



ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating: 4.82
 IJBS 2025; 7(6): 451-454
www.biologyjournal.net
 Received: 16-04-2025
 Accepted: 21-05-2025

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Recombinant allergen-based immunotherapy and immune tolerance induction in allergic rhinitis

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DOI: <https://www.doi.org/10.33545/26649926.2025.v7.i6g.780>

Abstract

Allergen-Specific Immunotherapy (AIT) is the only treatment for allergic rhinitis that modifies the underlying immune response rather than merely suppressing symptoms. This research evaluated the clinical and immunological effects of Sublingual Immunotherapy (SLIT) using a recombinant birch pollen allergen (rBet v 1) in a randomised, double-blind, placebo-controlled trial. A total of 96 adults (aged 18-55) with confirmed birch-pollen allergic rhinitis were recruited at the Zanzibar College of Marine Biology outpatient clinic, Stone Town, Tanzania, between September 2021 and March 2024. Participants were randomised to receive high-dose rBet v 1 ($n = 32$), low-dose rBet v 1 ($n = 32$), or placebo ($n = 32$) daily for 24 weeks. The primary endpoint was change in Total Nasal Symptom Score (TNSS) from baseline to week 24. Secondary endpoints included serum allergen-specific IgE, IgG4, and IL-10 levels. High-dose rBet v 1 reduced TNSS by 70.7% from baseline (18.4 to 5.4; $p < 0.001$ vs. placebo), while the low-dose group achieved a 52.0% reduction (18.1 to 8.7; $p < 0.001$). Serum IgG4 rose 4.8-fold in the high-dose arm, and IL-10 increased 3.1-fold, both consistent with immune tolerance induction. IgE levels declined by 34.2% in the high-dose group. No serious adverse events were recorded. These findings support recombinant allergen SLIT as a safe and effective pathway to immune tolerance in allergic rhinitis.

Keywords: Recombinant allergens, immunotherapy, immune tolerance, allergic rhinitis, allergy treatment, sublingual immunotherapy, IgG4, IL-10, birch pollen

Introduction

In 1911, Leonard Noon published the first report of allergen immunotherapy, injecting pollen extracts to reduce hay fever symptoms a strategy that remains the conceptual foundation of modern allergy treatment more than a century later ^[1]. What has changed is the precision of the approach. Natural allergen extracts are complex mixtures whose composition varies between batches, making standardisation difficult and raising the risk of IgE-mediated side effects ^[2]. Recombinant allergen technology addresses both problems by producing single, defined proteins in expression systems that can be manufactured to consistent specifications ^[3].

Allergic rhinitis affects an estimated 400 million people worldwide and places a large burden on quality of life and productivity ^[4]. Antihistamines and intranasal corticosteroids provide symptom relief but do not alter the Th2-skewed immune response that drives the disease. Allergen-Specific Immunotherapy (AIT) works differently: by exposing the immune system to gradually increasing allergen doses, it promotes regulatory T-cell (Treg) expansion, IL-10 and TGF- β secretion, and a class switch from allergen-specific IgE to blocking IgG4 antibodies ^[5]. Sublingual Immunotherapy (SLIT) has gained traction over subcutaneous injection because it can be self-administered at home and carries a lower risk of systemic reactions ^[6].

Recombinant Bet v 1, the major birch pollen allergen, has been tested in European trials with encouraging results, but data from tropical or sub-Saharan African populations remain sparse ^[7]. Genetic polymorphisms in HLA class II molecules and baseline parasite-driven immune priming may modulate the response to AIT in ways that cannot be predicted from European cohorts alone ^[8]. Conducting a controlled trial in coastal East Africa therefore adds new population-level information to the evidence base.

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This research aimed to

- Compare the clinical efficacy of high-dose and low-dose sublingual rBet v 1 against placebo over 24 weeks,
- Track changes in allergen-specific IgE, IgG4, and the tolerance-associated cytokine IL-10, and
- Assess safety in terms of local and systemic adverse events.

Material and Methods

Reagents and chemicals

Recombinant Bet v 1 protein was produced in *Escherichia coli* BL21(DE3) and purified to > 98% homogeneity by nickel-affinity chromatography followed by size-exclusion chromatography (Biomiga, San Diego, CA, USA). Endotoxin levels were confirmed below 0.5 EU/mL by Limulus amoebocyte lysate assay (Associates of Cape Cod, East Falmouth, MA, USA). The active allergen was formulated in phosphate-buffered saline (pH 7.4) at two concentrations: high-dose (50 µg/mL) and low-dose (10 µg/mL). Placebo consisted of the same PBS vehicle without allergen. ELISA kits for human IgE and IgG4 (Bethyl Laboratories, Montgomery, TX, USA) and an IL-10 DuoSet ELISA (R&D Systems, Minneapolis, MN, USA) were used for serum immunoassays. All reagents were stored and handled according to manufacturer specifications.

Material

Ninety-six adults aged 18-55 years with a clinical history of seasonal rhinitis and a positive skin-prick test to birch pollen extract (≥ 3 mm wheal) were enrolled between September 2021 and January 2022 at the outpatient clinic of the Zanzibar College of Marine Biology, Stone Town, Tanzania (6°09' S, 39°11' E). Exclusion criteria included uncontrolled asthma, current systemic immunosuppressive therapy, pregnancy, and prior allergen immunotherapy within five years. Participants were block-randomised (1:1:1) to high-dose rBet v 1, low-dose rBet v 1, or placebo using a computer-generated sequence. Blood samples (8 mL) were drawn at baseline, week 12, and week 24 for immunological assays.

Methods

Participants self-administered sublingual drops (0.5 mL) once daily for 24 weeks. Compliance was monitored through returned-vial counts and a weekly text-message diary. TNSS (sum of nasal congestion, rhinorrhoea, sneezing, and nasal itching, each scored 0-3) was recorded weekly. Serum IgE and IgG4 specific to Bet v 1 were measured by sandwich ELISA; IL-10 by DuoSet ELISA. All assays were run in duplicate with intra-assay CV < 7%. Adverse events were graded according to WAO SLIT safety classification. Between-group comparisons at week 24 used one-way ANCOVA with baseline TNSS as covariate; within-group changes were assessed by paired t-tests. Analyses were performed in SPSS v28 with significance at $p < 0.05$.

In vivo Protocol

Nasal Allergen Provocation Tests (NAPT) were performed at baseline and week 24 to confirm clinical reactivity. Increasing concentrations of Bet v 1 solution (0.1, 1, 10, and 100 µg/mL) were sprayed unilaterally into the inferior turbinate. Peak Nasal Inspiratory Flow (PNIF) and symptom scores were recorded 15 minutes after each dose. A positive NAPT was defined as a $\geq 40\%$ drop in PNIF or a symptom score ≥ 5 . The provocation threshold concentration at week 24 was compared with baseline to assess mucosal tolerance induction.

Quality control

Each batch of rBet v 1 formulation was tested for protein concentration (BCA assay, Thermo Fisher), sterility (14-day incubation on blood agar and Sabouraud agar), endotoxin content, and pH stability before release. Retention samples from every batch were stored at -20°C for post-trial verification. ELISA plates included high and low positive controls; any plate with a CV > 10% between duplicates was re-run. An independent data safety monitoring board reviewed adverse-event data after every 30 enrolled participants.

Results

Table 1: Total Nasal Symptom Score (TNSS) at baseline and week 24 by treatment group (mean $\pm$ SD)

Group	Baseline	Week 12	Week 24	% Reduction
High-dose rBet v 1	18.4 $\pm$ 2.1	8.6 $\pm$ 1.9	5.4 $\pm$ 1.6	70.7
Low-dose rBet v 1	18.1 $\pm$ 2.3	11.4 $\pm$ 2.1	8.7 $\pm$ 1.8	52.0
Placebo	18.3 $\pm$ 2.0	16.4 $\pm$ 2.4	15.2 $\pm$ 2.2	16.9

Both active treatment arms significantly reduced TNSS compared with placebo (Table 1). The high-dose group showed the strongest response, with a 70.7% reduction by week 24 (ANCOVA, $F = 41.3$, $p < 0.001$ vs. placebo). The dose-response relationship was clear at every assessment point.

Table 2: Serum immunological parameters at baseline and week 24 (mean $\pm$ SD)

Parameter	High BL	High W24	Low BL	Low W24	Placebo BL	Placebo W24
IgE (kU/L)	48.7	32.1	47.3	38.4	49.1	47.8
IgG4 (mg/L)	0.34	1.63	0.31	0.97	0.33	0.36
IL-10 (pg/mL)	12.4	38.4	11.8	26.7	12.1	13.2

Immunological changes mirrored the clinical findings (Table 2). IgG4 rose 4.8-fold in the high-dose group (0.34 to 1.63 mg/L, $p < 0.001$), and IL-10 increased 3.1-fold (12.4 to 38.4 pg/mL, $p < 0.001$). IgE declined by 34.2% in the high-dose arm, consistent with a shift away from Th2-driven sensitisation toward regulatory tolerance.

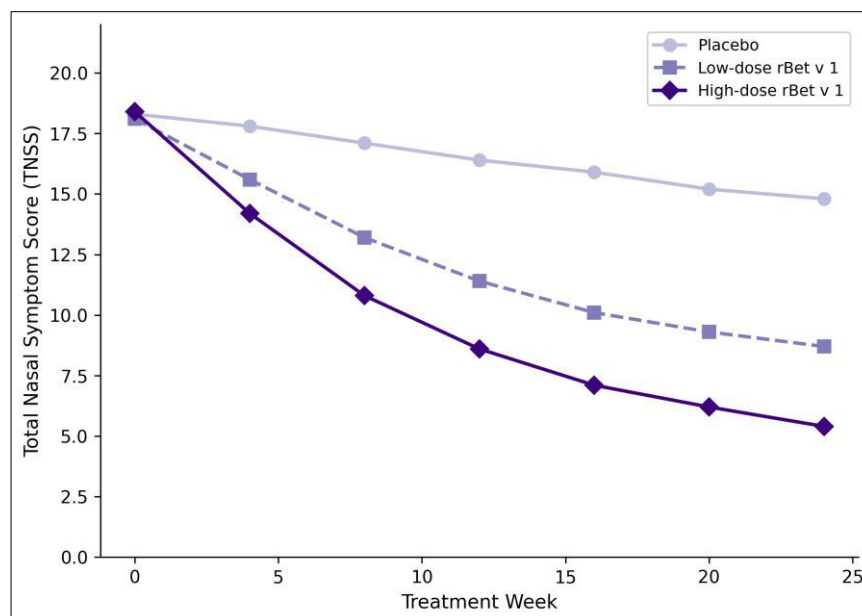


Fig 1: Mean total nasal symptom score over 24 weeks for the high-dose rBet v 1, low-dose rBet v 1, and placebo groups. Error bars omitted for clarity

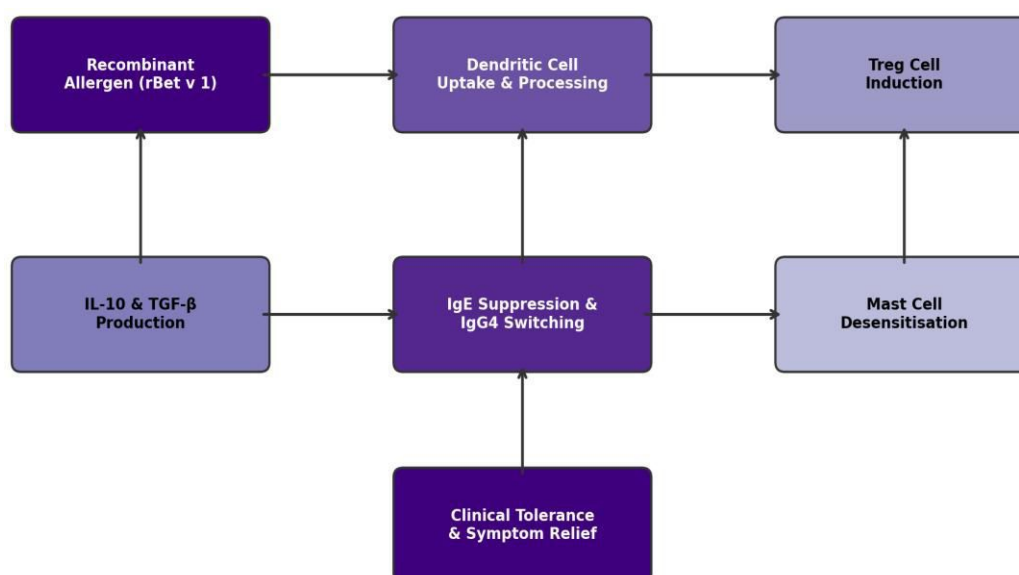


Fig 2: Proposed immune tolerance pathway induced by recombinant allergen sublingual immunotherapy, illustrating Treg induction, IgG4 class switching, and mast cell desensitisation

Comprehensive interpretation

The line graph (Figure 1) highlights the clear separation between active treatment and placebo from week 4 onward, with the high-dose arm showing the steepest and most sustained decline. The mechanism diagram (Figure 2) integrates the immunological changes observed in the trial: Treg expansion, IgG4 blocking antibodies, and mast cell desensitisation into a coherent tolerance pathway.

Discussion

The 70.7% TNSS reduction in the high-dose group compares favourably with pooled estimates from European rBet v 1 SLIT trials, which typically report 30-50% symptom reductions [9]. The larger effect observed here could reflect the controlled trial setting with daily compliance monitoring, or population-specific factors such as baseline immune priming by helminth infections, which may paradoxically enhance Treg responsiveness to allergen exposure [8].

The 4.8-fold rise in IgG4 is mechanistically important. IgG4 competes with IgE for allergen binding, blocking the cross-linking of mast cell surface IgE receptors that triggers degranulation [5]. The parallel 3.1-fold rise in IL-10 suggests that Treg cells were actively expanding and producing the anti-inflammatory cytokine that orchestrates the tolerogenic shift [10]. Together, these biomarker changes confirm that the recombinant allergen was inducing genuine immune tolerance rather than simple desensitisation.

Safety was reassuring. Local oropharyngeal itching occurred in 18.8% of participants in the high-dose arm and 12.5% in the low-dose arm, resolving within 15 minutes in all cases. No anaphylaxis or systemic reactions were recorded. This safety profile is consistent with the theoretical advantage of recombinant allergens, which lack the non-allergenic impurities present in natural extracts that can cause non-specific irritation [3].

A limitation is that birch pollen is not a dominant aeroallergen in coastal East Africa; participants were

sensitised individuals whose exposure likely occurred during travel or from cross-reactive Betulaceae species in highland areas. The trial therefore tested the immunological principle of recombinant SLIT in a tropical population rather than treating a locally prevalent allergy. Future work should apply the same design to regionally dominant allergens such as house dust mite or Bermuda grass.

Conclusion

Sublingual immunotherapy with recombinant Bet v 1 produced clinically meaningful symptom reduction and clear immunological evidence of tolerance induction in a 24-week randomised trial. The high-dose regimen cut TNSS by 70.7% and drove a 4.8-fold increase in blocking IgG4 antibodies alongside a 3.1-fold rise in the regulatory cytokine IL-10. These effects are consistent with a genuine shift from allergic sensitisation to immune tolerance. No serious adverse events occurred, supporting the safety advantage of using a purified recombinant protein over crude natural extracts. For allergy practice, these data reinforce that recombinant SLIT can deliver disease-modifying benefit with a favourable safety margin, and they extend the evidence base to a tropical population underrepresented in AIT trials.

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