(1) 小中学生のアレルギー性鼻炎の実態に関する調査

A. 研究目的

アレルギー性鼻炎の罹患率は近年増加傾向にあり、およそ全国民の 1/3 が罹患していると推測されている。このようにアレルギー性鼻炎は国民病といっていいほど罹患率が高いのみでなく発症年齢も低年齢化しており、学校生活や学業にも支障を来すことが少なくない。しかしながら、週日は授業や課外活動等で忙しく、また急性発作などがある喘息などと異なり緊急性が乏しいことからアレルギー性鼻炎をもつ生徒は医療機関を必ずしも受診していない可能性がある。このような場合には適切なアレルギー性鼻炎の治療を受けていないことも考えられる。そこで①平成 23 年度は中学生を、②平成 24 年度は小学生を、対象にアレルギー性鼻炎の治療法とその効果とを明らかにすることを目的とした。

B. 研究方法

千葉市内の①６カ所の中学校（総生徒数4880名)、10カ所の小学校(総生徒数8359名)に依頼し、毎年全国の学校で行われている保健調査票にて医師からアレルギー性鼻炎の診断を受けている①中学生とその保護者/②小学生とその保護者に①平成23年10月（中学生対象)、②平成24年10月（小学生対象）にアンケート調査を行った。調査項目は、性別、鼻症状の出現時期、検査で判明しているアレルゲン、最近１年間の病院・薬局からの薬物療法および健康食品・民間療法の有無、治療薬の内容、免疫療法・代替医療への評価、今までの治療法に対する不満点、今後の治療法への期待、などである。

（倫理面への配慮）

本研究はアンケート調査のみであり、また匿名で個人情報の保護に関しても問題ないものと考えられる。

C. 研究結果

1) ①中学生969名（男児543名、女児420名、性別未記載6名)、②小学生1517名（男児848名、女児669名）から解析可能なアンケートを回収した。鼻症状の出現時期は①中学生：2-5年前が35%、6-10年前が40%、10年以上前が21%、②小学生：1年前が4%, 2-3年前が34%、4-6年前が44%、7年以上前が23%と大部分が幼稚園から小学校低学年の頃に発症していた。

2) 症状のある時期では、①中学生：通年性が42%、季節性が55%であり、②小学生：通年性が45%、季節性が54%であった。検査で陽性になっているアレルゲンについては①中学生：ダニ単独17%、スギ単独19%、ダニ、スギ両者34%、不明30%であった。②小学生:ダニ単独22%、スギ単独22%、ダニ、スギ両者36%、不明20%であった。

3) 直近１年間にアレルギー性鼻炎の治療は①中学生：病院を受診した児は65%、薬局・市販品などで治療薬を購入した児は25%、未治療が20%、②小学生：病院を受診した児は85%、薬局・市販品などで治療薬を購入した児は10%、未治療が10%であった。複数回答であるために病院受診と薬局で市販薬を購入した児も存在したが（10%)、①およそ80%の中学生②およそ85%の小学生は何らかの治療を必要としたと考えられる。本調査では、健康食品などを利用した児は数%であり、治療の大部分が薬物と思われた。服薬内容としては、①中学生：病院受診者では90%が内服薬を、60%が点鼻薬を、40%が点眼薬を処方され、②小学生：病院受診者では80%が内服薬を、50%が点鼻薬を、40%が点眼薬を処方されていた。

4) 使用薬剤の効果に対する評価は、①中学生②小学生ともに病院受診者の2/3が有効と回答した。代替医療などの効果を感じているのは1/4程度であり、医療機関を受診した児に比較すると明らかに低かった。

5) ②平成24年小学生を対象としたアンケートでは免疫療法についての質問項目があったが、全体の2%が免疫療法治療経験あり、38%が治療経験なしと返答した。60%がこの質問に未回答であり、免疫療

法の認知度が低いと予測された。

6) これまでのアレルギー性鼻炎の治療（主に薬物療法）には①中学生②小学生ともに半数が大きな不満がないが、半数は不満があると回答していた。不満の理由としては、治療効果が乏しいと答えたのは①中学生26.3%、②小学生25.9%であり、費用（中学生：28.5%、小学生：25.4%）や通院が面倒（中学生31.8%、小学生28.3%）といった理由のほうがより多かった。一方、眠気などが困ると答えた児は中学生18%、小学生12%であり、およそアレルギー性鼻炎患者の1/5～1/10は薬物の副作用により学校生活に支障を来している可能性がある。

7) 今後希望する治療としては、病院受診しての薬物療法がもっとも多いのは当然だが(中学生・小学生ともに50%)、免疫療法が中学生10%、小学生およそ30%であった。

D. 考察

千葉市のアレルギー性鼻炎を有する中学生・小学生を対象としたアンケート調査から、通年性アレルゲンであるダニよりもむしろ花粉をアレルゲンとする鼻炎が多いことが示唆された。また大部分が幼稚園から小学校低学年の頃に発症しており、大部分が薬物療法を必要としていた。今後、発症や重症化の予防を考えると標的となる年代は小学生以下であると考えられる。現在の薬物療法等に満足する児は約半数であり、今後は従来の薬物療法主体の治療のみでなく、薬物療法以外の治療を望む患者が多いことを示している。しかしながら、免疫療法（減感療法）に関する希望・質問からは本治療法の認知がまだ低い可能性も考えられる。根本的な治療法であり、また発症予防にも効果がある可能性が示されている免疫療法の普及を図るべきと考える。

E. 結論

千葉市内のアレルギー性鼻炎患者およそ1000名のアンケート調査から、1）大部分の児はなんらかの治療を必要としている、2）病院での薬物療法をきちんと受けることが市販の治療薬よりも有効である、3）半数の患者は現在の治療法には満足しておらず、廉価で頻回の通院治療の必要性のない他の治療法を期待している、4）免疫療法はエビデンスもあり今後の治療として期待されるが認知度はまだ高くなく適切な情報提供が必要である、と考えられた。

G. 研究発表

1. 論文発表

なし

2. 学会発表

1. 森田慶紀、下条直樹、中野泰至、井上祐三朗、有馬孝恭、河野陽一、岡本美孝　千葉市内中学生を対象とするアレルギー性鼻炎の治療法とその効果に関するアンケート調査　第24回日本アレルギー学会春季臨床大会　大阪　2012年5月12日

2. 森田慶紀、下条直樹、千葉浩輝、中野泰至、井上祐三朗、有馬孝恭、河野陽一、岡本美孝アレルギー性鼻炎の小学生患者の治療法と今後の課題：アンケート調査より第25回日本アレルギー学会春季臨床大会　横浜　2013年5月11日

H. 知的財産権の出願・登録状況（予定を含む。）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

(2)免疫療法に関する小児科医の意識に関する研究

A. 研究目的

アレルギー性鼻炎の罹患率は近年増加傾向にあり、およそ全国民の1/3が罹患していると推測されている。このようにアレルギー性鼻炎は国民病といっていいほど罹患率が高いのみでなく発症年齢も低年齢化しており、学校生活や学業にも支障を来すことが少なくない。薬物療法は有効であるが対症療法に過ぎず、根本的治療法である免疫療法の普及が望まれてきた。しかしながら、わが国で従来から行われている皮下注射に

よるダニやスギの免疫療法は痛みや煩雑さが妨げとなって小児のアレルギー性鼻炎の治療法としては広く行われてはいない。近年、欧米を中心に舌下免疫療法が保険診療として行われており、わが国でも近々吸入アレルゲンに対する舌下免疫療法が認可される予定である。しかしながら、アレルギー性鼻炎を診療している第一線の小児科医の舌下免疫療法に対する認知はまだ高くない可能性がある。そこで、本研究では、実地医家を中心とする小児科医のアレルゲン舌下免疫療法に対する意識調査を目的とした。

B. 研究方法

千葉県小児科医会会員医師（総数450名）に郵送でアンケートを送付し調査を行った。調査、質問項目は、以下の9つである。専門科、勤務先、医師経験年数、アレルギー専門医資格の有無、現在のスギ花粉症の薬物治療に対する患者満足度、皮下注射による免疫療法の経験の有無、スギ舌下免疫療法への関心、舌下免疫療法の実施への対応、免疫療法を実施する医師の資格について、である。

（倫理面への配慮）
本研究はアンケート調査のみであり、また匿名であり、個人情報の保護に関しても問題ないものと考えられる。

C. 研究結果

207名の会員から解析可能なアンケートが回収された。このうち、
1) 90.8%が小児科、10.1%が内科であった（重複も含む）。(図1)
2) 勤務先はクリニックが64.3%で、病院が32.8%、その他が2.9%であり、実地医家が2/3を占めていた。（図2)
3) 経験年数では21年以上が86.5%であり、11年から20年以下が11.1%であり、以前に皮下注射免疫療法の経験がある医師も多いと考えられた。（図3)
4) 83.6%は非アレルギー専門医の資格を持っていなかった。（図4)
5) 63.8%の医師が現行の薬物療法に患者は満足していないと考えていた。(図5)
6) スギ舌下免疫療法に対しては62.3%の医師が関心がある、11.6%が存在は知ってはいるが関心がない、24.2%が知らないと回答した。（図6)
7) 舌下免疫療法を自ら行う希望のない（あるいは不明な）医師はおよそ30%であった。一方で、18.5%がぜひ実施したい、51.2%が場合によっては自分での実施を考えると回答していた。すなわち、およそ70%の医師は自ら実施することを考えていた。（図7)
8) 舌下免疫療法に関心を持つ医師（129名）の52.7%が、非専門医でも講習を受ければ実施して良いと回答した。一方で31%は、専門医が行うべきと回答した。(図8)

D. 考察

千葉県小児科医会会員医師（小児科医がおよそ9割）に対するアンケート調査の結果から、
およそ7割の医師はスギ舌下免疫療法に関心があり、実地医家の非アレルギー専門医にもスギ舌下免疫療法は支持されて施行される可能性が高いと思われる。
舌下免疫療法は皮下注射法に比較して安全性ははるかに高いと考えられるが、その適応、副作用などを適切に理解した上での施行が望ましい。そのためには耳鼻科、アレルギー科などの専門医による講習等を十分に行う必要があると思われる。また、患者に対しても舌下免疫療法についての情報提供を行うことが本治療法の安全で有効な施行のために望まれる。

E. 結論

千葉県小児科医会会員医師に対するアンケート調査の結果、スギ舌下免疫療法はアレルギーを専門としない一般小児科医の多くが施行を希望する可能性が高いことが明らかとなった。今後、舌下免疫療法の適正な施行の点からも一般医師ならびに患者への情報提供が必要と思われる。

F. 研究発表

1. 論文発表
なし

2. 学会発表
なし

G. 知的財産権の出願・登録状況(予定を含む)
1.特許取得
なし
2.実用新案登録
なし
3.その他

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（分担）研究報告書

# スギ花粉症に対する早期介入の効果と効果評価に関する研究

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**研究要旨**
スギ花粉症の早期介入効果を検討する目的で、スギ花粉症に対する皮下免疫療法が気管支喘息など他のアレルギー疾患の新規発症を予防しうるのか検討した。さらにスギ花粉症における最小持続炎症の検出を試みた。また臨床的に意味のあるQOLスコアの最小変動値（Minimal clinically important difference: MCID）を算出した。免疫療法を行った患者の内、6年間のフォローアップでは4.9%にのみ気管支喘息の新規発症がみられた。スギ花粉誘発無発症者でも鼻汁中ECPの上昇を認めた。5シーズンを通じた総QOLスコアの平均MCIDは6.804となった。以上より、スギ花粉症に対する皮下免疫療法は新規喘息発症を予防する可能性、スギ花粉症においても最小持続炎症が存在すること、JRQLQにおいて総QOLスコアで6.8の変化は臨床的に意味のある差と思われた。

## A. 研究目的

アレルギー疾患に対する免疫療法や薬物治療による早期介入の重要性が指摘されているがスギ花粉症では未明な点が多い。今回我々は、スギ花粉症に対する早期介入の効果と評価を解析する目的で以下の検討を行った。すなわち、①スギ花粉症に対する皮下免疫療法の気管支喘息など他のアレルギー疾患の新規発症の予防効果、②スギ花粉症における最小持続炎症の検出、③臨床的に意味のあるQOLスコアの最小変動値（Minimal clinically important difference: MCID）を算出、について検討した。

## B. 研究方法

①標準化スギ花粉エキスを用いた皮下免疫療法を行ったスギ花粉症患者のうち、2005年～2006年に維持療法を施行した患者44例を対象とした。2011年4月に面接あるいは電話調査を行い、気管支喘息やスギ花粉症以外のアレルギー性鼻炎を新規に発症したか問診した。②スギ花粉の非飛散期である2012年8月に、通年性鼻炎を有さないスギ花粉症患者を対象に、スギ花粉エキス付着ディスクによる連続鼻粘膜誘発反応を行った。誘発5分後の発症の有無を観察した。さらに鼻汁中ECP濃度を比較した。③2009年～2013年に実施した6件の臨床試験のJRQLQデータを基に、フェーススケールの1変動に応じた総QOLスコアの変化値を算出した。

（倫理面への配慮）
被験者に対しては学術的な意義について十分な説明を行い、同意・協力が得られた上で行った。

## C. 研究結果

①フォローアップのできた41例（93.2%）のうち、2例（4.9%）にのみ気管支喘息の新規発症がみられた。また新規に通年性鼻炎を合併した患者はおらず、新規の季節性鼻炎を発症したものは41例中2例(4.9%)であった。気管支喘息を新規に発症した群と対照群との間に年

齢、性、治療前血清総IgE量、治療前スギ特異的IgE抗体価、維持抗原量、維持期間での有意な差はみられなかった。②1日目の誘発反応では、20名中5名（25%）が発症した。1日目誘発の陽性者と陰性者を比較すると、2日目対照ディスク中のECP濃度には2群間で差を認めず、また1日目誘発陰性者であっても1日目対照ディスクと比較して2日目対照ディスク中のECP濃度は有意に亢進した。③フェーススケールの１変動に応じた総QOLスコアの変化値を算出した。2009年でのMCIDは10.469であった。2010年は2件の臨床試験を行ったが、MCIDはそれぞれ6.026および5.441であった。2011年は6.396、2012年は6.953、2013年は5.540であった。これらの総QOLスコアの平均MCIDは6.804となり、1項目当たりでは0.400となった。試験方法の違いや実薬とプラセボ薬でのMCID値に有意な差を認めなかった。さらに、総花粉飛散数とMCID値との間には有意な相関関係を認めなかった。

D. 考察

①これまでに、アレルギー性鼻炎に対する皮下免疫療法が喘息など他のアレルギー疾患の発症を予防することを示した報告がある。例えばPAT-study（Moller C, et al. J Allergy Clin Immunol 2002）では、喘息のない小児花粉症患者を対象とし、3年間の免疫療法の有無による治療終了2年後の喘息発症率を比較したところ、免疫療法を施行しなかった群での喘息発症率（44.4%）に比較して免疫療法群では喘息発症率が24.1%と半減することが示されている。今回の検討では、PAT-studyなどとも比較して喘息の新規発症率はごく軽度であり、スギ花粉症に対する免疫療法は喘息の新規発症を予防する効果が期待できることが示唆された。②海外では、発症に要する抗原濃度の1/100であっても鼻粘膜誘発によって鼻汁中ECP濃度が亢進することが報告されている（Roquat A, et al. 1996）。今回の結果では、誘発陰性、すなわち未発症であっても鼻汁中ECP濃度が亢進することが明らかとなり、スギ花粉症においても最小持続炎症が確認された。③国際的なアレルギー性鼻炎のQOL調査票であるRQLQに関しては包括的質問票をアンカーとしたMCIDが算出され、1項目あたり約0.5のQOLの差は臨床的に有意義ということが報告されている（Juniper EF, et al. J Allergy Clin Immunol 1996）。RQLQとJRQLQは項目数や尺度に違いがあるが、ほぼ同様のMCIDを示すことが明らかとなった。

E. 結論

G. 研究発表

1. 論文発表


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H. 知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得
なし

2. 実用新案登録
なし

3. その他

研究成果の刊行に関する一覧表

雑誌

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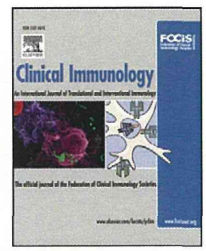

# Increase of regulatory T cells and the ratio of specific IgE to total IgE are candidates for response monitoring or prognostic biomarkers in 2-year sublingual immunotherapy (SLIT) for Japanese cedar pollinosis

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**KEYWORDS**
Allergic rhinitis;
Biomarker;
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Regulatory T cell;
Sublingual immunotherapy

**Abstract** The aims of this study were to examine the therapeutic effects of sublingual immunotherapy (SLIT) and to identify potential biomarkers that would predict the therapeutic response in a randomized, double-blind, placebo-controlled clinical trial. The trial was carried out over two pollinosis seasons in 2007 and 2008. Carry-over therapeutic effects were analyzed in 2009. SLIT significantly ameliorated the symptoms of pollinosis during the 2008 and 2009 pollen seasons. Cry j 1-specific cytokine production in a subgroup of patients with mild disease in the SLIT group was significantly attenuated. The ratio of specific IgE to total IgE before treatment correlated with the symptom-medication score in the SLIT group in 2008. Patients with increased

*Abbreviations:* DBPC, double-blind, placebo-controlled; ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunospot assay; iTreg, induced regulatory T cells; ITT analysis, intention-to-treat analysis; JAU, Japanese allergy unit; N.S., not significant; OT analysis, on-treatment analysis; PBMCs, peripheral blood mononuclear cells; RAST, radioallergosorbent test; SLIT, sublingual immunotherapy; SMS, symptom-medication score; Treg, regulatory T cells; QOL, quality-of-life.

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Cry j 1-iTreg in the SLIT group had significantly improved QOL and QOL-symptom scores. In summary, the specific IgE to total IgE ratio and upregulation of Cry j 1-iTreg are candidates for biomarker of the clinical response to SLIT.


## 1. Introduction

Japanese cedar (*Cryptomeria japonica*) pollinosis is a common allergy in Japan, with a prevalence estimated to be 26.5% in a nationwide survey conducted in 2008 [1].

A 2000 Japanese allergy unit (JAU) sample of standardized extract from Japanese cedar pollen is the only available allergen for subcutaneous and sublingual immunotherapy (SLIT) against pollinosis in Japan. The 2000 JAU extract contains 1.5 to 4.2 μg of the major allergen, Cry j 1 [2]. The common monthly cumulative dose for SLIT is 8000 JAU, which contains approximately 10 μg of Cry j 1. This maintenance dose is 200-fold higher than that used in traditional subcutaneous immunotherapy using 0.2 ml of a 200 JAU/ml extract, which contains approximately 50 ng of Cry j 1. Despite using a low dose of the major allergen compared with that in European trials, positive effects on pollinosis have been shown in randomized double-blind, placebo-controlled (DBPC) studies, in which SLIT significantly ameliorated the symptom score, symptom-medication score (SMS), and quality-of-life (QOL) score [3,4].

SLIT induces Cry j 1-specific IgG4 production and attenuates the seasonal increase in the number of Th2 cells specific to epitopes from Cry j 1 and Cry j 2 [3]. Involvement of antigen-specific Tr1 cells or regulatory T cells (Treg) in the therapeutic mechanism has also been suggested [5,6]. We previously found that SLIT increased the levels of Cry j 1-specific induced Treg cells (Cry j 1-iTreg; $IL10^+Foxp3^+$ cells in $CD25^+CD4^+$ leukocytes) and that the increase in Cry j 1-iTreg after the pollen season may serve as a response monitoring biomarker that correlates with a positive therapeutic effect based on the QOL-symptom score and distinguishes responders from non-responders after SLIT [6].

In this report, we examined the reproducibility of the positive therapeutic effects and safety of SLIT and upregulation of iTregs as a response monitoring biomarker, with the goal of confirming our previous results in a larger randomized DBPC study. Therefore, the safety and clinical effect of SLIT for Japanese cedar pollinosis were used as the primary endpoint, and carry-over effects, immunological changes, and biomarkers for a positive clinical effect induced by SLIT were secondary endpoints.

## 2. Materials and methods

### 2.1. Study population

The study was conducted as a randomized, DBPC, parallel-group, single center trial in subjects with Japanese cedar pollinosis. This study was performed for two pollen seasons between September 2006 and May 2008, with follow-up in the pollen season in 2009. We recruited 130 participants in September 2006. Diagnosis of Japanese cedar pollinosis was based on clinical history and the presence of IgE specific to Japanese cedar pollen of at least class 2 (CAP-RAST method, Phadia, Tokyo, Japan). Participants with a history of immunotherapy or a diagnosis of asthma, or those who were pregnant, were excluded from the study. Patients who suffered seasonal or chronic rhinitis that required medical treatment were also excluded.

A total of 103 patients were eligible for the study, and all had moderate or severe symptoms in the previous pollen season [7]. We anticipated that some participants in the SLIT group would drop out from the study due to side effects and we planned to evaluate the risk of mild or severe side effects due to the vaccination. Therefore, we randomly divided the patients into treatment (SLIT) and placebo groups with a ratio of 6:4 according to the table of random numbers prepared by the Department of Pharmacy at Chiba University Hospital (Fig. 1). The sample size was determined based on a previous study [3]. Briefly, we planned to have 50 patients in each group with anticipation of dropout. We set 1.0 as a magnitude for the difference of average SMS between that from the SLIT and placebo groups and 1.5 as a standard deviation according to the result of previous study. Therefore, when the power was set to 0.8 and the $\alpha$-error to 0.05, the number of required cases was 35 in each group. A person who was not directly involved in the study was responsible for group allocation. To prevent leakage of information, the allocation table was kept by this person and a member of the ethics committee who was also not directly involved in the study, until accessed with the key after completion of the study. The protocol was approved by the Ethics Committee of Chiba University, and written informed consent was obtained from each patient prior to participation in the study.

### 2.2. Clinical protocols

The SLIT group included 58 patients who received standardized Japanese cedar pollen extract (Torii Pharmaceutical Co. Ltd., Tokyo, Japan) [8], and the placebo group included 45 patients who received an inactive placebo. The protocol consisted of treatment with graded courses of the extract in 50% glycerol, followed by maintenance therapy [6]. Briefly, the extract was graded in three strengths: 20, 200, and 2000 JAU/ml. Patients received increasing doses with each vial, beginning with 0.2 ml from the 20 JAU/ml vial and increasing by 0.2 ml a day for 5 days per week. The vaccine was taken sublingually, kept in place for 2 min without a retention reagent, and then spit out. The procedure was repeated until the maximum dose (1.0 ml of 2000 JAU/ml) was reached. The maintenance dose was 1.0 ml of 2000 JAU/ml given once a week until the end of May 2008. The patients in the placebo group received inactive 50% glycerol in saline. All participants were allowed to take symptom-reducing drugs as needed.

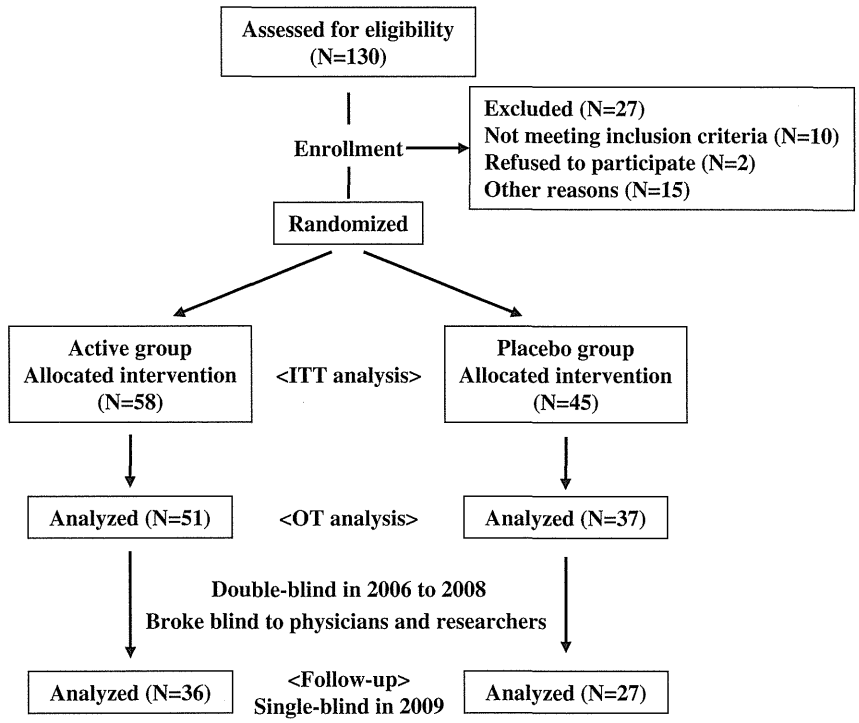


**Figure 1** Flow diagram for groups and individuals in the phases of the randomized trial. Fifteen participants from the SLIT ($N=7$) and placebo ($N=8$) groups were lost to follow-up due to reasons such as moving house and transfer. The double-blind status was maintained until completion of analysis of all clinical and immunological parameters (December 2008). Follow-up analysis in 2009 was undertaken in a single-blind manner.

## 2.3. Clinical symptoms and safety measurements

The patients completed a pollinosis diary to record their nasal symptoms and use of symptom-reducing drugs in the 2007, 2008, and 2009 pollen seasons. The total amounts of pollen scattered from Japanese cedar and Japanese cypress (*Chamaecyparis obtusa*) in Chiba prefecture were 2777, 6596, and 5486 grains/cm$^2$ during the 2007, 2008, and 2009 pollen seasons, respectively, based on measurements with a Durham pollen sampler. The duration and amount of scattered Japanese cedar pollen differed greatly among these years, but the daily amount of scattered pollen typically followed a wide-based bell-shaped curve over the whole pollen season from the middle of January or early February to the middle or end of May. The duration of the peak pollen season was relatively constant in the 3 years, and therefore, we analyzed the SMS during the peak period. The peak pollen season was defined as the period from the first day that the pollen count was $\geq$20 grains/cm$^2$/day for 3 consecutive days until the last day that the pollen count was $\geq$20 grains/cm$^2$/day before a period in which the pollen count was <20 grains/cm$^2$/day for 7 consecutive days.

The daily SMS was calculated as described previously [3]. Briefly, daily episodes of sneezing and nose blowing were rated as 0–4: none, 0; 1–5 episodes, 1; 6–10 episodes, 2; 11–20 episodes, 3; >20 episodes, 4. Daily medication was recorded based on drug types and duration of usage using the following guidelines: antihistamines, mast cell stabilizers, and vasoconstrictors, 1; topical ocular or nasal steroids, 2. Patients with an average daily SMS in the peak pollen season of $\leq$4 were judged to have mild symptoms based on guidelines for allergic rhinitis [7].

In the middle of the 2007 and 2008 pollen seasons, the participants completed the Japanese Allergic Rhinitis QOL Standard Questionnaire No.1 (JRQLQ No.1) for assessment of QOL-symptom and total QOL scores [9]. These scores were calculated as previously described [4,6]. The total QOL-symptom score was calculated as the sum of each component score: none, 0; mild, 1; moderate, 2; severe, 3; and very severe, 4. Nasal and ocular symptoms covered by the questionnaire included runny nose, sneezing, nasal congestion, itchy nose, itchy eyes, and watery eyes. Adverse events were graded using Common Terminology Criteria for Adverse Events (CTCAE) v.3.0 [10]. Briefly, adverse events were graded as mild, grade 1; moderate, grade 2; severe, grade 3; life threatening, grade 4; death, grade 5 according to a category for allergy/immunology in the CTCAE v.3.0 scoring system.

## 2.4. Blood samples

Peripheral blood was obtained from each patient before treatment (September to October 2006) and before and after the pollen seasons in 2007 (December 2006 to January 2007, and May to June 2007, respectively) and 2008 (November to December 2007, and May 2008, respectively). Peripheral blood mononuclear cells (PBMCs) were isolated, frozen, and stored in liquid nitrogen [6]. However, the PBMCs isolated before treatment, and before and after the 2007 pollen season were damaged during storage and we were unable to

analyze their immunological responses. Therefore, immunological data were obtained only from PBMCs collected before and after the 2008 pollen season.

### 2.5. Total and antigen-specific immunoglobulin titer

The Cry j 1-specific IgE and IgG4 titers in plasma were measured by ELISA [3,11]. Total IgE and specific IgE titers for Japanese cedar, orchard grass, mugwort, and house dust mites were evaluated by the CAP-RAST method (Phadia).

### 2.6. Flow cytometric analysis

The levels of Cry j 1-iTreg were analyzed by flow cytometry [6]. Briefly, PBMCs were cultured with or without Cry j 1 for 3 days, followed by a culture with 10 ng/ml phorbol 12-myristate 13-acetate, 1 μM ionomycin, and 2 μM monensin for 6 h. The PBMCs were stained with PE-Cy7-anti-CD4 antibody, APC-anti-IL10 antibody (BD Biosciences, San Diego, CA, USA), PE-anti-CD25, and FITC-anti-Foxp3 (clone: PCH101) using a Foxp3 staining buffer set (eBioscience, San Diego, CA, USA).

### 2.7. Analysis of the number of IL4-producing cells and the concentration of cytokines

The number of IL4-producing cells stimulated with Cry j 1 was determined by enzyme-linked immunospot (ELISPOT) assay, and the concentrations of IL2, IL5, and IL13 in the culture supernatant were measured using a BD™ Cytometric bead assay (CBA) Flex system (BD Biosciences) [6]. Briefly, a 96-well sterile filter plate (Millipore, Billerica, MA, USA) was coated with monoclonal antibody to human IL4 (Mabtech AB, Nacka Strand, Sweden). The plate was pre-incubated with AIM-V medium at 37 °C for 1 h. The medium was discarded, and then PBMCs ($3\times10^5$ cells/well) were cultured with fresh medium alone or with 10 μg/ml Cry j 1 for 17 h at 37 °C in AIM-V medium containing 5% human AB serum (Sigma-Aldrich, St. Louis, MO, USA). The plates were then incubated with a biotinylated monoclonal antibody to human IL4 for 2 h, and then with streptavidin-conjugated alkaline phosphatase for 1 h at room temperature. After washing with PBS, the plates were incubated with BCIP/NBT$^{PLUS}$ (Mabtech) for 5 min at 37 °C. For the CBA, isolated PBMCs were cultured at $2.5\times10^6$ cells/ml with or without 5 μg/ml Cry j 1 for 3 days at 37 °C in AIM-V medium containing 5% human AB serum (Sigma-Aldrich). After centrifugation at 300×*g* for 10 min, the supernatant was divided into aliquots and stored at −20 °C until the cytokine assay was performed.

### 2.8. Data representation

The full analysis set ($N=103$) was used for the intention-to-treat (ITT) analysis and per protocol populations ($N=88$) were used for on-treatment (OT) analysis (Fig. 1). Cry j 1-specific cytokine production is shown as the difference between cells stimulated with Cry j 1 and controls stimulated with medium only. Changes after the 2008 pollen season are shown as differences between pre- and post-pollen season values.

### 2.9. Statistical analysis

Two-group comparisons were performed using a Wilcoxon *t*-test or Mann–Whitney *U*-test to determine the significance of differences, or using an unpaired *t*-test as indicated. *P*-values $<0.05$ were considered to be significant.

## 3. Results

### 3.1. Clinical effects and adverse events

A total of 103 patients were included in the overall analysis of efficacy for the 2007 and 2008 pollen seasons. These patients were randomly divided into the SLIT ($N=58$) and placebo ($N=45$) groups at a ratio of 6:4. Diaries and QOL questionnaires for 88 patients were available at the end of the DBPC study. The overall randomized population was considered to be the ITT population. The SMS in the SLIT group did not differ significantly from that in the placebo group in ITT analysis after 2-year SLIT (*P*=N.S.; Student *t*-test, data not shown).

The final sample size included 88 subjects for OT analysis (SLIT; $N=51$, placebo; $N=37$, ratio 4:3). The demographic characteristics of the OT population before treatment are shown in Table 1. The SMS in the SLIT group did not differ significantly from that in the placebo group in the 2007 peak pollen season (February 19 to March 31, *P*=N.S.; Student *t*-test). However, the average SMS in the 2008 peak pollen season (February 29 to April 1) was significantly ameliorated in the SLIT group compared with the placebo group (4.2 vs. 5.3, $P=0.02$; Student *t*-test). The percentages of subjects with mild symptoms (SMS ≤ 4) were 55% and 28% in the SLIT and placebo groups, respectively, in the peak pollen

**Table 1** Clinical data of participants at the start of the study.

| Group | SLIT | Placebo | *P*-value |
|---|---|---|---|
| Number | 51 | 37 | |
| Sex (M/F) | 17/34 | 8/29 | N.S.[a] |
| Mean age | 44.4 | 42.3 | N.S.[b] |
| Range | 16–73 | 19–70 | |
| Total IgE [IU/ml] | 198 | 258 | N.S.[b] |
| Range | 6.8–1480 | 8.6–2090 | |
| Specific IgE[c] | 27 | 29 | N.S.[b] |
| Range | 0.8–100 | 1.5–100 | |
| Class [mean] | 3.5 | 3.8 | N.S.[b] |
| Range | 2–6 | 2–6 | |
| Other allergies[d] (%) | | | |
| Orchard grass | 16 (31%) | 11 (30%) | N.S.[e] |
| Mugwort | 5 (10%) | 3 (8%) | <0.05[f] |
| House dust mite | 24 (47%) | 13 (35%) | N.S.[e] |

[a] Yates2×2 Chi-squared test.
[b] Student *t*-test.
[c] Specific IgE to Japanese cedar pollen; CAP-RAST raw value [kAU/L], mean.
[d] Number of subjects with specific IgE of at least CAP-RAST class 2.
[e] 2×2 Chi-squared test.
[f] Fisher exact probability.

season (Fig. 2A). QOL-symptom and total QOL scores were also significantly ameliorated in the SLIT group compared to those in the placebo group in the middle of the 2008 pollen season (Fig. 2B).

There were no severe adverse events that required a patient to withdraw from the study; however, some subjects reported adverse events of mild discomfort: six of grade 2 (oral pruritus: 2; gingivostomatitis: 2; asthma: 1; rash in nasal cavity: 1) in the SLIT group (6/51; 11.8%); and one of grade 1 (bitter taste) in the placebo group (1/37; 2.7%).

### 3.2. Immunoglobulin production

There were no significant differences in Cry j 1-specific IgE and IgG4 production between patients in the SLIT and placebo groups before treatment, or before and after the pollen seasons. The SLIT group was divided into subgroups based on the SMS in the 2008 peak pollen season: a mild subgroup with SMS $\leq 4$ (classified as responders; $N=28$) and a severe subgroup with SMS $>4$ (non-responders; $N=23$). IgE and IgG4 production in patients in the mild subgroup were both similar to those in patients in the severe subgroup and in the placebo group at various time points (data not shown).

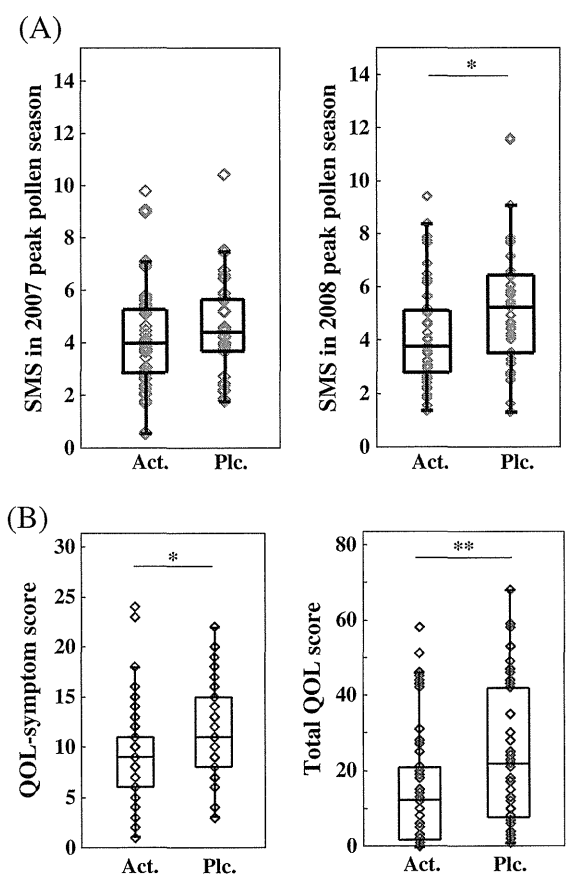


**Figure 2** Clinical scores after 2-year SLIT. (A) Average daily symptom-medication scores (SMS) in the SLIT (Act.; $N=51$) and placebo (Plc.; $N=37$) groups in the 2007 and 2008 peak pollen seasons. (B) QOL-symptom and total QOL scores from the QOL questionnaire were plotted for the SLIT (Act.; $N=51$) and placebo (Plc.; $N=37$) groups in the middle of the 2008 pollen season. Each diamond shows a value for an individual. Two-group comparisons were performed using an unpaired Student $t$-test. $^{*}P<0.05$, $^{**}P<0.01$.

### 3.3. Cry j 1-specific cytokine production

IL2, IL5, and IL13 levels were analyzed in the culture supernatant. The number of IL4-producing cells was measured by ELISPOT because IL4 was undetectable in the supernatant. There were no significant differences between the SLIT and placebo groups in the production of each cytokine following stimulation with Cry j 1 (Fig. 3A). IL5 was significantly increased after the pollen season in all groups ($P<0.05$; Wilcoxon $t$-test), and the IL2 and IL13 levels and the number of IL4-producing cells were significantly increased after the pollen season in the SLIT and placebo groups and in the severe subgroup ($P<0.05$; Wilcoxon $t$-test). Patients in the mild subgroup (responder to SLIT) did not show significant increase of IL2 and IL13 or of IL4-producing cells after the pollen season ($P$=N.S.; Wilcoxon $t$-test). The increases in the number of IL4-producing cells and IL5 level after the pollen season in the mild subgroup were significantly less than those in the severe subgroup (non-responders) and the placebo group. The increase of IL13 in the mild subgroup was significantly less than that in the severe subgroup and showed a tendency to be attenuated compared with the placebo group ($P$=N.S.; Mann–Whitney $U$-test). The increase of IL2 in the mild subgroup was significantly less than that in the placebo group ($P<0.05$) and showed a tendency to be attenuated compared with the severe subgroup ($P=0.053$; Mann–Whitney $U$-test, Fig. 3B).

### 3.4. Prognostic biomarkers for clinical effects

The average ratio of Japanese cedar pollen-specific IgE to total IgE (sIgE/tIgE ratio) in all patients in the study was 0.193 before treatment. The SLIT group was divided into subgroups with a sIgE/tIgE ratio $\leq 0.19$ (low, $N=28$) and $>0.19$ (high, $N=23$) before treatment. Similar subgroups were established in the placebo group. The SMS in the 2008 peak pollen season for the low subgroup was significantly improved compared to that in the high subgroup in the SLIT group ($P=0.02$; Mann–Whitney $U$-test); however, in the placebo group, the low and high subgroups had comparable SMSs ($P$=N.S.; Mann–Whitney $U$-test, Fig. 4A). Furthermore, the SMS was correlated with the sIgE/tIgE ratio in the SLIT group ($Rs=0.39$, $P<0.01$; Spearman correlation analysis), but not in the placebo group ($Rs=0.08$, $P$=N.S.; Spearman correlation analysis, Fig. 4B).

### 3.5. Upregulation of Cry j 1-iTreg levels as a response monitoring biomarker

A population of $IL10^{+}Foxp3^{+}$ cells in $CD25^{+}CD4^{+}$ leukocytes was evaluated as a potential marker for iTreg after stimulation with Cry j 1 or medium only before and after the pollen season in 2008. Neither the changes in Cry j 1-iTreg levels after stimulation with and without Cry j 1 nor the upregulation of Cry j 1-iTreg from pre- to post-pollen season differed significantly different between the groups (data not shown).

We previously reported that upregulation of Cry j 1-iTreg is a candidate biomarker that may distinguish SLIT responders from non-responders based on QOL-symptom scores [6]. Therefore, we divided the SLIT group into subgroups based on an increase ($N$=24) or decrease ($N$=27) in Cry j 1-iTreg levels from before to after the pollen season in 2008. QOL-symptom and total QOL scores in the increased iTreg subgroup significantly improved compared with those in the placebo group. In contrast, the scores in the decreased iTreg subgroup were similar to those in the placebo group (Fig. 4C).

### 3.6. Carry-over effects in the year after treatment

A total of 63 patients completed a pollinosis-symptom diary during the 2009 pollen season; 1 year after the 2-year SLIT treatment (Fig. 1). All participants remained blinded to their treatment with SLIT or a placebo. The SMS in the peak pollen season in 2009 (February 15 to March 6) in the SLIT group ($N$=36) was significantly attenuated compared to the placebo group ($N$=27, $P$=0.03). The average SMSs for the SLIT and placebo groups were 3.5 and 4.5, respectively, in the peak pollen season (Fig. 5).

## 4. Discussion

The primary endpoint of this randomized DBPC trial was the therapeutic effect evaluated in ITT analysis. No significant positive effect was observed between the SLIT and placebo groups after exchanging the perceived improvement of patients who dropped out with each median score from the counter group. In OT analysis, the SMS in the SLIT group was

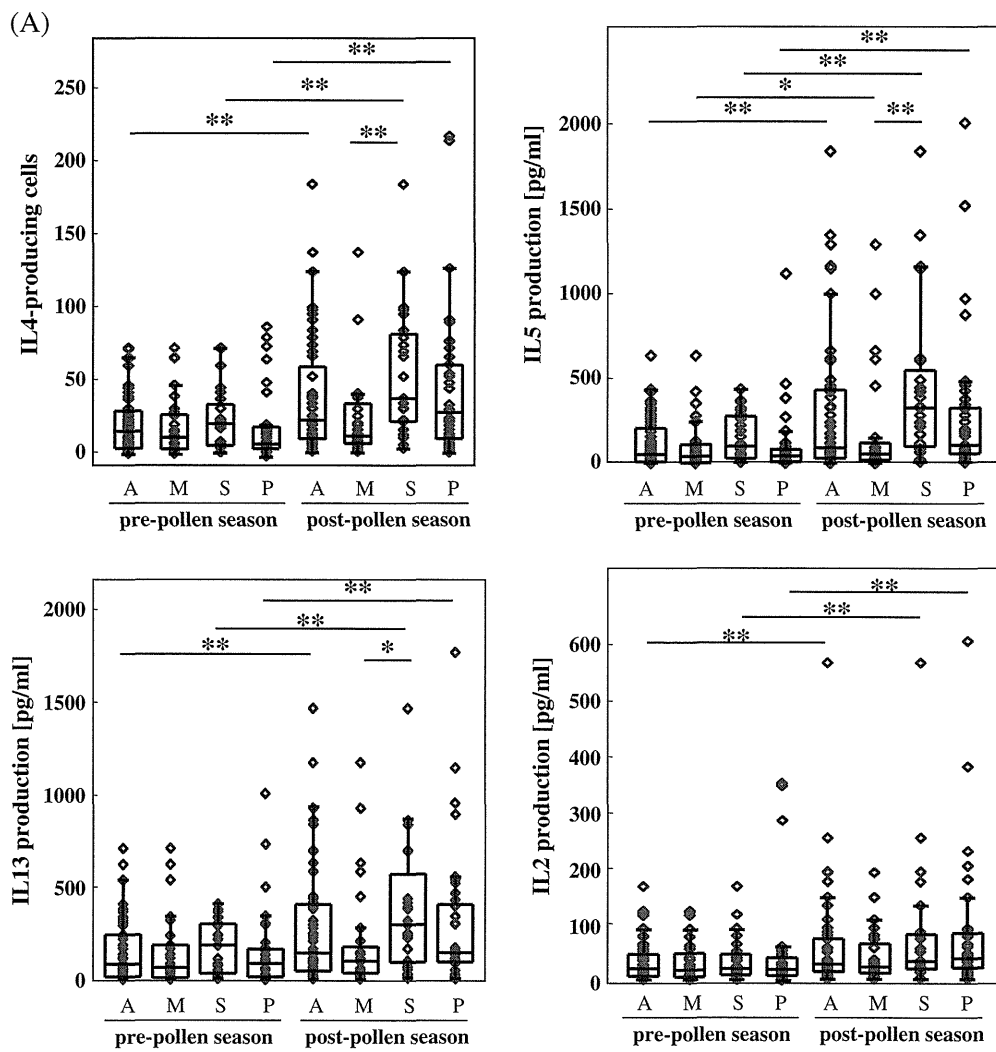


**Figure 3** Cytokine production from PBMCs. (A) Number of Cry j 1-specific IL4-producing cells and Cry j 1-specific cytokine levels in the SLIT group (A; $N$=51), the mild subgroup of the SLIT group (M; $N$=28), the severe subgroup of the SLIT group (S; $N$=23), and the placebo group (P; $N$=37) at before and after the 2008 pollen season. Comparisons with a significant difference are indicated as * and **; otherwise, comparisons are not significantly different ($P$=N.S.). (B) Increases in the number of Cry j 1-specific IL4-producing cells and Cry j 1-specific cytokine levels occurred from before to after the 2008 pollen season in the SLIT group (Act.; $N$=51), the mild subgroup of the SLIT group (Mild; $N$=28), the severe subgroup of the SLIT group (Sev.; $N$=23), and the placebo group (Plc.; $N$=37). Each diamond shows the value for an individual. Two-group comparison was performed using a Mann–Whitney $U$-test. *$P$<0.05, **$P$<0.01.

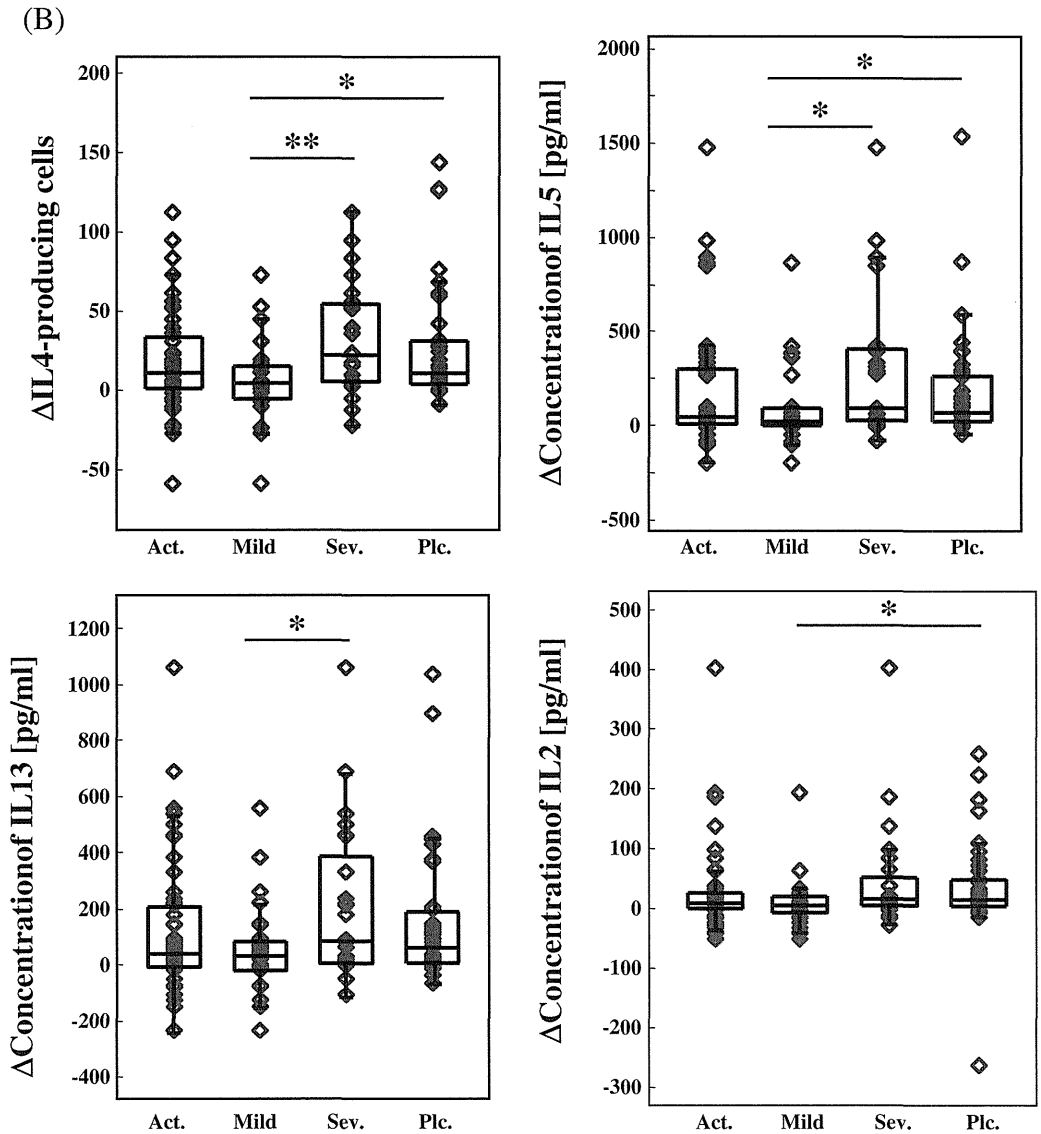


Figure 3 (*continued*).

significantly ameliorated compared to the placebo group in 2008. The percentage of mild subjects (SMS ≤ 4) in the SLIT group was 28% higher than that in the placebo group (SLIT, 55%; placebo, 27%), and the SMS was reduced by approximately 21% in the SLIT group compared with the placebo group (SLIT, 4.2; placebo, 5.3). This percentage of mild subjects differ significantly between the SLIT and placebo groups ($P$=0.009; 2×2 Chi-squared test). These effects following 2-year treatment were comparable to those in a trial of 1-year daily treatment using grass pollen tablets [12]. The low dose of the extract (about 1/40th of that used in Europe) may be one reason for the poor clinical outcome in the first year [13]. An extract of concentration >2000 JAU is not available for clinical use in Japan, and the clinical effects, safety, and optimum schedule for administration of an extract with a much higher allergen concentration remain unclear.

Positive clinical therapeutic effects were not obtained following 1-year treatment in our study, even in OT analysis (data not shown). In contrast, two previous reports demonstrated positive therapeutic effects after 1-year SLIT for Japanese cedar pollinosis [3,4]. However, in these studies, the annual pollen count (1154 grains/cm$^2$/season) [3] was less than in our study, and daily SMS was significantly attenuated on only 4 days in the pollen season [4]. The severity of SMS is affected by the amount of Japanese cedar pollen in the total and peak pollen season. Natural resolution and tolerance are not usually induced by natural exposure to Japanese cedar pollen, regardless of the amount of pollen [14].

Whether there are detectable alterations in peripheral T-cell responses after specific immunotherapy is still under debate [15–18]. The Cry j 1-specific cytokine profile from the SLIT group did not differ significantly from that in the placebo group. However, the increases in IL2, IL4, IL5, and IL13 production in the mild subgroup in the SLIT group were significantly attenuated (or showed a tendency to be attenuated) compared to the severe subgroup and the placebo

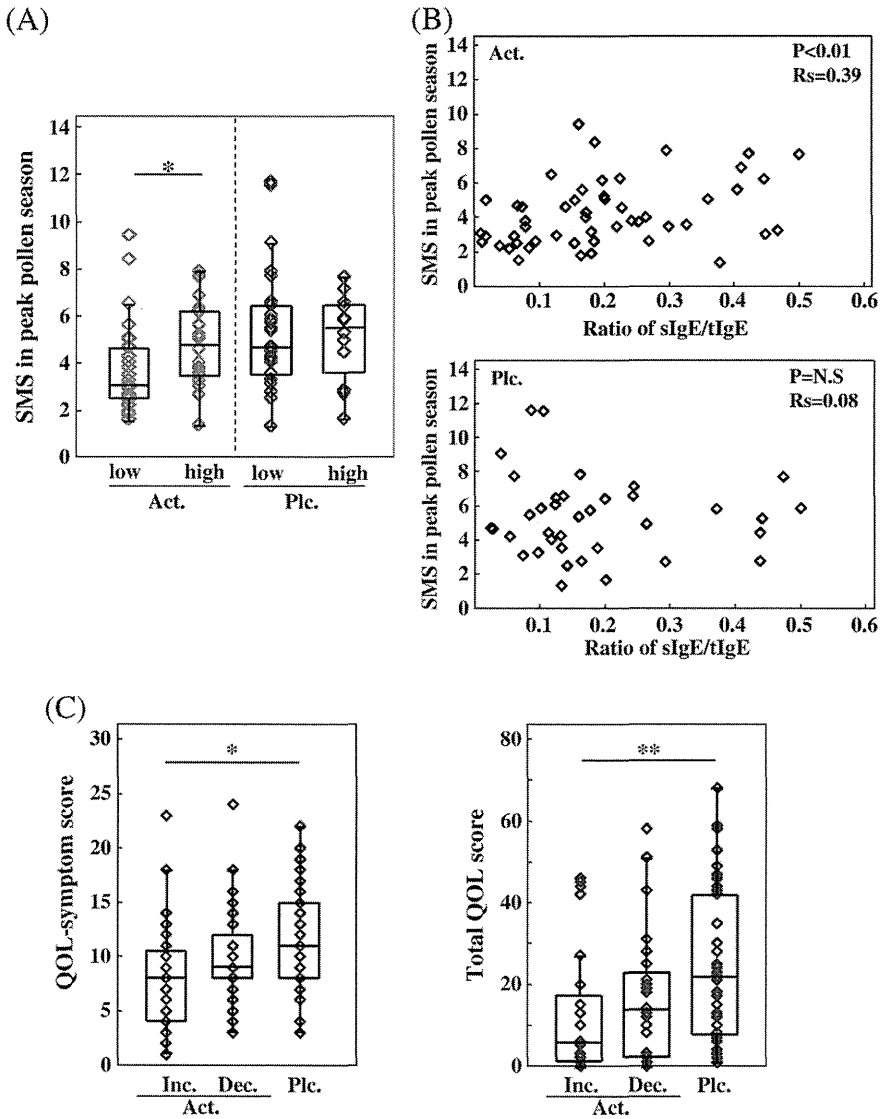


**Figure 4** Biomarkers for positive therapeutic effects following SLIT. (A) SMSs in the 2008 peak pollen season for patients with low (low; $N=28$) and high (high; $N=23$) sIgE/tIgE ratios in the SLIT group (Act.), and for those with low ($N=25$) and high ($N=12$) sIgE/tIgE ratios in the placebo group (Plc.). *$P<0.05$. (B) Correlation between SMSs in the 2008 peak pollen season and sIgE/tIgE ratios before treatment in the SLIT (Act.; $N=51$) and placebo (Plc.; $N=37$) groups. Statistical data were obtained with Spearman correlation analysis. (C) QOL-symptom and total QOL scores from the QOL questionnaire plotted for a subgroup with increased Cry j 1-iTreg in the SLIT group (Inc.; $N=24$), a subgroup with decreased Cry j 1-iTreg in the SLIT group (Dec.; $N=27$), and the placebo group (Plc.; $N=37$) in the middle of the 2008 pollen season. Each diamond shows the value for an individual. *$P<0.05$, **$P<0.01$.

group (Fig. 3B). The SMS in all patients in the study correlated with the seasonal increases in IL4 ($R=0.35$, $P<0.01$), IL5 ($R=0.35$, $P<0.01$), and IL13 ($R=0.36$, $P<0.01$). The discrepancy in our current results and the results of previous studies with regard to downregulation of cytokine production from PBMCs may depend on the extent of the therapeutic effects achieved in each clinical trial.

Cry j 1-specific IgE production was not changed by treatment, even in the mild subgroup, as also found in our preliminary study [6]. We speculate that more time is required for changing antibody production following the changes of antigen-specific T cell profiles, because the alteration of T cell profiles strongly influences subsequent class switch recombination of B cells and antibody production. Another possibility is that the dose for SLIT used in this study was not high enough to alter the antibody profiles.

The sIgE/tIgE ratio has been found to be significantly higher in responders than in non-responders following 4-year immunotherapy [19]. In our trial, this ratio did not differ significantly between responders and non-responders ($P$=N.S.; Mann–Whitney $U$-test). However, subjects with a low sIgE/tIgE ratio before treatment were more likely to be responders to 2-year SLIT, and the ratio correlated with the SMS only in patients treated with SLIT (Fig. 4A, B). This suggests that SLIT was more effective in patients with a low sIgE/tIgE ratio than in those with a high sIgE/tIgE ratio. The range of total IgE levels for the participants were relatively wide (6.8–2090 IU/ml in all patients); however, the change of the total IgE for each

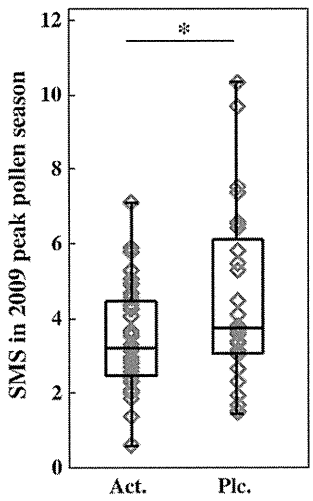


**Figure 5** Carry-over effects following 2-year treatment with SLIT. SMSs in the 2009 peak pollen season were plotted for the SLIT (Act.; $N=36$) and placebo (Plc.; $N=27$) groups. Each diamond shows the value for an individual. Two-group comparisons were performed using an unpaired $t$-test.

individuals after 2-year treatment was not significantly different compared to before treatment (1.5±1.0 times higher, $P$=N.S.; paired $t$-test). Therefore, the wide range of total IgE levels was due to the variability on the allergic status for individuals, but not on method for measurement. The serum IgE level may affect the surface IgE level on effector cells such as mast cells and basophils, and Tregs can downregulate activation of mast cells and eosinophils [20,21]. We speculate that effector cells with a low specific IgE level are less likely to be activated by antigen crosslinking or are more susceptible to downregulation by Tregs than those with a high specific IgE level. It is also possible that the symptoms of patients with a low sIgE/tIgE ratio may be more readily attenuated by suboptimal potentiation of iTreg induced by SLIT.

We previously reported that an increased count of Cry j 1-iTregs was a candidate biomarker that could be used to distinguish between responders and non-responders to SLIT, as evaluated by the QOL-symptom score. In this report, the subgroup with increased Cry j 1-iTregs showed significant amelioration of the QOL-symptom and total QOL scores compared to the placebo group, while the subgroup with decreased Cry j 1-iTregs did not show this response (Fig. 4C). However, there was no significant difference in Cry j 1-specific cytokine production from PBMCs among patients with increased iTregs and decreased iTregs, and those in the placebo group (data not shown). Foxp3-expressing $CD25^+CD3^+$ cells and IL10-expressing $CD3^+$ cells, which are induced in the nasal mucosa after subcutaneous immunotherapy, have been linked to the clinical efficacy and suppression of seasonal inflammation [22]. Immunotherapy using an Amb a 1-immunostimulatory oligodeoxynucleotide conjugate also induced $CD4^+CD25^+$ T cells and IL10-producing cells in the nasal mucosa after the pollen season [23]. These data suggest that iTregs may downregulate effector cells at local sites of inflammation to suppress clinical symptoms. Induction of iTregs in the nasal mucosa and functional analysis of these cells may be necessary to determine the regulatory mechanisms affected by SLIT. Mucosal biopsy in the peak pollen season is useful for evaluation of local induction of iTregs and downregulation of effector cells. However, nasal biopsy in the pollen season significantly influences the daily SMS in the peak pollen season. Mucosal biopsy outside the pollen season after exposure using an artificial pollen chamber may be a powerful tool for evaluation of local regulatory mechanisms induced by SLIT [24]. Upregulation of iTregs in nasal mucosa may be difficult to determine since the evaluation may be painful for patients. However, upregulation of iTregs in peripheral blood is simple to analyze and may be a useful biomarker because an increase of peripheral Cry j 1-iTregs is correlated with QOL and QOL-symptom scores in the pollen season, as discussed here and elsewhere [6].

Cry j 1-specific IgG4 production was not induced by SLIT in this study to the same extent as that in our previous study [6]. A clinical trial showing that daily 2500 SQ-T (14 μg Phl p 5 per 4 weeks) tablets failed to induce IgG production supports our current results [13]. A change in the immunoglobulin profile may require a higher allergen dose or longer duration of exposure. However, our study suggests that detectable quantitative changes in IgG4 are not essential for the amelioration of clinical symptoms.

In summary, we suggest that the sIgE/tIgE ratio and upregulation of iTregs may be considered as prognostic and response monitoring biomarkers, respectively, for SLIT. However, further investigation of induction of iTregs at local inflammatory sites and downregulation of inflammatory cells is needed. Furthermore, validation studies with larger sample size would be required before either biomarkers should be applied widely in the clinical management of pollinosis patients. Development of a more effective vaccine and better protocols may reveal more significant differences in the Cry j 1-specific cytokine profiles and iTreg induction, and these results may increase our understanding of the roles of iTregs or Tr1 in the therapeutic mechanisms underlying the efficacy of SLIT.

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3 years of life, but not thereafter. The observed sex differences were larger for asthmatic wheeze than for total wheeze, suggesting that sex differences are stronger for asthma than for transient symptoms.

Young boys are thought to have smaller airway diameters in proportion to their total lung volume than girls, predisposing them to airway obstruction and wheeze.[1,6] Our results suggest that sex differences in asthma may partly be explained by the higher prevalence of atopy in boys and cannot be explained by a stronger effect of perinatal risk factors in boys.

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## Characterization of pollen antigen–induced IL-31 production by PBMCs in patients with allergic rhinitis

*To the Editor:*

Japanese cedar/cypress pollinosis (JCCP) is the major phenotype of allergic rhinitis in Japan and has a prevalence of 29.8%, with a substantial impairment of quality of life (QOL).[1] JCCP is mainly caused by exposure to Japanese cedar *(Cryptomeria japonica)* pollen and Japanese cypress *(Chamaecyparis obtusa)* pollen. The cypress pollen disperses after the cedar pollen in spring. Because cedar and cypress pollen contain several cross-reactive components, pollinosis-related symptoms can last for as long as 4 months, from February to May. On the other hand, species-specific components and epitopes for IgE, T cells, or both have been identified.[2]

IL-31 is a novel cytokine produced by $CD4^+$ T cells, particularly $T_H2$ cells and skin-homing $CD45RO^+$ cutaneous lymphocyte–associated antigen–positive cells.[3,4] Thus the role of IL-31 in patients with pruritic skin diseases, including atopic dermatitis, has been examined.[3-6] On the other hand, the role of IL-31 in the pathogenesis of respiratory allergic diseases remains unclear.[7-9] IL-31 enhances epidermal growth factor, vascular endothelial growth factor, and CCL2 production by human bronchial epithelial BEAS-2B cells.[8] However, a murine model of $T_H2$-biased pulmonary inflammation suggests that IL-31 is a negative regulator in this type of inflammation.[7]

In the present study we investigated the production of IL-31 in pollen antigen–stimulated PBMCs from subjects with and without JCCP. Details on the methods are available in the Methods section and Fig E7 of this article's Online Repository at www.jacionline.org.

PBMCs from the healthy control group did not produce IL-31 in response to pollen antigens. On the other hand, the JCCP group included both positive and negative responders. The detection limit of the ELISA (7.8 pg/mL) was used as a cutoff for discriminating $IL\text{-}31^-$ from $IL\text{-}31^+$ JCCP. Of PBMCs from patients with JCCP not treated with specific immunotherapy (SIT), 62.1% ($P = .002$ compared with control subjects, Fisher exact probability test), 63.0% ($P = .002$), and 34.6% ($P = .060$) produced IL-31 in response to Cry j 1, cedar crude antigen, and cypress crude antigen, respectively. This might be the first report of the induction of IL-31 protein production by means of allergen stimulation in human subjects. Among the SIT-treated patients with JCCP, only 25.0% ($P = .011$ compared with patients not treated with SIT), 21.1% ($P = .005$), and 16.7% ($P = .166$) of PBMCs produced IL-31 in response to Cry j 1, cedar crude antigen, and cypress crude antigen, respectively. Overall, the median amounts of IL-31 produced in response to Cry j 1 and cedar crude antigen, but not cypress crude antigen, were significantly higher in patients with JCCP not treated with SIT compared with those seen in healthy control subjects and SIT-treated patients with JCCP (Fig 1). Increased expression levels of IL-31 protein, mRNA, or both in sera, PBMCs, and inflamed tissues in other allergic diseases have been reported for both human subjects and mice.[4-6,9] The present results are consistent with the previous reports and suggest that the increased expression of IL-31 might be a common feature in patients with atopic allergic diseases.

The amounts of Cry j 1–induced, cedar crude antigen–induced, and cypress crude antigen–induced IL-31 production were significantly and positively correlated with the production of IL-5 and IL-13, but not IFN-γ, in response to the respective antigens in patients with JCCP without SIT treatment (see Fig E1 in this article's Online Repository at www.jacionline.org). In addition, PBMCs from patients who produced IL-31 in response to Cry j 1, cedar crude antigen, and cypress crude antigen produced significantly higher amounts of IL-5 and IL-13 by means of stimulation with the respective antigens compared with PBMCs from patients who did not produce IL-31 (see Fig E2, *A*, *B*, *D*, *E*, *G*, and *H*, in this article's Online Repository at www.jacionline.