

One hundred years of immunotherapy: Review of the first landmark studies

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ABSTRACT

In 2011, one hundred years of allergen immunotherapy was celebrated. Several landmark studies date from the first decades of experience with this treatment and are still cited today, often without analysis of the original articles. Original articles of the oldest landmark studies on subcutaneous immunotherapy (SCIT) and sublingual immunotherapy were sought and reviewed in detail, together with some publications on their authors' historical background. Details that might be of importance to the present allergists are highlighted in this article. Study design, preparation of allergen extracts used for immunotherapy and clinical findings of the following studies are discussed. For the European school, Noon 1911 was the first report of successful application of grass pollen extract; Frankland 1954 was the first double-blind placebo-controlled randomized trial (DBPC-RCT) in SCIT. For the European school: Noon published the first report of successful application of grass pollen extract (1911); Frankland was the first double-blind placebo-controlled randomized trial (DBPC-RCT) in SCIT (1954). For the American line: Clowes published the first successful trial of ragweed SCIT (1913); Cooke used skin prick testing as diagnostic method (1915); Loveless used venom immunotherapy with purified venom (not whole body extract) (1956); in 1961/1968 Johnstone showed in a DBPC-RCT dose-effect of an allergen mix and highly significant asthma reduction after up to 14 yrs of treatment of asthmatic children; Lowell and Franklin did the first DB-RCT demonstrating ragweed pollen efficacy as part of a multi-allergen mix and 1967 ragweed pollen extract dose response. We discuss the first studies for SLIT in 1927 from Black (oral-IT versus SCIT) and 1986 from Scadding, DBPC-RCT with house dust mite extract. We conclude that an in-depth review of investigators' observations, methods, and thoughts, however, can also be enriching for investigators in the field today.

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The year 2011 is the centenary year of allergen immunotherapy, because the first publication on the successful use of this form of treatment was from 1911, by Leonard Noon in London.¹ The first attempts of active immunization against hay fever by vaccination with aqueous pollen extract were performed by William Dunbar in 1903 in Hamburg, but failed because of too high dosing ($\pm 10,000$ times). The hay fever patient—Dunbar himself—received the pollen extract injection from Prausnitz, but he had such a violent systemic reaction that he decided to shift from direct vaccination to passive procedures.² Some years later Noon and Freeman succeeded in considerably alleviating the condition of sufferers from European or spring hay fever by injecting small doses of timothy pollen extract.²

At present, allergic rhinitis (AR) is the fifth most common chronic disease in the United States with an estimated prevalence of over 20% of the population.³ A clear rise in the annual costs of AR in the United States

could be detected over the 5-year period between 2000 and 2005.⁴ In our improved understanding of the pathophysiology,⁵ apart from allergen avoidance measures, allergen immunotherapy is still today the only treatment of allergic rhinoconjunctivitis that corrects the underlying immune deviation. Similarly allergic asthma, venom hypersensitivity, and, lately, atopic dermatitis—in selected patients—have also been proven to improve with allergen immunotherapy.^{6,7} As such, it is a well-recognized treatment in the United States⁸ with clear guidelines, updated frequently in the Practice Parameters.⁹ In this article details of some of the first landmark studies and events around allergen immunotherapy in Europe and the United States are reviewed (see Table 1).

EUROPEAN LINE

1911: Leonard Noon (1878–1913) and John Freeman (1877–1962)

In the June 10th 1911 edition of the *Lancet*, Noon describes successful out-of-season desensitization with *Phleum pratense* pollen extract, citing the detailed observations on conjunctival reactivity of two cases. He prepared pollen with Dunbar's method adding freshly distilled water to pollen and submitting it to several freeze-thaw cycles, before boiling it for 10 minutes, which did not destroy the activity of the pollen ex-

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Table 1 Milestone publications on clinical aspects of allergen-specific immunotherapy

Year	Author	Finding
SCIT track		
1903	Dunbar	First experiments of grass pollen SCIT; failed because of severe systemic adverse reactions
1911	Noon	First successful experiments in hay fever patients of grass pollen SCIT
1913	Clowes	First successful trial of ragweed SCIT
1915	Freeman	First successful in-season treatment of grass pollen SCIT
1915	Cooke	Skin-prick testing as diagnostic method; allergy is hereditary
1954	Frankland	First DBPC trial of grass-pollen SCIT
1956	Loveless	Venom immunotherapy with purified venom and not whole-body extract
1961	Johnstone	First dose-response study with allergen mix in asthmatic children: low dose is no more effective than placebo, highest dose is most effective
1965	Lowell	First DB-RCT showing clinical efficacy of ragweed pollen extract
1967	Franklin	Ragweed dose-response in multimix: high dose more effective; no placebo
1968	Johnstone	First successful long-term asthma trial in children
1976	Hunt	First controlled trial with hymenoptera venom vs whole-body extract
1999	Durham	Long-term efficacy after 3 yr grass pollen SCIT
2006	Werfel	First successful randomized dose-response study showing efficacy of SCIT in atopic dermatitis
2007	Jacobsen	Long-term efficacy after 3 yr pollen SCIT and asthma prevention at 7 yr posttreatment
2010	Hankin	Large trial on SCIT cost-effectiveness in Florida children
SLIT track		
1927	Black	First SCIT oral administration trial, using very high doses of oral allergen
1986	Scadding	First DBPC-RCT of grass pollen SLIT
1998	Passalacqua	First DBPC-RCT of allergoid SLIT tablet
2006	Pajno	First DBPC-RCT of successful SLIT for atopic dermatitis
2010	Durham	Long-term efficacy after 3 yr grass pollen SLIT
Alternative track		
1996	Norman	Peptide: T-cell reactive cat peptid safely improved allergic responses
1998	Passalacqua	Allergoid: House-dust mite allergoid SLIT (see above)
2004	Niederberger	Recombinant: First trial of recombinant allergen SCIT in patients with birch allergy
2006	Creticos	Adjuvant added: Ragweed + Toll-like receptor 9 agonist SCIT
2008	Valenta/Pauli	First DBPC-RCT from Pauli on recombinant allergens
2008	Kündig	Intralymphatic applications: Fewer injections of much lower doses
Immunologic findings track		
1935	Loveless (Cooke)	Description of production of blocking antibodies in post-SCIT serum (inhibiting type of substance is present in post-SCIT serum)
1980	Rocklin	Generation of antigen-specific suppressor cells during SCIT
Onward	Many	Many investigators have made and are still making landmark contributions to this field that eventually shall lead to even further improvement of allergen-specific immunotherapy

DBPC-RCT = double-blind placebo-controlled randomized trial; SCIT = subcutaneous immunotherapy; SLIT = sublingual immunotherapy.

tract.¹ He defines the Noon-unit of pollen extract (1 unit = 1:1000 dilution of 1 mg of pollen) and uses it to quantify his primary efficacy variable: the amount of allergen needed to elicit a conjunctival reaction. Noon did the up-dosing experiment deliberately during the

off-season of the year, "as the scheme of dosage was then not liable to be upset by spontaneous absorption of toxin from the air (by the patients)." He describes that at the beginning—in July—small frequent doses are needed, but that after a while the conjunctival

reactivity only can be reduced further with larger doses and a dosing interval of about 15 days. At the end of his first publication he states that because of his enforced absence from work the following months, the description of the patients' symptoms throughout the following hay fever season is left in hands of his colleague, Freeman. Noon knew his advanced tuberculosis would keep him from finishing his work. So, finally, his close friend, Freeman, published these in-season findings of hay fever patients seen at St. Mary's Hospital in the next issue of the *Lancet*.¹⁰ As such, the allergy clinic at St. Mary's would be the first allergy clinic, in the beginning (1911), only functioning during the summer months. About 40 years later, the first double-blind placebo-controlled (DBPC) trial in allergen immunotherapy would be performed in the very same clinic by William Frankland.¹¹

Although Noon was the first to describe a successful desensitization, the theory behind the experiment was not correct. Strongly influenced by Dunbar's thoughts on hay fever, Noon also considered the disease to be caused by the exposure to a toxin, present in pollen, which could even induce antitoxin when injected into rabbits or horses. Administering little quantities of pollen extract to patients would actively immunize them.

1954: A. William Frankland (1912-) and Rosa Augustin

Frankland and Augustin conducted the first DBPC trial in allergen immunotherapy.¹¹ Two hundred patients were randomized into four groups to receive coseasonally either one of the two active preparations (50–50% crude extract or purified pollen after ultrafiltration) or one of the two inactive extracts (50–50% the liquid substance left over after ultrafiltration of the pollen: the ultrafiltrate or saline-phenol). The symptom improvement was highly significant with $p = 0.001$, in favor of both active groups. Frankland presented a separate analysis of patients with seasonal asthma, similarly improving under treatment. Moreover, Frankland clearly describes the 32% of patients with good–excellent results in the nonactive groups, a placebo effect of the same dimension as found in recent trials. Skin reactivity was another outcome measure that was reduced in the actively treated subjects. Another question Frankland sought to answer with the trial was if certain fractions of the pollen extract might contain the active substance and if this could be deduced from skin prick test reactivity. The trial results are in line with this hypothesis, because the ultrafiltrate was not clinically active or did not give skin reactivity as opposed to the purified protein fraction. The number of adverse events, “general reactions,” was quite high: 24–36% in the active group. The adequate

number of patients, the exact description of randomization, blinding, and of dropouts makes this study even today being rated as of moderately high scientific quality.

As an exquisite detail in his “in retrospect” notes, Dr. Frankland reminds the public that Dr. Fleming's laboratory was just above the allergy laboratory at St. Mary's.¹ In 1924 after a lecture of Storm van Leeuwen on the effect of climate and molds on asthma the allergy laboratory started to cultivate certain molds, among which was *Penicilium*. Mold contamination of Fleming's plates would have broad consequences for mankind.

U.S. LINE

1913: George A.H. Clowes

In 1913 Clowes from Buffalo published his preliminary communication on the treatment of American autumnal hay fever by vaccination with an aqueous extract of the pollen of ragweed.¹² He used Dunbar's method of pollen extraction, but he based his coseasonal dosing on the findings of Noon and Freeman, applying 1 mL of a 1:500,000 dilution, which is about the ragweed quantity still used today in up-dosing. According to the investigator, all eight cases treated experienced a marked alleviation of general symptoms.

1915: Robert Anderson Cooke (1880–1960)

Cooke himself suffered from intractable asthma as a child, which—as he later learned when leaving home to enter a boarding school—was caused by exposure to the horses and other environmental factors present at their family estate farm.¹³ During his years as a medical student he presented exacerbations of his asthma every time he had to ride in the ambulance (in those days a horse cart). Moreover, he collapsed and was unconscious for ten days after he had received a prophylactic horse serum antitoxin injection, while assisting a diphtheria patient's tracheotomy. After these personal experiences of severe asthma and anaphylactic shock it was no surprise his attention was drawn to the just starting field of allergy. After he joined Arthur Fernández Coca (1875–1959), who had developed a method for preparation of allergen extracts with a special protein-extracting fluid, Cooke introduced the intracutaneous technique of skin testing and its advantage over the conjunctival method in his 1915 milestone publication. Moreover, he stressed the hereditary origin of “protein sensitivity,” as allergy was called in those days. He was the founder of one of the first allergy clinics in the United States at the New York Hospital, when it had just been affiliated with the Cornell University. This unique opportunity brought diagnostic and therapeutic units together; the clinic

Table 2 Results of the primary efficacy outcome of the Johnstone studies of SCIT in asthmatic children: Patients with no asthma over the last year before evaluation, with “no asthma” being defined as no asthma attacks

	<i>n</i>	No asthma last year	Asthma attack last year	Significance
At 4 yr treatment				
Placebo groups	91	16 (18%)	75 (82%)	$p < 0.0001$
SCIT groups	82	57 (70%)	25 (30%)	
Medium dose	39	23 (58%)	16 (42%)	$p < 0.05$
Highest tolerated dose	43	35 (81%)	8 (19%)	
At 16 yr of age (after 2–14 yr of treatment)				
Placebo groups	64	14 (22%)	50 (78%)	$p < 0.0001$
SCIT groups	67	48 (72%)	19 (28%)	
Medium dose	35	23 (66%)	12 (34%)	NS
Highest tolerated dose	32	25 (78%)	7 (22%)	

Source: Refs. 14 and 15.

SCIT = subcutaneous immunotherapy.

would be the cradle of the first generations of allergists in the United States.

1961: Douglas E. Johnstone and Crump/

1968: Johnstone and Arthur Dutton

Johnstone *et al.* conducted the first dose-response study with a multiallergen mix in a DBPC randomized design.¹⁴ Two hundred asthmatic children (2–15 years) attending the asthma clinic were recruited, 173 concluded the 4-year study. After up-dosing the children received subcutaneous immunotherapy (SCIT) every 28 days for 4 years with a personalized extract mix, containing all allergens to which a child reacted on skin testing. After up-dosing there were three dosing groups: the 10^{-7} group (receiving 0.5-mL injections of a 1/10,000,000 w/v dilution of each allergen), the 1/5000 group (receiving 0.5 mL of 1/5000 w/v dilutions of each allergen), and the highest tolerated dosing group (receiving 1/250 w/v dilutions, or the highest tolerated dose). The fourth treatment group received only diluent injections. Patients, parents, and investigators were all blinded to the received treatment. Johnstone showed that newly diagnosed AR, wheezing on exertion, and wheezing with upper respiratory illnesses were all strongly reduced in the two high-dose groups during the last year of the study; the very low dose group did no better than placebo. The ratio of patients with no asthma attacks the last year in the highest- compared with the medium-dose group suggested a dose-response effect, although no proper statistics were run at this time. The same data rerepresented 7 years later reported significance was reached at the level of 5% (sic) (Table 2). At the end of the article Johnstone engages in an interesting discussion on the placebo effect.

Seven years later, Johnstone published the results of practically the same group of children who had continued injection treatment (see aforementioned data for the three dosing groups) until their 16th year of age (!).¹⁵ Again, great effort was taken to carefully blind all personnel involved in the evaluation of the children. For statistical analysis the very low dose and placebo patients were grouped into one “placebo” group: the final report was on 64 placebo- and 67 SCIT-treated patients. The primary end point, “loss of asthma” defined as no asthma attacks the last year, was reached in more treated patients than in placebo patients. The difference was highly significant (see Table 2).

Several interesting points can be mentioned of these studies, which make them unique and not replicable. First of all the blinding of the patients was perfect, because parents and patients did not know they were in a study; no informed consent rules applied in those days. Placebo injections were given to one-fourth of all children and almost placebo injections (very low dose) to another 25%, so approximately half of the children did not receive immunotherapy, which makes for an adequate sample size. Finally, immunotherapy was administered until the children turned 16, so some children receive immunotherapy for over ten years. These details make the study unique, because, today, it would be impossible to reproduce. A detailed description of reasons for dropout reduces bias in this aspect and strengthens the conclusion: treatment of asthmatic children with SCIT containing several mixes of allergens, specific per patient, in moderate–high doses augments the probability of no asthma at the age of 16 years. The fact that allergens in those days were non-standardized is another interesting observation considering the found efficacy, as is the finding that more

Design Lowell and Franklin ragweed studies in the 1960s

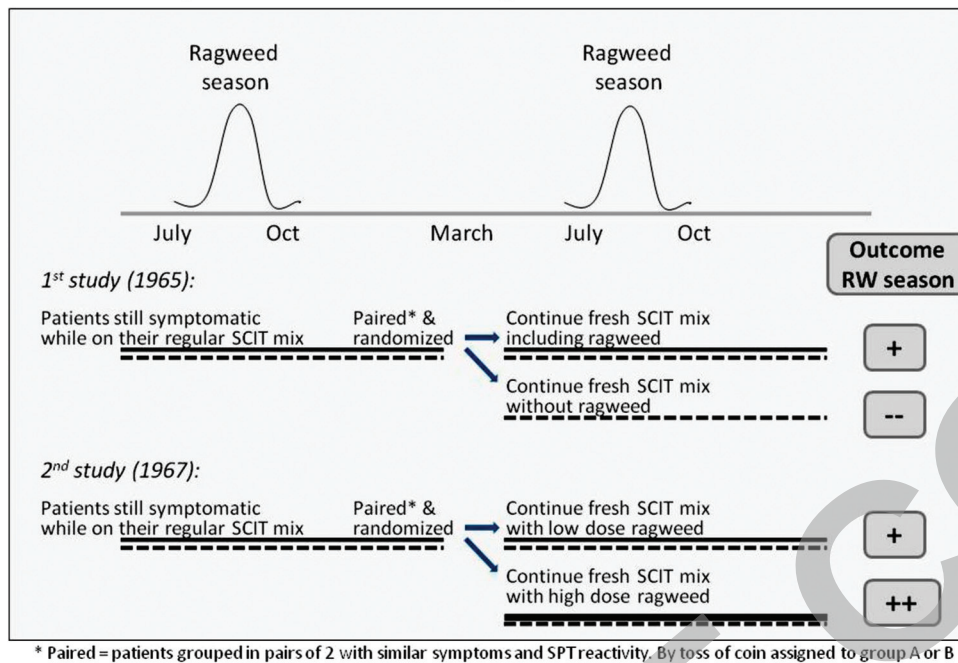


Figure 1. Design of the ragweed trials conducted by Lowell and Franklin in the 1960s. In the first trial (1965) ragweed was omitted from the immunotherapy mix in the study year, leaving only half of the patients treated with ragweed. In the second study (1967) half of the patients received a low dose and half of the patients a high dose of ragweed allergen, while the rest of the immunotherapy allergen-mix stayed unchanged.

severe asthmatic patients at study start showed a more pronounced effect of the treatment—although no statistical significance was reached. Contrarily, previous hay fever increased the likelihood of a child's asthma persisting into adolescence.

1965: Frank Lowell (1909–1979) and William Franklin (1919–2010)

In his presidential address in 1959, taking on the Presidency of the American Academy of Allergy, Lowell referred to the lack of controlled trials in the field of "injection therapy." Subsequently, and in cooperation with Franklin, he led several ragweed pollen immunotherapy trials at the Massachusetts General Hospital in Boston. Their publications indicated, first (1965),¹⁶ the effectiveness and specificity of injection therapy in ragweed hay fever in a double-blind trial and 2 years later the dose-effect relationship of ragweed SCIT.¹⁷ The design of both of these landmark studies is interesting: patients already on immunotherapy with a mixture of pollens, including ragweed, were selected to participate in the study based on continued presence of seasonal symptoms and intense intracutaneous skin testing positivity to ragweed. Patients were grouped in pairs according to symptom severity and skin reactivity. In the first trial in March each one of a pair was assigned to either continue with a fresh mix of the same allergens, including ragweed, or excluding ragweed. Symptoms and medication scores were followed during the ragweed season (Fig. 1). In the second trial, again, all 25 selected patients continued their accustomed pollen mix SCIT during the off-season, trying to

reach the maximum tolerated dose. In May 1964 each one of a pair was randomized to receive a high or 20 times lower dose of ragweed pollen extract, while all of the other extracts in the mix were maintained at the same dosage. Symptoms and medication scores showed the statistically significant superiority of the immunotherapy containing ragweed (study 1) and of the higher ragweed dose (study 2). No placebo group was introduced, but great care was taken of correct blinding, adjusting the color of the solutions with caramelized glucose. These trials prove ragweed immunotherapy is effective when given as part of a multiallergen mix; it is specific and efficacy improves with a higher dose.

1935 + 1956: Mary Hewitt Loveless (1899–1991)

The list of landmark studies would not be complete without the inclusion of the female contribution in the name of Loveless. She was the very first—1935—to discover blocking antibody and in 1937 she described the thermostable property of inducible blocking antibodies. This enabled Cooke's team to study them separately, although she never got the official credit for it.¹⁸ Apart from many other contributions in the field of the immunologic basis of allergy and immunotherapy, she was also highly interested in hymenoptera allergy and desensitization, to the degree that she cultivated wasp nests in her garden and even decades after her retirement kept on treating patients with a controlled, once-a-year sting. Already in 1956 she published the clear superiority of venom immunotherapy with purified venom, which she and her students carefully ex-

tracted from venom sacs of stinging insects, over whole-body wasp/honeybee extracts.^{19,20}

THE HISTORY OF SUBLINGUAL IMMUNOTHERAPY

1927: J. Harvey Black (1884–1958)

After his first case reports of symptom improvement by high-dose oral immunotherapy in 1927, Black published the first comparative trial of coseasonal oral versus hypodermic grass pollen immunotherapy, conducted in his new practice in Texas in 1928.²¹ The 3-mL oral doses were administered three times daily compared with the once-weekly injection therapy (oral weekly dose = 105× SCIT weekly dose). He documented superiority in efficacy of SCIT over oral immunotherapy and its lower cost, but he also describes the benefits of oral immunotherapy over SCIT: no severe systemic reactions and children and patients from out of town can take it easily. He even mentions how some patients were changed from oral to subcutaneous (because of intestinal problems) and *vice versa* (because of systemic reactions) and discusses issues related with both treatments that are still of value today.

1986: Glennis Scadding and Brostoff

Although the first clinical reports of oral desensitization to pollen date back to the 1920s,²¹ and there have been some anecdotal reports of sublingually administered allergens by a group of midwestern physicians, headed by Dr. Herbert J. Rinkel,²² it was not until the 1970s that the interest in the mucosal route was retaken by a group of German investigators.²³ In the mid-1980s some patients came to the Allergy Clinic run by Scadding-Brostoff in London telling about a low-dose sublingual extract they were receiving with t.i.d. dosing (from Latin: *ter in die*, three times daily). A small DBPC trial was started, without any funding. It had a crossover design: 2 weeks active or placebo, 2 weeks washout, and 2 weeks active or placebo. The maximum subcutaneous tolerated dose of an house dust mite extract was given sublingually three times daily. Against all expectations of the investigators, improvement in symptoms and titrated nasal inspiratory peak flow could be shown. This resulted in the first DBPC trial published on sublingual immunotherapy (SLIT) in 1986 by Scadding *et al.*²⁴ At the same time, the sublingual route was investigated by Italian allergists in an effort to make immunotherapy accessible to many more allergic patients, without the restrictions of the subcutaneous route.

1998: Giovanni Passalacqua

Since then, the scientific allergy world has been looking for ways to improve on SLIT. Combining what was learned from clinical trials on SLIT with both positive and negative outcome, it became clear that SLIT was more likely to be effective if given daily and at higher doses. The only pharmacokinetic study on SLIT done by Bagnasco *et al.*,²⁵ moreover, showed that just a very small fraction of the sublingual extract was taken up into the superficial layer of the sublingual mucosa. In search of a way to augment local uptake tablet SLIT and fast-dissolving tablet SLIT have been introduced. The first DBPC randomized controlled trial with allergoid SLIT tablets was conducted by Passalacqua in 1998.

CONCLUSIONS

I reviewed only some of the seminal publications from the first decades of the last century, during the starting area of allergy and allergen immunotherapy. The more recent studies have not been discussed in detail, because their publications can easily be retained with electronic search instruments. In contrast, the very first trials, although often quoted, are generally not known in detail. An in-depth review of investigators' keen observations, methods, and thoughts, however, can be enriching also for investigators in the field today.

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