

Sublingual versus injective immunotherapy in grass pollen allergic patients: a double blind (double dummy) study

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Summary

Background Injective immunotherapy is a well-known and recognized treatment for allergic diseases, but its safety has been questioned during recent years. Alternative administration routes have been proposed and there is a growing interest and experience in sublingual therapy. The safety of alternative routes is nonetheless a real advantage, so long as it is not counterbalanced by a loss of clinical benefit.

Objective We have compared the efficacy of the same biologically standardized grass pollen extract administered through the injective or the sublingual route, in a group of 20 patients followed for two pollen seasons.

Methods Both therapies were administered for 12 months according to a double-blind (double-dummy) plan; at the end of the trial the cumulative dosage of the sublingual therapy was 2.4 times higher than that of the injective therapy. Data about skin reactivity, symptoms and drugs scores during the pollen season, as well as total specific IgG and specific IgG4, during and after the trial, were obtained.

Results Our data show that sublingual and injective therapy are equally effective according to subjective clinical parameters, with a statistically highly significant reduction of symptoms and drugs ($P=0.002$ for symptoms and drugs in SLIT-treated patients; $P=0.002$ for symptoms and $P=0.0039$ for drugs in patients given injections). On the other hand, objective parameters (total specific IgG, specific IgG4, skin reactivity) changed only in patients treated with active injective therapy, with $P<0.001$, $P<0.001$ and $P=0.021$, respectively.

Conclusions The discrepancies observed could be interpreted as a consequence of different mechanisms of action of the two therapies or to the lack of close relationships between the clinical and the objective parameters which were considered here.

Keywords: sublingual immunotherapy, injective immunotherapy, grass pollen allergy, skin reactivity, specific IgE, specific IgG4, total specific IgG

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Introduction

Specific immunotherapy (SIT), traditionally administered by means of subcutaneous injections, is the only specific therapy of recognized efficacy for allergic diseases [1,2,3,4,5,6]. It may nonetheless produce side-effects, some of which are very serious [7]. In the evaluation of the risk : benefit balance, more importance is now being given to the intrinsic risk of

injective administration. The proposal to use SIT as a preventive treatment [8] must therefore be carefully evaluated, taking these drawbacks into account. On the other hand, the pharmacological therapy, even with the present availability of more potent and effective drugs, seems to be unable to avoid or to interfere with the spontaneous evolution of the allergic pathology and/or obtain a stable improvement of the patient [8]. In other words, while SIT seems to be able to achieve benefits lasting at least some years after the last administration [8], we would like to point out that drugs are effective only as long as they are regularly taken, and they

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may also provoke important side-effects, mainly in patients in long-term treatment [9].

This situation has encouraged many investigators to try non-subcutaneous administration routes, with the aim of avoiding the most life-threatening side-effects to the patients. The nasal topical, the bronchial topical, and the oral route have therefore been tried. The greatest attention has traditionally been paid to the oral route. In our opinion, clinical trials employing this route can be classified under four different headings: (1) oral SIT with gastric absorption (aqueous allergen solution to be swallowed) with allergen concentrations similar to those employed in traditional injective therapy; (2) oral SIT with enteric absorption (allergen tablets insoluble in gastric juice but soluble in enteric juice) with allergen dosages some 100 times higher than those employed in traditional injective therapy; (3) oral SIT with sublingual absorption (aqueous allergen solution to be kept in the mouth for a few minutes, with or without subsequent swallowing) with very low allergen amounts, corresponding to the cutaneous end-point found by means of the intradermal test; and (4) oral SIT with sublingual absorption, as above, but with allergen amounts similar to those used for traditional injective immunotherapy.

The results of these studies, although inconclusive, have shown fundamental differences among the four possibilities. When animal models were used, the immunological system reacted differently to sublingual administration than to gastric administration of the allergen [10]; opposite results have also been reached in humans following practically identical experimental conditions but administering the allergen sublingually in one case [11] and with gastric absorption in the other [12], with favourable results only for the sublingual administration. High-dosage, gastric-coated birch allergen tablets turned out to be effective, but with important side-effects from the clinical point of view and insoluble practical problems because of the very high allergen amount needed to run the therapy [13,14,15]. Similar experiences with grass pollen extracts gave negative results [16, 17]. Only one double-blind study with oral therapy was able to show a benefit, but only at the end of a 3-year treatment [18]. For low dose sublingual therapy, only one double-blind placebo-controlled study is available; the results are in favour of the active therapy but the experimental plan was very unusual and very short [19]. A number of sublingual trials with allergen dosages similar to those used for injective therapy have been published; all but one are in favour of this route [11,20,21,22,23,24]. All of these considerations have led us to check the tolerance and efficacy of sublingual therapy in comparison to traditional injective therapy. As the two therapies involve two clearly different methods of administration and dosage schedules, we have run our trial according to a double-dummy plan, the only one able to compare two different

routes of administration while avoiding the psychological effects of such differences. In our experience with allergenic extracts of grasses, side-effects are quite uncommon with sublingual therapy, while only a few local side-effects are normally seen with depot injective therapy; we have therefore decided not to add to either placebo preparations any substance, like histamine, able to give local side-effects after each administration.

Material and methods

We have conducted our trial in two steps. Step 1: selection of patients, observation during the first pollen season and balancing of experimental groups. Step 2: treatment according to a double-blind schedule and observation during the second pollen season.

Step 1

Patient selection Twenty grass-allergic patients living in the Milan area were enrolled between January and March 1992; they are described in Table 1. The inclusion criteria were the following: clinical history suggesting a grass pollen sensitization; grass-specific IgE class 2–4 RAST; prick-test positive to grass pollen extract; and age between 12 and 45 years. Patients presenting with at least one of the following conditions — chronic asthma, previous treatment with SIT, sensitization to other inhalant allergens, pregnancy, other contraindications [25] — were excluded. All were informed about the aim and the steps of the trial and gave their consent.

In vivo and in vitro diagnosis *In vivo* diagnosis was done by means of a prick-test employing a grass mix extract (*Dactylis glomerata*, *Festuca pratensis*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*). Each allergen had been purified and biologically standardized according to the BU protocol developed by Alergia e Immunologia Abellò S.A., Madrid, Spain. Prick test potency was 100 BU/mL, corresponding to an expected wheal size of 75 mm² on average in patients sensitized to grasses [26,27]. This biological potency corresponds also to about 10 mcg/mL of grass major allergen (group V), according to the producer's quality control methods [28].

Prick tests were judged positive if the wheal obtained was similar to or larger than the wheal elicited by the positive control (histamine hydrochloride 10 mg/mL). *In vitro* diagnosis for grass-specific IgE was performed by means of commercial kits according to the manufacturer's instructions (CAP System, EIA method, Pharmacia AB, Uppsala, Sweden).

Monitoring of patients All patients enrolled were monitored

Table 1. Demographic data of patients enrolled in the trial. R = patient suffering from rhinitis, C = conjunctivitis and A = asthma

Therapy	Age	Sex	Pathology	Years with pathology
Sublingual	27 (17–35)	5 M + 5 F	2 RC + 7 RCA + 1 A	6.7 (1–12)
Injective	27 (13–39)	4 M + 6 F	2 RC + 7 RCA + 1 A	2.8 (2–4)

during the 1992 pollen season before the allergen administration. They were allowed to use, when necessary, the following drugs: terfenadine tablets 60 mg; astemizole tablets 10 mg; beclomethasone dipropionate spray; and salbutamol spray. We decided to give our patients the choice between two antihistamines because, in our experience, each drug is not equally effective and well tolerated in all subjects. Patients were instructed to keep a diary, during the same period, for the daily evaluation of symptoms according to a 0–3 grading (0 = no symptoms 3 = severe symptoms), covering nasal symptoms (itching, sneezing, nasal discharge, nasal obstruction), eye symptoms (itching and redness), cough, symptoms of bronchial obstruction, and wheezing. The patients had to note down if they used drugs, and a specific daily score was given (1 point for each drug administration). Patients who had reported asthmatic symptoms when enrolled, were given a peak flow meter (Pocket Peak Flow Meter, Ferraris Medical Ltd, London, UK) and instructed to perform PEF determination and registration twice a day during the pollen season (08.00 and 20.00).

Pollen counts Grass pollen counts for the 1992 season were obtained from Associazione Italiana di Aerobiologia and are the mean of the 14 aerobiology stations in Lombardy.

Balancing of experimental groups After monitoring of patients during the 1992 grass pollen season, 10 matched pairs were created, taking into account age, gender, type and importance of symptoms. Asthma was considered as the most important criterion for balancing because 16 out of 20 patients suffered from this symptom, in 14 cases associated with rhinoconjunctivitis (Table 1).

Step 2

Experimental plan From September 1992 to September 1993 each subject was submitted to both sublingual and injective therapy, according to a code which allowed one of each pair to be treated with active sublingual and placebo injective therapy and the other with placebo sublingual and active injective therapy. The key code was unknown both to the patient and to the allergologists performing the treatment. Thus, 10 patients were treated with active

sublingual therapy and placebo injective therapy, and the other 10 patients were treated with placebo sublingual therapy and active injective therapy.

Allergens Both therapies were performed by means of a grass-mix extract, as above. The sublingual and the injective therapies were prepared from the same batch of freeze-dried grass allergen extract, according to the following presentations, identical to the commercial products. Injective therapy: three 5 mL vials with a concentration of 0.25, 2.5 and 25 BU/mL respectively, in physiological saline with 0.4% W/V of phenol and aluminium hydroxide to obtain a depot effect. Sublingual therapy: five 3 mL vials with a concentration of 0.04, 0.2, 1.0, 5 and 25 BU/mL in physiological saline with 50% v/v of glycerol and 0.4% w/v of phenol.

Placebo preparations, both for injective and for sublingual administration, were identical to the active therapy in composition, appearance, presentation, taste (for sublingual) and colour, but obviously without the allergens. The treatment schedules were those of the products normally on sale. This means for the injective therapy the administration of weekly injections of 0.1, 0.2, 0.4 and 0.8 mL of the first vial, then of the second and then of the third vial. A maintenance therapy was then performed administering the maximum tolerated dose every 3 weeks.

For the sublingual therapy, the first doses were 1, 2, 3, 4 and 5 drops from the vial of strength 0.04 BU/mL, each administered for two consecutive days; after this first phase, drops were to be taken daily starting from one drop of the strength 0.2 BU/mL and increasing by one drop every day up to 5 drops of the same vial and then repeating the procedure with the subsequent vials. The top dose (5 drops from the vial of strength 25 BU/mL or the maximum tolerated dose) was then administered three times a week. Each dose had to be taken in the morning before breakfast. To exclude therapeutic- or side-effects linked to absorption by the intestines, the drops of the allergen had to be kept in the mouth for at least 2 min without swallowing and then anything remaining in the mouth had to be spat out.

During the pollen season, the dosages were lowered to 40–60% of the maximum amount reached for both

therapies. According to the schedule, at the end of the trial an average cumulative dose of 510 BU of allergens was administered to each patient submitted to active sublingual therapy, while each patient who had undergone injective active therapy received about 210 BU of allergens.

Control parameters

Skin reactivity Duplicate prick-tests, with the same diagnostic criteria used for selection, were performed, and wheal areas registered, before the beginning of immunotherapy (T0, September–October 1992), after 6 months (T1, immediately before the 1993 pollen season), and after 12 months (T2).

Monitoring during the 1993 pollen season During the 1993 pollen season, all patients were monitored for symptoms, drug consumption and PEF, according to the criteria already given for the 1992 season.

Immunologic parameters Grass-specific IgE, total grass-specific IgG and grass-specific IgG4 were determined at the end of the trial by means of commercial kits according to the supplier's instructions (CAP System, EIA method, Pharmacia AB, Uppsala, Sweden) in samples of sera taken at T0, T1, T2.

Pollen counts Grass pollen counts for the 1993 pollen season were obtained from Associazione Italiana di Aerobiologia and are the mean of the same 14 aerobiology stations in Lombardy as for the previous year.

Statistical analysis

At the end of the trial all data but PEF data for four asthmatic patients were available. Symptom and drug intake data have been statistically analysed by means of non-parametric tests; the Wilcoxon sum rank test has been used for intragroup analysis and the Mann–Whitney *U*-test for intergroup analysis (BMDP Statistical Software, Cork, Ireland). Grass-specific IgE, total grass-specific IgG, grass-specific IgG4 and wheal areas of prick-tests, were statistically analysed by means of parametric tests (ANOVA) after having checked the data for normal distribution.

Results

All patients completed the study. No patient under active sublingual immunotherapy reported local or systemic side-effects during the treatment. A few patients under active injective therapy reported only minor local side-effects with no need of variations of the therapeutic schedule; no systemic side-effect was reported or detected. Pollen count data show some differences: the 1992 season had higher absolute counts but for a relatively short period; the 1993 season had lower absolute counts but a longer period with relatively high counts (Fig.1). The pollen load of the two seasons was estimated through the comparison of the area under each curve; a difference as low as 6.8% in favour of the 1992 season was found.

The comparison of drug and symptom scores during the pollen season before immunotherapy (1992) and during the pollen season after the beginning of immunotherapy (1993) (Tables 2 and 3), showed a statistically significant decrease

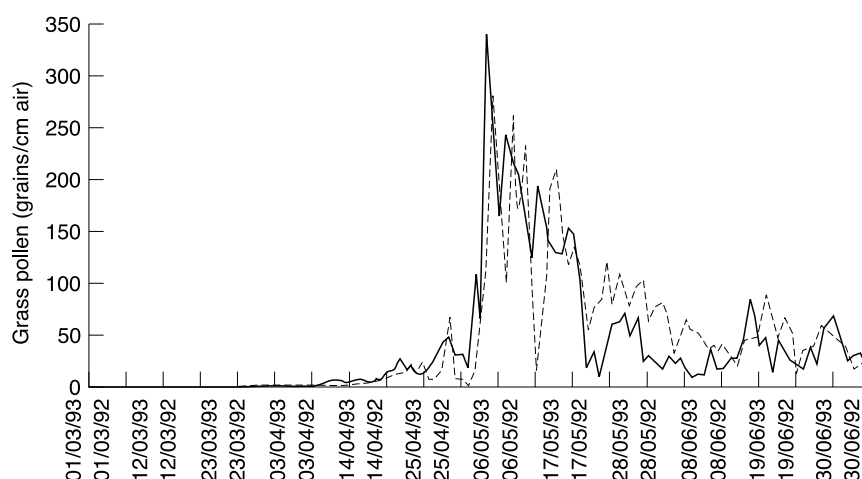


Fig. 1. Average data on grass pollen gathered by 14 aerobiology stations in Lombardy. Spring–Summer 1992 and Spring–Summer 1993. — = 1992; - - - = 1993.

Table 2. Individual symptoms and drugs scores of patients during the pollen season, before (1992) and after (1993) the SLIT treatment

SLIT patients	Symptoms		Drugs		Total	
	1992	1993	1992	1993	1992	1993
1	329	209	180	116	509	325
2	479	169	250	94	729	263
3	680	367	136	76	816	443
4	631	285	492	144	1123	429
5	320	191	132	86	452	277
6	583	190	168	112	751	302
7	294	175	187	84	481	259
8	464	281	381	223	845	504
9	774	399	310	52	1084	451
10	461	239	214	164	675	403

(P between 0.002 and 0.0039) for both symptoms and by drugs for the two groups of patients (intragroup analysis); the same result could be obtained comparing the drug-plus-symptom scores in the same two periods for both therapies (Table 4). On average, patients of both groups experienced a reduction of symptom and drug scores of at least 50% (Tables 2 and 3). No difference could be shown by inter-group analysis either for 1992 or for 1993; details are given in Table 5.

A statistically significant decrease of wheal areas obtained by means of a prick-test could be shown by comparing data throughout the treatment period for patients submitted to active injective therapy ($P = 0.021$), but the same could not be shown for patients treated with active

sublingual therapy ($P = 0.36$), as shown in Tables 6 and 7. No significant variation of specific IgE concentration was detected, either for sublingual or for injective therapy; an increase of specific total IgG and specific IgG4 could be shown for injective ($P < 0.001$) but not for sublingual therapy. The complete analysis of this data is given in Table 8.

PEF data were not analysed because they were only available for 12 patients.

Discussion

The evidence in favour of the injective immunotherapy is very strong, and its effectiveness is not a matter of debate, if

Table 3. Individual symptoms and drugs scores of patients during the pollen season, before (1992) and after (1993) the injective treatment

Injective therapy patients	Symptoms		Drugs		Total	
	1992	1993	1992	1993	1992	1993
1	783	428	146	62	929	490
2	478	284	248	142	726	426
3	320	145	102	70	422	215
4	920	302	506	214	1426	561
5	880	558	488	238	1328	796
6	480	215	244	88	724	303
7	734	285	336	162	1070	447
8	328	218	62	66	390	284
9	358	135	124	70	482	205
10	465	215	140	84	605	299

Table 4. Mean values and statistics of symptoms and drugs scores of patients, before (1992) and after (1993) the treatment (Wilcoxon test)

	Average value		<i>P</i>
	1992	1993	
<i>SLIT</i>			
Symptom score	501.5	250.5	0.002
Drug score	245	115	0.002
Total score	746.5	365.5	0.002
<i>Injective therapy</i>			
Symptom score	574	278	0.002
Drug score	239.6	119.6	0.0039
Total score	813.6	397.6	0.002

Table 5. Mean values and statistics of symptoms and drugs scores of the SLIT treated patients versus the injective therapy treated patients for years 1992 and 1993 (Mann–Whitney *U*-test)

	Average value		<i>P</i>
	Injective	SLIT	
<i>1992</i>			
Symptom score	574	501.5	0.3845
Drug score	239.6	245	0.5967
Total score	813.6	746.5	0.9397
<i>1993</i>			
Symptom score	278	250.5	0.5795
Drug score	119.6	115.1	0.6774
Total score	397.6	365.6	0.8206

diagnosis, administration and patient monitoring are correctly carried out [for a complete revision see ref. 8]. The safety and efficacy of sublingual immunotherapy has only recently been investigated in double-blind placebo-controlled studies; only six reports on this topic have therefore been published: five of them reached the evidence of clinical improvement [11,21,22,24,25], one failed [24], but all concluded in favour of safety. In two trials, grass-allergic patients have been studied, showing evidence of clinical improvement for allergic rhinitis. Certainly, we need more experience, which must include more patients and more allergens, with regard to this new kind of immunotherapy. In our opinion, the absolute effectiveness of alternative therapies with reference to placebo, is nonetheless only one part of the problem, the other being the relative effectiveness in comparison to injective therapy. The safety of an alternative therapy — in our case of the sublingual therapy — is a real advantage only if it is not counterbalanced by a loss of clinical benefit.

All of the subjective (symptoms, drug scores) and objective (wheal areas, specific IgE, total specific IgG, specific IgG4) data obtained with our experimental plan allow some comparisons: each group before versus after the treatment; and one group versus the other, before and after the treatment. First of all, the two experimental groups were correctly balanced both from the demographic and from the clinical point of view (Table 1 and Table 5, 1992 pollen season); this is said to be the best way of balancing experimental groups [8]. Sublingually treated patients received a cumulative dose about 2.4 times higher than the cumulative dose reached by patients treated with injective therapy; considering this relative amount and that the allergen not absorbed had to be spat out, we can reasonably exclude any significant absorption of allergens by the digestive tract. On the other hand, oral immunotherapy by the digestive route is said to require doses 50–200 times greater than those administered with the classical injective route [13,25].

Intra-group analysis before and after the treatment for scores, shows a significant difference in favour of both

Table 6. Mean values of wheal size variation at times T0, T1, T2 (i.e. before, after 6 months, after 12 months) of treatment

	Wheal size (mm ²), average (± SD)		
	T0	T1	T2
Injective	144.1 ± 107.82 (SEM = 34.10)	102.3 ± 60.47 (SEM = 19.12)	77.10 ± 45.75 (SEM = 14.47)
SLIT	135.3 ± 136.25 (SEM = 43.09)	135.6 ± 67.58 (SEM = 21.37)	101.7 ± 62.89 (SEM = 19.89)

Table 7. Statistics of wheal size variation at times T0, T1, T2 (i.e. before, after 6 months, after 12 months) of treatment (ANOVA)

	Wheal size			
	T0-T1-T2 (<i>P</i>)	T0-T1 (<i>P</i>)	T0-T2 (<i>P</i>)	T1-T2 (<i>P</i>)
Injective	0.021	0.082	0.030	0.182
Sublingual	0.361	0.992	0.297	0.071

treatments (Table 4: *P* between 0.002 and 0.0039 for symptoms and drugs, separately or combined, 1993 versus 1992 pollen season), with the same average decrease (more than 50%) of symptoms and drug consumption, under a similar allergenic pressure (Fig. 1, grass pollen counts in the two seasons). The comparison of the objective data (Tables 6, 7 and 8), shows a clear difference between SLIT treated patients and patients treated with injective therapy: no statistically significant variation in the SLIT group for skin reactivity, total specific IgG, specific IgG4, while all these parameters changed in the injective group.

A decrease in skin sensitivity to the allergen during the first months of treatment and an increase of IgG antibodies is common when injective therapy is employed [5,29,30,31,32,33]; few data are available for sublingual therapy, because most of the published trials investigated only clinical parameters. Accordingly, in one case [21] a

decrease in skin sensitivity and an increase in IgG antibodies was found, in another [1] no change in skin sensitivity was shown. It must be underlined, however, that in the first case 58 patients were studied for 18 months, in the second 41 patients were studied for 105 days: the differences in the sample size and in the length of the two trials must therefore be taken into account. From the statistical point of view, obviously, studies involving many patients and with a long period of treatment give the highest chance of reaching the evidence of changes in the investigated parameters. In our case, a decrease of skin reactivity near to statistical significance (*P* = 0.071, Table 6) was obtained only after between 6 and 12 months of sublingual therapy, with a sample size of only 10 patients.

The meaning of these parameters and their relationship with the clinical outcome is otherwise not clear and established, and clinical evaluations are judged as the only

Table 8. Mean values and statistics of immunoglobulins at times T0, T1, T2 (i.e. before, after 6 months, after 12 months) of treatment (ANOVA)

Immunoglobulins		Average $\pm$ SD			
		T0	T1	T2	T0-T1-T2
SLIT	IgEs	15.29 $\pm$ 4.94 (SEM = 1.49)	14.96 $\pm$ 5.53 (SEM = 1.91)	15.49 $\pm$ 4.64 (SEM = 1.40)	<i>P</i> = 0.317 (ns)
	Total IgGs	16.91 $\pm$ 4.57 (SEM = 1.38)	14.91 $\pm$ 3.83 (SEM = 1.16)	16.64 $\pm$ 5.87 (SEM = 1.77)	<i>P</i> = 0.393 (ns)
	IgG4	13.64 $\pm$ 4.80 (SEM = 1.45)	12.0 $\pm$ 2.97 (SEM = 0.89)	14.64 $\pm$ 6.10 (SEM = 1.84)	<i>P</i> = 0.429 (ns)
Injective	IgEs	12.76 $\pm$ 7.02 (SEM = 2.12)	11.68 $\pm$ 5.39 (SEM = 1.63)	14.55 $\pm$ 5.22 (SEM = 1.57)	<i>P</i> = 0.190 (ns)
	Total IgGs	17.27 $\pm$ 4.63 (SEM = 1.40)	31.64 $\pm$ 16.42 (SEM = 4.95)	28.27 $\pm$ 13.90 (SEM = 4.19)	<i>P</i> < 0.001 ***
	IgG4	11.45 $\pm$ 4.97 (SEM = 1.5)	21.64 $\pm$ 14.38 (SEM = 4.49)	22.82 $\pm$ 11.51 (SEM = 3.47)	<i>P</i> < 0.001 ***

method which truly reflects clinical efficacy [8]. Our results, showing the same clinical efficacy of the two treatments but no changes in objective parameters with SLIT, while the same parameters changed with injective therapy, are in favour of a different, independent evolution of the clinical parameters versus the objective parameters investigated in our study. The aim of our trial was indeed the comparison of the clinical advantages, in a balanced group of patients exposed to the same environmental allergenic pressure. The comparison was carried out after the administration of a sublingual specific immunotherapy or an injective specific immunotherapy taken as a standard reference therapy, and employing the identical purified and biologically standardized grass extract.

Although a larger experience is needed to draw definitive conclusions, our pilot study in grass allergic patients strongly suggests the possibility of similar clinical benefits from injective or sublingual specific immunotherapy, the latter being obviously cheaper and very well accepted by the patient, mainly for short-term pollinosis.

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