

in acute allergic reactions. It also began Larry's life-long passion for the role of the human basophil in allergy and inflammation. This work resulted in several seminal papers and was the basis for his PhD, which he received from Johns Hopkins in 1965. One of us (D.A.L.) became a postdoctoral fellow in Abe Osler's lab in 1962, where he used the human leukocyte-histamine release system to measure human reaginic antibody and thus became well acquainted with Larry's early work.

During his years as a postdoctoral fellow, Larry also took his turn seeing patients in the Hopkins Allergy Clinic, an experience that allowed him to become a clinical allergist. To round out his clinical training, after receiving his PhD, he spent the following year as Assistant Resident on the Osler Medical Service. After morning rounds, he would return to his lab on Blalock 10 to plan the day's experiments, which were usually performed by his first technician, Anne Kagey-Sobotka, who later earned her own PhD under Larry's mentorship. Many Hopkins medical students opted to work in Larry's laboratory during free quarters and summers, including one of us (N.F.A.) who began projects on penicillin allergy which continued into his Osler medical residency.

It was during his years in Osler's lab (1961-1965) that Larry began to collaborate with Phil Norman. Their first study together applied the use of the *in vitro* basophil histamine release model to quantify sensitivity and response to specific immunotherapy with a standardized ragweed extract. This landmark study was published in 1966 in *The Journal of Clinical Investigation* (45:1126-36), more than a year ahead of the Ishizakas' classic *Journal of Immunology* article identifying "reagin" as IgE. Norman and Winkewer organized the clinical trial, and Lichtenstein and Osler did the laboratory assessments of immunologic sensitivity. Three additional clinical papers by this pioneering team, all published in 1968, reported on the results of a controlled study of immunotherapy for ragweed hay fever, the value of adjuvants in boosting IgG antibody responses, and a comparison of treatment with "antigen E" (now Amb a 1), the principal ragweed allergen in whole ragweed extract. In 1969, Larry and Phil wrote a review article for *The American Journal of Medicine* (46:163-73) summarizing their work and view of human allergic reactions at that time.

Phil and Larry would go on in the 1970s to preside jointly over a separate Division of Clinical Immunology that would be housed in the newly built O'Neill Research Laboratories at the affiliated Good Samaritan Hospital across town. At the same time, Kimishige and Teruko Ishizaka were recruited from Denver where they had "discovered" IgE. This was followed by a long lineage of recruits and retained trainees that now constitutes one of the world's largest faculties devoted to the study of allergic diseases. In 1989, the Division moved to the new Johns Hopkins Asthma and Allergy Center, where the Norman and Lichtenstein legacy continues to this day.

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LEGENDS IN ALLERGY: PHILIP S. NORMAN AND LAWRENCE M. LICHTENSTEIN—THE HOPKINS EXPERIENCE

Dr Philip Norman began his medical career at Washington University School of Medicine in St Louis, Mo, where he graduated *cum laude* in 1951. During his formative years in medical school, he gravitated toward the emerging field of immunology and has remained in its forefront ever since, through his endeavors in allergen characterization, allergen immunoassays, allergen provocation, and clinical trial design.

After completing his internship the following year, at Barnes Hospital in St Louis, and subsequently his residency in 1954, at Vanderbilt University in Nashville, he received a highly sought after fellowship in New York at the Rockefeller Institute for Medical Research, under Dr Merrill Chase. It was here that he first explored allergen characterization in the laboratory with Dr T. P. King, an association that led to several important publications on the principle protein allergens isolated from ragweed.¹⁻⁴

He was initiated into the Hopkins community in 1956, becoming an Assistant Professor in 1959, and subsequently an Associate Professor in 1964. He was asked to establish a formal Division of Clinical Immunology in 1970. During these early years, he assembled a core team composed of Lawrence Lichtenstein, Martin Valentine, and David Marsh, and began to focus on the underlying pathophysiology and treatment of allergic respiratory disease. The nucleus of this team expanded through the 1970s with the addition of Thomas Van Metre, N. F. Adkinson Jr, and Anne Kagey-Sabotka. The Division grew rapidly in the 1980s as Robert Schleimer, Don McGlashan, Robert Naclerio, Stephen Peters, Edward Schulman, and Peter Creticos completed their fellowships and immersed themselves in the limited confines of the Howard Hughes Institute at the Good Samaritan Hospital, within which the incubating Division began to flourish.

As codirectors of the Division of Clinical Immunology, Drs Norman and Lichtenstein envisioned the establishment of a center to meet the national demand for advances in treating allergies, asthma, and related respiratory disorders. In 1989, the culmination of their

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vision led to the christening of the Johns Hopkins Asthma and Allergy Center.

This 5-story facility incorporates wings for research and diagnostic laboratories and outpatient care and clinical research. A major goal of the center is to train physicians who wish to specialize in the treatment of allergic and respiratory disorders. Funded through governmental and private sources, more than 150 fellows who populate academia, government, and industry as well as clinical practice owe a large debt of gratitude to the keen foresight, wisdom, and guiding hands of Philip Sidney Norman and Lawrence M. Lichtenstein.

THE EARLY YEARS: THE 1960s: CHARACTERIZATION OF RAGWEED

In 1962, Drs Norman and King isolated the first allergenic protein (antigen E [Ag E]; Amb a 1) from a known allergy-causing substance,¹ thus paving the way for standardization of allergens for both diagnostic and treatment purposes. Indeed, on the basis of this ability to characterize and measure the major allergenic moiety of a defined and clinically relevant allergen, Norman and Lichtenstein were the first to use modern immunoassay techniques, including gel and liquid chromatography, leukocyte (basophil) histamine release assays, antibody titer measurements, and RAST inhibition studies, to evaluate the effectiveness of allergen desensitization therapy, a cornerstone of the allergist's armamentarium for treatment of patients with allergy since the early 1900s, and ushered in the new era of "allergen immunotherapy," as coined by Norman.

The initial work by King and Norman isolated the allergenic activity of ragweed to specific protein-rich fractions (III, IV, V).¹⁻³ They used standard laboratory methods to extract and concentrate the active moieties from ragweed pollen. They isolated and characterized the active protein moiety in fraction IV and designated this 37,800-kd protein Ag E.² In their 1967 publication, they isolated and defined the second most active allergenic moiety in ragweed pollen, antigen K (Ag K; Amb a 2), in protein fraction V.³ Ag E accounted for 6% of the ragweed pollen's weight, whereas Ag K represented 3% of the pollen's weight. The 2 proteins appeared to be antigenically independent on the basis of immunodiffusion studies of Ag E and Ag K against rabbit antisera to ragweed. On the other hand, these 2 active allergenic moieties appeared to share common antigenic determinants. Comparative skin test studies demonstrated that both fractions were more reactive on the skin than whole ragweed extract (100-fold to 1000-fold). Furthermore, the Ag E fraction was more active in most patients (n = 30), with the Ag K fraction more active in only 1 study subject. Comparative histamine release assays demonstrated that the allergenic activity of the Ag K fraction was approximately 50% less than that ascribed to fraction IV (Ag E).^{3,4}

Antigen E took center stage as an important diagnostic and potential therapeutic tool.

DEVELOPMENT AND APPLICATION OF *IN VITRO* IMMUNOASSAYS TO EVALUATE CLINICAL RESPONSE

In 1921, Prausnitz and Kustner identified a "factor" in serum from a patient with allergy that had the capacity to transfer the "allergic" wheal and flare reaction from one individual to another.⁵ In 1968, Kimishi and Terri Ishizaka, from their laboratory in the Department of Microbiology at the University of Colorado Medical School, identified a serum immunoglobulin, which they termed IgE, as the reagenic antibody responsible for this passive transfer property.⁶

Studies by Cooke et al⁷ in 1935 and Loveless⁸ in 1943 demonstrated that patients with allergy undergoing allergen desensitization produced immunoglobulin "blocking antibodies" that had the capacity to block the passive transfer reaction in the skin. In 1968, Lichtenstein, Holtzman, and Burnett⁹ described the immunoglobulin and chromatographic characteristics of this human blocking antibody.

It was demonstrated that blocking antibody and IgG eluted together, leading to their conclusion that "blocking activity" was essentially a function of IgG molecules. A subsequent publication by Lichtenstein, Norman, and Winkenwerder¹⁰ the same year made the observation that the blocking activity of the sera from immunized patients increased several 100-fold above control levels.

Tangential to these studies, Norman, Lichtenstein, Winkenwerder, and Osler¹¹ published a landmark paper in 1966 in which specific *in vitro* methods were used to study the changes in cellular and humoral activity associated with specific desensitization. This work demonstrated that the sensitivity of an allergic donor's leukocytes to an offending allergen (ragweed) correlated significantly with the degree of symptomatology the patient had during the ragweed season. Furthermore, the serum from these ragweed-treated patients was shown to contain antibodies capable of binding the antigen and thereby decreasing the *in vitro* allergic response, and that, as contrasted with the placebo-injected group, the ragweed-immunized group demonstrated an increase in the level of antibody with antigen-neutralizing capacity, which increased proportionally to the quantity of ragweed Ag E administered with treatment (*vis-à-vis* IgG antibody).

In 1967, Lichtenstein, Norman, and Connell¹² used measurements of *in vitro* leukocyte sensitivity to ragweed Ag E and Prausnitz-Kustner titrations of skin-sensitizing antibody as indices of severity in ragweed hay fever. These studies clearly showed that cell sensitivity, as defined by the concentration of ragweed antigen required to release 50% of histamine content from leukocytes isolated from the blood of allergic donors, strongly correlated with symptom severity and

was a more accurate measure of the disease state than skin-sensitizing antibody.

In a 1971 paper, Levy, Lichtenstein, Goldstein, and Ishizaka¹³ reported that clinical improvement in ragweed-immunized children correlated best with decreased leukocyte sensitivity and reactivity to ragweed Ag E and that the observed decreased cell sensitivity was associated with lower serum reagenic antibody levels.

The pertinent corollary to use of these immunologic assays is the validation of their results. In this regard, in 1973, Norman, Lichtenstein, and Ishizaka¹⁴ reported their finding on the intercorrelations between these newer immunoassays and *in vivo* skin test sensitivity and clinical symptoms. In a study of 87 patients with grass and ragweed allergy assessing these diagnostic tests, the authors reported significant agreement between quantitative skin test titration and the *in vitro* methods to measure specific IgE antibody against an allergen, and basophil histamine release (a nonspecific assay dependent both on the amount of IgE antibody fixed to the surface of basophils and the releasability of the cells themselves). Furthermore, there was significant agreement between the degree of positivity with each of these diagnostic tests and the reciprocal patient symptomatology.

That same year, the group and Hill¹⁵ also expounded on the effects of ragweed immunotherapy on these same parameters and made the following hallmark observations: (1) antiragweed IgE antibodies rise in untreated patients as they are naturally exposed to ragweed pollen during the season; (2) in immunized patients, treatment induces an initial rise in IgE antibodies, which over time declines and is not boosted with subsequent seasonal exposure; (3) the treatment-induced increase in IgG blocking antibody correlates with the observed decrease in serum IgE antibody; (4) IgE antibody on basophils appears to correlate with the untreated patients' serum IgE; and (5) in untreated patients, symptom scores strongly correlate with both their serum IgE and the sensitivity of their basophils to Ag E-induced histamine release.

The earlier studies cited on cell sensitivity^{11,12} combined with the published results demonstrating the ability to measure antibody response after allergen immunotherapy^{10,11,15,16} set the stage for a series of definitive studies of immunotherapy with ragweed allergen published by the Hopkins group from 1968 to 1972.¹⁷⁻²⁰

BRIDGING TO CLINICAL STUDIES OF EFFICACY: ALLERGEN IMMUNOTHERAPY USING A QUALITATIVELY DEFINED RAGWEED EXTRACT

In 1963, Lowell and Franklin²¹ reported that in 24 patients with ragweed allergy and seasonal fall hay fever symptoms, a significant reduction in symptoms was noted during the peak of the ragweed season by

the patients on active therapy compared with the placebo group ($P = .05$).

Subsequently, in 1967, Franklin and Lowell²² undertook a study of 25 patients with allergy already on multiple allergen therapy and compared the efficacy of continued high-dose ragweed therapy (range, 0.07-0.5 mL of a 1:50 concentration; mean, 0.3 mL) to that of a low-dose ragweed regimen, where the administered dose was reduced to 1/20th (range, 0.1-0.4 mL of a 1:1000 concentration; mean, 0.3 mL). Analysis of diary scores showed that patients with ragweed allergy receiving high-dose allergen treatment ($n = 13$) reported excellent symptom control (median score, 20), whereas the low-dose group ($n = 13$) reported inadequate symptom relief (median score, 300; $P = .025$). These trials used a crude ragweed extract, and no attempt was made to define protein content in the allergen extract. However, the studies demonstrated the importance of an adequate treatment dose.

In this same timeframe, Norman, Winkenwerder, and Lichtenstein embarked on a series of studies incorporating the new immunologic techniques described to evaluate the efficacy of allergen immunotherapy in a more precise and objective fashion. They anticipated that a well characterized ragweed extract, one quantified on the basis of Ag E protein content, would allow better characterization of the immunologic changes associated with immunotherapy and might lead to more effective clinical relief. These studies provided important treatment parameters related to dose requirements and length of treatment necessitated to achieve clinical benefit.

In 1968, Lichtenstein, Norman, and Winkenwerder¹⁷ published an initial double-blind study comparing the treatment effect of preseasonal immunotherapy with a ragweed Ag E preparation (provided by the National Institutes of Health Committee on Standardization of Allergens) versus placebo injections in 40 patients with ragweed allergy matched by cell sensitivity. This study addressed the relationships between symptoms, cell sensitivity, and "blocking antibody" in these subjects and expanded on preliminary observations, confirming that treated patients reported lower symptom scores ($P < .01$) than matched placebo patients (affirming the usefulness of balancing patient groups by cell sensitivity) and that patients with higher antibody measurements, induced by treatment, likewise had better clinical outcomes. As predicted, the antibody response correlated significantly with the amount of Ag E administered.

Next, Norman, Winkenwerder, and Lichtenstein published their findings of a controlled, double-blind, 4-year study of preseasonal immunotherapy of patients with ragweed-allergic hay fever randomized to treatment with whole ragweed extract (defined by Ag E content), purified ragweed Ag E, or placebo.¹⁸ In this preseasonal study, the patients were given weekly injections (May to early August), with doubling doses unless local or systemic reactions necessitated a slower build-up.

This study highlights several important findings. First, an adequate dose must be achieved before evidence of clinical relief is noted—hence, during the first 2 years of this study, the lower cumulative preseasonal dosage levels of purified Ag E (1963, 3.7 µg; 1964, 32 µg) and whole ragweed (1964, 3.1 µg Ag E) were not effective in ameliorating symptoms. However, as higher cumulative doses of ragweed were attained in 1965 and 1966, a stepwise improvement in clinical symptoms was demonstrated in both treatment groups compared with placebo.

In fact, the patient group that received whole ragweed reported peak season symptom scores that were 44% lower than those of placebo-treated patients during the final year of study. Clinical improvement was also demonstrated in the group that received purified Ag E (1965, 252 µg; 1966, 36 µg), where symptom diary scores were reduced by 31% compared with the placebo group. Fig 1 plots the average daily symptom scores for the patients in each group and clearly displays the significant improvement in clinical symptoms achieved by the actively immunized groups compared with placebo groups during the peak of the ragweed pollen season (whole ragweed vs placebo, $P = .004$; purified Ag E vs placebo, $P = .03$). These results demonstrate that the efficacy of preseasonal therapy with ragweed pollen immunotherapy is dependent on the dose of antigen administered. The question of whether a purified Ag E extract could supplant a whole ragweed extract containing other relevant major allergens (Ag K/Ra3/Ra5) was not conclusively answered by this study.

Expanding on these observations, Lichtenstein, Norman, and Winkenwerder¹⁹ in 1971 reported the results of a clinical trial designed to determine whether a more aggressive regimen that allowed patients to achieve the same final cumulative dose in 1-year instead of 3 years could achieve the same clinical benefit. In this placebo-controlled trial, in which purified pollen extracts (Amb a 1, Amb a 1 + Ag K) were compared with crude (whole) ragweed, and which used the same preseasonal study design as outlined, patients receiving weekly injections (March 6 to August 6) with doses doubled unless local or systemic reactions necessitated a slower build-up.

The mean total cumulative dose of crude (whole) ragweed extract was 33.4 µg Ag E (median, 26.7), and the mean cumulative dose of the purified Ag E extract was 1000 µg (median, 742). There was an approximately 40% reduction in clinical symptoms (symptom index [SI]) for each of the treated groups (whole ragweed SI, 7.8, vs placebo SI, 11.3; $P < .01$; purified Ag E SI, 7.2, vs placebo SI, 11.3; $P < .01$). The authors recommended pushing to a “maximally tolerated” dose because, at that time, as shown in their 1970 publication, commercial extracts varied in their Ag E content.²³

An interesting corollary from this study,¹⁹ also observed in the previous study,¹⁸ was that the crude (whole) ragweed preparation and the purified extract

preparations were fairly comparable in efficacy. Of importance, this single year of therapy produced a degree of symptom relief comparable to that seen with the 3-year course of immunotherapy reported in the 1968 study, and provided solid evidence that a more aggressive regimen that allowed a comparable dose to be achieved in 1 year could be safely administered and could achieve clinical success in a more rapid timeframe.

This study also incorporated the newly defined *in vitro* techniques to characterize further the immunologic parameters influenced by immunotherapy. A decrease in leukocyte sensitivity, as assessed by basophil histamine release, accompanied the symptomatic improvement in some but not all patients. A rise in IgG blocking antibodies was demonstrated in the actively immunized populations, and the measured antibody titer correlated in a positive fashion with the dose of allergen administered. However, as observed with the histamine release studies, some patients obtained good clinical relief without significant IgG antibody production, and conversely, some patients demonstrated high IgG antibody production but exhibited poor clinical benefit.

Next a perennial injection regimen with boosters administered at 6-week intervals was initiated in a group of patients with ragweed (RW) hay fever who had participated in the 1963 to 1966 preseasonal study.¹⁸ This led to a publication in 1971 by Lichtenstein, Norman, and Winkenwerder¹⁹ that demonstrated a sustained clinical improvement in both treated patient groups immunized perennially with ragweed (whole RW and Ag E treatment cohorts) over the 2-year study period (1967-1968), compared with the placebo (PL) group's mean daily symptom index scores ($P < .01$). Of note, the booster immunization regimen incurred a reaction rate similar to that observed during the last year of preseasonal therapy.

These properly designed clinical trials, using ragweed allergen, validated this long-standing treatment modality and ushered in the modern era of allergen immunotherapy, in which well characterized or standardized allergens, administered in an adequate dose, and administered for an appropriate duration were shown to induce clinical benefit that could be substantiated in an objective manner with specific immunoassays.

SPECIFICITY OF IMMUNOTHERAPY AND COMPARATIVE STUDIES ON ADEQUATE DOSE

The 1965 study by Lowell and Franklin²⁴ addressed not only the efficacy of ragweed immunotherapy but also its specificity. In that study, patients initially received multiple-allergen immunotherapy on the basis of their clinical sensitivities. In the subsequent year, patients who had reported clinical symptoms on their diaries, in spite of immunotherapy, were then randomized in a blind fashion to receive either an extract with

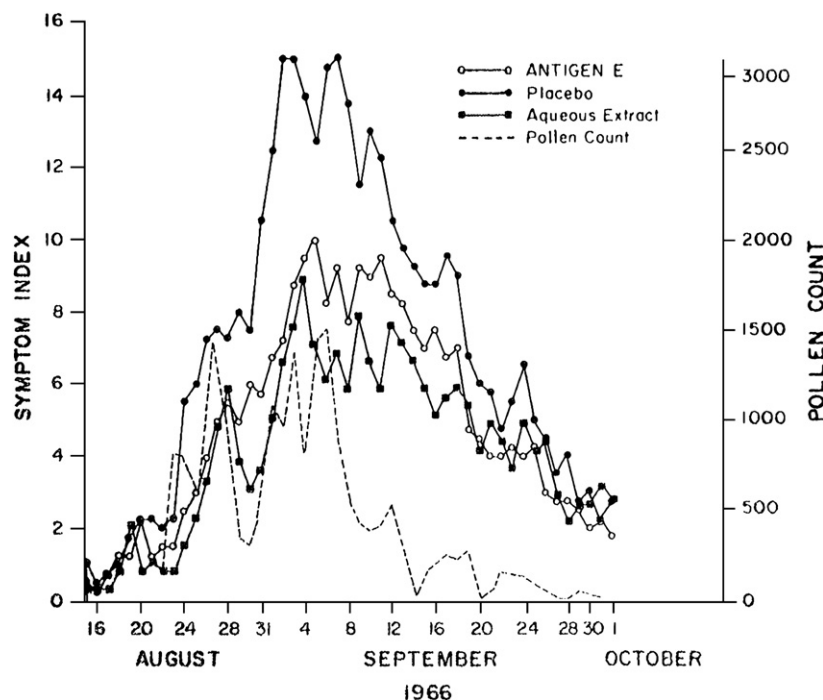


FIG 1. Average daily symptom scores of groups of patients with hay fever treated with antigen E, whole ragweed extract, or placebo in 1966. Reprinted with permission from Norman PS, Winkenwerder WL, Lichtenstein LM. Immunotherapy of hay fever with ragweed antigen E: comparisons with whole pollen extract and placebos. *J Allergy* 1968;42:93-108.¹⁸

ragweed (ragweed-included) or an extract from which ragweed had been removed. Over the course of the next ragweed season, the group no longer receiving ragweed had a recrudescence of symptoms, whereas the (ragweed-included) treatment group continued to report good control of clinical symptoms.

The clinical and immunologic specificity of immunotherapy was likewise demonstrated by Norman and Lichtenstein²⁵ in a 1978 study of patients allergic to both ragweed and grass. Only the group of patients immunized to ragweed showed a clinical improvement in their fall hay fever on graded symptom medication diaries, whereas the nonimmunized group reported typical fall symptoms on their seasonal diary records. The effects of immunotherapy were shown to be specific for the allergen to which the patients were immunized, because neither group received grass immunotherapy injections, and both groups reported similar spring pollen symptoms.

In 1982, Van Metre, Adkinson et al²⁶ reported a study that compared the standard weekly injection regimen (with immunotherapy) to that of a clustered (immunotherapy) regimen in which multiple doses were given at 3-week intervals. Again, it was demonstrated that a whole ragweed extract delivering a median cumulative dose of 70 μg Amb a 1 provided effective clinical relief (SIT, 3.8, vs PL, 11.1; $P = .01$). In this study, the authors reported that single maintenance doses of about 9.4 μg Amb a 1/injection compared favorably with their calculations of maximally tolerated doses from reviewed studies, in which

maintenance doses ranged from 2.7 to 46.8 μg Amb a 1/injection.

In contrast with standard immunotherapy, an alternate form of low-dose treatment, termed *Rinkel immunotherapy* (RIT), was also practiced in the 1970s. In 1980, Van Metre, Adkinson, Lichtenstein et al²⁷ published a critical evaluation of this form of immunization, in which the dose of allergen injected was 1/1000 the customarily recommended dose (median maintenance dose, 0.006 μg Amb a 1/injection; mean cumulative dose, 0.094 μg Amb a 1; range, 0.004-0.151 μg). The symptom medication diary scores indicated that the low-dose RIT method of immunotherapy was no more effective than placebo (symptom medication diary score, RIT, 9.8, vs placebo, 8.1).

In 1980, these investigators also published their comparative study of the Rinkel method of immunotherapy vs SIT and conclusively demonstrated the efficacy and superiority of SIT, with symptom scores reduced ~50% compared with RIT (SIT, 2.9, vs RIT, 5.8; $P < .01$).²⁸ In addition, SIT was shown to be statistically superior to placebo ($P < .01$) with no difference observed between the RIT and placebo groups (RIT, 5.8, vs PL, 5.0; $P = .3$; Fig 2). In this maximally tolerated protocol, the patients ($n = 15$) on standard immunotherapy received a median maintenance dose of 11 μg Amb a 1/injection (range, 3.7-46.8 μg) with a median cumulative dosage over the treatment interval (February to October) of 84.9 μg Amb a 1 (range, 18.1-351.2). In contrast, the RIT group received only 0.001 μg Amb a 1 (Fig 2). As would

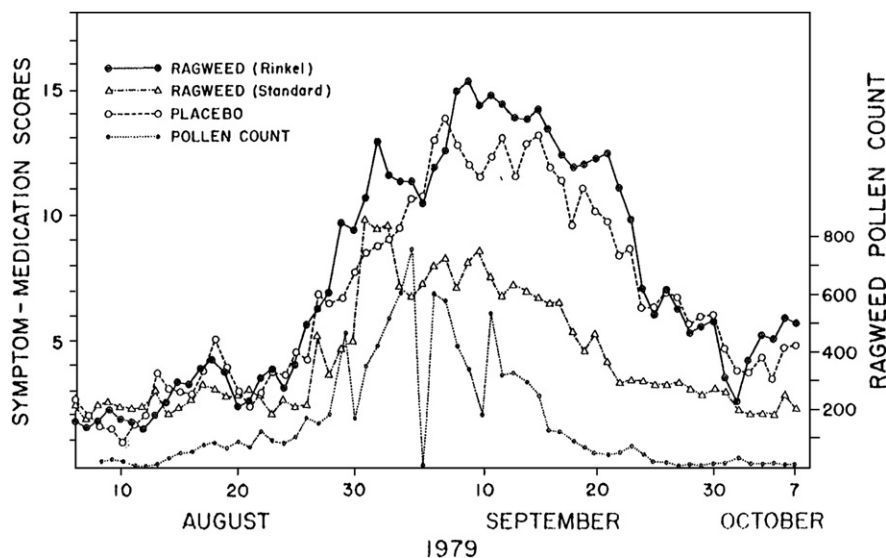


FIG 2. Arithmetic mean of daily symptom-medication scores for the groups indicated compared with the pollen count reported as ragweed pollen grains per cm^2 of roto-slide. Rise and fall of symptom medication scores were significantly correlated with rise and fall of ragweed pollen counts for standard ragweed group (Spearman correlation coefficient, $r_s = .696$, $P < .01$), placebo group ($r_s = .626$, $P \leq .01$), Rinkel 78/79 group ($r_s = .718$, $P < .01$), and Rinkel 79 group ($r_s = .603$, $P < .01$). Symptom medication scores of standard group were significantly less ($P < .01$) than those of Rinkel and placebo groups; those of Rinkel and placebo groups were not significantly different ($P = .30$). Reprinted with permission from Van Metre TE, Adkinson NF, Amodio FJ, Lichtenstein LM, Mardiney MR, Norman PS, et al. A comparative study of the effectiveness of the Rinkel method and the current standard method of immunotherapy for ragweed pollen hay fever. *J Allergy Clin Immunol* 1980;66:500-13.

be predicted, SIT produced a significant increase in anti-ragweed IgG antibody and decrease (blunting) of the seasonal rise in antiragweed IgE antibody; in contradistinction, RIT and placebo demonstrated neither of these surrogate immunologic changes previously shown to be associated with appropriate immunizing dose regimens.

DEVELOPMENT AND APPLICATION OF A MODEL OF RAGWEED PROVOCATION

Norman and Lichtenstein envisioned that a model of nasal allergen provocation could provide a unique tool with which to characterize the allergic response, and implicitly to study the effects of immunotherapy on the cellular events, inflammatory responses, and immunologic changes that occur in allergic rhinitis. The standardized incremental inhalation of allergen by nasal provocation induces clinical symptoms that correlate with the stepwise appearance of a variety of inflammatory mediators (histamine, kinins, prostaglandins, and leukotrienes) in nasal secretions.²⁹⁻³¹

In 1985, Creticos, under Norman's tutelage,³² published the initial study by the Hopkins group that defined the utility of the method for the study of immunotherapy. This comparison of 12 patients with ragweed allergy (on long-term [3-5 years], moderate-dose immunotherapy [6 μg Amb a 1/injection]) with a group of 27 patients not previously immunized revealed that significantly fewer of the immunized patients developed an immediate-phase allergic reaction

as characterized by clinical symptoms or mediator release after nasal ragweed provocation.

In the follow-up study by Creticos et al,³³ the 27 previously untreated patients were randomized in a blind fashion to 2 different ragweed immunotherapy products. In the patients placed on standard ragweed immunotherapy, evaluation of the clinical, immunologic, and biochemical mediator response to nasal pollen challenge was performed when each of 3 predetermined levels (0.6 μg , 12.4 μg , 24.8 μg RW Amb a 1/injection) of immunotherapy had been reached over the 18-month treatment period. The low dose of immunotherapy provided no protection against nasal provocation. However, both the 12.4- μg and 24.8- μg doses afforded protection as evidenced by more pollen being required to elicit mediator appearance in nasal secretions, and the absolute amount of mediator released at each stage was reduced. Furthermore, patient seasonal symptom-medication scores over the 3 ragweed seasons evaluated demonstrated significant clinical improvement during the 2 years patients were on immunotherapy compared with the pretreatment year. These studies defined an optimal individual treatment dose for ragweed immunotherapy at 6 to 12.4 μg Amb a 1/injection.

In a later study of 41 subjects with ragweed allergy from the group, Iliopoulos et al³⁴ showed that ragweed immunotherapy not only diminished the immediate-phase allergic reaction but also significantly attenuated the late-phase component of the IgE-mediated allergic reaction. Recognizing that the late-phase reaction is dependent on the recruitment of a secondary wave of inflammatory cells, including eosinophils, basophils,

and neutrophils, into tissue, Furin et al³⁵ demonstrated that immunotherapy significantly decreased eosinophil migration into the nasal cavity. Then Majchel et al³⁶ showed that immunotherapy prevented the increased responsiveness of the nasal mucosa to a nonspecific irritant (histamine) in patients with allergy during the season.

These studies convincingly demonstrated that conventional immunotherapy, administered at an optimal therapeutic dose, had a positive effect on each component of the allergic diathesis—the acute (immediate) allergic response, the late-phase allergic response, the nonspecific mucosal reactivity, and the underlying cellular basis of the disease, and that these objective mechanistic observations were associated with clinical improvement.

SUMMARY

Through a research career that spanned 50 years, this prodigious collaboration provided the allergy community with the tools to design and perform clinical studies of immunotherapy based on sound scientific principles. Their studies of ragweed immunotherapy provide a framework for assessment of therapeutic efficacy based on measurable objective immunologic parameters: (1) a rise in serum IgG blocking antibodies. (2) a suppression of the typical seasonal rise in IgE antibodies followed by a steady decline in the level of specific IgE antibodies with a continued maintenance immunotherapy regimen, (3) an increase in blocking antibodies in nasal secretions, and (4) inhibition of allergen-induced histamine release from isolated human leukocytes (reduced basophil sensitivity and reactivity). Furthermore, their acumen in clinical trial design defined equally important clinical parameters that defined clinical success: (1) use of a well characterized allergen extract standardized based on major protein content (Amb a 1); (2) defining an optimal immunizing dose which minimized the systemic anaphylaxis engendered with maximally tolerated dose regimens; (3) use of immunotherapy for an adequate length of time through incorporation of a perennial maintenance immunotherapeutic regimen to induce lasting protection; and (4) defining the specificity of immunotherapy.

In conclusion, their enormous body of work has advanced the discipline of allergy from what most would term an art into a defined science, based on sound scientific principles, and provided the practicing allergist-immunologist with a more precise practice of medicine.

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REFERENCES

- King TP, Norman PS. Isolation studies of allergens from ragweed pollen. *Biochemistry* 1962;1:709-20.
- King TP, Norman PS, Connell JT. Isolation and characterization of allergens from ragweed pollen, II. *Biochemistry* 1964;3:458-68.
- King TP, Norman PS, Lichtenstein LM. Isolation and characterization of allergens from ragweed pollen, IV. *Biochemistry* 1967;6:1992-2000.
- King TP, Norman PS, Lichtenstein LM. Studies on ragweed pollen allergens, V. *Ann Allergy* 1967;25:541-53.
- Prausnitz C, Kustner H. Studies on supersensitivity. *Zentralbl Bakteriol* 1921;86:160-9.
- Ishizaka K, Ishizaka T. Human reaginic antibodies and immunoglobulin E. *J Allergy* 1968;42:330-63.
- Cook RA, Barnard JH, Hebal S, Stull A. Serologic evidence of immunity with coexisting sensitization in a type of human allergy (hay fever). *J Exp Med* 1935;62:733-50.
- Loveless MH. Immunological studies of pollinosis, IV: the relationship of thermostable antibody in the circulation and clinical immunity. *J Immunol* 1943;47:165-80.
- Lichtenstein LM, Holtzman NA, Burnett LS. A quantitative *in vitro* study of the chromatographic distribution and immunoglobulin characteristics of human blocking antibody. *J Immunol* 1968;101:317-24.
- Lichtenstein LM, Norman PS, Winkenwerder WL. Clinical and *in vitro* studies on the role of immunotherapy in ragweed hay fever. *Am J Med* 1968;44:514-24.
- Lichtenstein LM, Norman PS, Winkenwerder WL, Osler AG. *In vitro* studies of human ragweed allergy: changes in cellular and humoral activity associated with specific desensitization. *J Clin Invest* 1966;45:1126-36.
- Lichtenstein LM, Norman PS, Connell JT. Comparison between skin-sensitizing antibody titers and leukocyte sensitivity measurements as an index of the severity of ragweed hay fever. *J Allergy* 1967;40:160-7.
- Levy DA, Lichtenstein LM, Goldstein EO, Ishizaka K. Immunologic and cellular changes accompanying the therapy of pollen allergy. *J Clin Invest* 1971;50:360-9.
- Norman PS, Lichtenstein LM, Ishizaka K. Diagnostic tests in ragweed hay fever. *J Allergy Clin Immunol* 1973;52:210-24.
- Lichtenstein LM, Ishizaka K, Norman PS, Sobotka AK, Hill BM. IgE antibody measurements in ragweed hay fever: relationship to clinical severity and the results of immunotherapy. *J Clin Invest* 1973;52:472-82.
- Lichtenstein LM, Norman PS, Winkenwerder WL. Antibody response following immunotherapy in ragweed hay fever: Allpyral vs. whole ragweed extract. *J Allergy* 1968;41:49-57.
- Lichtenstein LM, Norman PS, Winkenwerder WL. Clinical and *in vitro* studies on the role of immunotherapy in ragweed hay fever. *Am J Med* 1968;44:514-24.
- Norman PS, Winkenwerder WL, Lichtenstein LM. Immunotherapy of hay fever with ragweed antigen E: comparisons with whole pollen extract and placebos. *J Allergy* 1968;42:93-108.
- Lichtenstein LM, Norman PS, Winkenwerder WL. A single year of immunotherapy for ragweed hay fever: immunologic and clinical studies. *Ann Intern Med* 1971;75:663-71.
- Norman PS, Winkenwerder WL, Lichtenstein LM. Maintenance immunotherapy in ragweed hay fever. *J Allergy* 1971;47:273-82.
- Lowell FD, Franklin W. "Double-blind" study of treatment with aqueous allergenic extracts in cases of allergic rhinitis. *J Allergy* 1963;34:165-82.
- Franklin W, Lowell FC. Comparison of two dosages of ragweed extract in the treatment of pollinosis. *JAMA* 1967;201:95-7.
- Baer H, Godfrey H, Maloney CJ, Norman PS, Lichtenstein LM. The potency and antigen E contents of commercially prepared ragweed extracts. *J Allergy* 1970;45:347-54.
- Lowell FD, Franklin W. A double-blind study of the effectiveness and specificity of injection therapy in ragweed hay fever. *N Engl J Med* 1965;273:675-9.

25. Norman PS, Lichtenstein LM. The clinical and immunologic specificity of immunotherapy. *J Allergy Clin Immunol* 1978;61:370-7.
26. Van Metre TE Jr, Adkinson NF Jr, Amodio FJ, Kagey-Sobotka A, Lichtenstein LM, Mardiney MR Jr, et al. A comparison of immunotherapy schedules for injection treatment of ragweed pollen hay fever. *J Allergy Clin Immunol* 1982;69:181-93.
27. Van Metre TE Jr, Adkinson NF Jr, Lichtenstein LM, Mardiney MR Jr, Norman PS, Rosenberg GL, et al. A controlled study of the effectiveness of the Rinkel method of immunotherapy for ragweed pollen hay fever. *J Allergy Clin Immunol* 1980;65:288-97.
28. Van Metre TE Jr, Adkinson NF Jr, Amodio FJ, Lichtenstein LM, Mardiney MR, Norman PS, et al. A comparative study of the effectiveness of the Rinkel method and the current standard method of immunotherapy for ragweed pollen hay fever. *J Allergy Clin Immunol* 1980;66:500-13.
29. Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NF Jr, Meyers DA, Norman PS, et al. Mediator release after nasal challenge with allergen. *Am Rev Respir Dis* 1983;128:597-602.
30. Creticos PS, Peters SP, Adkinson NF Jr, Naclerio RM, Hayes EC, Norman PS, et al. Demonstration of peptide-leukotriene release following nasal antigen challenge in vivo. *N Engl J Med* 1984;310:1626-30.
31. Proud D, Togias AT, Naclerio RM, Crush SA, Norman PS, Lichtenstein LM. Kinins are generated in vivo following nasal antigen challenge of allergic individuals with allergen. *J Clin Invest* 1983;72:1678-85.
32. Creticos PS, Adkinson NF Jr, Kagey-Sobotka A, Proud D, Meier HL, Naclerio RM, et al. Nasal challenge with ragweed pollen in hay fever patients. *J Clin Invest* 1985;76:2247-53.
33. Creticos PS, Marsh DG, Proud D, Kagey-Sobotka A, Adkinson NF, Friedhoff L, et al. Responses to ragweed pollen nasal challenge before and after immunotherapy. *J Allergy Clin Immunol* 1989;84:197-205.
34. Iliopoulos O, Proud D, Adkinson NF Jr, Creticos PS, Norman PS, Kagey-Sobotka A, et al. Effects of immunotherapy on the early, late, and rechallenge nasal reaction to provocation with allergen: changes in inflammatory mediators and cells. *J Allergy Clin Immunol* 1991;87:855-66.
35. Furin MJ, Norman PS, Creticos PS, Naclerio RM. Immunotherapy: decreases antigen-induced eosinophil migration into the nasal cavity. *J Allergy Clin Immunol* 1991;88:27-32.
36. Majchel AM, Proud D, Freidhoff L, Creticos PS, Norman PS, Naclerio RM. The nasal response to histamine challenge: effect of the pollen season and immunotherapy. *J Allergy Clin Immunol* 1992;90:85-91.