

Histologic studies of human skin test responses to ragweed and compound 48/80

III. Effects of alternate-day steroid therapy

Burton Zweiman, M.D., Robert I. Slott, M.D.,* and Paul C. Atkins, M.D.
Philadelphia, Pa.

Our previous studies have shown depressed eosinophil responses in skin test reactions to pollen antigens and compound 48/80 in those just completing a 1-wk course of daily steroids. Wheal reactions were unaffected. In this study, 6 ragweed-sensitive atopic subjects were studied before and on the seventh day ("day on") and day 8 ("day off") of a course of alternate-day steroids. Blood neutrophil levels rose on day 7 and were similar to baseline on day 8, whereas blood eosinophil levels were significantly reduced on both days 7 and 8. Neutrophil responses in skin test reactions were depressed on day 7 and normal on day 8. In contrast, the tissue eosinophil responses were depressed significantly, and to similar degree, on both days 7 and 8. These findings are of potential significance in evaluating the clinical effects of steroids in allergic diseases.

In previous studies in this laboratory and by others, some of the characteristics and kinetics of the cellular infiltrations in the sites of immediate hypersensitivity reactions in the skin have been defined. By the use of skin window¹⁻⁵ or biopsy⁶ techniques, it has been shown that eosinophil accumulations start after the time of peak-wheal-and-flare reactivity and become prominent over the next hours. During this latter time period, there is generally little if any gross evidence of an inflammatory response.

It was subsequently shown by us⁷ that such eosinophil accumulation in skin test sites was markedly decreased in those previously treated for one week with moderate daily oral doses of corticosteroids, even though the wheal-and-flare reactivity was undiminished. Peripheral blood eosinophil levels were also decreased significantly after one week of oral steroids.

Recent studies⁸⁻¹¹ have shown that steroids lead to transient effects on blood granulocyte and leukocyte levels, with normal neutrophil responses in skin windows applied on the "off" day of alternate-day steroid therapy. However,

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Reprint requests to: Burton Zweiman, M.D., 512 Johnson Pavilion, University of Pennsylvania, School of Medicine, Philadelphia, Pa. 19174.

*Present address: Columbus-Cuneo-Cabrini Medical Center, Department of Medicine, 2525 N. Lakeview, Chicago, Ill. 60614.

TABLE I. Wheal reactions—ragweed and compound 48/80 skin tests

Subject	Day 1	Day 7	Day 8
Wr			
Ragweed	15*	15	13
48/80	15	25	25
Ru			
Ragweed	12	11	12
48/80	20	23	25
De			
Ragweed	9	8	10
48/80	20	25	25
Ir			
Ragweed	13	10	9
48/80	25	30	25
Or			
Ragweed	14	15	14
48/80	24	20	25
Fa			
Ragweed	14	13	10
48/80	25	18	14

*Mean diameter wheal reaction at 20 min (mm).

there has been little study of effects of alternate-day steroids on the kinetics of eosinophil responses in immediate hypersensitivity reactions. This report describes such studies, which may enhance our understanding of the therapeutic effects of alternate-day steroids in allergic and other immunologic disorders.

MATERIALS AND METHODS

Subjects

Six volunteer healthy ragweed-sensitive subjects were studied outside of the ragweed pollenating season. None of the subjects had been treated with hyposensitization and were on no other medication for at least two weeks prior to study. Informed consent was obtained in every case.

Treatment program

All subjects received methyl prednisolone (kindly provided as Medrol, 4 mg tablets, by The Upjohn Co.) in daily doses of 48 mg at noon of day 1 and 7 A.M. of days 3, 5, and 7 of the study period. The noon medication time on day 1 was chosen to permit baseline studies described below. No medication was taken on days 2, 4, 6, and 8.

Skin tests and dermal biopsies

At 9 A.M. on days 1 (baseline), 7, and 8, skin tests were performed by intradermal injections at different sites on the dorsal surface of the upper arm. Wheal responses were measured 20 min after injection of 0.02 ml of the following reagents: (1) ragweed extract (prepared in the Allergy and Immunology Clinic, Hospital of the University of Pennsylvania), 1,000 PNU/ml; (2) compound 48/80 (Burroughs Wellcome & Co., Research Triangle Park, N. C.), 35 mg/ml; and (3) control diluting solution. In each subject the mean wheal diameter of the ragweed skin test site at 20 min was at least 8 mm and 3 times greater than that of the control site.

Two hours following the intradermal injection, dermal biopsies 3 mm in diameter were performed at the injection sites and fixed; 5- μ paraffin sections of the tissue were coded and stained with May-Gruenwald-Giemsa, and the number of neutrophils and eosinophils were counted in an area of dermis in one plane of focus covered by ten squares of an overlying grid starting at the dermal-epidermal junction, as described previously by us.^{6,7} Eosinophils were easily distinguished by their bright large red granules.

TABLE II. Blood eosinophil and neutrophil levels

Subject	Eosinophils			Neutrophils		
	Day			Day		
	1	7	8	1	7	8
Wr	96*	16	32	2,400*	3,900	2,112
Ru	112	96	128	—	—	—
De	416	192	224	1,386	2,976	2,100
Ir	308	192	256	1,377	2,080	1,440
Or	256	176	192	2,688	3,900	2,950
Fa	640	528	324	3,608	5,104	4,302

*Cells per cubic millimeter.

TABLE III. Eosinophil responses in skin test reaction sites

Subject	Test	Day 1	Day 7	Day 8
Wr	Ragweed	13*	1	0
	48/80	35	0	4
Rv	Ragweed	13	3	0
	48/80	41	16	0
De	Ragweed	16	23	0
	48/80	38	44	6
Ir	Ragweed	10	0	0
	48/80	0	1	0
Or	Ragweed	10	0	0
	48/80	26	0	0
Fa	Ragweed	87	12	10
	48/80	17	1	6

*Number of eosinophils per 10 grid squares (see text).

Blood leukocyte levels

At 9 A.M. on days 1, 7, and 8, immediately before the intradermal injections, blood specimens were obtained for total and differential leukocyte counts and for total eosinophil levels (Unopipette, Becton, Dickinson & Co.).

Analysis of data

A comparison of each of the following parameters was made between day 1 (baseline), day 7 ("day on steroids"), and day 8 ("day off steroids"): (1) blood eosinophil and neutrophil levels; (2) skin test eosinophil responses; and (3) skin test neutrophil responses. Comparative findings in each subject were analyzed by the paired *t* test method.

RESULTS

Wheal-and-flare reactivity

No significant differences in the mean diameter of wheal reactions 20 min after injection of ragweed or 48/80 were seen in individual subjects studied at the three time periods (Table I).

Peripheral blood granulocyte levels

A modest increase in blood neutrophil levels was seen in most of the subjects on day 7 compared to the pretreatment baseline measurements made at the same time on day 8 (Table II). Blood neutrophil levels on day 8 were not different from those seen in the baseline period. A different pattern was seen in blood eosinophil levels, which were decreased significantly ($p < 0.01$), and to approximately similar degree, on both days 7 and 8 (Table II).

TABLE IV. Neutrophil responses in skin test reactions

Subject	Test	Day 1	Day 7	Day 8
Wr	Ragweed	32	4	25
	48/80	25	0	19
Rv	Ragweed	20	5	30
	48/80	57	4	40
De	Ragweed	36	50	11
	48/80	10	16	22
Ir	Ragweed	8	0	12
	48/80	10	6	10
Or	Ragweed	16	3	14
	48/80	12	4	25
Fa	Ragweed	18	4	18
	48/80	42	9	11

Skin test eosinophil responses

The numbers of eosinophils at the sites of injection of ragweed and compound 48/80 on days 1, 7, and 8 are compared for each subject in Table III. The number of eosinophils in ragweed reaction was significantly lower on both days 7 and 8 than on day 1 ($p < 0.05$). If anything, the eosinophil responses on day 8 were lower than those on day 7, although not significantly so. A similar pattern was seen in sites of compound 48/80 injection, in that eosinophil responses were significantly lower on both day 7 ($p < 0.05$) and day 8 ($p < 0.01$), as compared to day 1.

The pattern of changes was a bit variable among the subjects. No eosinophils were seen in the 48/80 site of Subject Ir, even during the baseline period. In Subject De, eosinophil responses to both ragweed and 48/80 were slightly higher on day 7, but considerably decreased on day 8, as compared to day 1.

Skin test neutrophil responses

A different pattern was seen in the tissue neutrophil responses to ragweed and compound 48/80 (Table IV). The findings were more variable among the subjects. However, for the group as a whole, tissue neutrophil levels were significantly lower than baseline in both ragweed and 48/80 injection sites on day 7 ($p < 0.05$) but not on day 8.

DISCUSSION

The findings of this study help to characterize the complex effects of steroids on the cellular inflammatory responses in immediate hypersensitivity reaction sites. The neutrophil responses seen here are similar to those reported in previous studies in the human using other experimental models. It has been shown^{8, 9} that a single parenteral or oral dose of steroids is followed by transient increase in blood neutrophils, returning to normal by 24 hr. In comparative skin window studies¹¹ of the inflammatory responses during daily and alternate-day steroid therapy, it was found that the exudate neutrophil reaction was depressed during both daily steroid administration and the "on" day of alternate-day steroids, but not significantly lower on the "off" day. Although neutrophil responses were somewhat variable in the skin test sites in this study, a similar conclusion could be drawn.

The pattern of eosinophil responses seen here suggests a much more prolonged effect on both the blood levels and tissue responses of this cell type. It has been shown that blood eosinophil levels decrease significantly within hours after a single intravenous dose of steroids,¹² but the kinetics over a 48-hr period of the effect of a single oral steroid dose have not been well characterized.

The mechanisms underlying these effects are not well defined. It has been shown that the interaction of antigen and basophils or mast cells sensitized with antibodies of the IgE class results in the liberation of a number of mediators, including histamine, a low molecular weight eosinophil factor chemotactic for eosinophils (ECF-A) existing preformed in granules of these cells¹³ and a neutrophil chemotactic factor.¹⁴ Such release could be responsible for the observed whealing and neutrophil/eosinophil responses at the ragweed skin test sites, mimicked by injection of compound 48/80.⁶ There is no firm evidence that steroids, in doses employed here, inhibit the release of histamine. Indeed, there was no change in whealing reaction in these subjects, as well as in those previously studied more extensively by us before and after administration of daily steroids.¹⁵ It is conceivable that steroids could selectively inhibit liberation of chemotactic factors at the tissue level without associated depression of histamine release, but this would require additional studies to define.

It is more likely that the tissue inflammatory responses seen here reflect differing steroid effects on the neutrophil and eosinophil responding population. The paradoxical decrease in tissue neutrophil responses in the face of elevated blood neutrophil levels during the "on" day of alternate-day steroids has been thought by some investigators^{9, 11} to be due to a combination of increased mobilization into the circulating pool of neutrophils from the marginal pool, and possibly from the bone marrow as well, while there is decreased migration of these cells into inflammatory sites.

As summarized by Honsinger, Silverstein, and Van Arsdel,¹⁶ the mechanisms underlying corticosteroid-induced eosinopenia in humans have been variously postulated as (1) decreased release of eosinophils from the bone marrow,¹⁷ (2) increased peripheral destruction in the reticuloendothelial system,¹⁸ or (3) reversible sequestration in some unknown tissue compartment.¹⁸ Herion and co-workers²⁰ have reviewed the limited mathematical studies of the patterns of decrease in human blood eosinophil levels after steroids and did not find a consistent pattern. In animal studies,^{21, 22} the half-life of the eosinophil in the circulation has been found significantly shorter than that of the neutrophil, but the eosinophil was thought to exist for relatively long time periods in a tissue phase.

There is additional limited information suggesting that the decrease in tissue eosinophil responses in immediate hypersensitivity reactions may be due to factors other than just a decrease in the levels of blood eosinophils available for emigration into the inflammatory site. In a recent analysis,²³ we have found no correlation in individual subjects between the blood eosinophil levels at the time a skin test was performed and the intensity of the eosinophil response in the resultant inflammatory reaction. Felarca and Lowell¹⁹ found that cortisol, introduced along with pollen antigens in a chamber applied to an abraded skin area of sensitive atopic subjects, inhibited the expected eosinophil response over the next

24 hr. Only a single cortisol concentration was employed, considerably higher than would likely be achieved with the usual orally administered therapeutic doses. Therefore, local toxic effects on emigrating eosinophils and regional effects of steroids absorbed through the abraded surfaces might be other factors playing a role.

Additional studies will be required to further characterize the mechanisms involved in the somewhat unexpectedly long suppressive effect of steroids on the eosinophil responses in immediate hypersensitivity reactions reported here. Since cellular inflammatory responses may play a significant role in the pathogenesis of allergic inflammation, such understanding of steroid effects may permit more effective use of these potent therapeutic agents.

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