

In vitro studies of rye grass pollen antigens

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The activity of rye grass Group I and II antigens, which constitute the major allergens of this pollen, was investigated by the in vitro technique of histamine release from the leukocytes of sensitive donors. We found that: (1) In rye grass pollen, as distinct from ragweed pollen, there is no cross-reactivity between the two major allergenic proteins. (2) There is no allergenic or haptenic activity in dialysates of rye grass pollen such as that described by Malley and associates in timothy grass pollen. (3) The major allergens of rye grass and ragweed pollens (Group I antigens and antigen E) show no antigenic or allergenic cross-reactivity.

Marsh and collaborators¹ have shown by chromatographic analysis that two groups of antigenic proteins account for a large proportion of the allergenic activity of rye grass pollen. These antigens, later designated Group I and Group II, have molecular weights of about 30,000 and 10,000, respectively.^{1, 2} In skin testing with these preparations it was found that almost all patients who reacted to the whole pollen extract (WPE) also had a positive reaction to Group I antigens; about 70 per cent of patients were also positive to Group II antigens.³ Dialysates of the WPE had little or no activity. Of particular interest was the recent observation that Group I and II antigens are common to most of the allergenic species of grass pollens, notable exceptions being Bermuda pollen and pollens of the tribe Paniceae,^{4, 14} in which both groups of allergens are absent.

The leukocyte histamine release system has been used to assay the allergenic activity of various antigens derived from ragweed pollen; it has the advantage of being 20 to 100 times as precise as skin testing.^{5, 6} We felt that the increased accuracy of this technique would be useful in exploring several aspects of the allergenicity of grass pollens. A first question involved the possible allergenicity or hapten-like activity of dialyzable components of grass pollens, considered most recently by the studies of Malley and associates.⁷ Another question which seemed pertinent in view of the recent availability of relatively clean antigens from

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Supported by Grants Nos. AI 07290, AI 08270, and AI 1399-11 from the National Institute of Allergy and Infectious Diseases.

Received for publication Jan. 27, 1969.

*American College of Physicians Research and Teaching Scholar.

**Recipient of a grant from Hollister-Stier Laboratories and a travel grant from Wellcome Foundation.

both ragweed and grass pollens was that of the cross-reactivity between these pollens, which was reported most recently by Lidd and Farr.⁸ Finally, the question of the allergenic relationship between grass pollen antigens in Groups I and II was of interest. The present report examines these questions.

MATERIALS AND METHODS

Rye grass pollen antigens

The preparation of these allergens has been described in detail¹; briefly, ether-defatted perennial rye grass pollen was extracted with 10 times its weight of one per cent NH_4HCO_3 , dialyzed extensively against 0.002M NH_4HCO_3 , and lyophilized. This material is referred to as dialyzed WPE. The lyophilized material was fractionated on DEAE-cellulose and Sephadex G-75 or G-100 columns to yield Group I or Group II allergens possessing a high degree of purity according to physical, chemical, and immunological criteria.^{1, 2}

Rye grass pollen dialysate

A 20 per cent (w/v) extract of rye pollen (200 ml.) was dialyzed in Visking $1\frac{1}{2}$ inch diameter tubing against 0.002M NH_4HCO_3 (1,800 ml.) for 24 hours at 4° C. The dialysate was lyophilized, giving a highly hygroscopic solid (yield: 3.8 mg. per milliliter of dialysate). In order to eliminate the possibility of traces of high molecular weight contaminants in the dialysate, a solution of the first dialysate was redialyzed for a much shorter period. The first dialysate (1.83 Gm.) dissolved in water (17.5 ml.) was dialyzed for 3 hours at 4° C. against distilled water and lyophilized (yield: 5.9 mg. per milliliter of dialysate). Sixty-four per cent of the theoretical amount of the dialyzable material was found to have passed through the dialysis sac on termination of the second dialysis. Antigen E was kindly donated by Dr. T. P. King⁹ of The Rockefeller University.

Antisera

The human anti-antigen E was a pool obtained from approximately 20 allergic donors after a series of 14 weekly injections of antigen E. The guinea pig anti-Group I was prepared by immunizing a guinea pig in the hind footpads with the allergen adsorbed on alum. A primary immunization of 20 μg antigen was followed by a secondary immunization (10 μg) after one month, and the guinea pig was bled 2 weeks later.

Histamine release studies

The details of leukocyte preparation, antibody measurements, and the fluorometric assay of histamine have been reported.^{10, 11} The cell donors used in these studies were allergic but otherwise healthy adults.

RESULTS

Fig. 1 presents a typical assay of the grass pollen preparations on the cells of a sensitive individual. Group I antigens usually have the highest specific activity; the potency of the Group II antigens is often considerably lower, and, as will be shown, they may be totally inactive. The dialyzed WPE is more potent (in terms of histamine-releasing ability per microgram of antigen) than the

WPE. This is consistent with the observation that constituents which were dialyzable had no activity in this and the remainder of the 6 patients tested with concentrations of the dialysate up to 10 γ per milliliter. Thus, if the dialysate has any allergenic activity, it represents less than 0.01 per cent of the dialyzed WPE. In this patient Group I antigens had a specific activity four- to fivefold greater than that of the dialyzed WPE and twentyfold greater than that of the WPE. The ratio between the activity of Group I antigen and WPE was, of course, variable from person to person since the WPE contained multiple antigens to which the patients were differentially sensitive. In the 6 patients tested, the ratio of specific activities of Group I antigens to dialyzed WPE varied between about 4 to 17. The dialyzed WPE was found to have about 5 times the specific activity of the WPE. The theoretical increment in activity expected after the removal of inactive material by dialysis is sevenfold.¹ The reason for this discrepancy is not clear but may be accounted for by losses in prolonged dialysis or lyophilization.

In view of Malley's⁷ report on the hapten-like behavior of a dialyzable low molecular weight antigen from timothy pollen, we attempted to demonstrate a similar type of activity in our rye grass preparation. To this end cells from a grass-sensitive donor were exposed to the dialysate of WPE and then to the WPE or Group I antigen. The concentration of the dialysate was 5 γ per milliliter, more than 10,000-fold greater than that of the Group I antigens at the point of 50 per cent histamine release. The histamine release from these cells was compared with that obtained with the same antigens from the same suspension of cells which had not been pretreated with the dialysate. The percentage of histamine released was the same from both sets of cells (Fig. 2). Similar

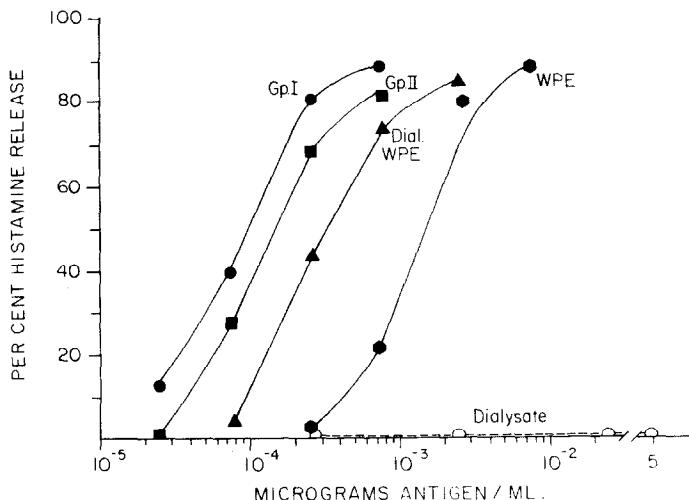
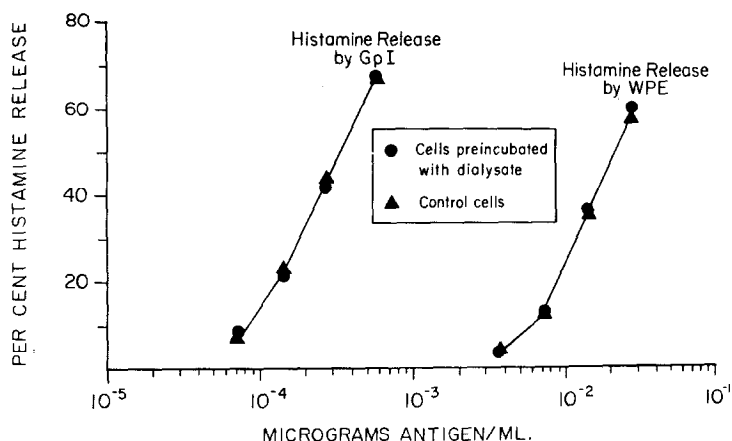


Fig. 1

Typical dose-response curves with antigenic preparations from rye grass pollen assayed on the leukocytes of a sensitive donor. In this and subsequent figures the dialysate or crude whole extracts of ragweed or grass pollens are expressed as the weight of total dry solids per milliliter.

**Fig. 2**

The effect of preincubation of sensitive leukocytes with 5 µg per milliliter of rye grass pollen dialysate on the subsequent histamine release by WPE or Group I antigens. See text for details.

results were found in experiments with the cells of several different grass-sensitive donors. If the dialysate was capable of combining with cell-bound antibody as a hapten, it should have inhibited the histamine release caused by the active allergens.

The allergenic relationship between Group I and Group II antigens is illustrated in Fig. 3. Dose-response curves were run on the cells of 2 different grass-sensitive donors. The data on the left is from a patient who was atypical in that she was more sensitive to Group II than to Group I; the reverse is true in most cases. The data on the right is from a patient who did not respond at all to Group II antigens. These data suggest that there is no allergenic cross-reactivity between Group I and Group II antigens. Thus, if Group II cross-reacted with Group I to the extent of one part in 10,000 or if the Group II preparation were contaminated with Group I antigens to this extent, there should be detectible histamine release with the Group II preparation. A complete lack of reactivity to Group II antigens was found in about half of the several cell preparations from grass-sensitive donors.

Fig. 4 deals with the possibility of cross-reaction between the major antigens of grass and ragweed pollens. Shown are data from two patients, one releasing histamine with grass WPE and Group I antigens and not with ragweed antigen E or whole ragweed pollen extract, the other showing the opposite pattern. We have observed several donors quite sensitive to ragweed antigens who did not release any histamine even when exposed to high concentrations of grass antigens and several more donors who were sensitive to grass antigens but not to ragweed. Once again, quantitative considerations based on the curves in Fig. 4 suggest that if the allergenic cross-reactivity between grass and ragweed antigens exceeded one part in 10,000, histamine release would have been seen in the first patient with ragweed antigens and in the second patient with rye grass antigens.

Another approach to this question is illustrated in Figs. 5A and 5B. In this

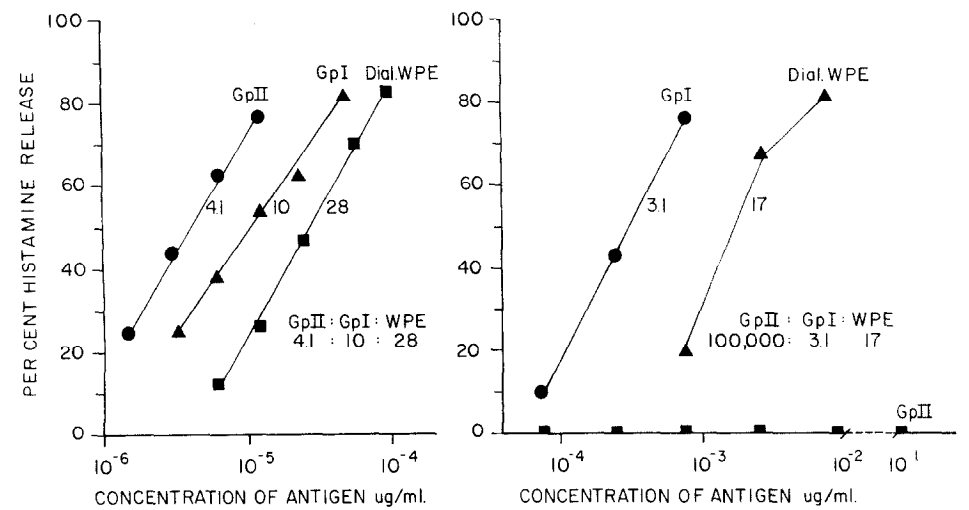


Fig. 3
An assay of the allergenic activity of dialyzed rye grass pollen extract and Group I and II antigens on the leukocytes of 2 sensitive donors. The numbers on the graph refer to the concentration of antigen (*left*, micrograms $\times 10^{-6}$ per milliliter; *right* micrograms $\times 10^{-4}$ per milliliter) required for 50 per cent histamine release.

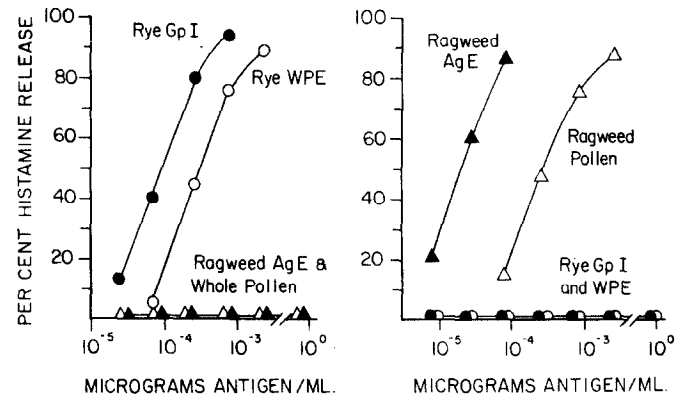


Fig. 4
Assay of grass and ragweed pollen antigens on the leukocytes of 2 donors, one sensitive only to grass and the other only to ragweed.

type of experiment we used the cells of individuals sensitive to both grass and ragweed antigens. Aliquots of a cell suspension were exposed to a concentration of ragweed antigen E or grass Group I antigens adequate to release 70 to 80 per cent of the total cellular histamine (control). The same concentrations of both antigens were also incubated, before addition to the cells, with serial dilution of an antiserum obtained from a pool of individuals immunized for therapeutic purposes with ragweed antigen E (Fig. 5A). The per cent of histamine release in each tube was obtained, and the per cent of inhibition, relative to the control,

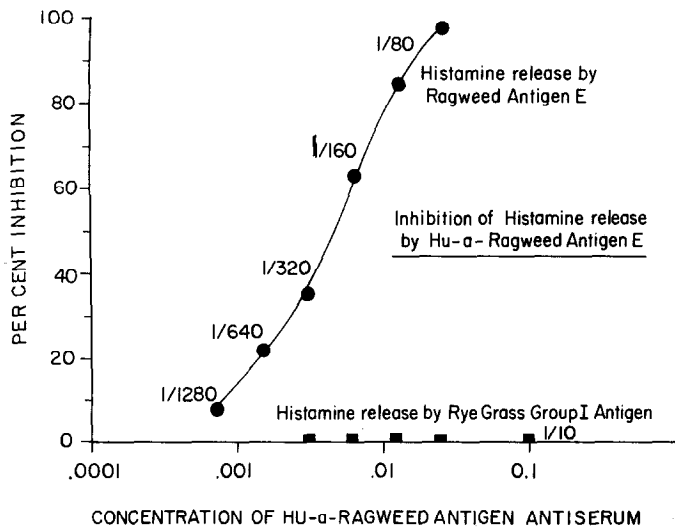


Fig. 5A
Inhibition of histamine release by a pool of human antiragweed antigen E sera. There is complete inhibition of antigen E-induced histamine release, but no activity is demonstrated against Group I antigens.

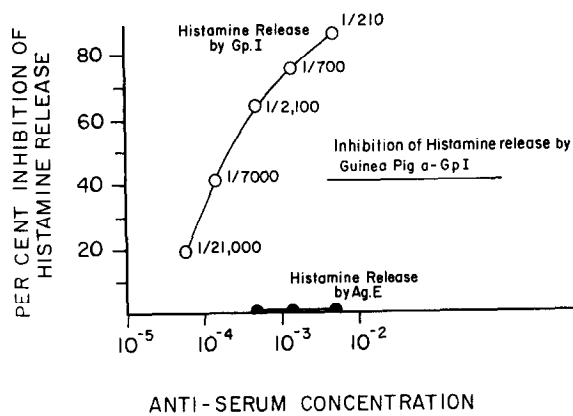


Fig. 5B
Inhibition of histamine release by guinea pig anti-Group I sera. The inhibition pattern is the opposite of that shown in Fig. 5A.

was calculated for each of the tubes containing the antiserum. The antiserum against antigen E inhibited antigen E-induced histamine release at dilutions of up to $\frac{1}{1,280}$, while this antiserum had not inhibiting effect on the histamine release induced by Group I antigens even at a concentration of $\frac{1}{10}$. The same type of experiment was carried out with the use of a guinea pig antiserum made against Group I as an inhibiting agent. This antiserum inhibited Group I-induced histamine release at dilutions of up to $\frac{1}{21,000}$ but did not inhibit antigen E-induced histamine release at a concentration of $\frac{1}{210}$ (Fig. 5B).

DISCUSSION

These experiments confirm the observation that rye grass pollen contains two antigens which do not have appreciable allergenic cross-reactivity. This is different from the situation found with the antigens purified from ragweed pollen. In the latter case antigens K and Ra₃, while separable by physical means from the major antigen, cross-react extensively with antigen E.^{6, 12} In our studies of 6 individuals reacting to rye Group I, only 2 reacted to Group II; in one of these Group II was more allergenic than Group I on a weight basis. The results from earlier more extensive skin testing on 50 patients with rye Groups I and II indicated that about 95 per cent of grass-allergic patients were skin sensitive to Group I and 60 to 70 per cent to Group II.^{3, 4} Some individuals were found to be more sensitive to Group II than Group I, although the reverse was more often the case. From a therapeutic point of view, the existence of at least two separate antigens emphasizes the unsatisfactory nature of immunization with crude whole pollen extracts. If many patients are not sensitive to Group II antigens, there is no therapeutic benefit derived from having this material in the immunization mixture. We recognize, however, that at the present time there is no practical, available alternative to the use of whole extracts.

Malley and associates^{7, 13} have carried out a detailed examination of a hapten-like material isolated from the dialysate of timothy grass pollen. This material, designated antigen D, is rather heterogeneous and has an average molecular weight of approximately 5,000. It acts as a hapten in that it inhibits, at a hapten to antigen molar ratio of about 1,000, the precipitin reaction between antigen B (a nondialyzable timothy allergen of molecular weight 10,500) and antibody prepared in rabbits or guinea pigs against timothy WPE. Antigen D blocks the PCA reaction and systemic anaphylaxis in guinea pigs but can cause skin reactions in some timothy-allergic human beings, generally at higher concentrations than required with the nondialyzable timothy allergens. We could find no allergenic activity in the dialysate of rye WPE nor could we demonstrate haptenic activity of his material, as judged by its failure to inhibit histamine release by rye antigens. The divergence between our results and those of Malley and associates is probably explained by the observed lack of immunological cross-reactivity between timothy antigen B (supplied by Malley) and rye Groups I and II. Other possible contributing factors are: (1) the pH of 10.4 used to extract timothy pollen perhaps causing partial degradation of certain nondialyzable timothy allergens into dialyzable "haptenic fragments," a process unlikely to occur at the pH of 7.5 to 8.0 used to extract rye pollen; and (2) differences in our methods of assaying for hapten-like activity.

Lidd and Farr⁸ were able to show antigenic cross-reactivity between certain components present in whole grass and whole ragweed pollen extracts. The cross-reactivity was observed principally when sera of rabbits hyperimmunized with emulsions of either whole grass or whole ragweed pollen extracts were used in cross-inhibition experiments, although one patient treated with grass pollen extract produced an antiserum showing cross-reaction with ragweed. In the present studies, no antigenic or allergenic cross-reactivity was detected between the highly purified major allergens of rye and ragweed pollens; no allergenic

cross-reactivity was found between the corresponding whole extracts. The quantitative limit on the degree of allergenic cross-reactivity detectable by our methods was under 0.01 per cent. In experiments more closely related to Lidd and Farr's work we found that a pool of human antisera to antigen E was able to inhibit the histamine release caused by this antigen but was not active in the system when histamine release was caused by rye Group I antigens. We did not have a pool of human anti-Group I antigens, but with guinea pig anti-Group I sera we found the same failure of cross-reactivity. It must be emphasized that our assay system pertains only to antigens which have allergenic activity in man, whereas Lidd's and Farr's work concerns antigens which may or may not have this activity. Moreover they were dealing with antisera produced against whole pollen extracts, whereas our antisera were against the purified antigens. These two differences in technique are adequate to explain the apparent difference between our results. In fact, working with an antiserum similar in specificity to that used by Lidd and Farr, we found that a pool of human antisera against crude ragweed extract had some inhibiting activity against rye WPE and Group I antigens (3 per cent and 1 per cent, respectively, as compared with the activity of the antiserum against ragweed antigen E). This may well be due to contamination of the crude ragweed extract with grass pollens. In any event, the major antigens of ragweed and grass pollens show neither allergenic nor antigenic cross-reactivity.

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