

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

II. Effects of corticosteroid therapy

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In a controlled study, a 1-week course of a moderate dosage of corticosteroids given to ragweed-sensitive subjects was associated with a statistically significant decrease in blood eosinophil levels and tissue eosinophil, but not mast cell, responses to ragweed antigen and compound 48/80. This therapy did not affect IgE-class anti-ragweed antibody levels or gross whealing responses to antigen or compound 48/80. While these findings suggest indirectly that a major component of the corticosteroid effect is likely an alteration of the mobilization of eosinophils to the inflammatory site, additional studies will be required to more directly characterize the mechanisms involved.

Infiltration of eosinophils is prominent in sites of immediate hypersensitivity reactions in a variety of tissues, including the respiratory mucosa and skin. In previous studies, using skin windows¹⁻⁵ and dermal biopsy⁶ techniques, eosinophils were observed accumulating over periods of hours to days at the site of immediate wheal-and-flare skin test response. Findings concerning changes in the tissue mast cells and/or basophils have been more variable; in previous studies in this laboratory,⁷ it was found that there was a moderate decrease in stainable mast cells in the skin test sites at a time when eosinophil accumulation was becoming prominent.

Corticosteroid treatment of atopic subjects frequently leads to a marked decrease in peripheral blood eosinophil levels. Observations from skin window studies² have shown decreased eosinophil exudation in response to antigen application in sensitive subjects who had previously been treated systemically with corticosteroids. An inhibitory effect on this exudation of eosinophils was also observed by one group⁸ when steroids were applied topically at the skin window site. However, skin window assays measure only those cells that adhere readily to the glass coverslip employed in this technique. Indirect evidence has suggested

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that steroids may affect cell adhesiveness, so that the decreased numbers of eosinophils in steroid-treated subjects reported in the previous skin window studies might reflect predominantly, if not entirely, an effect of the drugs on the adhesiveness to such coverslips. Further clarification of the drug effects could come from histologic studies of the inflammatory skin test sites. The effects of systemic corticosteroid administration on the cellular response at the immediate hypersensitivity reaction site have not been studied systematically by histologic techniques. The present report compares the effects of corticosteroid and placebo therapy on mast cell and eosinophil histologic responses in skin test reactions to the stimuli. These findings are related to simultaneous measurement of gross skin test reactivity and blood levels of eosinophils and IgE-class anti-ragweed antibodies.

MATERIALS AND METHODS

Subjects

Fifteen atopic male volunteer subjects were studied. Informed consent was obtained in all cases. All individuals were clinically sensitive to ragweed and exhibited an immediate wheal response of at least 8 mm. in diameter (and at least three times the diameter of the reaction to a negative control injection) 20 minutes following the injection of 20 protein nitrogen units (PNU) of crude ragweed extract. One subject showed similar clinical and skin test responses to an extract of mixed grasses, and studies utilizing this antigenic extract were carried out in this subject.

None of the subjects was receiving immunotherapy. All studies were performed at times other than the ragweed pollinating season. All subjects had received no medication for at least 1 week prior to this study.

Treatment programs

All subjects received a 1-week course of methyl prednisolone (kindly provided as Medrol, 4 mg., by the Upjohn Company, Kalamazoo, Michigan) in a single daily dose of 24 mg. each morning. Ten of the subjects served as their own control subjects, receiving a similar 1-week course of an identical-appearing placebo given at another time.

Skin tests and dermal biopsies

Before and immediately following the 1-week treatment periods, skin test studies were carried out. Intradermal injections were performed on the dorsal surface of the upper arm, with a particular skin site used for only one test. Wheal responses were measured 20 minutes 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 per milliliter (10 PNU per milliliter were used in 2 subjects who were highly reactive on screening scratch tests); (2) compound 48/80 (35 mg. per milliliter, Burroughs-Wellcome Company, Research Triangle Park, North Carolina).

Two hours following the intradermal skin test, dermal punch biopsies 3 mm. in diameter were performed, and the 5 μ sections of the tissue were fixed and stained with May-Gruenwald-Giemsa solution by methods described previously by us.⁷ The only modification made was that each stained biopsy section was covered with a small piece of nylon mesh prior to mounting of the coverslip. Biopsies were obtained at the sites of skin tests with compound 48/80 and ragweed. The same concentration of ragweed was employed in individual subjects before and after each of the treatment programs. All skin tests were carried out at 9 A.M.; the skin biopsies were carried out 2 hours thereafter because we had found in previous studies⁷ that eosinophil infiltration was generally maximal or near maximal at this time.

The nature of the treatment program used at the particular time was unknown to the

TABLE I. Effect of placebo and steroids on eosinophil tissue responses to ragweed

Subject	Placebo		Steroids*	
	Before	After	Before	After
R. B.	ND†	ND	135	0
J. B.	10‡	4	58	0
C. B.	ND	ND	34§	2
E. G.	85	7	7	0
J. K.	4	4	4	0
J. L.	4	78	78	0
J. M.	12	23	23	0
R. M.	104	122	122	65
F. M.	ND	ND	29	24
M. M.	ND	ND	12	71
H. N.	0	0	0	0
J. P.	ND	ND	57	1
K. R.	0	14	14	0
R. S.	0	0	0	0
E. T.	18	16	16	0
Significance of difference		p < 0.5	p < 0.05	

*Steroid dosage, methyl prednisolone, 24 mg. per day (see text).

†Not done.

‡Number of eosinophils per 10 grid squares after injection of 20 PNU of ragweed antigen.

§Response to mixed grass—one subject.

||Two highly reactive subjects—skin testing begun with 0.2 PNU of ragweed.

patient and the individual reading the skin test. All biopsy specimens were coded and read blind. The use of an overlying grid permitted quantitation of the eosinophils and mast cells in the dermis by a method similar to that in performing a blood leukocyte count, where counting was started at the dermal-epidermal junction and followed uniformly at one plane of focus in each biopsy into the area of the deep dermis. The number of cells per 10 squares was calculated, since this total area of 2.4 by 2.4 mm. encompassed practically all of the dermis in the large majority of biopsies studied.

Total eosinophil counts of the peripheral blood

These counts were obtained with the Unopipette (Becton, Dickinson & Co., E. Rutherford, New Jersey). Serum levels of IgE-class antiragweed antibodies were assayed by the RAST technique (performed on coded specimens sent to the Pharmacia Company, Piscataway, New Jersey).

Analysis of the data

The findings before and after either steroids or placebo were analyzed in two ways: (1) mean and standard deviation of these responses were calculated for each type of measurement and the difference between means was analyzed by Student's *t* test; (2) the responses for each individual were compared before and after the treatment programs and the differences analyzed by the paired *t* test method.

RESULTS

Gross skin test reactivity

Neither a 1-week course of placebo or steroid had any statistically significant effect on the gross whealing responses to ragweed or compound 48/80. These findings have been described in detail by us recently.⁹

TABLE II. Effect of placebo and steroids on eosinophil tissue responses to compound 48/80

Subject	Placebo		Steroids*	
	Before	After	Before	After
R. B.	ND†	ND	36	0
J. B.	6‡	75	143	4
C. B.	ND	ND	85	1
E. G.	110	126	126	2
J. K.	9	9	9	0
J. L.	42	124	124	0
J. M.	52	18	18	2
R. M.	54	92	92	31
F. M.	ND	ND	84	105
M. M.	ND	ND	10	0
H. N.	0	0	0	0
J. P.	ND	ND	135	3
K. R.	102	18	18	0
R. S.	0	0	0	0
E. T.	8	45	45	3
Significance of difference		p < 0.4	p < 0.01	

*Steroid dosage methyl prednisolone 24 mg. per day (see text).

†Not done.

‡Number of eosinophils per 10 grid squares after injection of 0.02 ml. of 35 mg. per milliliter solution of a single lot of compound 48/80.

Tissue eosinophil responses

Ragweed skin test sites. There was no significant change in the eosinophils present in 10 square areas of the dermis before and after placebo in individual subjects (Table I).

In contrast, similar measurements (Table I) before and after 1 week of corticosteroid therapy showed a significant decrease in the tissue eosinophil responses of these subjects ($p < 0.05$). It should be noted that this decrease was not seen in two of the 15 subjects (F. M. and M. M.).

Compound 48/80 skin test. While 1 week of placebo therapy was not associated with a significant effect on the number of eosinophils in the compound 48/80 skin test reaction of the subjects, there was a marked and statistically significant decrease in the tissue eosinophil response to 48/80 following 1 week of steroid therapy ($p < 0.01$). This significant decrease was observed in 14 of the 15 subjects (Table II).

Mast cell responses

Ragweed skin test sites. There was no statistically significant difference in the number of stainable mast cells in the antigen skin test sites obtained before and after placebo ($p < 0.5$) or steroids ($p < 0.5$) (Table III).

Compound 48/80. There was a modest decrease in the number of stainable mast cells at compound 48/80 reaction sites following 1 week of placebo therapy in some subjects (Table IV) but this change was not statistically significant for the group as a whole. Likewise, 1 week of steroid therapy was not associated with any significant difference in mast cell responses following injection of this agent.

TABLE III. Effect of placebo and steroids on mast cell responses to ragweed

Subject	Placebo		Steroids*	
	Before	After	Before	After
R. B.	ND†	ND	12	5
J. B.	34‡	42	20	27
C. B.	ND	ND	13§	10
E. G.	4	6	6	13
J. K.	11	22	22	14
J. L.	32	28	28	8
J. M.	15	9	9	22
R. M.	2	4	4	6
F. M.	ND	ND	10	10
M. M.	ND	ND	10	3
H. N.	8	16	16	21
J. P.	ND	ND	3	1
K. R.	8	16	16	9
R. S.	42	8	8	12
E. T.	7	20	20	19
Significance of difference		$p < 0.5$	$p < 0.5$	

*Steroid dosage, methyl prednisolone, 24 mg. per day (see text).

†Not done.

‡Number of stainable mast cells per 10 grid squares after injection of 20 PNU of ragweed antigen.

§Response to mixed grass—one subject.

||Two highly reactive subjects—skin testing begun with 0.2 of PNU ragweed.

Total eosinophil counts in the peripheral blood

The use of placebo for 1 week was associated with a small but not significant increase in the mean total eosinophil counts in the 10 subjects (186 before, 217 after). By contrast, there was a striking and significant ($p < 0.001$) decrease in eosinophil counts following 1 week of steroid administration (236 before, 93 after) in the 14 subjects studied (table previously published⁹).

Serum antiragweed antibody levels

Considerable variation in levels of these antibodies, as measured by the RAST technique, was noted from week to week in these subjects. However, when analyzed by the paired *t* test, there were no significant differences in the antibody levels before and after placebo, and before and after steroid administration (Table V).

DISCUSSION

Intensive investigations¹⁰⁻¹⁵ of the *in vivo* eosinophil responses to a variety of immunologic stimuli have been carried out in animal models with varied interpretations as to the dynamics and mechanisms of this cellular response. Prominent eosinophil infiltration of tissues and secretions involved in human allergic diseases has long been recognized, yet much has to be learned about the *in vivo* dynamics of such human cellular responses and the factors affecting them.

Because of the availability of the skin for repeated study, and the clinical importance of the wheal-and-flare skin test in atopic disorders, much of the *in vivo* investigation in man has centered in this area. Prior studies by skin win-

TABLE IV. Effect of placebo and steroids on mast cell responses to compound 48/80

Subject	Placebo		Steroids*	
	Before	After	Before	After
R. B.	ND†	ND	10	11
J. B.	27‡	28	7	15
C. B.	ND	ND	5	13
E. G.	25	3	3	28
J. K.	25	16	16	26
J. L.	14	3	3	14
J. M.	8	8	8	28
R. M.	14	10	10	3
F. M.	ND	ND	10	11
M. M.	ND	ND	8	11
H. N.	6	5	5	4
J. P.	ND	ND	18	4
K. R.	6	13	13	3
R. S.	12	9	9	12
E. T.	12	5	5	7
Significance of difference		p > 0.05	p > 0.1	

*Steroid dosage, methyl prednisolone, 24 mg. per day (see text).

†Not done.

‡Number of stainable mast cells per 10 grid squares following injection of 0.02 ml. of 35 mg. per milliliter solution of a single lot of compound 48/80.

dow¹⁻⁵ and dermal biopsy^{6, 7} techniques have shown prominent accumulation of eosinophils over hours to days after the immediate hypersensitivity reaction to a pollen antigen in a sensitive subject. The exact source(s) and trafficking pattern of the eosinophils appearing in these skin test reactions have not been well characterized. Studies in animals¹⁶ have suggested that a relatively short period of less than one day of the 8 to 12 day life cycle of the eosinophil is spent in the peripheral blood. Most of the cell tenure appears to consist of a "tissue phase."

The effect of systemic corticosteroids in reducing eosinophil levels in peripheral blood of man has been known for many years.¹⁷ The findings of the present study include yet another confirmation of that phenomenon, including the fact that poststeroid eosinopenia was not observed in every subject. In addition, the eosinophil infiltration of wheal-and-flare skin test reactions was significantly diminished in steroid-treated subjects when compared to the responses measured before steroid administration. At the same time, no significant effect of the steroid treatment on the gross whealing reaction was noted. The effects of steroid treatment on the histologic patterns in the wheal-and-flare reaction site observed here parallel the previously reported² effects of steroid treatment on eosinophil exudation as measured by skin window techniques. In addition, these histologic findings rule out the likelihood that the decrease in eosinophils seen in the skin windows of steroid-treated subjects in the previous study² reflects just an in vivo effect of steroids on eosinophil adherence to the glass coverslip used as the "window." Additional data from the present study also permit some speculation concerning the possible mechanisms involved in the observed steroid effect.

One way in which steroids could depress eosinophil responses would be by alteration of the reaction between the intradermally injected antigen and the

TABLE V. Effect of placebo and steroids on serum IgE antiragweed antibody levels

Subject	Placebo		Steroids	
	Before	After	Before	After
R. B.	ND	ND	973	1,268
J. B.	293*	260	233	255
E. G.	946	1,105	1,105	2,049
J. K.	283	295	295	345
J. L.	6,928	7,875	ND	ND
J. M.	227	645	645	359
R. M.	5,193	5,108	5,108	9,627
M. M.	ND	ND	202	233
H. N.	922	1,349	1,349	3,252
K. R.	225	214	214	223
R. S.	335	455	455	514
E. T.	283	255	255	309
Significance of difference		p > 0.05	p > 0.1	

*CPM measured by RAST technique (Pharmacia Laboratories Inc., Piscataway, N. J.):

Score	CPM
0	< 534 negative—no IgE antibodies
1	534 to 719 doubtful, insignificant levels of IgE antibodies
2	719 to 2,274 weakly positive, significant but low levels of IgE antibodies
3	2,274 to 9,950 moderately positive, moderate levels of IgE antibodies
4	> 9,950 strongly positive, very high levels of IgE antibodies

cell-bound reagin antibodies. There appears to be little evidence that the steroid treatment, as used in this study, affects the level and binding of IgE-class antibodies directed against the ragweed antigens in the skin test material. No significant decrease was observed in the blood levels of anti-antigen E antibodies following a week of steroid administration in the study. In addition, the finding of unaltered whealing response to ragweed following steroid treatment of our subjects also suggests that the interaction of the antigen with reagin bound to mediator-rich cells (presumably mast cells) is unchanged. The observation that tissue eosinophil responses to injected compound 48/80 were also depressed following steroid administration points to other mechanisms of steroids on the cellular inflammatory responses, independent of any effect on antigen-reagin interaction.

Another mechanism by which steroid therapy could depress tissue eosinophil responses would involve altered release of eosinophilotactic mediators from cells following the interaction of antigen and antibody. Varied effects of steroid therapy on levels of human and animal basophils and mast cells have been reported.^{18, 19}

In this study, no significant differences were found during the pre- and poststeroid treatment studies as to the number of stainable mast cells at the skin test sites 2 hours after antigen injection. Technical limitations of the light microscopic technique utilized, however, do not permit a reliable quantitative estimate of the degree of partial degranulation of such cells. It has already been noted in this report that the wheal-and-flare response to antigen or compound 48/80 (presumably mediated in part, if not entirely, by histamine) was unaltered following a week of steroid administration. Actual measurement of

mediators released in vivo at the skin test sites, when technically feasible, would add considerably to knowledge about the effects of steroids on mast cell activation in tissues.

A third mechanism to explain the inhibitory effect of steroids on the tissue eosinophil response is related to the action of these agents on the in vivo migration of eosinophils. The majority of prior evidence²⁰ suggests that the steroid-induced eosinopenia in the peripheral blood is due not to eosinophil destruction, but to a margination of such cells in pools not measured by the usual peripheral blood eosinophil counts. This margination appears to involve increased adherence of eosinophils to the intima of certain vessels. In the present study, prominent tissue eosinophil responses to both antigen and compound 48/80 were generally limited during the baseline study period to those with blood eosinophil levels of at least 150 per cubic millimeter, although the degree of tissue eosinophil response did not correlate precisely with blood eosinophil levels in individual patients. Likewise, following steroid treatment, there was not a precise correlation between the tissue eosinophil responses and the blood levels of these cells measured concomitantly. This raises the question as to whether the steroid therapy might also result in decreased emigration of eosinophils through vessel walls in the reaction site. Comparison of localization patterns of eosinophils within biopsies of this study showed no consistent restriction of these cells to intravascular or perivascular areas of skin test sites following steroid administration. It seems, therefore, that a likely major effect of corticosteroid therapy in reducing the number of eosinophils seen in the wheal-and-flare inflammatory reactions of this study relates to altered delivery of eosinophils to the test site. How much of this effect is due to reduction of a circulating pool of eosinophils and how much is due to a drug effect on emigration of cells from the vascular compartment to the interstitium of the dermis cannot yet be stated with certainty.

Information forthcoming from these and additional studies investigating the mechanisms of eosinophil response may be of considerable importance in understanding how corticosteroids benefit diseases like asthma. The prominent inflammatory cellular infiltrate (including many eosinophils) in the bronchial walls of patients with severe asthma and the time lag in the onset of steroid-induced clinical improvement in this disease are well known. Additional study may help determine whether the depression of the tissue cellular inflammatory response by steroids is related to the observed clinical improvement.

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