

ORIGINAL ARTICLE

Use of A-type CpG oligodeoxynucleotides as an adjuvant in allergen-specific immunotherapy in humans: a phase I/IIa clinical trial

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Summary

Background B-type CpG oligodeoxynucleotides (ODN) is currently used in clinical trials because of its prolonged half-life, which is due to its phosphorothioate backbone. A-type CpG ODN is a stronger inducer of IFN but has an unstable phosphodiester backbone that has so far prohibited its clinical use. However, upon association with virus-like particles (VLP) consisting of the bacteriophage Q β coat protein, A-type CpG ODN can be stabilized and can become an efficient adjuvant in mice. Therefore, the phase I/IIa study presented represents the first test of A-type CpGs in humans.

Objective To test the safety, tolerability and clinical efficacy of QbG10 as an adjuvant for subcutaneous immunotherapy with a house dust mite (HDM) allergen extract in allergic patients.

Methods A single centre, open-label phase I/IIa study evaluated the safety, tolerability and clinical efficacy of QbG10 as an adjuvant to immunotherapy with a subcutaneous HMD allergen extract in 20 patients suffering from HDM allergy. Twenty-one patients were enrolled between March and July 2005. Individual immunotherapy lasted 10 weeks. Clinical end-points included questionnaires, conjunctival provocation, skin prick tests and the measurement of allergen-specific IgG and IgE.

Results QbG10 was well tolerated. Almost complete tolerance to the allergen was observed in conjunctival provocation testing after treatment with QbG10, and symptoms of rhinitis and allergic asthma were significantly reduced. Within 10 weeks of therapy, patients were nearly symptom-free and this amelioration lasted for at least 38 weeks post-treatment. Following injections of QbG10 and HDM allergen extract, allergen-specific IgG increased, while there was a transient increase in allergen-specific IgE titres. Skin reactivity to HDM was reduced.

Conclusion The subcutaneous application of HDM allergen, together with A-type CpG ODN packaged into VLP, was safe. All patients achieved practically complete alleviation of allergy symptoms after 10 weeks of immunotherapy. This promising clinical outcome calls for larger placebo-controlled phase II studies.

Keywords allergy, house dust mite, specific immunotherapy, Type A CpG, virus-like particles

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Introduction

IgE-mediated allergies affect up to 35% of the population in westernized countries [1, 2]. Subcutaneous allergen-specific immunotherapy (SCIT) is an effective treatment for patients with allergic rhinitis (AR), conjunctivitis, allergic asthma and allergic reactions to insect venom [3]. SCIT confers a long-term benefit even after disconti-

nuation [4, 5], it may interrupt the expansion of allergic sensitization to multiple allergens [5] and stop progression from AR to asthma [5, 6]. However, only a few allergy patients choose SCIT, mainly because of the long treatment duration (3–5 years).

SCIT modulates allergen-specific immune responses, shifting the balance of T lymphocyte subsets from a T-helper type 2 (Th2) phenotype towards a Th1 phenotype,

followed by increased IFN- γ and decreased IL-4 and IL-5 production [7], production of allergen-specific IL-10 by CD4⁺CD25⁺ T-regulatory cells [8] and increased serum levels and affinity of allergen-specific IgG antibodies [9, 10].

Traditionally, aluminium hydroxide (alum) is used as an adjuvant in SCIT. Alum enhances the overall immunogenicity and reduces the risk of anaphylaxis, but is often reported to promote Th2 responses in mice [11, 12], although the relevance of this in humans is debatable [13]. In contrast, oligodeoxynucleotides (ODN) rich in non-methylated CG motifs (CpG), which bind to toll-like receptor 9 (TLR9) expressed within endosomes of human plasmacytoid dendritic cells (pDC) and B cells, promote Th1 responses [14], making them promising for SCIT.

Three types of CpG ODN can be distinguished based on their immunological activities on human pDC and B cells [15, 16]. B-type CpG ODN (also known as K-type) has a phosphorothioate backbone. It strongly stimulates B cells and promotes maturation and activation of pDC but induces only small amounts of IFN- α/β . A-type CpG ODN (also known as D type) has a central palindromic CG motif-containing sequence flanked by polyG and a phosphodiester backbone. It is relatively weak at activating B cells, but stimulates APC maturation and induces high amounts of IFN- α/β in pDC. Finally, C-type CpG ODN is known to stimulate both B cells for secretion of IL-6 and IgM and pDC for secretion of IFN- α .

While B- and C-type CpG ODN are stabilized by a phosphorothioate backbone and therefore remain in tissues with a half-life of 2 days [17, 18], the phosphodiester backbone of A-type CpG ODN is rapidly digested *in vivo* by DNase type I, and hence is less effective in clinical use. Packaging A-type CpG ODN into virus-like particles (VLP) protects it against digestion by DNase I, and further stabilizes the CpG due to the presence of DNA-binding domains on the Qb subunits that point towards the inside of the particle. A particle size of around 30 nm enhances transport into draining lymph nodes [19], and enhances phagocytosis, thus delivering the CpG directly to TLR9 in the endolysosome [20]. Another advantage of packaging CpGs into VLPs is the reduction in their systemic adverse events (AE) [20]. Unpackaged CpG ODNs may distribute systemically and have significant systemic adverse effects, including splenomegaly and toxic shock [21, 22].

The present open-label, single-dose phase I/IIa clinical trial tested the safety and tolerability of packaged CpG at enhancing allergen-specific immunotherapy by a subcutaneous injection of A-type CpG ODN contained in VLPs (QbG10) together with a house-dust mite (HDM) allergen extract. In this study, we chose HDM as our allergy model, as it is highly prevalent and its management is difficult [23, 24]. However, SCIT gradually reduces allergic and asthmatic symptoms, and it reduces the required use of symptomatic medication [25, 26].

Materials and Methods

Study population

Twenty-one subjects with a history of mild to moderate perennial allergic rhinoconjunctivitis with asthma due to sensitization with HDM *Dermatophagoides pteronyssinus* (Der p) or *farinae* (Der f) were enrolled from March through July 2005 in the study performed at the University Hospital Zurich, Switzerland. Asthma and allergy were confirmed by forced expiratory volume in 1 s and bronchial methacholine provocation tests as well as by skin prick and conjunctival provocation tests with an aqueous allergen solution (Der p and Der f) from Allergopharma (Reinbek, Germany).

Study design

This single-centre, open-label study evaluated the specific immunotherapy with an allergy vaccine consisting of the synthetic oligodeoxynucleotide G10 contained in bacteriophage Qb capsid VLPs (QbG10) admixed with a conventional HDM allergen extract (Novo-Helisen[®] Depot, Allergopharma); the study design is outlined in Table 1. QbG10 was produced by Cytos Biotechnology (Schlieren, Switzerland) following GMP/ICH ICH guidelines. The patients first received the allergen without QbG10 according to a standard dose-escalating cluster regimen. In two sessions, they attended the clinic once weekly and received three subcutaneous injections of the allergen at increasing doses at 30-min intervals. If the cumulative dose on a given day was well tolerated, this dose was used as the starting dose for further up-titration 1 week later. The immunotherapy regimen with QbG10 consisted of six subcutaneous injections at intervals of 1–2 weeks.

Clinical assessment of the patients was made by a physician before inclusion as well as 12, 34 and 48 weeks after starting the treatment. The expected clinical endpoints were increased allergen thresholds in the conjunctival provocation test, reduced allergic and asthmatic symptoms, improved quality of life and a decrease of the weal and flare areas in the skin prick test (SPT). Safety was monitored throughout the study. The study was approved by the local Ethical Review Committee and notified to the Swiss legal agency for therapeutic products (Swissmedic). Informed consent was provided by all participating subjects in writing. (clinicaltrials.gov Identifier: NCT00652223)

Allergen preparation and immunotherapy

The standardized HDM extract used for immunotherapy (Novo-Helisen[®] Depot) contains equal amounts of Der p and Der f allergens adsorbed to alum. Allergen concentrations of 5, 50, 500 and 5000 UT/mL were used. During

Table 1. Outline of the study design

Intervention/analysis	Time (week)	Comment
Conjunctival provocation and skin prick tests	-2	
Self assessment (questionnaires)	-2	Allergy scores
Vaccination 1: Novo-Helisen [®] Depot	1	5, 10 and 20 U
Serum for antibody assessment	1	
Vaccination 2: Novo-Helisen [®] Depot	2	40, 50 and 100 U
Vaccination 3: Novo-Helisen [®] Depot+QbG10 ^a	3	200, 400 and 400 U
Serum for antibody assessment	3	
Vaccination 4: Novo-Helisen [®] Depot+QbG10 ^a	4	3 times 1000 U
Vaccination 5: Novo-Helisen [®] Depot+QbG10 ^a	5	2000, 2000, 3000 U
Vaccination 6: Novo-Helisen [®] Depot+QbG10 ^a	6	5000 U
Serum for antibody assessment	6	
Vaccination 7: Novo-Helisen [®] Depot+QbG10 ^a	8	5000 U
Vaccination 8: Novo-Helisen [®] Depot+QbG10 ^a	10	5000 U
Serum for antibody assessment	10	
Self assessment (questionnaires)	10-12	Allergy scores
Conjunctival provocation, skin prick tests and serum for antibody assessment	12	
Self assessment (questionnaires)	32-34	Allergy scores
Conjunctival provocation, skin prick tests and serum for antibody assessment	34	
Self-assessment (questionnaires)	46-48	Allergy scores
Conjunctival provocation, skin prick tests and serum for antibody assessment	48	

their first two visits, the patients received increasing amounts of the allergen preparations according to the cluster regimen. At their next six visits, the patients received injections of the allergen preparation combined with 300 µg QbG10, which were mixed immediately before the subcutaneous injection. Blood was sampled before, during and after immunotherapy for analysis of allergen-specific antibodies in serum.

Conjunctival provocation

Before treatment, and 12, 34 and 48 weeks after treatment initiation, a conjunctival provocation test was performed using the aqueous Der p and Der f mixed test solution (Allergopharma); the test was not performed during periods of acute conjunctivitis any other ocular symptoms, or anti-allergic therapy, and contact lenses were removed. Starting with 50 µL of the allergen solvent (negative

control; Allergopharma), increasing amounts of the allergen solution were administered into the lower conjunctival sac of left or the right eye, respectively. Allergen doses of 2.5, 25, 250 and 2500 BE/mL were then applied at intervals of 10 min, and conjunctival hyperaemia, tearing, itching, burning and swelling of eyelids were evaluated after 10 min as absent (0 points), mild (1 point), moderate (2 points) or severe (3 points). The outcome measure was defined as the threshold concentration of the allergen solution that produced a total score of 3 or more of a maximum of 15. The allergic reaction was stopped by administration of the ocular antihistamine Opatanol (Alcon GmbH, Mannheim, Germany).

The treatment took place from March to September 2005. The baseline CPT was performed at screening from December 2004 to July 2005. Post-treatment CPT was performed 12, 34 and 48 weeks later, hence between March and October 2005, August 2005 and February 2006 and November 2005 and June 2006, respectively.

Skin reactivity

A quantitative SPT was performed with standard Der p and Der f mixed prick test solutions (Allergopharma) according to EAACI recommendations [27]. Thirty micro-litre of solutions containing 25, 250, 2500 and 25 000 BE/mL extract were applied on the volar forearm. A lancet with a 1 mm point was used to prick the solution into the skin. Histamine dihydrochloride (1 mg/mL) was used as positive control and a mixture of glycerol/saline as negative control (Allergopharma). The test was evaluated after 15 min and considered positive if the weal diameter was at least 3 mm or at least 25% of the weal induced by simultaneous histamine pricking. Weals were outlined on the skin with a pen, blotted onto a cellophane tape and stored on paper. The areas of weals were calculated by planimetry [28].

Patient self-assessment of allergy symptoms and medication

The patients used questionnaires to record the incidence and severity of seven rhinoconjunctivitis symptoms, i.e., runny, congested and itchy nose, sneezing, itchy and red eyes and itchy throat as absent (0 points), mild (1 point), moderate (2 points) or severe (3 points), and a total score was calculated (maximum possible score 21). The records were made before inclusion as well as 12, 34 and 48 weeks after the start of treatment over a 2-week period. For assessment of asthma symptoms during daily life, dry cough and asthma bronchiale were recorded similarly, with a maximum possible score of 6. Similarly, whether (1 point) or not (0 points) rhinitis or asthma affected daily life activities was determined, i.e., daily life symptom annoyance, sleeping disorders, work or school restriction

as well as leisure-time restriction were recorded by the patients (maximum possible score 4). Finally, the patients recorded their use of rescue medications such as antihistamines, steroids and β -agonists.

Determination of antibodies

For the determination of HDM-specific IgE, serum from each patient was prepared and analysed for Der f and Der p-specific IgE with the ImmunoCAP system as described by the provider (Phadia, Uppsala, Sweden).

ELISAs to detect Der f and Der p-specific IgG1, IgG2, IgG3 or IgG4 antibodies were performed according to standard operating procedures on a robotic liquid handling system (Hamilton, Bonaduz, Switzerland). ELISA plates were coated with a 1:1 mixture of Der p and Der f and blocked with Superblock (Pierce, Rockford, IL, USA) and incubated with study sera for 2 h. Anti-human IgG1-, IgG2-, IgG3- or IgG4-specific horse-radish peroxidase conjugate was added for 1 h, and the plates were developed with *o*-phenylenediamine (Fluka, Steinheim, Germany) for 4.5 min and stopped by addition of sulphuric acid. The antibody levels were reported as units, which correspond to the OD at 492 nm value multiplied by factor 1000. A responder was defined as a subject whose post-treatment IgG serum titre was larger than the average baseline titre plus three standard deviations of the triplicates.

Statistical analyses

The sample size and the primary outcome parameters of this study were based on published data on conventional SCIT schemes [25, 29, 30]. The goal of the primary outcome was an increase of the threshold dose in the conjunctival provocation test between baseline and post-treatment assessment by a factor of 10 and a decrease of the weal size by 30%. With 20 study subjects, the immunotherapeutic effects of this size may be detectable with a significance level of $\alpha = 0.05$ and a power of 90%.

All within-group comparisons were calculated with the exact two-tailed Wilcoxon's signed-rank test comparing baseline values with successive values or the Friedman test for more than two related variables.

Results

Study population

Twenty-one patients with self-reported allergic rhinoconjunctivitis were enrolled. One patient withdrew consent for personal reasons after 2 weeks, but was kept in the evaluation for safety. The remaining 12 males and eight females included in the efficacy evaluations had a median age of 34 years (18–56) and a median 8-year (1–40) history of mild to moderate perennial allergy and asthma.

Ten patients were mono-sensitized to Der p or Der f, while 10 were additionally sensitized to other aeroallergens. One patient was of Asian ethnicity, and the remaining 19 patients were of Caucasian ethnicity. One male patient discontinued treatment due to local side-effects and had no post-treatment evaluation. He was included in the efficacy analysis at week 12 with a 'last observation carried forward' approach using his baseline values. At weeks 34 and 48, only the 19 patients available for follow-up were included in the analysis.

Safety and tolerability

The incidence of SIT-associated AE was assessed from the start of the treatment until 2 weeks after the last injection (Fig. 1). Twelve out of 21 study subjects (57.1%) reported a total of 41 AEs, of which 59% were rated as mild, 41% as moderate and none as severe. The most frequent AE was asthma, which was reported by six subjects with seven events, pruritus (three subjects, seven events) and hypersensitivity (three subjects, six events). Fatigue, peripheral oedema and rhinitis were reported by two subjects with two events each. Pyrexia, dizziness and headache were reported by one subject with two events each. Cough, pharyngitis, rhinorrhoea, injection site urticaria, atopic dermatitis, paraesthesia, tachycardia, conjunctivitis and oral pruritus were reported once each. The majority of related symptoms occurred during the dose escalation of the allergen up to week 4 of allergen administration (second dose of QbG10 combination) and thereafter declined despite increasing allergen doses, indicating an early onset of desensitization already during the up-titration phase. No treatment-related AEs were reported following the last dose of QbG10 and HDM allergen extract.

Six systemic allergic reactions required treatment with i.v. corticosteroids and i.v. antihistamines (125 mg

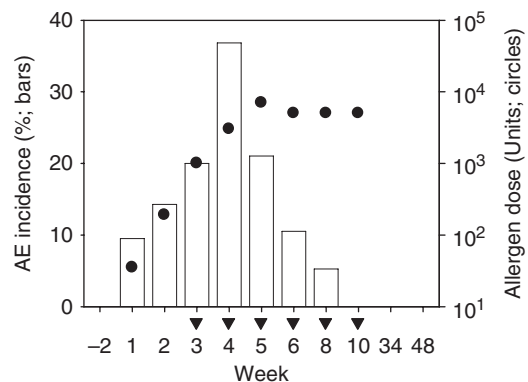


Fig. 1. Incidence of drug-related adverse events. Adverse events (AE) that were considered to be associated with the study treatment were recorded throughout the study (% of subjects with any AE). Closed circles show the amount of allergen applied (right y-axis) and triangles indicate when QbG10 (300 µg) was co-administered ($n = 21$).

Methylprednisolone, Solumedrol[®], Pfizer, Zurich, Switzerland and 2 mg Clemastine, Tavegil[®], Novartis, Basel, Switzerland). Two cases of pruritus were treated with oral corticosteroids and oral antihistamines (100 mg Prednisone, Spiricort[®], Spirig, Egerkingen, Switzerland and 8 mg Acrivastin, Semprex[®], GlaxoSmithKline, Münchenbuchsee, Switzerland). Eight asthmatic reactions required inhalation with corticosteroids and/or bronchodilators (100 µg Salbutamol, Ventolin[®], GlaxoSmithKline, 200 µg Budesonide and 6 µg Formoterol, Symbicort Turbuhaler 200/6[®], AstraZeneca, Zug, Switzerland). All the above treatments were single dose and all the allergic reactions resolved within the 2-h time period of observation, except for one case of asthma that required a second dose of a bronchodilator the next day.

Local reactions at the site of injection were mostly mild and transient, with the highest incidences reported for pain, erythema and swelling after the second or the third dose of QbG10. Three days after the third dose, swelling was observed in 56% of patients, pain in 28%, erythema in 33% and induration in 28%. Local reactions declined despite continued immunotherapy. Three days after the eighth and the last injection, swelling and erythema were observed only in one patient, induration in four patients and pain was no longer reported.

Clinical laboratory parameters including routine serum chemistry, haematology and urinalysis showed few, minor and mostly clinically nonsignificant deviations from normal ranges, and no pattern indicative of an adverse effect of immunotherapy was observed (data not shown). Antinuclear antibody (ANA) titres were either negative or considered not clinically significant if detectable. In particular, there was no switch from negative to clinically significant ANA titres in any subject.

Der f- and Der p-specific immunoglobulin G and immunoglobulin E titres

The IgG subclass titres at week 3 after injection of low doses of allergens were similar to the ones measured in pre-study sera. After 6 weeks of immunotherapy, i.e., after three injections of QbG10 in the presence of HDM allergen extract, titres of allergen-specific IgG subclasses increased and peaked 10–12 weeks after starting SIT (Fig. 2). All patients showed substantial increases in Der f/ Der p-specific IgG1 and IgG4, while the responder rates for IgG2 and IgG3 were 95% and 47%, respectively. Der f- and Der p-specific IgE levels increased slightly during the 10–12 weeks of SIT (Fig. 3). After the end of the treatment, the IgE levels decreased again.

Conjunctival allergen provocation tests

Eleven out of 20 patients reacted during conjunctival provocation with a score ≥ 3 at the second lowest dose

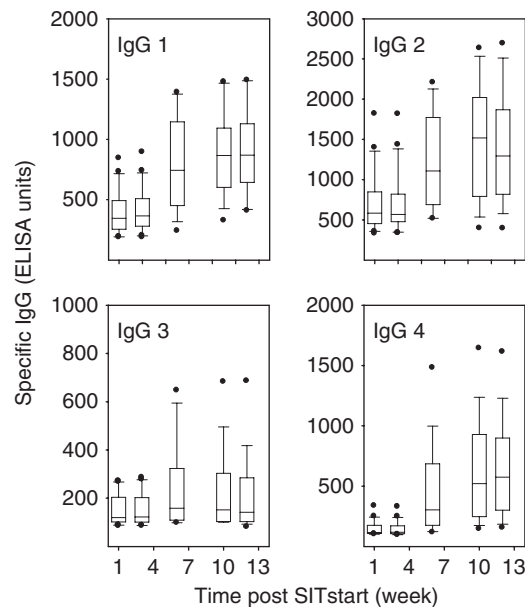


Fig. 2. Der f- and Der p-specific IgG1, IgG2, IgG3, IgG4. Der f- and Der p-specific antibodies were determined by sandwich ELISA from sera collected at the indicated time-points after SIT initiation. Average anti-Der f and Der p titres determined from triplicate analysis of each serum were calculated. The box plots show the median, the 10th, 25th, 75th and 90th percentiles, and outliers.

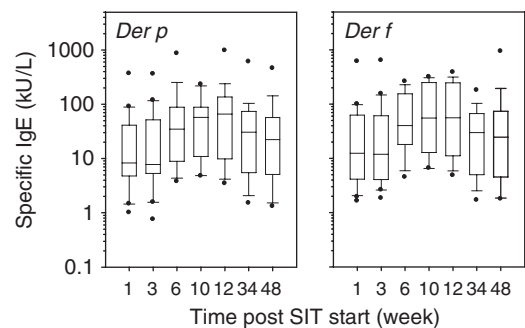


Fig. 3. Der f- and Der p-specific IgE antibody responses. Der f- and Der p-specific antibodies were determined by ImmunoCAP FEIA from sera collected at the indicated time-points after SIT initiation. The box plots show the median, the 10th, 25th, 75th and 90th percentiles, and outliers.

and one patient at the lowest dose before inclusion. At 12 weeks, only two out of 19 patients reacted at the highest dose. The follow-up analysis at 34 weeks revealed that nine patients reacted at the highest and one at the second highest dose. After 48 weeks, only three out of 19 patients reacted at the highest and one patient reacted at the second highest dose (Table 2). The overall increase in allergen tolerance before and after treatment was highly significant ($P < 0.0001$). The median threshold dose was reduced from 25 BE/mL before starting treatment to 25 000 BE/mL ($P < 0.0001$) after 12 weeks, to 2500 BE/mL ($P < 0.0001$) after 34 weeks and to 25 000 BE/mL ($P < 0.0001$) after 48 weeks. Out of 19 patients finishing treatment, one showed a 10-fold tolerance increase, nine

Table 2. Conjunctival provocation test

Time point (weeks)	Number of patients reacting in CPT with threshold concentration (BE/mL):				
	2.5	25	250	2500	25 000*
0	1	11	7	1	0
12	0	0	0	2	17
34	0	0	1	9	9
48	0	0	1	3	15

The study subjects were challenged with increasing doses of a standard HDM allergen by direct ocular application. Allergic symptoms (conjunctival hyperemia, tearing, itching, burning and swelling of eyelids) were recorded by a physician as absent (0 points), mild (1 p.), moderate (2 p.) or severe (3 p.). The threshold concentration was defined as the allergen solution producing a total score of 3 or more of a maximum of 15. The table shows the number of patients with threshold concentration of 2.5, 25, 250, 2500, and 25 000 BE/mL in the CPT performed before and 12, 34 and 48 weeks after initiation of SIT with HDM allergen extract and QbG10.

*If patients did not react to the highest provocation dose of 2500 BE/mL, a threshold value of 25 000 BE/mL was given.

HDM, house dust mite.

Table 3. Recorded symptoms of rhinoconjunctivitis (RcSDL) and asthma (AsSDL) in daily life as well as recorded scores for the daily-life consequences of rhinitis (CoR) and asthma (CoA): median (minimum and maximum)

	Time of measurement			
	Baseline	12 weeks	34 weeks	48 weeks
RcSDL	10.5 (5–17)	1.5 (0–7)	3 (0–12)	2 (0–8)
AsSDL	2 (0–6)	0 (0–3)	0 (0–3)	0 (0–3)
CoR	3 (1–4)	0 (0–3)	1 (0–2)	0 (0–2)
CoA	3 (0–4)	0 (0–4)	0 (0–4)	0 (0–3)

patients showed a 100-fold, eight patients showed a 1000-fold and one patient showed a 10 000-fold tolerance increase. Overall, the median individual increase in allergen tolerance was 100-fold after the treatment.

Symptom scores

Four different symptoms scores were assessed to test the efficacy of the vaccination protocol. A highly significant impact of vaccination on rhinoconjunctivitis symptoms in daily life symptoms was observed (Table 3). The median score was reduced from 10.5 at inclusion to 1.5 two weeks after treatment termination ($P < 0.0001$). The reduction in score was maintained for 48 weeks (median score 2, $P < 0.0001$). Similarly, the score for asthma symptoms in daily life was reduced from 2 at inclusion to 0 after 12 ($P < 0.0001$), 34 ($P = 0.0007$) and 48 ($P < 0.0001$) weeks (Table 3). The median scores for the daily-life consequences of rhinitis (CoR) and of asthma (CoA), i.e., the

implication of rhinitis and asthma on daily-life activity were reduced from baseline levels of 3 (for a maximum score of 4) to 0 as a function of vaccination ($P < 0.0001$; Table 3). After 34 and 48 weeks, the median CoR score was 1 and 0, respectively, both significantly lower than at baseline ($P < 0.0001$). Similarly, the CoA scores remained 0 throughout the assessment period ($P \leq 0.0001$).

At baseline, nine patients had reported intermittent use of allergy medications within the last 2 weeks. After the treatment, only four patients reported intermittent use of their medications within the last 2 weeks before the 12-week, the 34-week and the 48-week visit.

Skin prick test

SPTs to HDM were positive for all patients before the immunotherapy. The median weal area (25th–75th percentiles) was 52 mm² (37–96 mm²) when tested with the 25 000 BE/mL HDM allergen extract. The vaccination protocol caused a significant reduction of the skin reactivity at the highest dose (Fig. 4). The median weal areas were 27 mm² (16–38 mm²), 36 mm² (20–48 mm²) and 39 mm² (20–50 mm²) when tested after 12 ($P = 0.0002$), 34 ($P = 0.0062$) and 48 weeks ($P = 0.0014$), respectively.

Discussion

Despite the high success rate of SCIT [3, 31], only few allergy patients choose to undergo SCIT, mainly because of the time-consuming and laborious procedure. Strategies to improve the efficiency of SCIT include the use of recombinant allergens, allergen derivatives, as well as T- and B-cell epitope-containing peptides, or DNA vaccination [32], which all allow better product standardization than for natural allergen extracts. Moreover, sublingual and oral immunotherapy has so far been proven to be safe, and self-administration makes these methods attractive for the patient [33]. However, the duration of treatment has not been shortened [34]. Much effort has therefore been focused on improving the adjuvant. The traditionally utilized alum was introduced to SIT already 70 years ago [35], has a good safety record and reduces the anaphylactic properties of the injected allergens [36]. However, alum may cause contact sensitivity [37], local granuloma formation [38] and may induce Th2-like immune responses [12]. Hence, new generations of adjuvants are being investigated that favour Th1 responses and allow the differentiation of regulatory T cells. One such adjuvant that is currently undergoing clinical trials is monophosphoryl lipid A [39, 40].

CpG ODNs are also strong inducers of Th1 responses and therefore promising adjuvants in allergen immunotherapy. In mice, co-administration of allergen and CpG ODNs promotes Th1 responses and inhibits IgE production [41]. In sensitized mice, immunotherapy with

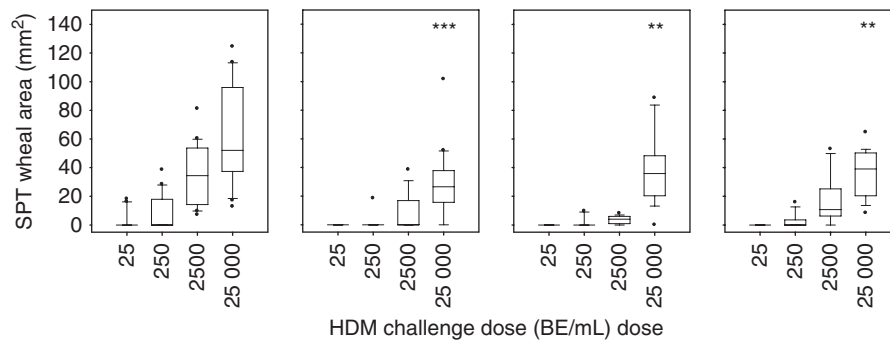


Fig. 4. Skin prick test (SPT). The sensitization status of the patients was tested by their intradermal reactivity to the house dust mite (HDM) allergens using a standard SPT. The wheal diameter was evaluated 15 min after application to the forearm, and the test was considered positive if the wheal diameter was larger than three millimetres or at least 25% of the histamine prick control. The four panels illustrate tests performed before as well as 12, 34 and 48 weeks after initiation of SIT with HDM allergen extract and QbG10. The box plots show the median, the 10th, 25th, 75th and 90th percentiles, and outliers. *P*-values are indicated when SPT values differed from baseline (**P* < 0.01; ***P* < 0.001).

covalently linked allergen and CpG ODN, which allows targeting to the same APC, promotes Th1 responses and decreases allergen-specific IgE [42]. A similar Th1 shift was observed in humans [43–45], although the effects on symptoms have been conflicting [43, 44].

Three distinct types of CpG ODNs have been identified based on their capacity to stimulate APCs. CpG ODN can also be distinguished based on NK cell activation and on IFN- γ production as an indirect effect of platelet-derived growth factor stimulation [46, 47]. While the preferable CpG for allergen SIT due to enhanced IFN- α and IFN- γ production may be of the A-type, the *in vivo* efficiency of A-type CpG ODNs is hampered by their short half-life, unless administered repeatedly [48]. For this reason, CpG ODNs currently used in clinical trials have a phosphorothioate-stabilized backbone.

The present open-label phase I pilot trial evaluated the safety and, in part, the efficacy of HDM-specific immunotherapy by admixing an A-type CpG ODN contained and stabilized in VLPs (QbG10) with the HDM allergen extract. The combination of QbG10 with HDM allergen extract was safe and well tolerated. Most AE observed were of a typical allergic nature and attributed to the injected allergen extract. The AE that may be expected from CpG ODN injection would be flu-like symptoms. None of the treated patients reported such symptoms. Another effect of CpG ODN injection may be leucocytosis. Assessments before as well as 1 and 2 weeks after each injection of QbG10 revealed no significant changes in white blood cell counts. Because TLR9 was found to control ANA production in mice, ANA titres were measured. No elevation was observed.

Yet, the immunotherapeutic regime of QbG10 and HDM allergen extract strongly stimulated protective allergen-specific immune responses, e.g., amelioration of allergy symptoms and increase of HDM-specific IgG antibodies. Because this was an open-label pilot trial, neither a

placebo effect nor an effect of the HDM extract alone, or QbG10 alone can be excluded. However, the extent and speed of the responses were very encouraging. Allergy symptoms recorded at home and upon conjunctival provocation practically disappeared after only 10 weeks of immunotherapy. Typically, comparable improvements are reached after at least 2 years of conventional SCIT [25, 49] or SLIT [50] using allergen extracts alone. To our knowledge, such an increase in allergen tolerance with an almost complete alleviation of allergy symptoms has never been reported when similar cluster regimens for HDM SCIT had been used. Consequently, if the time required to desensitize allergic patients could be reduced from 2–3 years to only 2 months, more patients would be willing to complete the otherwise laborious treatment. Moreover, only one drop-out in 20 patients was recorded, while a major problem of conventional SIT or SLIT trials is the high drop-out rate [49, 50].

It is also possible that the CpG alone had an ameliorating effect on both rhinitis and asthma. As suggested by the hygiene hypothesis, stimulation of innate immunity and Th1 responses by such bacterial products may be able to restore the Th1/Th2 balance. It has been shown in mice that CpG can ameliorate the asthmatic airway response (reviewed in [51]). In humans, the situation is not that clear [52]. The first data in a follow-up placebo-controlled clinical trial programme aiming to disentangle the relative contributions of allergen alone and CpG alone, however, confirm that CpG on its own has an ameliorating effect and thus likely contributed to the effect observed in this study (G. Senti et al. manuscript in preparation).

Also, it is highly unlikely that a confounding effect of other aeroallergens can explain the marked improvement in CPT scores and symptoms. For those 10 patients with additional sensitization to other aeroallergens, CPTs were performed only during symptom-free intervals. An effect of additional pollen allergy due to subclinical irritation of

the mucosa would therefore have been present not only during baseline measurements during December to July but also during the 12 week (March–October) and the 34 week (November–June) follow-up, and can therefore hardly explain the improvement observed in CPT scores. The same holds true for the allergy symptoms, which patients were asked to record during the 2 weeks before their visits.

In closing, this is the first human trial using A-type CpG ODN as an adjuvant. Subcutaneous immunotherapy of HDM-allergic patients with CpG in VLP (QbG10) admixed with allergen was safe. A nearly complete alleviation of allergy symptoms was observed after only 10 weeks of treatment. These highly encouraging observations call for larger placebo-controlled clinical trials. Because only a low percentage of allergy patients choose to receive the presently existing but laborious allergen-specific immunotherapies, use of QbG10 as an adjuvant could reduce the number of necessary injections, thus making immunotherapy a more attractive alternative in treating allergy and asthma.

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