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Interaction of paramyxoviruses with human basophils and their effect on histamine release

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We have demonstrated that human peripheral blood basophils released histamine on direct incubation with paramyxoviruses in vitro. Most histamine release occurred during the first 15 to 30 minutes after challenge, depending on the dose of virus used; release initiated by virus was complete by 1 hour. At a virus/cell ratio of 1:1, Sendai virus caused 41 ± 9% histamine release, whereas parainfluenza type 3 (PI-3) virus caused 25 ± 5% release and respiratory syncytial (RS) virus caused 19 ± 5% release. Sendai, but not PI-3 or RS, also caused a decrease in cell number and release of lactic dehydrogenase; however, this apparent cell lysis did not account for all the histamine released. Incubation of cells with virus desensitized them to subsequent triggering by viruses but did not affect response of cells to other stimuli. Histamine release was dependent on the virus/cell ratio, temperature, and metabolic energy, but it was not strictly dependent on the presence of calcium in the extracellular medium. Histamine release was not affected by preincubation of cells with colchicine, suggesting that microtubules were not involved in the release process. Basophils desensitized by anti-IgE in the absence of calcium or treated with lactic acid to dissociate IgE molecules from membrane receptors released amounts of histamine similar to that of control basophils; thus, release was not initiated through Fc receptors. It was found, however, that histamine release by these viruses was greatly reduced when concanavalin A was used for desensitization. These data demonstrate that the respiratory viruses studied can cause direct nonimmunologic release of histamine from human basophils. Our findings provide evidence for another mechanism by which respiratory viruses can initiate inflammation. (J ALLERGY CLIN IMMUNOL 1989;84:538-46.)

Viral respiratory infections are a major cause of illness in infancy and childhood. More than 10% of children less than 1 year of age experience clinically significant airway obstruction with respiratory viral infections. This rate decreases to approximately 6% in the second year of life and continues >1% throughout elementary school ages. In developed countries, as many as 10% of children younger than 5 years of age admitted to hospital with acute respiratory infec-

Abbreviations used
HSA: Human serum albumin
Con A: Concanavalin A
PI-3: Parainfluenza type 3 virus
RS: Respiratory syncytial virus
HEPES: N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HACM: HEPES buffered saline, pH 7.4, containing 0.3 mg/ml of human serum albumin, 2 mmol/L of Ca++, and 1 mmol/L of Mg++
HA: HEPES buffered saline, pH 7.4, containing 0.3 mg/ml of human serum albumin
LDH: Lactic dehydrogenase
MEM: Minimum essential medium
FBS: Fetal bovine serum
2-DG: 2-Deoxyglucose
UV: Ultraviolet

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Received for publication Feb. 13, 1989.
Revised May 1, 1989.
Accepted for publication June 5, 1989.
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children 6 to 16 years old. Viral infections are a major cause of exacerbations of asthma in children. There also is considerable evidence indicating that asthma and other lung abnormalities often are the consequence of acute viral infection of the lower respiratory tract in children.

The symptoms of respiratory tract infection appear identical to symptoms that occur in allergic rhinitis and asthma, in which release of histamine and other mediators of hypersensitivity from mast cells and basophils are responsible for the symptoms observed. Indeed, we and other investigators have demonstrated histamine in nasal secretions during certain acute respiratory infections. Welliver et al. have identified IgE antibodies specific for viral antigens, suggesting that mediator release from respiratory tract mast cells and basophils could be the result of IgE-mediated hypersensitivity to viral agents. We previously demonstrated that RS can activate complement in vitro, and Kaul et al. identified C3b on sloughed nasal epithelial cells from patients with acute respiratory infections. These data together imply that concomitant generation of anaphylatoxins from complement by viruses also might initiate mediator release. Sugiyama demonstrated that Sendai virus, which is not pathogenic to humans, will induce histamine release in vitro from rat mast cells. However, other investigators have been unable to document histamine release from human basophils incubated with viruses that cause human respiratory tract infections.

In the present study, we have demonstrated the ability of three paramyxoviruses to cause the direct release of histamine from human basophils. These observations indicate that a direct interaction of paramyxoviruses with mediator-containing cells might be important during respiratory tract infection.

MATERIAL AND METHODS

Reagents

HEPES, Ficoll-Hypaque (Histopaque, density 1.077), human serum albumin, polyethylene glycol (molecular weight 3000), penicillin, streptomycin, amphotericin B, MEM, FBS, trypsin, Con A, 2-DG, calcium ionophore A23187, goat antimouse IgG, and antimycin A were obtained from Sigma Chemical Co. (St. Louis, MO.). Goat antihuman IgE was obtained from Meloy Laboratories (Springfield, Va.). Anti-T cell monoclonal antibodies (anti-Leu-1 and anti-HLA-DR) were obtained from Becton-Dickinson (Mountain View, Calif.).

Viruses

The following viruses were used: parainfluenza type 1, Sendai strain; PI-3, C-243 strain; RS, Long strain; influenza types A and B, strains A/PR/8/34 and B/LEE/40; human coronavirus, strains 229E and OC-43; and poliovirus types 1, 2, and 3, Sabin strain. These viruses were kindly provided by Dr. J. Hierholzer (Respiratory and Enteric Virus Division, CDC, Atlanta, Ga.), obtained from the American Type Culture Collection (Rockville, Md.), or Leeder Laboratories (Pearl River, N.Y.).

Buffers and media

Eagle’s MEM was supplemented with penicillin (50 U/ml), streptomycin (50 μg/ml), amphotericin B (0.025 μg/ml), and 2% or 10% FBS heat inactivated at 56°C for 30 minutes. Human epithelial cells (HEp-2, American Type Culture Collection) were maintained in MEM with 2% FBS. HACM buffer was prepared with 10 mmol/L of HEPEs buffer (pH 7.4) containing 137 mmol/L of NaCl, 5 mmol/L of KCl, 0.3 mg/ml of human serum albumin, 2 mmol/L of Ca++, and 1 mmol/L of Mg++. When it was necessary, Ca++ and Mg++ ions were omitted (HA buffer).

Viruses preparation

Sendai virus was propagated and assayed by inoculation of the allantoic fluid of embryonated hen’s eggs. PI-3 and RS viruses were propagated and assayed in HEp-2 cells. Infected and uninfected preparations of cells and allantoic fluids were clarified at 800 g for 10 minutes. Supernatants were mixed with polyethylene glycol (7% wt/vol) and kept for 12 hours with constant agitation at 4°C. These fluids were resuspended in HA or HACM buffer to one fifth the original volume. Virus pools containing the following titers were used throughout the study: Sendai, 3 x 10^5 50% egg infectious dose, per milliliter; PI-3, 3 x 10^5 50% (median) tissue culture infective dose, per milliliter; and RS, 8 x 10^8 plaque-forming units, per milliliter. Virus-cell ratios were calculated from these titers.

Preparation of peripheral blood leukocytes

Venous blood from healthy adult volunteers was drawn into plastic syringes and anticoagulated with 10 mmol/L of ethylenediaminetetraacetic acid. Erythrocytes were sedimented in hydroxethyl starch at room temperature for 30 minutes. The leukocyte-rich supernatant was removed, layered over Histopaque (density, 1.077), and centrifuged at 800 g for 30 minutes. The visible cell band and leukocytes below were removed by aspiration, washed three times with buffer, and resuspended in HA or HACM. Final preparations containing 2% to 2.5% basophils were possible by this method, and hemadsorption of viruses to red blood cells could be avoided. In some experiments, basophils were enriched by negative selection according to the method of Landry and Findlay, obtaining preparations containing 20% to 30% basophils.

Histamine-release assay

Leukocytes (0.3 ml) were added to polyethylene tubes, warmed to 37°C, and incubated with viruses and/or stimuli for 60 minutes. A virus/cell ratio of 1:1 was used throughout the study unless it is otherwise indicated. After incubation, leukocytes were centrifuged at 800 g for 5 minutes. Supernatants were assayed for histamine with an automated fluorometric technique, as used previously. The net percentage release was calculated by subtracting histamine re-
Degranulation of mast cells and basophils and subsequent release of histamine and other mediators of inflammation can be initiated both immunologically, through Fcε receptors, and nonimmunologically, through receptor-specific and nonspecific mechanisms. Sugiyama demonstrated previously that incubation of rat peritoneal mast cells with Sendai virus will cause histamine release from these cells. We found that Sendai and two other paramyxoviruses reproducibly caused histamine release from human basophils. At a concentration of one infective viral particle per cell, histamine release after incubation for 1 hour was 41% (mean ± SD) with Sendai virus (n = 19), whereas release with PI-3 was 25% (n = 18) and release with RS was 19% (n = 19). Measurable histamine release was detected with virus to cell ratios as low as 0.2. No release of histamine was detected with uninfected culture supernatants or allantoic fluids as controls. We also were unable to detect any release of histamine after incubation of basophils with the following viruses: influenza A or B, poliovirus types 1, 2, or 3, or coronaviruses 229-E and OC-43 (data not presented).

These data suggest that histamine release is a generalized consequence of paramyxovirus interaction with human basophils. To begin to understand the potential mechanisms for paramyxovirus-induced histamine release, we explored the time course of release from virus-treated basophils. At a virus/cell ratio of 10:1, Sendai-induced histamine release was rapid, such that three fourths of release occurred during the first 15 minutes, and the rate of release slowed during the next 45 minutes (Fig. 1). Interestingly, lower virus/cell ratios caused a decreased rate of release as well as total amount of histamine released. We detected no further release of histamine after the first hour of incubation at any virus/cell ratio tested. Similar results were observed with PI-3 and RS. The changes in rate of histamine release over time suggested that release was the result of degranulation of basophils rather than nonspecific leakage of cell contents or cell lysis.

We further explored this important distinction with leukocyte preparations enriched in basophils. Aliquots of cells were incubated with viruses at a virus/cell ratio of 1 or with buffer for 1 hour at 37° C. Total
We next performed experiments to define the optimal conditions for histamine release induced by these viruses. As presented in Table III, histamine release was temperature dependent. At temperatures <37°C, release was inhibited considerably. Omitting calcium from the buffer decreased but did not block histamine release, indicating that extracellular calcium ions were not necessary to obtain degranulation of human basophils by these paramyxoviruses.

The time course of histamine release suggested that release initiated by these viruses was not the result of activation of Fcε receptors. To study further this possibility, we used basophils that had been rendered unresponsive to IgE-mediated stimuli with two different approaches. First, basophils were desensitized with either anti-IgE (1 × 10^-1 mg/ml) or Con A (5 μg/ml) for 30 minutes at 37°C in calcium-free buffer. After cells were washed, they were resuspended in media containing calcium and incubated with a paramyxovirus, anti-IgE, or Con A. As illustrated in Fig. 2, release induced by anti-IgE or Con A was completely blocked by preincubation with either agent in calcium-free buffer. However, no change in histamine release by these paramyxoviruses was observed when leukocytes had been desensitized with anti-IgE. It also should be noted that preincubation of cells with paramyxoviruses did not affect their subsequent response to triggering with anti-IgE (Table I). Second, cell aliquots were suspended in 0.01 mol/L of lactic acid buffered isotonic saline at pH 3.9 for 2.5 minutes at room temperature to dissociate IgE molecules from basophil membrane receptors. After cells were washed, they were resuspended in HA buffer, pH 7.4, cells were either maintained in this buffer or passively sensitized with autologous serum and then incubated with anti-IgE or a paramyxovirus. As illustrated in Fig. 3, lactic acid treatment of cells reduced histamine release by anti-IgE from 51 ± 14% (mean ± SD: n = 3) to 3 ± 3% (n = 3).
Leukocytes were incubated with viruses at a virus/cell ratio of 1:1, or with anti-IgE antibody (0.1 mg/ml), or calcium ionophore A23187 (0.3 μg/ml) at 37°C in buffer containing 2 mmol/L Ca++ for 1 hour. After incubation for the first hour, either control media or an additional aliquot of stimulus was added to the incubation mixtures, and the reactions were permitted to proceed for a second hour. Percent histamine released into cell supernatants at the end of incubation for the first and second hour are expressed as mean ± SD of three experiments.

*p < 0.05 compared with results of the second hour of incubation with control media.
†p < 0.01 compared with results of the second hour of incubation with control media.
‡p < 0.001 compared with results of the second hour of incubation with control media.

TABLE II. Histamine release by Sendai, PI-3, or RS viruses or by anti-IgE or calcium ionophore A23187 from cells after initial triggering by these stimuli

| First stimulus | % Histamine release after first hour | Control | RS | PI-3 | Sendai | Anti-IgE | Calcium ionophore A23187 |
|----------------|------------------------------------|---------|----|------|--------|---------|--------------------------|
| RS             | 18 ± 4                             | 20 ± 6  | 20 ± 1 | 14 ± 3 | 18 ± 2  | 46 ± 2†   | 38 ± 2†                   |
| PI-3           | 20 ± 5                             | 21 ± 2  | 17 ± 1 | 22 ± 1 | 23 ± 2  | 40 ± 2‡   | 25 ± 3‡                   |
| Sendai         | 32 ± 4                             | 35 ± 3  | 25 ± 3 | 23 ± 2 | 24 ± 3  | 34 ± 3‡   | 45 ± 3‡                   |
| Anti-IgE       | 27 ± 2                             | 32 ± 3  | 31 ± 3 | 33 ± 4 | 29 ± 4  | 28 ± 3    | 52 ± 4‡                   |
| Calcium ionophore A23187 | 21 ± 3                         | 25 ± 1  | 30 ± 6 | 29 ± 4 | 26 ± 6  | 76 ± 3‡   | 44 ± 2‡                   |

**TABLE III. Effect of temperature, calcium concentration, colchicine, and metabolic inhibitors on histamine release by Sendai, PI-3, and RS viruses**

| Effect of | Sendai | PI-3 | RS | Anti-IgE |
|-----------|--------|------|----|----------|
| Temperature | 4°C     | 1 ± 1 | 1 ± 1 | 1 ± 2 | 1 ± 1 |
|            | 25°C    | 19 ± 3 | 10 ± 3 | 11 ± 2 | 16 ± 2 |
|            | 37°C    | 40 ± 5 | 8 ± 2 | 24 ± 1 | 38 ± 6 |
| Calcium    | None    | 16 ± 1 | 21 ± 2 | 17 ± 3 | 2 ± 1 |
|            | 2 mmol/L| 40 ± 3 | 22 ± 3 | 18 ± 2 | 39 ± 6 |
| Inhibitors | None    | 37 ± 4 | 20 ± 6 | 16 ± 5 | 32 ± 3 |
|            | Colchicine | 36 ± 5 | 19 ± 6 | 17 ± 3 | 11 ± 1 |
|            | 2-DG plus | 19 ± 6 | 9 ± 6 | 7 ± 4 | 8 ± 3 |

Leukocytes were incubated with viruses at a virus/cell ratio of 1:1 or with anti-IgE as the control. Results listed are percent histamine release at 37°C in buffer containing 2 mmol/L of Ca++ or at temperatures, Ca++ concentrations, or with inhibitors of release as indicated. Results are corrected for spontaneous release from untreated cells and are expressed as mean ± SD for three experiments.

7 ± 3%, whereas resensitization of cells with autologous serum restored release to control levels (48 ± 12%). However, lactic acid treatment had no effect on histamine release by Sendai, PI-3, or RS. These results demonstrate that triggering of histamine release from human cells is not necessarily IgE-dependent. It is noteworthy that there was a decrease in histamine release from basophils that had been desensitized with Con A (Fig. 2), suggesting that this lectin may be binding to carbohydrates on the cell membrane as well as on IgE molecules.

Active release of histamine from mast cells and basophils is dependent on formation of microtubules, transport of intracellular granules containing mediators to the cell surface, fusion of granule membrane with the cell membrane, and subsequent degranulation. Preincubation of basophils with colchicine will block histamine release by IgE-dependent stimuli,
FIG. 2. Effect of desensitization of human basophils on virus-induced histamine release. Cells were desensitized by preincubation with anti-IgE (0.1 mg/ml) or Con A (5 μg/ml) in HA buffer for 30 minutes at 37° C followed by washing and further incubation with viruses for 60 minutes in HACM. Histamine release by cells preincubated only with HA buffer was used for controls. The histamine release to anti-IgE and Con A is represented on the right as evidence of effective desensitization.

FIG. 3. Effect of removal of IgE on virus-induced histamine release. Cells were placed in 0.01 mol/L of lactic acid/lactate-buffered isotonic saline at pH 3.9 (■, □), to remove IgE, or pH 7.4 (■, △), as the control, for 2½ minutes at 22° C, then washed in HA, pH 7.4. Next, cells were incubated in HACM (■, □) or in autologous serum (■, △) for 30 minutes at 37° C to resensitize them, washed in HACM, and then treated with viruses (virus/cell ratio, 1) or anti-IgE (0.1 mg/ml) for 60 minutes in HACM.

presumably by interfering with microtubule formation. To examine the role of microtubules in histamine release induced by these paramyxoviruses, basophils were preincubated with 1.5 × 10⁻³ mol/L of colchicine. This treatment did not change histamine release induced by viruses compared to release from untreated cells (Table III). In contrast, treatment clearly caused a significant reduction of histamine release by anti-IgE (Table III) and by the calcium ionophore A23187 (27% histamine release by calcium ionophore A23187 alone versus 4% release by calcium ionophore A23187 plus colchicine) used as positive
controls. Furthermore, metabolic energy was required by these viruses to induce histamine release. Basophils, incubated with 5 mmol/L of 2-DG and 50 mmol/L of antimycin A for 15 minutes at 37°C and then washed extensively, were found to demonstrate considerable decrease in histamine release on subsequent incubation with viruses compared to histamine release from untreated cells.

We also studied the ability of UV inactivated viruses to cause histamine release. Samples of each virus were exposed to UV light; aliquots were removed at intervals and assayed for infectivity, hemagglutinating activity (Sendai only), and ability to cause histamine release (Table IV). UV treatment of RS for 5 minutes caused a 2.7 log reduction in infective virus while it virtually ablated release of histamine. Similar treatment of PI-3 resulted in a 0.5 log reduction in infective virus while it decreased histamine release by 68%. UV treatment of Sendai caused a 1.6 log reduction in infective virus, but only a single tube dilution decrease in hemagglutination titer; histamine release was decreased by 58%. Thus, we found not only a difference in effect of UV treatment among these viruses but also a discrepancy between effect on infectivity and histamine-releasing capacity.

TABLE IV. Histamine release by UV-treated RS, PI-3, and Sendai viruses

| UV treatment (min) | 0   | 5   | 30  |
|-------------------|-----|-----|-----|
| RS PFU/ml         | 5 × 10⁶ | 1 × 10⁶ | 0   |
| % Histamine release | 17 ± 1 | 0   | 0   |
| PI-3 TCID₅₀/ml    | 3 × 10⁷ | 1 × 10⁷ | 0   |
| % Histamine release | 24 ± 4 | 7 ± 3 | 0   |
| Sendai EID₅₀/ml   | 3 × 10⁶ | 8 × 10⁶ | 0   |
| HA titer*         | 1:1024 | 1:512 | 0   |
| % Histamine release | 37 ± 5 | 15 ± 4 | 0   |

*Hemagglutination titer (lowest dilution of virus causing agglutination of 1% chicken erythrocytes in phosphate-buffered saline).

DISCUSSION

We have demonstrated that three different paramyxoviruses, Sendai virus, PI-3, and RS, will induce secretion of histamine from preparation of human basophils. The lack of significant LDH release after incubation with PI-3 or RS, the lack of detectable change in cell number, and the inability of additional virus to cause additional histamine release support our conclusion that histamine release is the result of degranulation rather than leakage or cell lysis. Although Sendai caused some damage to cell membranes, resulting in loss of cytoplasmic content (LDH), this alone was insufficient to explain the histamine release observed, indicating that Sendai also will cause degranulation of human basophils. Our data indicate that IgE molecules and Fc receptors do not necessarily play any role in histamine release induced by these viruses. Clearly, neither desensitization of cells with anti-IgE nor stripping IgE molecules from Fc receptors altered virus-induced histamine release, and triggering of cells with virus did not alter their subsequent response to anti-IgE. In fact, the kinetics of release is inconsistent with a receptor-mediated phenomenon. It is evident that triggering cells with either anti-IgE or calcium ionophore A23187 causes some alteration in cell function that interferes with subsequent triggering by viruses; this is unlikely to be related to Fc receptors.

Our data suggest that these viruses interact directly with the cell membrane to permit histamine release. It appears reasonable to speculate that this interaction is a result of the binding of virus-specific glycoproteins with carbohydrates on the cell surface, which is the usual mechanism for paramyxovirus attachment that leads to cell infection. These three paramyxoviruses all possess F (fusion) glycoproteins that permit insertion of the virus envelope into the cell membrane and, subsequently, also are responsible for formation of syncitia. In the case of human basophils, it is possible that this interaction permits the passive fusion of intracellular granules containing histamine and other mediators with the cell membrane, resulting in release of these mediators extracellularly. It has been suggested previously that this virus-cell interaction results
in an alteration in the permeability of cell membranes. An alteration in membrane permeability permitting influx of calcium would be expected to result in histamine release, and the augmentation of virus-induced histamine release by calcium suggests that this might occur. However, the lack of dependence of virus-induced histamine release on the presence of calcium in the buffer, the lack of inhibition by colchicine, and the kinetics of release all suggest that virus-induced histamine release is not primarily the result of calcium influx and cell triggering, which occurs with the calcium ionophore A23187. The loss of HA activity by Sendai treated with UV light suggests that this treatment can alter envelope glycoproteins; this effect may explain the loss of histamine-releasing capacity by UV-treated viruses.

We have provided strong evidence that, under the appropriate circumstances, certain paramyxoviruses will cause the direct release of histamine from human basophils. We were unable to detect direct histamine release by viruses other than paramyxoviruses, as in previous studies by other investigators. It should be noted that the emphasis of these studies was to demonstrate that viruses affect histamine release by indirect mechanisms. Ida et al. were unable to detect histamine release by Sendai, and Chonnaitree et al. were unable to detect histamine release by RS. In both studies, cells were incubated with viruses for 2 hours, washed, then resuspended in virus-free buffer; there was no histamine release from these cells after a subsequent 22-hour incubation. There was no indication of observation for histamine release after the initial 2-hour incubation by either group. We also were unable to demonstrate that histamine release occurs after the first 2 hours of exposure to the viruses studied. It is likely that respiratory viruses can trigger mast cells and basophils to release histamine by IgE-dependent mechanisms; however, these mechanisms are not necessary to achieve significant release of histamine by these viruses. Basophils from all donors studied so far have responded to these respiratory viruses. Small interdonor variability has been noted, which can be observed in the results presented above. We have been unable to identify differences in virus-induced responses of cells from atopic versus nonatopic donors. It should be noted that we did not study cells from children, who might be more susceptible to this effect. Further studies will be necessary to explore the relationship between host susceptibility to this direct virus effect or intrinsic releasability of histamine and clinical symptoms during acute infection with these viruses.

We believe our findings contribute further evidence to suggest that mediator-containing cells may play a role in the symptomatology of acute respiratory virus infection. Paramyxoviruses and their well-defined envelope glycoproteins provide yet another tool to study membrane function in histamine release. Our current experiments are designed to investigate these interactions in detail.

We thank Dr. John Hierholzer for his generous guidance and assistance in obtaining, purifying, and quantitating viruses, Dr. David Morrison for his encouragement, helpful suggestions, and review of our manuscript; Dr. Prescott Atkinson for help with illustrations, and Mrs. Lillie Dunlap for her patience in manuscript preparation.

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Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids

III. Efficacy and safety of unfractionated and high-molecular-weight preparations in rhinoconjunctivitis and asthma

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Specific immunotherapy with unmodified formalinized allergoids is effective in grass-pollen allergy, but systemic reactions have been observed. A high-molecular-weight formalinized allergoid (HMW-GOID) was fractionated by gel filtration, retaining molecules of >50,000 daltons in the expectation of improving safety without sacrificing efficacy. HMW-GOID and unfractionated allergoid (GOID) had a similar allergenic activity assessed by RAST inhibition, but the HMW-GOID preparation was 65 times less reactive when it was tested by skin prick test than the GOID preparation. A double-blind, placebo-controlled study was carried out in grass-pollen-allergic patients with placebo (14 patients), GOID (15 patients), and HMW-GOID (13 patients). An additional group of 18 patients was treated by a rush schedule with a standardized orchard grass-pollen extract. A similar mean cumulative dose was administered with both allergoids. The fractionated allergoid only elicited minor systemic reactions similar to reactions elicited by placebo, whereas 20% of patients treated by GOID and 5.5% of patients receiving the standardized extract had a severe systemic reaction. For rhinitis, conjunctivitis, and asthma, the HMW-GOID and the standardized extract had a similar efficacy, significantly greater than placebo. GOID was less effective than the other two active treatments but was significantly more effective than placebo treatment for asthma and conjunctivitis. (J ALLERGY CLIN IMMUNOL 1989;84:546-56.)

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Received for publication Dec. 13, 1988.

Revised March 29, 1989.

Accepted for publication April 10, 1989.

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Abbreviations used

DU: Biological unit
GOID: Unfractionated allergoid
HMW-GOID: High-molecular-weight allergoid
MW: Molecular weight
PNU: Protein nitrogen unit
SPT: Skin prick test
HPLC: High-performance liquid chromatography