

Histologic responses in human skin test reactions to ragweed

IV. Effects of a single intravenous injection of steroids

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A controlled study has been carried out dealing with the early effects of a single intravenous dose of either methylprednisolone or placebo on newly developed and ongoing cellular inflammatory responses in immediate hypersensitivity skin test reactions. There was no significant difference in the tissue eosinophil responses to ragweed injected 2 hr before and 2 hr after placebo; there was a significant rise ($101\% \pm 39$) from the second to fourth hour after antigen injection. By contrast, there was a marked decrease in the tissue eosinophil response to antigen injected 2 hr after steroids as compared to the pattern seen in the presteroid reaction. In addition, the eosinophil numbers not only did not increase from the second to fourth hour when steroids were injected at the second hour but decreased markedly. These findings suggest early suppressive effects on tissue eosinophil responses within 2 hr after steroid were administered intravenously. Also, there may be trafficking of eosinophils both into and out of these inflammatory sites during the first hours after intradermal antigen injection.

Although corticosteroids are commonly employed as therapeutic agents in the treatment of allergic disease, the exact mechanism of action underlying their efficacy remains unclear. In addition to suppression of clinical expressions of allergic reactions, corticosteroid therapy has been associated with a diminution in the abnormal accumulation of eosinophils in tissue sites of hypersensitivity reactions.^{1, 5}

Several mechanisms for the suppressive effects of steroid on tissue eosinophil accumulation have been postulated, with evidence presented for diminution in the blood eosinophil pool,^{2, 3} and on the migration rate of these cells into the inflammatory site,^{1, 3, 5} decreased circulating mediator-rich cells (i.e., basophils),^{6, 7} and possible inhibitory effect on mediator release itself.⁸

The limited studies of these effects of steroids in humans, including those in our own laboratory,¹ have concentrated on subjects treated for days to weeks

with orally administered agents. Additional valuable information about the eosinophil kinetics, and possibly the mechanism of steroid actions, could be gained from studies of the early effects of a single intravenous (iv) injection of a rapidly acting steroid preparation. These findings may also be of practical clinical value because of the frequent use of such preparations in the treatment of acute allergic reactions. This report describes studies of the effects of a single iv injection of steroid on the cellular events occurring in newly developing and ongoing inflammatory responses at the sites of intradermal pollen extract injections.

MATERIALS AND METHODS

Subjects. Five atopic male volunteer subjects (weighing 70 to 90 kg) were studied after obtaining their informed consent. All individuals were clinically sensitive to ragweed and exhibited an immediate-type wheal response of at least 10 mm in diameter (and at least 3 times the diameter of a reaction to a negative control injection) 20 min following the injection of 20 protein nitrogen units (PNU) of crude ragweed extract (Greer Laboratories, Lenoir, N. C.).

None of these subjects were receiving immunotherapy. Four of the five patients were studied at times other than the pollinating season; none were receiving immunotherapy, and all had discontinued other medications 2 wk prior to this study.

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Treatment program. Each subject reported here was studied on two occasions several weeks apart. On one occasion (at random) the subject received an intravenous injection of methylprednisolone sodium succinate (provided as Solu-Medrol by Dr. Sam Stubbs, The Upjohn Co., Kalamazoo, Mich.) in a dose of 1 mg/kg. On the other occasion an intravenous saline injection was given as a placebo.

Skin tests and dermal biopsies. On each of the occasions the subjects were studied, three skin tests were performed using 0.02 ml of ragweed extract (1,000 PNU/ml) in separate sites on the dorsal surface of the upper arm. Positive whealing responses were noted within 20 min at all sites. Three-millimeter diameter dermal punch biopsies were obtained at the designated times (see subsequent discussion) and were divided in half by a scalpel incision through the antigen injection site perpendicular to the skin surface. Five-micron sections were obtained at the cut surface, representing the center of the reaction site. These sections were coded, stained with May-Grünwald-Giemsa stain, and read in a blind fashion. The number of eosinophils in each of three adjacent sections of each biopsy specimen was quantitated with use of an overlying calibrated grid and expressed as the mean number of eosinophils per square millimeter in the three sections of a biopsy specimen.

Timing of studies (Fig. 1). Each of the five subjects was studied on two separate occasions at least 1 wk apart. During each of the two study periods, the individual received three separate skin tests as well as an iv injection during a single 6-hr time period. Each subject received steroid intravenously during one of the study periods and placebo intravenously during the other, in a random order for the study group.

At time 0 (8 A.M.), two ragweed skin tests were performed. At time 2 hr, skin test No. 1 was biopsied and a rapid iv injection (steroid or placebo) was given immediately thereafter. At time 4 hr, skin test No. 2 was biopsied, and skin test No. 3 was performed. At 6 hr, skin test No. 3 was biopsied. All skin tests were placed in the same geographic distribution in the posterior aspect of the upper arm, separated by distances that we had previously found to satisfactorily exclude effects of one reaction on adjacent skin test sites. Thus in each study period the biopsies of skin test Nos. 1 and 3 permitted a comparison of the histologic responses 2 hr after antigen injection in cases where antigen had been injected either 2 hr before (test No. 1) or 2 hr after (test No. 3) the iv treatment (steroids or placebo).

The histologic response to skin test No. 2, in comparison to those in test No. 1, allowed evaluation of the iv placebo or steroid effects on eosinophil accumulation in an ongoing reaction (i.e., eosinophil response from the second to the fourth hour after antigen injection).

Total eosinophil counts of peripheral blood. Total eosinophil counts were read blindly at times 0, 2, 4, and 6 hr (Fig. 1) using the Uno-pipette system (Becton, Dickinson & Co., East Rutherford, N. J.).

Analysis of data. The statistical analyses are based on paired t test determinations of changes in individual subjects. The "percent change" as presented in the last column

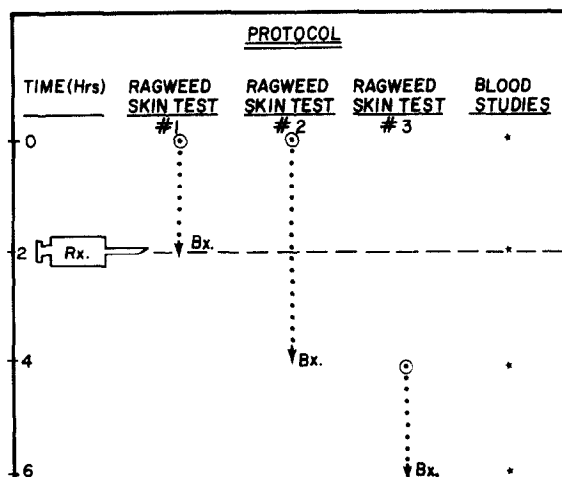


FIG. 1. Circled dot indicates antigen intradermal injection; dotted line, duration of skin test reaction; arrowhead, time of biopsy; asterisk, time of blood sample; and syringe, iv injection of steroid or placebo.

TABLE I. Tissue eosinophil response to antigen before and after iv placebo

Subject	Skin test No. 1: Preplacebo (eosinophils/mm ²)	Skin test No. 3: Postplacebo (eosinophils/mm ²)	Percent change
N. S.	32	36	+11%
M. M.	74	143	+93%
F. K.	84	29	-67%
W. S.	217	60	-72%
D. H.	51	46	-9%

Mean change = -9%, SD = ±67%, $p = >0.1$.

of Tables I to IV represents an index of difference expressed in percent.

$$\frac{B - A}{A} \times 100 = \% \text{ difference.}$$

RESULTS

Comparative eosinophil responses in skin tests performed before and after iv placebo or steroid. Table I shows the number of eosinophils/mm² in biopsies of skin tests Nos. 1 and 3 in the five subjects on the days when the iv placebo was given.

Previous extensive studies in our laboratory have shown no eosinophils immediately (within 30 sec) after antigen injection. Therefore the number of cells seen here reflects accumulation over a 2-hr period. It can be seen that responses were variable among the five subjects, with no consistent differences seen in the biopsies obtained before or after placebo.

In contrast, the eosinophil accumulations were consistently and significantly lower ($p < 0.05$) in skin test No. 3 as compared with skin test No. 1 on the day steroids were given intravenously (Table II).

TABLE II. Tissue eosinophil response to antigen before and after iv methylprednisolone

Sub- ject	Skin test No. 1: Presteroid (eosino- phils/mm ²)	Skin test No. 3: Poststeroid (eosino- phils/mm ²)	Per- cent change
N. S.	78	0	-100%
M. M.	212	11	- 94%
F. K.	108	6	- 94%
W. S.	440	15	- 96%
D. H.	138	16	- 88%

Mean change = -94%, SD $\pm$ 4, P = <0.05.

TABLE III. Tissue eosinophil responses to antigen at 2 and 4 hr in iv placebo-treated subjects

Sub- ject	Skin test No. 1: 2 hr (eosino- phils/mm ²)	Skin test No. 2: 4 hr (eosino- phils/mm ²)	Per- cent change
N. S.	32	101	+215%
M. M.	74	198	+167%
F. K.	89	93	+ 4%
W. S.	217	364	+ 67%
D. H.	51	79	+ 54%

Mean change = 101%, p = 0.05.

Comparison of eosinophil accumulation in skin tests biopsied at 2 and 4 hr after intradermal injection of antigen. Table III shows the number of eosinophils in biopsies obtained 2 hr (skin test No. 1) and 4 hr (skin test No. 2) after intradermal antigen injection on the day when placebo was injected at the second hour. It can be seen that the responses are greater at 4 hr than at 2 hr in every case (mean increase of 101% $\pm$ 39, p = 0.05).

A similar comparison of skin test No. 1 and skin test No. 2 responses on the day of steroid administration (Table IV) reveals that in four of the five subjects, eosinophil accumulation at the reaction site did not increase from the second to the fourth hour as seen with placebo treatment; in fact, the number of eosinophils decreased markedly. A statistically significant difference was found in comparing the percent changes of eosinophils from the second to the fourth hours in the placebo-treated group to those in the steroid-treated group (p = <0.05).

Total eosinophil counts in peripheral blood. In subjects receiving placebo there was a slight diminution in total eosinophil counts over the 6-hr study period (Table V). In contrast, the iv steroids induced a 62% mean diminution in blood eosinophil levels 2 hr after receiving steroids, becoming even more prominent 4 hr after drug administration (-89%).

TABLE IV. Tissue eosinophil response to antigen at 2 and 4 hr in iv methylprednisolone-treated subjects

Sub- ject	Skin test No. 1: 2 hr (eosino- phils/mm ²)	Skin test No. 2: 4 hr (eosino- phils/mm ²)	Per- cent change
N. S.	79	31	-60%
M. M.	212	56	-73%
F. K.	108	148	+36%
W. S.	440	105	-76%
D. H.	138	18	-86%

Mean change = -52%, p = >0.1.

TABLE V. Peripheral total eosinophil count

Sub- ject	Injec- tion	Hours*			
		0	2	4	6
N. S.	Placebo	264†	144	98	104
	Steroid	128	88	40	8
M. M.	Placebo	416	224	186	179
	Steroid	246	208	136	80
F. K.	Placebo	344	179	360	179
	Steroid	346	304	128	88
W. S.	Placebo	502	762	1040	912
	Steroid	1208	1056	298	8
D. H.	Placebo	800	375	376	517
	Steroid	352	216	104	24

*Steroid or placebo was injected at this time.

†Eosinophils/mm³.

There was no statistically significant correlation between tissue eosinophil response and peripheral blood eosinophil levels of individual study points in each subject, as determined by a regression coefficient analysis.

Concomitant changes in circulating leukocytes. The mean total leukocyte counts in the five subjects at time 0 and time 6 hr were 6,040/mm³ and 6,275/mm³, respectively, on the day placebo was given. By comparison, mean leukocyte levels of 6,032/mm³ and 8,410/mm³ were found at time 0 and 6 hr on the day steroids were administered to the same individuals.

The mean percent of neutrophils increased over the 6-hr period in the placebo-treated subjects from 48% $\pm$ 9.7 to 63% $\pm$ 7.8. During steroid treatment there was a mean increase from 49% $\pm$ 3.8 to 90% $\pm$ 4.

The percent of lymphocytes in the placebo-treated subjects decreased modestly from 43% $\pm$ 7 to 32% $\pm$ 5. When the same subjects were treated with steroids, the patients' lymphocytes diminished from 40% $\pm$ 6 to 8% $\pm$ 4 over the same time period.

DISCUSSION

Increased numbers of eosinophils are common in the peripheral blood and tissues involved in immediate hypersensitivity reactions, possibly due to liberation of one or more factors chemotactic for eosinophils.¹²⁻¹⁴ Sequential studies of such reactions in the skin of atopic humans have been carried out using the skin window technique.^{12, 15} More recently, similar studies on punch biopsies from positive skin test reactions to pollen antigens have been carried out⁹ showing an earlier eosinophil response than seen in most skin window studies.¹² Some findings in experimental animal models suggest a long residence of eosinophils in tissues but a shorter intravascular half-life.¹⁶⁻¹⁸ However, recently Dale and associates¹⁹ have presented evidence suggesting that the intravascular half-lives of eosinophils in humans with hypereosinophilic syndromes may be 3 to 4 times that of neutrophils. Too few eosinophils were obtainable from normal subjects to permit similar kinetic studies by those investigators.

Several studies, including those from our laboratory, have shown a gradual increase in the number of eosinophils over a period of hours in the sites of immediate hypersensitivity reaction.^{9, 12, 15, 20} There has been no correlation in individual subjects between the numbers of eosinophils appearing in such reaction sites and the blood eosinophil level at the time of antigen injection; however, few if any eosinophils were found in skin test sites of those subjects with blood eosinophil levels below 150/mm³.²¹

Despite extensive use and observed clinical efficacy of corticosteroids in allergic disorders, the precise mechanisms underlying these effects are not well defined. In previous studies in our laboratory, skin test eosinophil responses to antigen were remarkably decreased after 1 wk of oral steroids,¹ even though the whealing reaction was unaffected.²² Similar findings were subsequently observed in subjects treated with alternate-day steroids by Zweiman and associates.²³

The mechanism underlying the suppression of tissue eosinophil response is still not well delineated. A significant decrease in blood eosinophil levels following steroid administration has been noted for some time² and was seen in our own subjects as well. Several reasons for this eosinopenia have been postulated on the basis of studies in animals.²⁴ There has been no direct evidence in humans for steroid-induced destruction of eosinophils or redistribution of these cells to marginal pools. However, the current studies of Dale and associates¹⁹ in hypereosinophilic patients suggested that there may be movement of eosinophils between the circulating and marginal blood compartments.

The findings of the present study add to our knowledge about steroid effect in humans. As might be expected, iv steroids lead to a prominent blood eosinopenia within 2 hr. As Slott and Zweiman¹ noted previously, there is considerable variation of the degree of tissue eosinophil response not only among ragweed-sensitive subjects but from week to week in the same subject. However, a significantly different pattern emerged in comparisons of the tissue eosinophil responses to intradermal antigen following steroid or placebo injection in the same subject. Previous studies carried out in this laboratory have shown that intradermal injection of normal saline solution (control) or histamine does not produce a dermal eosinophil infiltrate, nor are the histologic patterns altered by appropriately placed adjacent pollen extract skin tests. Intradermal injection of antigen 2 hr after iv steroid induced a significantly lower eosinophil response than occurred in similar experiments when placebo was administered. However, another finding of this study suggests an even earlier effect of steroids on tissue eosinophil responses. Intravenous steroids given during an ongoing inflammatory response to antigen not only prevented the expected subsequent increase in tissue eosinophils from the second to the fourth hour but resulted in a marked decrease (-51%) in the number of these cells seen in the placebo-treated subjects at the fourth hour. Several mechanisms could be postulated to explain these findings. We think it is likely that the accumulation of eosinophils over the first hours after antigen injection reflects a greater number of eosinophils entering into the reaction site than those cells departing the area. The subsequent iv steroid quite rapidly blocks ingress of eosinophils into the site, whereas egress of these cells proceeds unabated. An alternative explanation, for which there is no evidence, is that steroids induce predominantly an increased departure of eosinophils from the inflammatory reaction sites.

If steroids do rapidly alter mobilization of eosinophils into the reaction site, then one of its possible actions might be attributed to its ability to suppress the release of eosinophil chemotactic factor that follows interaction of antigen with sensitized basophil or mast cells.^{26, 27} Although there is no direct evidence from studies in humans about this possibility, several studies have shown that steroids in the usual pharmacologic doses exert little inhibitory effect on aspects of immediate hypersensitivity reaction such as skin whealing²² or bronchoconstrictive response following suitable exposure to antigen.²⁸ Preliminary findings in our laboratory that the *in vitro* sensitivity of leukocytes to antigen for histamine release is unaffected after iv steroids would be compatible with

those previous studies. It should be noted that two groups have reported that *in vitro* addition of steroids inhibits antigen-induced tissue histamine release.^{8, 29}

Nevertheless, it appears that a major component in the steroid-induced depression of tissue eosinophil response is the result of the rapid effect of these drugs on the emigration of eosinophils from the blood into the tissue reaction site. Additional studies will further delineate these inhibitory effects.

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