-35 yr of age, received 60 mg of prednisone (Parke-Davis) orally at 8:00 a.m. Blood samples were taken just prior to administration and 5 hr afterward. In four of the subjects, this was repeated 1-3 mo later without administering the drug. White cell and differential counts of the blood samples were assayed by standard methods described previously.[16] Mononuclear cells were isolated from Ficoll-Hypaque gradients, treated with NGAO, and cultured for 48 hr. Both ^{3}H-TdR incorporation and giant SRBC rosettes of cultured samples were assayed.

Statistics

Results of several experiments were computed into averages ±SEM. Statistical comparisons were done by the paired observation *t* test. The ^{3}H-TdR incorporation and percent giant SRBC rosettes of samples cultured in the presence of drugs were also expressed as percent inhibition of those without the drugs:

$$\% \text{ Inhibition} = 100 - \frac{\text{Values of samples cultured with drugs}}{\text{Values of samples cultured without drugs}} \times 100.$$

RESULTS

Effect on Con A Stimulation

The ^{3}H-TdR incorporation of Con A–stimulated samples was higher in the presence of monocytes than in their absence ($p < 0.02$). ^{3}H-TdR incorporation was almost completely inhibited by 10^{-4} *M* hydrocortisone (OHC). The percent inhibition of ^{3}H-TdR incorporation by 10^{-6} *M* OHC was significantly higher in the absence of monocytes than in their presence ($p < 0.05$). (See Table 1.)

The percent of giant SRBC rosettes generated by Con A was not affected by the absence of monocytes ($p > 0.05$). OHC did not have any significant effect on the percentage of these rosettes ($p > 0.05$). (See Table 2.)

Effect on $NaIO_4$ Stimulation

Cells were stimulated with $NaIO_4$ and cultured in the presence of 10^{-4}–10^{-6} *M* OHC. No significant differences were detected in the numbers of viable cells recovered from the different samples after 48 hr of culture. The ^{3}H-TdR incorporation was decreased in the absence of monocytes ($p < 0.02$). ^{3}H-TdR incorporation was almost completely inhibited by 10^{-4} *M* OHC. In four of five experiments, the suppression by OHC was more in the absence of monocytes than in their presence. In the fifth experiment, 100% suppression was observed in all the samples. There was no significant difference when the averages of all five samples were compared (Table 3). The percent of giant SRBC generated was not affected by the presence of monocytes or hydrocortisone ($p > 0.05$). (See Tables 3 and 4).

Table 1. Effect of Hydrocortisone on the CMP of ^{3}H-TdR Incorporated by Lymphocytes Stimulated by Con A

Samples	Monocytes Present		Monocytes Absent	
	cpm	Inhibition (%)	cpm	Inhibition (%)
Control	4,800 ± 1,600	—	2,100 ± 3,000	—
Con A	119,000 ± 2,300	—	42,300 ± 11,100	—
Con A + OHC 10^{-4} M	30,900 ± 19,400	75.8 ± 18.7	8,800 ± 5,300	92 ± 7.7
Con A + OHC 10^{-5} M	81,300 ± 24,900	43.3 ± 20.8	19,500 ± 9,900	72.5 ± 16
Con A + OHC 10^{-6} M	97,100 ± 18,700	18.5 ± 10.8	24,700 ± 6,000	50.0 ± 10.7

Results are means ±SE of five separate experiments.

Table 2. Effect of Hydrocortisone on the Percent of SRBC and Giant SRBC-RFC of Con A–stimulated Lymphocytes

Samples	Monocytes Present		Monocytes Absent	
	SRBC-RFC	Giant SRBC-RFC	SRBC-RFC	Giant SRBC-RFC
Control	68.3 ± 4.2	0.4 ± 0.4	68.5 ± 1.5	0
Con A	74.1 ± 2.7	28.1 ± 8.5	74.2 ± 4.3	28 ± 7.2
Con A + OHC 10^{-4} M	73.1 ± 3.3	21.8 ± 4.2	75.2 ± 2.1	26.6 ± 6.8
Con A + OHC 10^{-5} M	69.9 ± 3.1	30.1 ± 7.2	72.7 ± 4.0	29.9 ± 7.4
Con A + OHC 10^{-6} M	72.4 ± 2.6	28.7 ± 6.2	72.5 ± 3.4	28.2 ± 7.5

Table 3. Effect of Hydrocortisone on the CPM of ^{3}H-TdR Incorporated by Lymphocytes Stimulated by $NaIO_4$

Samples	With Monocytes		Without Monocytes	
	cpm	Inhibition (%)	cpm	Inhibition (%)
Control	600 ± 60	—	860 ± 300	—
$NaIO_4$	23,700 ± 9,600	—	10,500 ± 5,600	—
$NaIO_4$ + OHC 10^{-4} M	1,200 ± 700	98.8 ± 1.2	300 ± 100	100 ± 0
$NaIO_4$ + OHC 10^{-5} M	8,800 ± 5,400	81.6 ± 9.7	2,100 ± 1,200	88.2 ± 6.8
$NaIO_4$ + OHC 10^{-6} M	13,000 ± 6,800	63.6 ± 14.1	4,050 ± 1,900	88.0 ± 5.4

Results are means ±SE of five separate experiments.

Table 4. Effect of Hydrocortisone on the Percent of SRBC and Giant SRBC-RFC of Samples Stimulated by $NaIO_4$

Samples	With Monocytes			Without Monocytes		
	SRBC-RFC	Giant SRBC-RFC	Recovery* × 10^6	SRBC-RFC	Giant SRBC-RFC	Recovery* × 10^6
Control	65.3 ± 3.8	4.4 ± 1.7	0.8 ± 0.1	67.2 ± 2.6	2.9 ± 1.1	0.8 ± 0.1
$NaIO_4$	63.8 ± 3.4	19.1 ± 1.4	0.7 ± 0.4	64.2 ± 3.8	21.2 ± 1.8	0.8 ± 0.1
$NaIO_4$ + OHC 10^{-4} M	64.5 ± 2.7	19.8 ± 1.4	1.0 ± 0	62.2 ± 3.8	21.5 ± 2.5	1.0 ± 0
$NaIO_4$ + OHC 10^{-5} M	62.5 ± 2.9	19.7 ± 1.9	1.1 ± 0.1	61.2 ± 3.8	20.0 ± 1.7	0.9 ± 0.1
$NaIO_4$ + OHC 10^{-6} M	61.3 ± 3.4	20.1 ± 2.0	1.0 ± 0.1	62.4 ± 4.3	21.0 ± 2.4	0.9 ± 0.1

Cells were cultured at 1 × 10^6/ml.

*Concentrations of viable cells after 48 hr of culture.

Table 5. Effect of Hydrocortisone on the CPM of ^{3}H-TdR Incorporated by Samples of Lymphocytes Stimulated by NGAO

Samples	With Monocytes		Without Monocytes	
	cpm	Inhibition (%)	cpm	Inhibition (%)
Control	1,200 ± 300	—	1,400 ± 500	—
NGAO	154,700 ± 31,300	—	54,900 ± 20,900	—
NGAO + OHC 10^{-4} M	24,500 ± 5,700	85.0 ± 2.7	3,700 ± 1,200	94.2 ± 1.9
NGAO + OHC 10^{-5} M	92,100 ± 20,600	41.2 ± 5.4	16,000 ± 5,000	75.5 ± 3.8
NGAO + OHC 10^{-6} M	120,000 ± 26,800	23.5 ± 5.1	24,700 ± 7,400	54.0 ± 7.0

Results are means ± SE of six separate experiments.

Table 6. Effect of Hydrocortisone on the Per Cent of SRBC and Giant SRBC-RFC of Cells Stimulated by NGAO

Samples	Monocytes Present			Monocytes Absent		
	SRBC-RFC	Giant SRBC-RFC	Recovery* × 10^6	SRBC-RFC	Giant SRBC-RFC	Recovery* × 10^6
Control	75 ± 3.6	0	0.8 ± 0.1	74.3 ± 7.2	0	0.8 ± 0.1
Neuraminidase alone	79.9 ± 1.4	4.2 ± 1.7	0.7 ± 0.1	76.8 ± 4.4	3.2 ± 1.0	0.7 ± 0.1
NGAO	82.7 ± 2.6	40.5 ± 4.2	0.8 ± 0.1	82.6 ± 4.5	39.2 ± 2.9	0.5 ± 0.1
NGAO + OHC 10^{-4} M	81.4 ± 1.9	40.0 ± 4.1	0.8 ± 0.1	80.1 ± 5.3	36.3 ± 2.8	0.6 ± 0.1
NGAO + OHC 10^{-5} M	80.7 ± 1.4	40.9 ± 3.2	0.8 ± 0.1	81.8 ± 4.2	37.8 ± 3.4	0.6 ± 0.1
NGAO + OHC 10^{-6} M	77.8 ± 1.6	38.6 ± 4.7	0.9 ± 0.4	80.5 ± 4.1	37.2 ± 3.0	0.7 ± 0.1

Cells were cultured at 1 × 10^6/ml.

*Concentrations of viable cells after 48 hr of culture.

Table 7. Percent Inhibition Induced by Methylprednisolone and Prednisolone on the Generation of Giant SRBC Rosettes and ^{3}H-TdR Incorporation of Cells Stimulated by NGAO

Drug Conc. (M)	Methylprednisolone		Prednisolone	
	Giant SRBC-RFC	^{3}H-TdR	Giant SRBC-RFC	^{3}H-TdR
10^{-4}	15.8 ± 11.2	91.5 ± 1.5	6.8 ± 9.4	94.0 ± 1.9
10^{-5}	14.1 ± 22.2	68.7 ± 4.6	−13.8 ± 16.6	48.6 ± 22.2
10^{-6}	9.7 ± 12.9	14.2 ± 17.2	−5.5 ± 6.2	34.9 ± 15.7
10^{-7}	7.0 ± 11.5	18.3 ± 7.3	−1.5 ± 1.5	16.8 ± 11.3
10^{-8}	1.3 ± 10.4	13.5 ± 10.9	−2.3 ± 8.8	14.9 ± 2.9

Effect on NGAO Stimulation

The numbers of viable cells recovered from the samples after 48 hr of culture were not affected by the presence of monocytes or of OHC. Again the amount of ^{3}H-TdR incorporated was increased by the presence of monocytes ($p < 0.05$). ^{3}H-TdR incorporation was almost completely inhibited by 10^{-4} *M* OHC. The suppression by 10^{-5} and 10^{-6} *M* OHC was increased by the absence of monocytes ($p < 0.001$). (See Table 5.). The percent giant SRBC rosettes generated was not affected by either change (Table 6). Three samples of NGAO-stimulated cells were cultured with 10^{-4}–10^{-8} *M* methylprednisolone. Significant suppression of ^{3}H-TdR incorporation was observed at 10^{-4} and 10^{-5} *M* ($p < 0.01$ and <0.01, respectively), but not in the percentages of giant SRBC rosettes (Table 7). Three other samples of NGAO-stimulated cells were cultured with 10^{-4}–10^{-8} *M* prednisolone. In two experiments significant suppression of ^{3}H-TdR incorporation was observed at 10^{-4}–10^{-6} *M*. When the averages of three experiments were taken into account, suppression was observed only at 10^{-4} *M* ($p < 0.05$). The percentages of giant SRBC-RFC were not affected (Table 7). Samples cultured with ethanol in quantities similar to those present in 10^{-4} *M* concentration were not inhibited.

Table 8. Effect of Prednisone Administration on Lymphocyte Concentration and Percent SRBC-RFC in Peripheral Blood

Subjects	Samples*	SRBC-RFC (%)	Lymphocytes/ cu mm
1	8:00 a.m.	73.7	2680
	1:00 p.m.	51.3	985
2	8:00 a.m.	85	2120
	1:00 p.m.	77.3	516
3	8:00 a.m.	73	2510
	1:00 p.m.	45	823
4	8:00 a.m.	74.7	2760
	1:00 p.m.	71	1040
5	8:00 a.m.	75.3	1650
	1:00 p.m.	59.3	660
Mean ± SEM	8:00 a.m.	76.3 ± 2.2	2344 ± 206
	1:00 p.m.	60.8 ± 6.0	805 ± 98

*Samples were assayed for SRBC-RFC prior to culture.

Table 9. Effect of Prednisone Administration on the Generation of Giant SRBC-RFC

Subjects	Samples*	^{3}H-TdR Incorporated (cpm)	SRBC-RFC (%)	Giant SRBC-RFC (%)
1	8:00 a.m. Control	500 ± 100	85	2
	N	700 ± 90	92	8
	NGAO	87,400 ± 5,800	95	75
	1:00 p.m. Control	300 ± 30	82	3
	N	200 ± 0	86	6
	NGAO	52,400 ± 2,900	96	58
2	8:00 a.m. Control	300 ± 20	81	3
	N	330 ± 20	87	3
	NGAO	97,860 ± 6,460	87	59
	1:00 p.m. Control	120 ± 10	77	1
	N	120 ± 20	80	2
	NGAO	27,200 ± 3,170	89	35
3	8:00 a.m. Control	5,200 ± 300	73	0
	N	1,100 ± 300	76	0
	NGAO	126,000 ± 8,500	89	40
	1:00 p.m. Control	300 ± 30	62	0
	N	1,000 ± 400	77	0
	NGAO	52,900 ± 6,600	88	12
4	8:00 a.m. Control	1,220 ± 120	55	0
	N	2,260 ± 1,270	66	0
	NGAO	70,900 ± 10,100	75	29
	1:00 p.m. Control	580 ± 140	84	0
	N	650 ± 70	80	0
	NGAO	21,000 ± 2,600	84	15
5	8:00 a.m. Control	1,080 ± 30	84	0
	NGAO	142,000 ± 7,000	83	55
	1:00 p.m. Control	910 ± 400	79	0
	NGAO	65,700 ± 1,000	83	32
Mean ± SEM	8:00 a.m. Control	700 ± 200	75.6 ± 5.6	1.0 ± 0.6
	N	1,100 ± 400	80.3 ± 5.8	2.8 ± 1.9
	NGAO	104,800 ± 12,900	85.9 ± 3.3	51.5 ± 7.9
	1:00 p.m. Control	400 ± 100	76.8 ± 3.9	0.8 ± 0.6
	N	500 ± 200	80.8 ± 1.9	2.0 ± 1.4
	NGAO	43,800 ± 8,500	88.0 ± 2.3	30.4 ± 8.3

*Samples were cultured for 44–48 hr prior to assay. Control: untreated samples. N: samples treated with neuraminidase. NGAO: samples treated with NGAO.

Effect of Administration of 60 mg of Prednisone

Five subjects received 60 mg of prednisone orally at 8:00 a.m. Blood samples were taken immediately prior to and 5 hr after administration. The peripheral blood lymphocyte concentration decreased from 2344 ± 206 to 805 ± 98/cu mm ($p < 0.001$). The percent SRBC-RFC decreased from 76.3 ± 2.2 to 60.8 ± 6.0 ($p < 0.001$). In response to stimulation by NGAO, the amount of ^{3}H-TdR incorporated by samples taken at 1:00 p.m. was significantly less than in those taken at 8:00 a.m. ($p < 0.001$). There was also a significant decrease in the generation of SRBC-RFC ($p < 0.001$). (See Tables 8 and 9.) Such changes were not observed in the control experiments (Table 10).

DISCUSSION

The immunosuppressive ability of corticosteroids is well accepted. Administration of sufficient doses suppresses both immunoglobulin production and antigenic skin reactivity.[17,18] Two modes of action are examined in this paper. First, their ability to suppress lymphocyte activation in vitro; and second, the induction of lymphopenia in vivo.

The present investigator has reported that stimulation of human lymphocytes by mitogens in vitro could lead to the appearance of giant SRBC-RFC. In general, when testing a range of concentrations of a mitogen, those which elicited relatively high rates of ^{3}H-TdR incorporation also generated high percentages of these rosette-forming cells.[15] This observation did not necessarily mean that such rosette-forming ability was of vital biologic importance to the immune response of the hosts. However, these results, and other evidence presented previously, suggested that the percent of rosettes could be used as an index of the degree of lymphocyte activation. This indicator had the advantage that it directly enumerated mitogen-affected lymphocytes.[14] The functional significance of these rosette-forming cells awaits further investigation.

As in the previous reports, stimulation of lymphocytes by Con A, NGAO, and $NaIO_4$ led to an increased amount of ^{3}H-TdR incorporation and generated 12%–40% of giant SRBC-RFC.[14] In addition, the amount of ^{3}H-TdR incorporated was significantly less in the absence of monocytes. Surprisingly the percentages of giant SRBC-RFC were not affected, indicating that the event which generated giant SRBC-RFC, unlike that of ^{3}H-TdR incorporation, did not require interaction with monocytes.

The presence of 10^{-4}–10^{-5} *M* hydrocortisone, methylprednisolone, and prednisolone significantly suppressed the mitogen-induced incorporation of ^{3}H-TdR. The suppression was enhanced by the absence of monocytes. Since the plasma corticosteroid levels reached after administration of 60 mg of prednisone were about 3×10^{-6} *M*,[19] this type of suppression may conceivably also occur in vivo. However, the generation of giant SRBC-RFC was unaffected by the presence of corticosteroids in vitro, even at 10^{-4} *M* when ^{3}H-TdR incorporation was almost completely inhibited. This finding suggests that a significantly large group of lymphocytes could still be activated in the presence of corticosteroids. This group could be distinguished from those in the unstimulated samples by their ability to form giant SRBC-RFC.

The effect of corticosteroids on lymphocyte activation has received intensive investigation. Under the influence of corticosteroids, the lymphocytes become unable to release certain lymphokines or increase their rates of protein and DNA synthesis.[2, 10] Nevertheless, the production of three types of lymphokines, the macrophage-aggregating factors, skin-reactive factors, and macrophage-

Table 10. Comparison of the Response of Lymphocytes to NGAO From Samples Taken at 8:00 a.m. and 1:00 p.m.

Subjects	Time	^{3}H-TdR (cpm)	SRBC-RFC (%)	Giant SRBC-RFC (%)
1	a.m.	283,000 ± 9,000	82.7	35.3
	p.m.	193,000 ± 1,700	83.9	44.0
2	a.m.	177,000 ± 4,000	84.7	47
	p.m.	231,000 ± 11,000	88.7	59
3	a.m.	16,773 ± 137	83.7	10
	p.m.	37,061 ± 234	90	17
4	a.m.	15,300 ± 1,000	77.6	24
	p.m.	14,900 ± 230	78.3	23

The results shown are those of samples stimulated by NGAO. Control samples were similar to those shown in Table 9 and are not shown.

inhibitory factors, is unaffected.[11 13] The fact that the lymphocytes are still able to form giant SRBC-RFC strengthens the suspicion that lymphocytes can be activated to a certain degree even in the presence of corticosteroids.

There is a possibility that the giant SRBC rosette-forming ability is not related to all events which occur after mitogens have reacted with the lymphocytes. This question has been examined in two previous reports. Two pieces of evidence appear to be against this. First, giant SRBC-RFC can be generated by a large variety of reagents which can activate lymphocytes in vitro. Second, the presence of ouabain can completely suppress such generation. Ouabain is an ATPase inhibitor. Presumably it inhibits the entry of K^+ into cells. The latter is one of the earliest events of lymphocyte activation. Its occurrence is a prerequisite for many of the subsequent events. Thus its suppression will prevent the appearance of mitosis, blastogenesis, and the increase in protein, DNA, and RNA synthesis. The fact that ouabain can suppress the generation of giant SRBC-RFC strongly suggests that these rosettes occur as a result of lymphocyte activation.[14,15]

Finally, even if the ability of forming giant rosettes does not reflect a vital biologic process, it still reflects a special cell surface event which occurs as a result of reaction of the lymphocytes with the mitogens. This particular cell surface event cannot be suppressed by corticosteroids. Presumably other biologically more important processes may also be unaffected.

In summary, lymphocytes are probably activated even in the presence of corticosteroids. These activated lymphocytes can be recognized by their ability to form giant SRBC-RFC.

This finding was utilized to examine a related problem. When human subjects receive the equivalent of 60 mg of prednisone they develop transient lymphopenia several hours later.[16] The percent SRBC-RFC in the postprednisone samples was less than in the preprednisone samples. Such differences became much smaller after 48 hr of culture. This change during culture might be due to selective cell death or change in surface markers. The reasons are not certain. Of interest, the lymphocytes obtained after drug administration respond poorly to mitogen stimulation.[20-21]

Two theoretical possibilities can account for this diminished response. The first is that the circulatory lymphocytes are being inactivated by the increased levels of corticosteroids in the blood. The second is that the lymphocytes that normally respond well to the mitogens have become sequestered outside the circulation. It has been quite well established that administration of corticosteroids to human subjects does not cause lympholysis but a redistribution of the recirculating lymphocytes away from the circulatory compartment.[22] In experimental animals, the lymphocytes that become sequestrated in the bone marrow are highly responsive to mitogen stimulation.[23]

To resolve this question, the ability of the lymphocytes to generate giant SRBC-RFC was examined. It was found that after prednisone administration, the circulatory lymphocytes generated less giant SRBC-RFC than prior to administration. This finding favored the hypothesis that corticosteroid administration led to partial sequestration of mitogen-responsive cells.

This sequestration may also prevent migration of these functionally more

capable cells into areas of immunologic challenges. It may constitute one of the immunosuppressive mechanisms of corticosteroids.

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