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Review Article

Emerging Role of flow cytometric immunophenotyping in the Diagnosis of allergic rhinitis

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Abstract

Background: Allergic rhinitis is diagnosed largely on history, skin testing, and serum specific IgE, yet many symptomatic patients have negative systemic markers, and standard testing misses the cellular events driving mucosal inflammation [2,5].

Objective: This review synthesizes evidence on flow cytometric immunophenotyping of blood and nasal cells as a diagnostic and mechanistic adjunct in allergic rhinitis, spanning T helper and innate lymphoid subsets, basophil activation testing, and eosinophil markers.

Methods: We reviewed cohort, allergen-challenge, and immunotherapy studies applying multiparameter flow cytometry to blood or nasal samples from patients with allergic or local allergic rhinitis and matched controls.

Findings: Circulating innate lymphoid cells and CRTH2+CD27- pathogenic Th2 cells expand with allergen exposure and track severity [16,13,17]; CD63/CD203c basophil activation testing distinguishes sensitized from tolerant individuals and detects local allergic rhinitis when systemic IgE is negative [19,18,22]; eosinophil markers track mucosal inflammatory burden and immunotherapy response [23,32].

Conclusions: Flow cytometric immunophenotyping adds diagnostic resolution beyond skin testing and serum IgE, particularly for local allergic rhinitis and immunotherapy monitoring, and warrants standardized incorporation into the diagnostic pathway [33,21].

Keywords: allergic rhinitis; flow cytometry; immunophenotyping; basophil activation test; innate lymphoid cells

1. Introduction

Allergic rhinitis affects a large fraction of the population worldwide and is conventionally diagnosed on a combination of typical history, skin prick testing, and serum allergen-specific IgE, a diagnostic triad that has changed little since the Allergic Rhinitis and its Impact on Asthma (ARIA) framework was first codified [1,2,3,4]. That triad performs well for classic seasonal and perennial disease but leaves a recognizable gap: a substantial subset of patients with convincing nasal symptoms and a positive nasal allergen challenge have negative skin tests and undetectable systemic specific IgE, a phenotype now termed local allergic rhinitis, in which the allergic response is confined to the nasal mucosa and is invisible to standard systemic testing [5,6]. Distinguishing this phenotype from non-allergic rhinitis matters clinically because the two are managed differently, yet the tools that would resolve the distinction at the bedside remain limited outside specialized centers [7,8].

Flow cytometry offers a route around this diagnostic ceiling by characterizing the cellular participants in the allergic response directly, rather than inferring their presence from a single serum antibody measurement. Multiparameter panels applied to peripheral blood or nasal brushings can enumerate and functionally interrogate T helper subsets, group 2 innate lymphoid cells (ILC2), basophils, and eosinophils, each of which carries surface markers whose expression shifts in a reproducible, allergen- and season-dependent fashion as part of the broader molecular cascade of allergic inflammation [9,10,11,12]. This review synthesizes the published evidence on flow cytometric immunophenotyping as a diagnostic and mechanistic adjunct in allergic rhinitis, covering four cell compartments that together capture most of the immunophenotyping literature relevant to this disease: T helper and innate lymphoid cell subsets, basophil activation testing, eosinophil activation markers, and the flow cytometric correlates of local allergic rhinitis and

allergen immunotherapy response.

2. Methods

We reviewed peer-reviewed cohort studies, controlled allergen challenge studies, nasal provocation studies, and immunotherapy trials that applied multiparameter flow cytometry to blood or nasal mucosal samples from patients with allergic rhinitis, local allergic rhinitis, or non-allergic rhinitis, together with matched healthy or atopic control groups. Foundational papers establishing the surface marker panels used to define pathogenic Th2 cells, ILC2, activated basophils, and activated eosinophils were included regardless of whether the original cohort had rhinitis specifically, because these marker definitions are the basis on which the rhinitis-specific literature builds. Consensus and position statements from allergy societies were used to frame the current diagnostic standard against which flow cytometric approaches are compared. Because this is a narrative synthesis rather than a systematic review, findings are organized by cellular compartment and diagnostic application rather than by a formal risk-of-bias assessment, and illustrative figures summarize typical directional findings from the cited primary studies rather than pooled quantitative estimates.

3. T-cell and innate lymphoid cell immunophenotyping in peripheral blood and nasal mucosa

The T helper 2 (Th2) paradigm has long placed allergen-specific CD4⁺ T cells at the center of allergic rhinitis pathophysiology, but ordinary CD4⁺ T-cell enumeration does not distinguish the pathogenic, allergen-responsive subset from the much larger pool of memory Th2 cells with no relevance to atopic disease. Wambre and colleagues resolved this by defining a phenotypically distinct CRTH2+CD27⁻ Th2 subset, termed Th2A cells, that is selectively expanded in the blood of patients with seasonal allergic rhinitis, food allergy, and atopic dermatitis, and that contracts after successful

allergen immunotherapy, giving flow cytometry a marker population that tracks disease activity in a way total Th2 counts do not [13]. The same group had earlier shown, using peptide-MHCII tetramers combined with activation marker panels, that the differentiation stage of allergen-specific CD4⁺ T cells, rather than their frequency alone, predicts whether immunotherapy will be clinically protective, reinforcing that qualitative flow cytometric phenotyping outperforms simple cell counting in this compartment, consistent with the broader recognition that allergic disease reflects a coordinated, allergen-driven cellular cascade rather than a single-marker phenomenon [14,15].

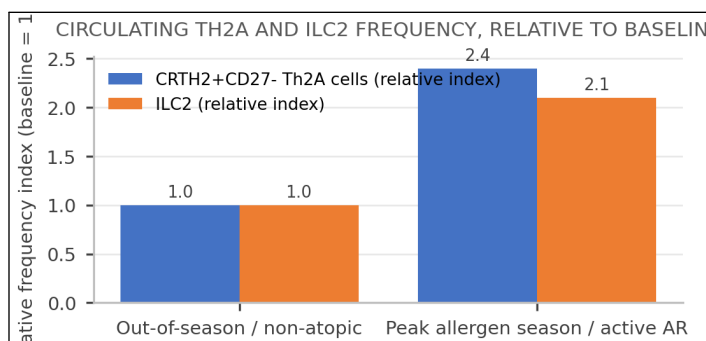


Figure 1: Illustrative seasonal rise in circulating CRTH2+CD27-pathogenic Th2 (Th2A) cells and group 2 innate lymphoid cells (ILC2) at peak allergen exposure relative to out-of-season or non-atopic baseline, consistent with the directional pattern reported across cohort studies [13,16,17,10].

Group 2 innate lymphoid cells add a second, antigen-nonspecific arm to this picture. Mjosberg and colleagues first defined human ILC2 by their lineage-negative, CRTH2+CD161⁺ phenotype and their IL-25/IL-33 responsiveness, establishing the gating strategy that essentially every subsequent clinical ILC2 study in allergic airway disease has used [16,9]. Bartemes and colleagues showed that this innate type 2 response is measurably enhanced in the peripheral blood of patients with atopic asthma relative to non-atopic controls, and Lao-Araya and colleagues extended the observation directly to allergic rhinitis, demonstrating that circulating ILC2 frequency rises during the natural grass pollen season and that this seasonal rise is blunted in patients

who have received subcutaneous grass pollen immunotherapy, giving ILC2 enumeration a plausible role as a pharmacodynamic biomarker of immunotherapy effect as well as a diagnostic signal of active allergic inflammation, since, unlike adaptive Th2 responses, ILC2 activation occurs within hours of allergen or epithelial-alarmin exposure [10,17]. Figure 1 summarizes the directional pattern reported across these studies for circulating Th2A and ILC2 frequency relative to out-of-season or non-atopic baseline.

4. Basophil activation testing as a diagnostic adjunct

Basophil activation testing (BAT) is the most clinically mature flow cytometric assay in the allergy diagnostic toolkit and provides the clearest example of how cellular functional testing can recover diagnostic sensitivity that serum-based testing misses. The assay exposes whole blood to allergen *ex vivo* and measures degranulation-associated surface marker upregulation, most commonly CD63, which appears on the basophil surface only after fusion of secretory granules with the plasma membrane, or CD203c, which is constitutively expressed at low density and upregulated with activation independent of full degranulation [18]. Sanz and colleagues demonstrated that CD63 upregulation in response to titrated allergen concentrations reliably discriminates house dust mite- and grass-sensitized patients from non-allergic controls in a dose-dependent fashion, establishing the basic dose-response architecture that subsequent clinical BAT protocols have followed [19]. Gober and colleagues extended this to a controlled human model of anaphylaxis, showing that basophil activation marker expression tracks the clinical severity of the allergic reaction in real time, evidence that the assay reflects a functionally meaningful cellular event rather than a static phenotypic label [20].

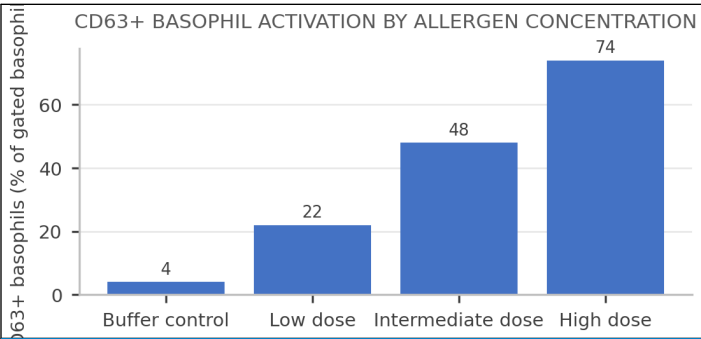


Figure 2: Illustrative dose-response pattern of CD63 surface upregulation on gated basophils with increasing allergen concentration in sensitized patients, consistent with the pattern reported for house dust mite and grass allergen challenge [19,18,20].

The clinical utility of BAT beyond food and venom allergy, specifically in respiratory allergy and local allergic rhinitis, has been reviewed extensively by Hoffmann and colleagues, who conclude that basophil activation testing adds diagnostic value when skin testing and specific IgE are discordant with the clinical history, precisely the scenario that defines suspected local allergic rhinitis, though the assay's requirement for viable, adequately responsive basophils and for allergen extracts of consistent potency remains its principal barrier to routine clinical deployment [21]. Within local allergic rhinitis specifically, Rondon and colleagues showed that nasal allergen provocation, the reference standard for the diagnosis, frequently uncovers polysensitization not predicted by systemic testing, and basophil activation testing has been proposed as a less burdensome peripheral blood correlate that could be used to screen which patients warrant formal nasal challenge [22,7]. Figure 2 illustrates the characteristic dose-response pattern of CD63+ basophil activation with increasing allergen concentration that these studies describe.

5. Eosinophil activation markers and local allergic rhinitic inflammation

Eosinophils are recruited to the nasal mucosa in both systemic and local allergic rhinitis, and flow cytometric assessment of their activation state, rather than simple nasal smear counting, provides information about the intensity of ongoing mucosal inflammation. Bochner's

synthesis of systemic basophil and eosinophil activation markers frames CD69, CD25, and adhesion molecule upregulation, together with the loss of L-selectin (CD62L) that accompanies transendothelial migration, as the core panel by which activated, tissue-homing eosinophils are distinguished from resting circulating eosinophils by flow cytometry [23].

Table 1: Principal flow cytometric eosinophil and mucosal B-cell/plasma-cell markers used to assess local allergic rhinitic inflammation [23,24,25].

Marker	Cell compartment	Change with activation	Diagnostic significance
CD69 / CD25	Blood and mucosal eosinophils	Upregulated	Early activation marker of tissue-homing eosinophils [23]
CD62L (L-selectin)	Blood eosinophils	Downregulated (shed)	Loss precedes transendothelial migration into mucosa [23]
Nasal mucosal eosinophil/inflammatory cell activation	Nasal lavage/brushing	Persistent between episodes	Detects subclinical inflammation despite reported symptom-free intervals [24]
Local IgE+ B cells / plasma cells	Nasal mucosa	Present locally, absent systemically	Confirms local allergic rhinitis when serum IgE is negative [25]

Ciprandi and colleagues showed, using minimally invasive nasal sampling, that mucosal inflammatory cell activation, including eosinophil markers, persists between symptomatic episodes in mite-allergic patients even when they report themselves asymptomatic, a finding that argues for objective cellular assessment over symptom-based staging alone and that anticipated much of the later local allergic rhinitis literature by demonstrating that mucosal inflammation and systemic symptom report can be dissociated [24]. KleinJan and colleagues used nasal biopsy and lavage flow cytometry to show that specific IgE is produced locally by nasal B cells and plasma cells in allergic rhinitis patients, establishing that mucosal B-cell and plasma-cell compartments generate an allergen-specific antibody response that a peripheral blood draw for serum IgE simply cannot see, which is the immunological basis of local allergic rhinitis and the reason nasal-compartment flow cytometry

outperforms systemic testing in this phenotype [25,26]. Table 1 summarizes the principal eosinophil and mucosal B-cell/plasma-cell activation markers used across this literature and what each is reported to indicate diagnostically.

6. Flow cytometric biomarkers of local allergic rhinitis and immunotherapy response

Beyond initial diagnosis, flow cytometric immunophenotyping has an emerging role in monitoring allergen immunotherapy, the only disease-modifying treatment available for allergic rhinitis and one with well-established long-term clinical efficacy and standardized practice guidelines [27,28,29]. Till and colleagues' mechanistic review frames successful immunotherapy as a shift away from allergen-specific Th2 effector responses toward a regulatory and Th1-skewed phenotype, a shift that is, in principle, directly measurable by flow cytometry rather than inferred indirectly from symptom scores [30]. Palomares and colleagues extend this mechanistic frame to regulatory T and B cells, describing how IL-10- and TGF-beta-producing regulatory lymphocyte subsets expand during tolerance induction, giving immunophenotyping panels a second, tolerogenic axis to track alongside the contraction of pathogenic Th2A cells and ILC2 already described [31,13,17].

Shamji and Durham's review of predictive biomarkers for allergen immunotherapy places flow cytometric cellular markers alongside antibody-based measures, concluding that no single marker yet has sufficient standalone predictive value but that panels combining allergen-specific Th2A cell frequency, basophil reactivity, and regulatory lymphocyte expansion outperform any single assay for forecasting clinical response [32]. Eguiluz-Gracia and colleagues' broader review of biomarkers for allergen immunotherapy across allergic diseases reaches a similar conclusion, and specifically highlights local allergic rhinitis as a phenotype where flow cytometric and nasal-compartment biomarkers are most

needed, precisely because the systemic biomarkers used elsewhere in allergy diagnosis are, by definition, unrevealing in this population [33,8]. Non-allergic rhinitis remains the essential negative comparator against which any proposed local allergic rhinitis flow cytometric signature must be validated, since symptom overlap between the two conditions is substantial and misclassification carries direct treatment consequences [6]. Table 2 summarizes how the flow cytometric markers discussed in this review shift across the diagnostic and treatment-response continuum from non-allergic rhinitis through systemic allergic rhinitis to successful immunotherapy.

Table 2: Flow cytometric marker trends across the diagnostic and treatment-response continuum from non-allergic rhinitis to successful allergen immunotherapy [6,30,31,32,33].			
Clinical state	Th2A / ILC2 signal	Basophil reactivity	Regulatory lymphocyte signal
Non-allergic rhinitis	Not elevated	Low	Baseline [6]
Local allergic rhinitis (systemic IgE negative)	Elevated, mucosal-predominant	Variable, may require nasal challenge	Baseline to modestly reduced [5,7]
Active systemic allergic rhinitis	Elevated	High, allergen dose-dependent	Reduced [13,32]
Successful allergen immunotherapy	Contracted toward baseline	Reduced reactivity	Expanded (IL-10/TGF-beta+ subsets) [30,31]

7. Discussion

Taken together, the flow cytometric immunophenotyping literature converges on a consistent picture: allergic rhinitis, including its local, skin-test-negative variant, is characterized by measurable, reproducible shifts in the frequency and activation state of Th2A cells, ILC2, basophils, eosinophils, and mucosal B/plasma cells, each of which can in principle be captured by a peripheral blood or nasal sample panel [13,16,19,23,25]. The strongest evidence base supports basophil activation testing, which already has defined protocols, dose-response characterization, and demonstrated clinical utility in discordant cases, positioning it as the

immunophenotyping assay closest to routine adoption [18,21]. ILC2 and Th2A enumeration are mechanistically compelling and track disease activity and immunotherapy response in research cohorts, but standardized gating, reference ranges, and inter-laboratory reproducibility data comparable to those available for BAT are still needed before these assays can be deployed outside specialized immunology laboratories [9,17].

A recurring theme across this literature is that the diagnostic value of flow cytometric immunophenotyping is greatest precisely where the conventional triad of history, skin testing, and serum IgE is weakest: local allergic rhinitis, where systemic markers are negative by definition, and immunotherapy monitoring, where a binary allergic/non-allergic classification is insufficient and a graded, mechanistic readout of treatment response is required [5,32]. This complementary relationship, rather than outright replacement of existing testing, is the most defensible near-term clinical role for these assays, and it is the framing adopted by recent biomarker reviews in the field [33,21]. Cost, the need for fresh or carefully preserved samples, operator-dependent gating, and the absence of harmonized commercial panels remain the principal barriers between the research literature summarized here and routine clinical laboratory deployment, and closing that gap is the logical next step for the field.

8. Conclusion

Flow cytometric immunophenotyping of Th2A cells, group 2 innate lymphoid cells, basophils, and eosinophils captures cellular events in allergic rhinitis that the conventional diagnostic triad of history, skin testing, and serum specific IgE cannot, and it is particularly valuable in local allergic rhinitis and in monitoring the immunological effect of allergen immunotherapy, two clinical scenarios where systemic testing is structurally limited [5,13,32]. Basophil activation testing is the most clinically mature of these assays and is already

recommended as a diagnostic adjunct in discordant cases, while ILC2 and Th2A enumeration remain promising research tools awaiting the standardization needed for routine use [21,9]. As multiparameter panels become more accessible and better standardized, incorporating flow cytometric immunophenotyping alongside, rather than instead of, existing allergic rhinitis diagnostics is likely to improve both diagnostic accuracy in atypical presentations and the precision with which immunotherapy candidates are selected and monitored.

9. References

1. Bousquet J, Van Cauwenberge P, Khaltaev N; ARIA Workshop Group (2001) Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol 108(5 Suppl): S147-334.
2. Bousquet J, Khaltaev N, Cruz AA, et al. (2008) Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update. Allergy 63(Suppl 86): 8-160.
3. Brozek JL, Bousquet J, Agache I, et al. (2017) Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines-2016 revision. J Allergy Clin Immunol 140(4): 950-958.
4. Pawankar R, Canonica GW, Holgate ST, Lockey RF, Blaiss MS, eds (2013) WAO White Book on Allergy: Update 2013. World Allergy Organization.
5. Rondon C, Campo P, Togias A, et al. (2012) Local allergic rhinitis: concept, pathophysiology, and management. J Allergy Clin Immunol 129(6): 1460-1467.
6. Powe DG, Jagger C, Kleinjan A, Carney AS, Jenkins D, Jones NS (2003) "Entopy": localized mucosal allergic disease in the absence of systemic responses for atopy. Clin Exp Allergy 33(10): 1374-1379.
7. Eguiluz-Gracia I, Fernandez-Santamaria R, Testera-Montes A, et al. (2017) The need to differentiate between local and systemic allergic rhinitis. Curr Allergy Asthma Rep 17(12): 84.
8. Campo P, Rondon C, Gould HJ, Barrionuevo E, Gevaert P,

- Blanca M (2016) Local allergic rhinitis. Immunol Allergy Clin North Am 36(2): 321-332.
9. Mjosberg J, Spits H (2016) Human innate lymphoid cells. J Allergy Clin Immunol 138(5): 1265-1276.
10. Bartemes KR, Kephart GM, Fox SJ, Kita H (2014) Enhanced innate type 2 immune response in peripheral blood from patients with asthma. J Allergy Clin Immunol 134(3): 671-678.
11. Galli SJ, Tsai M, Piliponsky AM (2008) The development of allergic inflammation. Nature 454(7203): 445-454.
12. Kay AB (2001) Allergy and allergic diseases. First of two parts. N Engl J Med 344(1): 30-37.
13. Wambre E, Bajzik V, DeLong JH, et al. (2017) A phenotypically and functionally distinct human TH2 cell subpopulation is associated with allergic disorders. Sci Transl Med 9(401): eaam9171.
14. Wambre E, DeLong JH, James EA, LaHood NA, Robinson D, Kwok WW (2012) Differentiation stage determines pathologic and protective allergen-specific CD4+ T-cell outcomes during immunotherapy. J Allergy Clin Immunol 129(2): 544-551.
15. Broide DH (2001) Molecular and cellular mechanisms of allergic disease. J Allergy Clin Immunol 108(2 Suppl): S65-71.
16. Mjosberg JM, Trifari S, Crellin NK, et al. (2011) Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CCR2 and CD161. Nat Immunol 12(11): 1055-1062.
17. Lao-Araya M, Steveling E, Scadding GW, Durham SR, Shamji MH (2014) Seasonal increases in peripheral innate lymphoid type 2 cells are inhibited by subcutaneous grass pollen immunotherapy. J Allergy Clin Immunol 134(5): 1193-1195.
18. Boumiza R, Debarb AL, Monneret G (2005) The basophil activation test by flow cytometry: recent developments in clinical studies, standardization and emerging perspectives. Clin Mol Allergy 3(1): 9.
19. Sanz ML, Sanchez G, Gamboa PM, et al. (2001) Allergen-induced basophil activation: CD63 cell expression detected by flow cytometry in patients allergic to Dermatophagoides pteronyssinus and Lolium perenne. Clin Exp Allergy 31(7): 1007-1013.
20. Gober LM, Eckman JA, Sterba PM, et al. (2007) Expression of activation markers on basophils in a controlled model of anaphylaxis. J Allergy Clin Immunol 119(5): 1181-1188.
21. Hoffmann HJ, Santos AF, Mayorga C, et al. (2015) The clinical utility of basophil activation testing in diagnosis and monitoring of allergic disease. Allergy 70(11): 1393-1405.
22. Rondon C, Campo P, Salas M, et al. (2011) Nasal allergen provocation test with multiple aeroallergens detects polysensitization in local allergic rhinitis. J Allergy Clin Immunol 128(6): 1192-1197.
23. Bochner BS (2000) Systemic activation of basophils and eosinophils: markers and consequences. J Allergy Clin Immunol 106(5 Suppl): S292-S302.
24. Ciprandi G, Buscaglia S, Pesce G, et al. (1995) Minimal persistent inflammation is present at mucosal level in patients with asymptomatic rhinitis and mite allergy. J Allergy Clin Immunol 96(6 Pt 1): 971-979.
25. KleinJan A, Vinke JG, Severijnen LW, Fokkens WJ (2000) Local production and detection of (specific) IgE in nasal B cells and plasma cells of allergic rhinitis patients. Eur Respir J 15(3): 491-497.
26. Gould HJ, Sutton BJ (2008) IgE in allergy and asthma today. Nat Rev Immunol 8(3): 205-217.
27. Durham SR, Walker SM, Varga EM, et al. (1999) Long-term clinical efficacy of grass-pollen immunotherapy. N Engl J Med 341(7): 468-475.
28. Cox L, Nelson H, Lockey R, et al. (2011) Allergen

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- immunotherapy: a practice parameter third update. J Allergy Clin Immunol 127(1 Suppl): S1-55.
29. Pfaar O, Bachert C, Bufe A, et al. (2014) Guideline on allergen-specific immunotherapy in IgE-mediated allergic diseases. Allergo J Int 23(8): 282-319.
30. Till SJ, Francis JN, Nouri-Aria K, Durham SR (2004) Mechanisms of immunotherapy. J Allergy Clin Immunol 113(6): 1025-1034.
31. Palomares O, Akdis M, Martin-Fontecha M, Akdis CA (2017) Mechanisms of immune regulation in allergic diseases: the role of regulatory T and B cells. Immunol Rev 278(1): 219-236.
32. Shamji MH, Durham SR (2017) Mechanisms of allergen immunotherapy for inhaled allergens and predictive biomarkers. J Allergy Clin Immunol 140(6): 1485-1498.
33. Eguiluz-Gracia I, Tay TR, Hew M, et al. (2018) Recent developments and highlights in biomarkers in allergic diseases: biomarkers for allergen immunotherapy. Allergy 73(9): 1763-1774.