

Intralymphatic immunotherapy for cat allergy induces tolerance after only 3 injections

Gabriela Senti, MD,^a Reto Cramer, PhD,^b Daniela Kuster, MD,^a Pål Johansen, PhD,^c Julia M. Martinez-Gomez, PhD,^c Nicole Graf, PhD,^a Martin Steiner, PhD,^d Ludwig A. Hothorn, PhD,^e Hans Grönlund, PhD,^f Christine Tivig, MD,^a Anna Zaleska,^b Ozge Soyer,^b Marianne van Hage, MD, PhD,^f Cezmi A. Akdis, MD,^b Mübeccel Akdis, MD, PhD,^b Horst Rose, PhD,^d and Thomas M. Küding, MD^c Zurich and Davos, Switzerland, Hannover, Germany, and Stockholm, Sweden

Background: Subcutaneous allergen-specific immunotherapy frequently causes allergic side effects and requires 30 to 80 injections over 3 to 5 years.

Objective: We sought to improve immunotherapy by using intralymphatic allergen administration (intralymphatic immunotherapy [ILIT]) and by targeting allergen to the MHC class II pathway.

Methods: Recombinant major cat dander allergen Fel d 1 was fused to a translocation sequence (TAT) and to part of the human invariant chain, generating a modular antigen transporter (MAT) vaccine (MAT–Fel d 1). In a randomized double-blind trial ILIT with MAT–Fel d 1 in alum was compared with ILIT with placebo (saline in alum) in allergic patients (ClinicalTrials.gov NCT00718679).

Results: ILIT with MAT–Fel d 1 elicited no adverse events. After 3 placebo injections within 2 months, nasal tolerance increased less than 3-fold, whereas 3 intralymphatic injections with MAT–Fel d 1 increased nasal tolerance 74-fold ($P < .001$ vs placebo). ILIT with MAT–Fel d 1 stimulated regulatory T-cell responses ($P = .026$ vs placebo) and increased cat dander-specific IgG₄ levels by 5.66-fold ($P = .003$). The IgG₄ response positively correlated with IL-10 production ($P < .001$).

Conclusion: In a first-in-human clinical study ILIT with MAT–Fel d 1 was safe and induced allergen tolerance after 3 injections. (J Allergy Clin Immunol 2012;■■■:■■■-■■■.)

Key words: Intralymphatic, cat dander allergy, immunotherapy, tolerance, vaccination, Fel d 1

Allergies have reached epidemic proportions.^{1,2} Allergen-specific immunotherapy (SIT) represents the only disease-modifying treatment.^{3,4} However, conventional subcutaneous SIT requires 30 to 80 allergen injections over 3 to 5 years^{3,4} and is variably associated with allergic side effects.⁵ The newer sublingual immunotherapy is more patient friendly, but treatment duration could not be shortened.⁶ Because of these disadvantages, less than 5% of allergic patients choose to undergo SIT, and a method that would render SIT both safer and faster would certainly be desirable.^{7,8}

There is good evidence that use of higher allergen doses enhances the efficacy of subcutaneous and sublingual immunotherapy.⁹ However, administration of high allergen doses through these routes is prohibitive because of local and systemic allergic side effects caused by mast cells and basophils. Our goal is to deliver high allergen doses to T and B cells through a combination of 2 strategies: targeting the allergen to the lymph node and to the MHC class II pathway.

The first strategy is to inject allergen directly into a lymph node, which is called intralymphatic immunotherapy (ILIT).¹⁰ ILIT with various types of immunotherapeutic agents, such as RNA,¹¹ DNA,¹² oligopeptides,¹³ proteins,^{14–17} whole cells,^{18,19} and adjuvants,^{20,21} strongly enhances immunologic responses in mice, dogs,^{14,22} horses,²³ monkeys,^{24,25} and human subjects.^{26,27} In a randomized controlled phase I/II clinical trial in patients with grass pollen-induced rhinoconjunctivitis, 3 intralymphatic injections with grass pollen allergen extract induced nasal tolerance.²⁷ The presented trial supports these findings in patients with cat dander allergy using a modified recombinant allergen. rFel d 1 was fused to the HIV-derived translocation peptide TAT, mediating cytoplasmic uptake of extracellular proteins.^{28–30} To enhance presentation through the MHC class II pathway, a truncated human invariant chain (Ii) was also fused to Fel d 1,^{31,32} targeting the allergen to MHC class II $\alpha\beta$ heterodimers assembled in the endoplasmic reticulum and thus circumventing phagosomal uptake and degradation.^{31–33} In mice modular antigen transporter (MAT)–Fel d 1 enhanced antibody and T-cell responses compared with nFel d 1 and protected mice sensitized to cat dander extract against anaphylaxis elicited by challenge with

From ^athe Clinical Trials Center and ^cthe Department of Dermatology, University Hospital Zurich; ^bthe Swiss Institute for Allergy and Asthma Research (SIAF), Davos; ^dImVisioN GmbH, Hannover; ^eInstitut für Biostatistik, Leibniz Universität Hannover; and ^fthe Department of Medicine, Clinical Immunology and Allergy Unit, Karolinska University Hospital Solna, Stockholm.

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Corresponding author: Thomas M. Küding, MD, Department of Dermatology, University Hospital Zurich, Gloriastrasse 31, 8091 Zurich. E-mail: thomas.kuending@usz.ch. 0091-6749/\$36.00

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Abbreviations used

IDT: Intradermal test
 ILIT: Intralymphatic immunotherapy
 MAT: Modular antigen transporter
 mRQLQ: Mini-rhinitis quality-of-life questionnaire
 NPT: Nasal provocation test
 SIT: Allergen-specific immunotherapy
 SPT: Skin prick test

cat dander extract.³³ MAT–Fel d 1 also induced less *in vitro* degranulation and leukotriene release of basophils from sensitized human subjects than nFel d 1, suggesting a safety benefit.³³ Clinical superiority testing of MAT–Fel d 1 versus rFel d 1 versus purified nFel d 1 would also have to include subcutaneous arms for all molecules and would be beyond the scope of a first-in-human clinical study with MAT–Fel d 1. Therefore the present phase I/IIa trial compared ILIT with MAT–Fel d 1 with placebo ILIT. The presented clinical study also represents the first immunotherapy trial with a recombinant cat allergen.

METHODS**Study population and objectives**

Patients with cat dander allergy were recruited by means of newspaper advertisement and enrolled in August and September 2008 to the study site at University Hospital Zurich, Switzerland.

The main inclusion criteria were a history of cat dander allergy, including rhinitis and conjunctivitis, age of 18 to 65 years, a positive reaction to at least 1 concentration of cat dander extract in skin prick tests (SPTs) and nasal provocation tests (NPTs), and detection of serum IgE specific to Fel d 1. General contraindications were serious immunologic diseases; serious cardiovascular diseases; malignancies; chronic infections; use of β -blockers, including eye drops; uncontrolled and severe asthma (FEV₁ during treatment <70%); pregnancy or nursing; severe psychiatric disorders; or inability to provide informed consent.

Of 25 patients screened, 20 (6 male and 14 female patients aged 20–54 years) were randomized to receive either MAT–Fel d 1 or placebo ILIT (Fig 1). The randomization list was provided by IKFE GmbH (Mainz, Germany). Study medication was packed and blinded by the hospital pharmacy, and thus both patients and physicians were blind. All patients completed the study.

Study objectives

The study objectives in this phase I/IIa trial were to evaluate the safety, immunogenicity, and efficacy of ILIT with MAT–Fel d 1. The primary efficacy objective was induction of tolerance to cat dander extract in a titrated NPT. Secondary efficacy objectives were induction of allergen tolerance in intradermal tests (IDTs) and SPTs and immunologic parameters.

Study design

At the first of 2 screening visits, patient eligibility was determined, blood was sampled, and a conjunctival provocation test was performed with cat dander extract. Also, a titrated SPT with cat dander extract was performed on one arm and a titrated SPT with MAT–Fel d 1 was performed on the other arm. Patients with a positive SPT response came for a second screening visit 5 ± 2 days later. Subsequently, an NPT with cat dander extract and an IDT with cat dander extract on one arm and an NPT with MAT–Fel d 1 and an IDT with MAT–Fel d 1 on the other arm were performed. After another 5 ± 2 days, patients were randomly allocated to receive either MAT–Fel d 1 ($n = 12$) or placebo ($n = 8$) ILIT and received the first of 3 injections. With intervals of 28 ± 3 days, patients received injections with either placebo or with 1, 3, and 10 μ g of MAT–Fel d 1. Blood was drawn before the first treatment, as well as 5 ± 2 days after the last treatment.

Another SPT was done 22 ± 4 days after the last ILIT. At the final visit 5 ± 2 days later, IDTs and NPTs with cat dander extract were performed. Throughout the study, all adverse events and concomitant medications were recorded. The study remained double blind. Emergency envelopes were unbroken. The code was broken after database lock, and thus all raw clinical and laboratory data were filed in the electronic data system before the unblinding procedure took place. The statistical analysis plan was finalized on March 1, 2009, and code break was on March 10, 2009. The study was quality controlled by a qualified monitor assigned by the CRO (IKFE GmbH). The study was approved by the local ethics review committee and Swissmedic. Written informed consent was provided by all participants (ClinicalTrials.gov no. NCT00718679).

Allergen and placebo preparation

MAT–Fel d 1, a recombinant nonglycosylated fusion protein consisting of 291 amino acids, was supplied by ImVisioN GmbH (Hannover, Germany), manufactured by Strathmann Biotec GmbH & Co KG (Hamburg, Germany), and stored at -80°C . Both MAT–Fel d 1 and placebo were incubated at room temperature with aluminum hydroxide (Alhydrogel 2%; Brenntag Biosector, Frederikssund, Denmark) for more than 60 minutes before injection. Cat dander allergen extract was purchased from Stallergenes (Antony, France). Placebo (saline in alum) and verum (MAT–Fel d 1 in alum) were visually indistinguishable.

Intranodal injection under ultrasonographic guidance

A superficial inguinal lymph node was identified for administration of 100 μ L of either MAT–Fel d 1 or placebo preparation. Injections were performed aseptically with a 1-mL hypodermic syringe with a 25-gauge needle. The procedure was controlled and documented by means of ultrasonography.²⁷

NPTs

Patients were adapted to room temperature for more than 15 minutes before baseline evaluation by means of rhinoscopy and rhinomanometry.³⁴ One hundred microliters of allergen solution (Alyostal; Stallergenes, Antony, France) was sprayed into the wider side of the nose. Symptoms were scored according to published guidelines.³⁵ The end point was defined as the allergen concentration inducing either (1) nasal flow reduction of greater than 40%, (2) flow reduction of greater than 20% combined with a score of 2, or (3) a score of greater than 3.

Mathematic fitted curves of individual scores versus concentration were calculated in a mixed model including the fixed factor treatment (with MAT–Fel d 1 or placebo) and random patient-specific slopes and intercepts of the log(score) by log(concentration) relationship. The readout parameter was set as the allergen concentration needed to reach the score threshold value of 2. On the basis of these estimated patient-specific allergen concentrations, the k-fold increase of the allergen concentration to reach the threshold is estimated for the paired design “posttest score versus baseline score” independently for the placebo- and MAT–Fel d 1–treated groups. For direct comparison between the treatment groups, the transformed end point “post minus pre concentrations on log scale” was estimated. These exploratory efficacy analyses were conducted for the full analysis set. The 2-sided *P* value from the permutation Wilcoxon test was used for statistical analysis.

SPTs

Serially diluted allergen solutions were pricked on the volar forearm.³⁶ Test sites were greater than 2 cm apart. Diluent solution was used as a negative control. Histamine was used as a positive control, with 1 mg/mL inducing a wheal 2 to 7 mm in diameter.³⁷ Wheal-and-flare reactions were recorded after 20 minutes, outlined on the skin with a pen, blotted onto cellophane tape, and stored on paper. Areas were assessed by means of computer-aided planimetry.³⁸ A wheal size of 7 mm² or greater was regarded as positive.³⁷ Reactions were scored as a percentage of positive controls. Evaluation of end point titrations was based on parallel-line bioassay and on median slope.³⁹

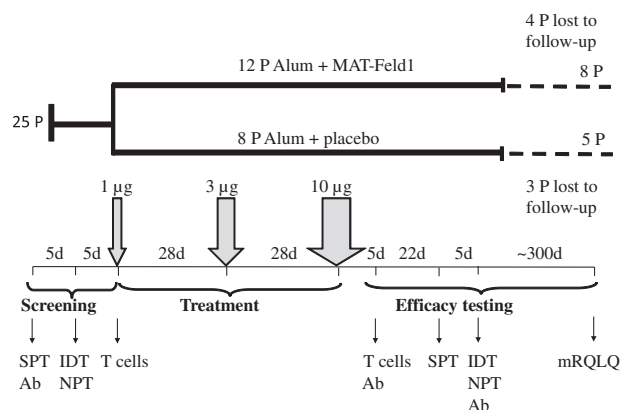


FIG 1. Trial design. The trial was composed of 2 screening visits, 3 treatment visits, 2 efficacy visits within a month of the last treatment, and 1 follow-up visit after another 300 days. Ab, Antibody measurement.

IDTs

The starting concentration was a 1000-fold dilution of the solution used for SPTs.⁴⁰ One hundred microliters was injected by using 0.5-mL syringes with 26-gauge needles placed at a flat angle to the skin. After 15 minutes, wheal-and-flare reactions were assessed as for the SPTs. Wheal reactions of 6 mm or greater in diameter were regarded as positive.

Determination of antibodies

IgE was analyzed by means of ImmunoCAP (Phadia, Uppsala, Sweden). ELISAs to detect cat dander-specific IgG, IgG₁, or IgG₄ antibody titers were performed according to standard in-house procedures. ELISA plates were coated with 1 IR/mL cat dander extract (Stallergenes), and biotinylated anti-human IgG, IgG₁, or IgG₄ was purchased from BD BioSciences (Basel, Switzerland).

Cellular antigen stimulation test

Peripheral blood was drawn into EDTA-coated vials according to provider protocols (Bühlmann Laboratories AG, Schönenbuch, Switzerland). Dextran was added for 90 minutes at ambient temperature, and leukocytes were isolated by means of Ficoll density separation and resuspended in stimulation buffer containing IL-3. In 96-well plates cells were stimulated with rFel d 1 (LoTox rFel d 1; Indoor Biotechnologies, Charlottesville, Va), MAT-Fel d 1, or nFel d 1 from cat dander extract (Stallergenes) at 37°C for 40 minutes. Allergens were tested at 5, 0.5, 0.05, and 0.005 nmol/L Fel d 1. Supernatants were assayed for soluble leukotrienes by means of ELISA.

T-cell analyses

Allergen-specific T-cell responses were determined by means of stimulation of 2×10^5 PBMCs with LPS-free rFel d 1 (LoTox rFel d 1, Indoor Biotechnologies) allergen in 200 µL of medium by using 96-well flat-bottom tissue-culture plates in triplicates. After 5 days, supernatants were collected, and the content of IL-2, IL-4, IL-5, IL-10, IL-17, and IFN-γ was analyzed by using an immunofluorescence method.

Mini-rhinoconjunctivitis quality-of-life questionnaires

At the follow-up visit around day 300, patients were asked to document their ocular and nasal symptoms during the last week by using standardized mini-rhinoconjunctivitis quality-of-life questionnaires (mRQLQs).⁴¹

TABLE I. Demographic data and baseline values by treatment group

Characteristic	MAT-Fel d 1 (n = 12)	Placebo (n = 8)
Age (y)	34.6 ± 11.9	27.0 ± 5.8
Sex (M/F)	4/8	2/6
Body mass index (kg/m ²)	26.2 ± 6.1	24.4 ± 6.9
White race	12	8
NPT (score)		
10 ⁻³	0.75 ± 1.22 (n = 12)	0.50 ± 0.53 (n = 8)
10 ⁻²	2.00 ± 1.94 (n = 9)	1.33 ± 1.21 (n = 6)
10 ⁻¹	2.71 ± 1.25 (n = 7)	3.25 ± 0.96 (n = 4)
10 ⁰	5.67 ± 0.58 (n = 3)	4.50 ± 2.12 (n = 2)
SPT (threshold concentration)		
10 ⁻³	0	1
10 ⁻²	7	4
10 ⁻¹	3	2
10 ⁰	2	1
IDT (threshold concentration)		
10 ⁻⁷	2	1
10 ⁻⁶	6	2
10 ⁻⁵	3	5
10 ⁻⁴	1	0
Cat IgG ₄ (titer)	528 ± 632	667 ± 1080
Cat IgE (kU _A /L)	13.8 ± 20.2	11.5 ± 15.6

Numeric parameters are presented as mean values ± SDs. All other parameters are shown as absolute frequencies.

Statistical analysis

The goals of this study were to test the safety, immunogenicity, and efficacy of MAT-Fel d 1. Because only limited information was available for quantitative valid sample size estimation, the present study was designed as an experimental pilot phase I/IIa study. A sample size of 20 randomized patients was estimated as sufficient to produce valuable information to determine the patient sample size for an upcoming phase II study, the primary objective of which will be to demonstrate dose-dependent efficacy by using the same clinical end points assessed in this study.

Nonparametric tests were used because the data are partly tied and skewed. Two-sided tests at an α value of .05 were used. Several end points in the paired design “post versus pre” were tested by using an exact version of the Wilcoxon signed-rank test separately within the MAT-Fel d 1 group and the placebo group. The comparison of the MAT-Fel d 1 group versus the placebo group in the randomized design was performed by using the exact version of the Wilcoxon test or the Welch *t* test. The correlation between changes in IgG₄ and IL-10 responses (post/pre) was analyzed by using a Pearson correlation coefficient (2-sided *P* value). All analysis was done with the statistical package R (Version 12.2).

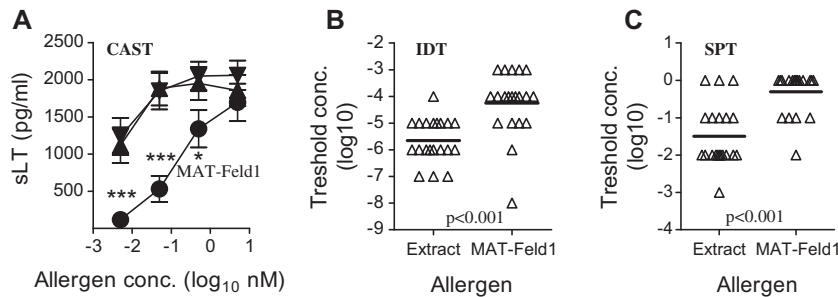
RESULTS

Patients' characteristics

Of the 25 patients screened, 20 (6 male and 14 female patients 20–54 years of age) were randomized to receive either MAT-Fel d 1 or placebo ILIT (Fig 1). There were no withdrawals or discontinuations. However, 4 verum-treated and 3 placebo-treated patients were lost to follow-up by the 300-day visit. At baseline, the 2 patient groups showed no statistically significant differences (Table I).

Allergenicity *in vitro*

Leukocytes were isolated from all patients and incubated with equimolar concentrations of rFel d 1, MAT-Fel d 1, or nFel d 1 isolated from cat dander extract. In this cellular antigen



stimulation test assay approximately 100-fold more MAT-Fel d 1 than rFel d 1 or nFel d 1 from cat dander extract was required to stimulate soluble leukotriene production from basophils (Fig 2, A).

Skin reactivity

We also tested the capability of MAT-Fel d 1 to induce skin reactions. Administration of MAT-Fel d 1 caused significantly reduced skin reactions than equimolar concentrations of nFel d 1 contained in cat dander extract. When injected intradermally, a 23-fold higher MAT-Fel d 1 dose than Fel d 1 was tolerated ($P < .001$; Fig 2, B). In the SPTs 16-fold more MAT-Fel d 1 was tolerated ($P < .001$; Fig 2, C).

Systemic adverse events

For safety reasons, the initial intralymphatic MAT-Fel d 1 dose was 1 μ g, whereas the second and third injections were 3 and 10 μ g, respectively. Intralymphatic injections proved practically painless and easily achieved by using ultrasonographic guidance.²⁷ The number of suspected drug-related adverse events was higher in the placebo group than in the MAT-Fel d 1 group, although the difference was not statistically significant (Table II). Pulmonary function tests revealed no evidence of treatment-associated asthma, and cardiologic, hematologic, and chemical tests on serum and urine showed no abnormalities (data not shown).

Humoral responses

Five weeks after the third immunotherapeutic injection, cat dander-specific IgG₄ levels increased by a median factor of 5.66 in patients treated with MAT-Fel d 1, whereas no median IgG₄ level increase was observed in placebo-treated patients (factor 1.0; Fig 3, A). No induction of allergen-specific IgG₁ was observed (data not shown), and treatment with MAT-Fel d 1 did not boost allergen-specific IgE titers (Fig 3, A).

T-cell responses

For analysis of T-cell responses, freshly isolated PBMCs were isolated before the first injections and 5 days after the last injection and were restimulated with rFel d 1. Production of IL-10 increased in MAT-Fel d 1- but not placebo-treated patients (Fig 3, B), and this allergen-specific IL-10 production correlated positively with

TABLE II. Comparison of reported local and systemic AEs observed up to 88 days after first injection

	ILIT/severity of AE					
	Placebo			MAT-Fel d 1		
	Mild	Moderate	Severe	Mild	Moderate	Severe
Local reactions						
Lymph node swelling*	2	1	0	3	1	0
Systemic reactions						
Rhinorrhea	1	1	0	0	0	0
Sneezing	1	1	0	0	0	0
Pruritus	1	1	0	0	0	0
Nausea	0	0	0	1	0	0
Rash	0	0	0	0	1	0
Dyspnea	2	0	0	1	0	0
Headache	1	1	0	0	0	0
Cough	1	0	0	0	0	0
Totals	9	5	0	5	2	0

AE, Adverse event.

*Lymph node swelling had resolved in all patients examined at day 300.

the increase in IgG₄ titers (Fig 3, C). Neither ILIT with MAT-Fel d 1 nor placebo caused significant changes in the production of IL-2, IL-4, IL-5, IL-17, and IFN- γ (data not shown).

Effect on nasal tolerance

The above immunologic changes are suggestive of successful immunotherapy, but the key question and primary end point of this clinical trial was whether ILIT with MAT-Fel d 1 could induce nasal tolerance to cat dander. Therefore patients received a nasal challenge with titrated amounts of cat dander extract 5 weeks after the third injection with MAT-Fel d 1 or placebo, and tolerated concentrations were compared with baseline levels obtained before ILIT. As shown in Fig 4, nasal tolerance in placebo-treated patients increased less than 3-fold ($P = .008$), whereas ILIT with MAT-Fel d 1 increased nasal tolerance by a factor of 74 ($P < .001$, $P < .001$ for placebo vs MAT-Fel d 1).

Effect on skin tolerance

Significant increases in skin prick and dermal (Fig 4) tolerance were observed in patients treated with MAT-Fel d 1 ($P = .047$ and

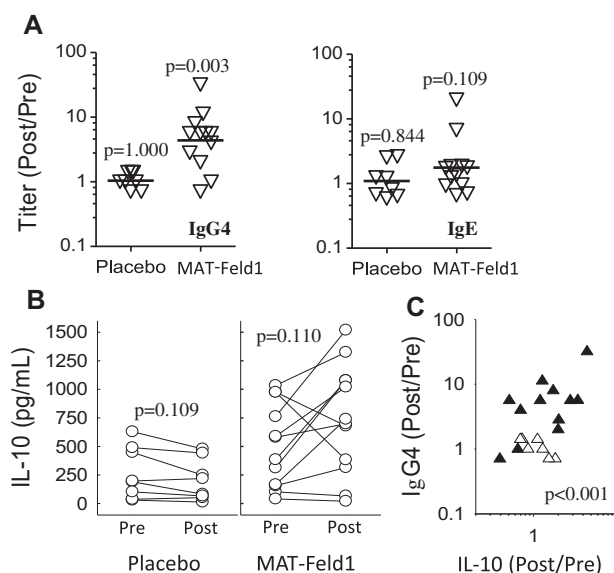


FIG 3. Immunologic testing. **A**, Cat dander-specific IgG₄ and IgE levels were analyzed at baseline (*pre*) and 5 weeks after final treatment (*post*). Changes (*post/pre* ratio) were analyzed by using a 2-sided exact Wilcoxon signed-rank test. **B**, Pretreatment and posttreatment production of IL-10 after *in vitro* restimulation of PBMCs with rFel d 1. The Welch *t* test was used to test the statistical significance between MAT-Fel d 1 and placebo treatment. **C**, Correlation between allergen-specific IgG₄ titer changes with changes in IL-10 production for the combined MAT-Fel d 1-treated (solid triangles) and placebo-treated (open triangles) patients after Pearson correlation analysis ($r^2 = 0.725$).

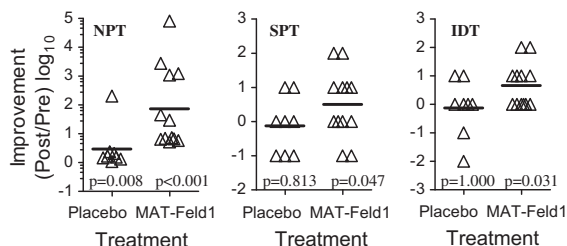


FIG 4. Efficacy testing: titrated NPTs, SPTs, and IDTs with cat dander extract. Changes in nasal allergen tolerance were calculated as the ratio of posttreatment to pretreatment allergen concentration required to induce a rhinitis symptom score of greater than 2. Concentration was calculated assuming random patient-specific slopes and intercepts of the $\log(\text{score})$ by $\log(\text{concentration})$ relationships ($P < .001$, placebo versus MAT-Fel d 1 by using the 2-sided exact Wilcoxon test). Changes in allergen tolerance in SPTs and IDTs were calculated as allergen concentrations required to induce a wheal after and before treatment (2-sided exact Wilcoxon signed-rank test).

$P = .031$, respectively). Posttreatment allergen tolerance in titrated SPTs and IDTs increased in the active group by a factor of 3.16 and 4.64, respectively. No significant increase in skin prick or dermal tolerance to cat dander allergens was observed in patients receiving placebo treatment ($P = .0813$ and $P = 1.000$, respectively).

Follow-up visit

An additional follow-up visit was scheduled after approximately 300 days to assess the longevity of the induced allergen tolerance. Eight of 12 MAT-Fel d 1-treated and 5 of 8 placebo-

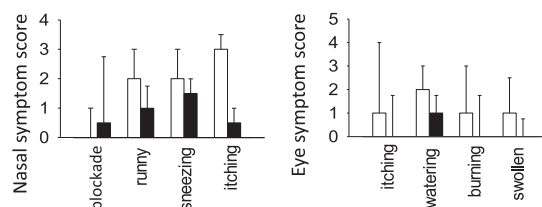


FIG 5. Follow-up analysis of quality of life. Patients receiving placebo or MAT-Fel d 1 ILIT were asked to document their symptoms during the last week in an mRQLQ. Eight of 12 MAT-Fel d 1-treated patients and 5 of 8 placebo-treated patients returned for this visit. Nasal and conjunctival symptoms approximately 300 days after treatment are shown. The error bars show the median scores and 75th percentiles for the allergen-treated (solid bars) and placebo-treated (open bars) patient groups. The Wilcoxon exact test showed no significant differences in the ocular ($P = .287$) or nasal ($P = .371$) sum scores.

treated patients returned for this visit. Brief clinical examination and lymph node palpation revealed no abnormalities. Patients were asked to complete a validated rhinitis quality-of-life questionnaire. Patients reported practically no impairment in the domains of activity limitations, practical problems, or non-nasal-ocular symptoms (data not shown). Reports on nasal and ocular symptoms, however, revealed that these were less in the MAT-Fel d 1-treated group than in the placebo-treated group (Fig 5). The improvement was not statistically significant, and it has to be taken into account that the statistical power is reduced because of the low patient number.

DISCUSSION

Allergy to cat dander is a common cause of rhinoconjunctivitis and asthma.⁴²⁻⁴⁴ The major allergen of cat dander, Fel d 1, an 18-kd heterodimer present in the cat's saliva and sebaceous glands, elicits symptoms in more than 90% of patients with cat allergy. The current study revealed that ILIT with MAT-Fel d 1 rendered allergic patients tolerant to nasal provocation with cat dander extract after only 3 injections. The measured 74-fold increase in nasal tolerance is comparable with or better than the degree of improvement reported in conventional subcutaneous immunotherapy involving numerous injections over several years.⁴⁵

Safety and tolerability are major concerns in SIT.³⁻⁵ Injected allergen inadvertently reaching the circulation can cause severe systemic allergic reactions by cross-linking surface-bound IgE on basophils and mast cells, leading to anaphylaxis. In contrast to this much-feared but rare adverse event, local allergic side effects caused by local mast cells and local late-phase responses, typically a swelling of the injected arm, are frequent. As previously demonstrated with MAT-Fel d 1 produced in our own laboratory,³³ also the batch produced under good manufacturing practice conditions used in the present study showed approximately 100-fold reduced mediator release of basophils from allergic patients compared with equimolar concentrations of rFel d 1 or nFel d 1 purified from cat dander extract. This is probably explained by means of rapid intracellular translocation of the MAT molecule,³¹⁻³³ which might reduce the amount of extracellular allergen able to cross-link surface IgE. Reduced basophil stimulation should reduce the risk of anaphylaxis. As demonstrated in titrated SPTs and IDTs, mast cell degranulation was also reduced (ie, 16-fold in SPTs and 23-fold in IDTs, respectively). In line with this, the local reaction to injection of MAT-Fel d 1 was not increased

compared with reactions to injections of alum alone. Also, no MAT–Fel d 1–related adverse events were observed in this study, neither local nor systemic, and pulmonary function tests revealed no therapy-induced measurable asthmatic airway response, a recognized adverse effect of cat dander immunotherapy.^{46,47}

Three injections of MAT–Fel d 1 induced a 5.66-fold increase of cat dander-specific IgG₄ titers, which is within the range of what has been reported after 1 or 3 years of conventional high-dose SIT with cat dander, ranging from 3- to 4-fold⁴⁸ to 8- to 10-fold.⁴⁹ Also, MAT–Fel d 1 did not boost IgE levels, an unwanted phenomenon frequently observed in patients undergoing conventional SIT.^{50,51}

Three injections of MAT–Fel d 1 were sufficient to induce a significant IL-10 response. Allergen-specific regulatory T-cell responses are today considered a hallmark of successful SIT.⁵² The IL-10 response correlated with allergen-specific IgG₄ levels.

Altogether, production of IgG₄ and IL-10 and lack of IgE boosting fit well with previous findings that IL-10 suppresses IgE while increasing IgG₄ production.⁵³

Whether the presented 3-shot regimen induced tolerance was assessed by using provocation tests with cat dander extract. Allergen tolerance of the skin, as assessed by using titrated SPTs and IDTs, was increased 3.16-fold and 4.64-fold, respectively, which is comparable with that seen after conventional 3-year SIT regimens.^{45,48}

At a follow-up visit after 300 days, symptom assessment with validated mRQLQs⁴¹ revealed less symptoms in the MAT–Fel d 1 group, albeit with reduced statistical power because of the low patient number. A further limitation is that symptoms were not assessed at baseline, and therefore a pre-existing bias cannot be excluded.

Although ILIT with MAT–Fel d 1 proved readily feasible, safe, and effective, there are limitations to the interpretation of these results. We used a novel molecule delivered through a novel route, and therefore the clinical data do not allow us to distinguish which aspect contributed more to the positive results. The contribution of MAT would have to be investigated in a superiority study comparing ILIT with MAT–Fel d 1 versus rFel d 1, whereas the contribution of ILIT would have to be examined by comparing subcutaneous versus intralymphatic administration of MAT–Fel d 1. We could not formally investigate this in a clinical trial. However, in preclinical murine models ILIT enhanced the immunogenicity of both Fel d 1 and MAT–Fel d 1 by orders of magnitude compared with subcutaneous administration (ie, subcutaneous immunotherapy required approximately 10⁴-fold higher allergen doses than ILIT to induce comparable IgG_{2a} titers).¹⁷ Furthermore, ILIT with MAT–Fel d 1 was significantly more effective than ILIT with rFel d 1.¹⁷

This is a first-in-human study using a novel allergen molecule delivered through a new route. Several parameters remain to be optimized. In light of the excellent safety observed here, the next phase IIb study will escalate the MAT–Fel d 1 dose and perform short- and long-term efficacy analyses in a validated experimental exposure chamber. Also, the time interval between injections and the number of injections remains to be optimized. In murine models 3 injections were found to be optimal, with a fourth injection not further boosting IgG_{2a} responses.¹⁷ Finally, NPTs and also testing in an experimental exposure chamber are considered merely supportive evidence for efficacy. Although their predictive value is high, further studies are required to assess efficacy (ie, symptom reduction and reduction of medication use).^{54,55}

Larger patient numbers in future studies will also allow a more definite assessment of the safety of ILIT with MAT–Fel d 1.

In conclusion, ILIT with MAT–Fel d 1 was safe and induced tolerance to nasal allergen provocation in allergic patients after only 3 injections. This is a major improvement over conventional subcutaneous or sublingual immunotherapy, which both require years of treatment. This vaccination approach might therefore represent an attractive treatment option, especially for all the allergic patients who are reluctant to undergo SIT because of time constraints and the risk of allergic side effects.

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Clinical implications: ILIT with MAT allergens might represent a promising safe and rapid treatment alternative for IgE-mediated allergies.

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