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Association between the type of allergen and T-helper 2 mediated inflammation in allergic reactions: a systematic review and a meta-analysis

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Abstract

Objectives: To determine whether the levels of T-helper (T_H) 2 cytokines (interleukin (IL)-4 and IL-5) in allergic reactions are allergen dependent and evaluate the impact of various treatment strategies on the levels of these cytokines.

Methods: The PubMed search engine was used from inception until January 2021. The random-effects residual maximum likelihood model was performed, and effect sizes were estimated using the Hedge's g statistic. All data analysis was performed using STATA 16.0 (StataCorp LP, TX, USA).

Results: Fourteen studies reporting on 794 participants were included in this study. House dust mite was associated with eliciting a stronger immune response mediated by both IL-4 and IL-5 when compared to pollen. Whereas a mixture of house dust mite and pollen was associated with IL-4-weighted inflammation. Comparisons of IL-4 and IL-5 levels amongst the allergens showed significant differences. The treatment with anti-corticosteroids or allergen-specific immunotherapy was effective in normalising the T_H 2 responses and alleviating allergy symptoms.

Conclusion: T_H 2-mediated inflammation in allergic reactions is allergen-dependent. Therefore, the type of allergen should be considered when using cytokine-targeting biologics in allergic reactions.

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Introduction

A balance between T-helper (T_H)1 and T_H 2 immune responses is important in modulating inflammation and maintaining immune homeostasis.¹ An alteration in the T_H 1/ T_H 2 ratio is a hallmark of several immune-mediated diseases, including immediate hypersensitivity disorders.^{2,3} In fact, allergic reactions are characterised by an exacerbated T_H 2-skewed immune response and the manifestation of allergic symptoms such as atopic dermatitis, allergic rhinitis and asthma.^{4,5} Notably, the resulting inflammatory milieu is modulated by interleukin (IL)-4 and IL-5, the two major cytokines that mediate T_H 2-mediated inflammation.⁶ The aggravated elevation of these cytokines is associated with increased activation of B-cells, eosinophils, basophils and the secretion of immunoglobulin (Ig)G and IgE in allergic reactions.⁷⁻⁹ As a result, several current therapeutic strategies aim to alleviate these symptoms and ameliorate T_H 2-mediated inflammation.¹⁰⁻¹³

Although allergic responses are characterized by a predominant T_H 2 response, there are some discrepancies with regards to the levels of IL-4 and IL-5 in patients with allergies. For instance, the allergic responses in patients hypersensitive to house dust mite (HDM) are associated with elevated serum IL-4 and IL-13- levels.^{14,15} Whereas increased IL-5 levels have been reported in patients allergic to pollen.¹⁶ A comparison between patients allergic to HDM and pollen showed that the levels of IL-5 are in fact elevated in the former group.¹⁷ In patients allergic to peanuts, the immune response is skewed towards IL-4,¹⁰ albeit others suggested it to be IL-5-weighted.¹⁸ Therefore, these inconsistencies suggest that the immune responses in allergic reactions may be dependent on the type of allergen.

The use of allergen-specific immune therapy, antihistamines, and corticosteroids in allergic reactions has been to a greater extent successful in alleviating T_H 2-mediated inflammation and the associated symptoms, albeit poor efficacy due to various factors such as age and obesity has been noted in some patients.^{19,20} Consequently, cytokine-targeting biologics are currently being explored as an alternative treatment approach in allergic reactions.^{11,21,22} In this systematic review and meta-analysis, we aimed at assessing available literature reporting on T_H 2-mediated inflammation in allergic reactions. The primary objective of this study was to investigate whether the levels of IL-4 and IL-5, the key cytokines that mediate inflammation in an allergic response, are allergen-dependant. The secondary objective was to assess the impact of treatment on the levels of these cytokines.

Methods

This systematic review and meta-analysis was prepared following the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines.²³ A protocol was designed and agreed upon by all authors before conducting the qualitative and quantitative synthesis (Appendix 1). The protocol was however not registered. We conducted a comprehensive and systematic search of available literature to answer the following research question: are T_H 2 immune responses in allergic reactions influenced by the type of allergens?

Sources of evidence and search strategy

A comprehensive search was designed and independently conducted by two reviewers (EPN and TMN) using the PubMed search engine from inception until January 2021. The following search terms were used to retrieve all relevant studies; “allergy”, “allergens”, “IL-4”, “IL-5”, “ T_H 2” and “type 1 hypersensitivity”. Moreover, grey literature and the reference lists of included studies were scanned to identify any additional studies. No language restrictions were applied on the search strategies, and a third reviewer (BBN) was consulted for arbitration in cases of disagreements. A detailed MEDLINE search strategy on PubMed search engine is shown in [Supplementary Table S1](#).

Eligibility criteria and study selection

Two independent investigators (EPN and FN), with the help of third reviewer (TMN), screened all titles and abstracts of the retrieved studies. Studies were included if they reported on the levels of both IL-4 and IL-5 in patients of all ages with allergies.

Inclusion and exclusion criteria

The following PICO was used in the inclusion criteria:

- Participants: patients of all ages with allergies
- Intervention: none
- Comparisons: healthy individuals without allergies (controls).
- Outcome: T_H 2-mediated inflammation

We excluded reviews, case studies, letters to the editor and animal studies from this systematic review and meta-analysis. In addition, studies that were not relevant to the topic of interest or with no suitable comparator were also excluded.

Data extraction and management

Two independent reviewers (EPN and FN) extracted detailed study information and characteristics using a pre-defined data extraction form adapted from the Cochrane Consumers and Communication Review Group data extraction for included studies template.²⁴ The extracted data consisted of names of authors, publication year, study design, study size, age and gender, types of allergens, IL-4 and IL-5 levels and the main findings of each study. The extracted data items were verified by FN, and disagreements were resolved through discussions or by consulting the third reviewer (TMN).

Assessment of risk of bias

The risk of bias in all included studies was independently assessed by two reviewers (EPN and FN) using the modified Downs and Black checklist.²⁵ The checklist consists

of four domains, reporting bias, external validity, internal validity and selection of bias. The studies were rated as follows: excellent (a score between 24-27), good (score of 19-23), fair (score of 13-18) or poor (score of <12). A third reviewer (PVD) was consulted for arbitration in cases of disagreements. The Cohen's kappa scores were used to measure inter-rater reliability, and a score of 0.00 was considered poor, slight (0.01-0.20), fair (0.21-0.40), moderate (0.41-0.60), substantial (0.61-0.80) and perfect (0.81-1.00).²⁶

Statistical analysis

The mean and standard deviation for each effect measure was either extracted or estimated using GetData Graph Digitizer software or calculated from the median range using Hoza et al.'s method.²⁷ The continuous outcome was reported as standardised mean differences and 95% confidence interval (CI), and effect sizes were estimated using the Hedge's *g* statistic to correct for small study bias. A random-effects residual maximum likelihood (REML) model was performed to minimise the bias of variance estimator. A sensitivity analysis was performed based on study designs to evaluate the robustness of the reported overall effect estimates. A *P* value of < 0.05 was considered significant. All data analysis was performed using STATA 16.0 (StataCorp LP, TX, USA).

Results

Selected studies

The search identified a total of 71 studies which were assessed for eligibility. A total of 50 studies were excluded at the abstract screening stage as these were not describing findings related to the outcomes of the present study. The remaining 21 studies were assessed for eligibility using full texts and a total of seven citations were excluded because five were not relevant to the topic of interest, while two studies had no suitable comparators. As a result, only 14 studies met the inclusion criteria and were included in this systematic review and meta-analysis (overall agreement, 97.62%; kappa=0.95), and only eight were included in the quantitative synthesis (Figure 1).

Characteristics of included studies

The included studies consisted of two randomised controlled trials (RCTs), three non-RCTs and nine observational studies published in peer-reviewed journals between 1997 and 2017. Of these studies, four were from Asia,^{12,15,28,29} three from Europe,^{13,30,31} five from North America^{10,14,16,18,32} and two from Oceania.^{17,33} A total of 794 participants with a male/female ratio of 0.53 were included in this systematic review and meta-analysis. Of which, 565 participants had allergies, and 229 were healthy controls. The cohort consisted of 64% of the participants being children (Table 1) and 36% adults (Table 2) with an average age of 11.10 ± 4.44

years and 44.03 ± 17.45 years, respectively. A total of 26% of patients from the allergic group were allergic to pollen, 29% to HDM, 4% to peanut and 13% consisted of a mixture of patients allergic to pollen and HDM, whilst 28% were not specified.

Study quality and risk bias

The median score range of included studies was 15 (14-24) out of a possible score of 27 (Supplementary Table S2).^{14,24} All studies were rated as fair^{10,12, 13-18, 28,31-33} except for one study which was scored as good.²⁹ Thus, the included studies had a moderate risk of bias. Assessments based on the four domains showed that the included studies had a low risk of reporting bias with a median score of 7.5 (6-10) out of the possible score of 11 (overall agreement, 92.85%; kappa=0.86) and internal validity bias, a median of 4 (3-6) out of the possible score of 7 (overall agreement, 92.85%; kappa = 0.86). However, the studies had high risk of external validity bias with a median of 1 (0-2) out of the possible score of 3 (overall agreement, 88.10%; kappa = 0.76) and selection bias with a median of 2.5 (2-6) out of the possible score of 6 (overall agreement, 97.62%; kappa = 0.95).

The levels of IL-4 in patients with allergies are influenced by the type of allergen

Most of the included studies (36%) involved patients allergic to pollen, followed by those allergic to HDM (29%) and peanuts (14%). Interleukin-4 shares common receptor and functional properties with IL-13,³⁴ and 57% of the included studies reported elevated levels of both cytokines. Allergic responses irrespective of the allergens were reported to be IL-4-weighted in 36% of the included studies,^{10,14,15,29,32} whereas a few reported undetectable low levels.^{17,18} Notably, the levels of IL-4 and IL-13 were associated with those of allergen-specific IgE^{10,31} and disease severity.¹² We performed a subgroup analysis based on the type of allergen and participants' characteristics. The test for subgroup differences was only significant in the allergen subgroup (*P* = 0.01), which showed that the type of allergen in these patients influenced the levels of IL-4 and had an impact on the reported effect size. Studies that included patients allergic to HDM (Hedges' *g*: 0.68 [95% confidence interval [CI]: 0.12, 1.24], *P* = 0.018) and those that involved a mixture of patients allergic to pollen and HDM (Hedges' *g*: 1.02 [95% CI: 0.39, 1.65], *P* = 0.002) had a large increase in IL-4 levels than healthy controls. However, pollen (*P* = 0.579) and unspecified allergens (*P* = 0.065) did not affect the reported effect measure of T_H2 immune responses (Figure 2). We further compared the standardised means of IL-4 levels in each antigen. Notable differences were observed in pollen versus HDM (*P* = 0.0430) versus pollen and HDM (*P* = 0.0009) and versus unspecified antigens (*P* = 0.0002). In addition, HDM vs. unspecified antigens (*P* = 0.0024) (Supplementary Table S3). We performed a sensitivity analysis based on the study design. Notably, Hedges's *g* did not change direction nor the magnitude of effect size (Supplementary Table S4).

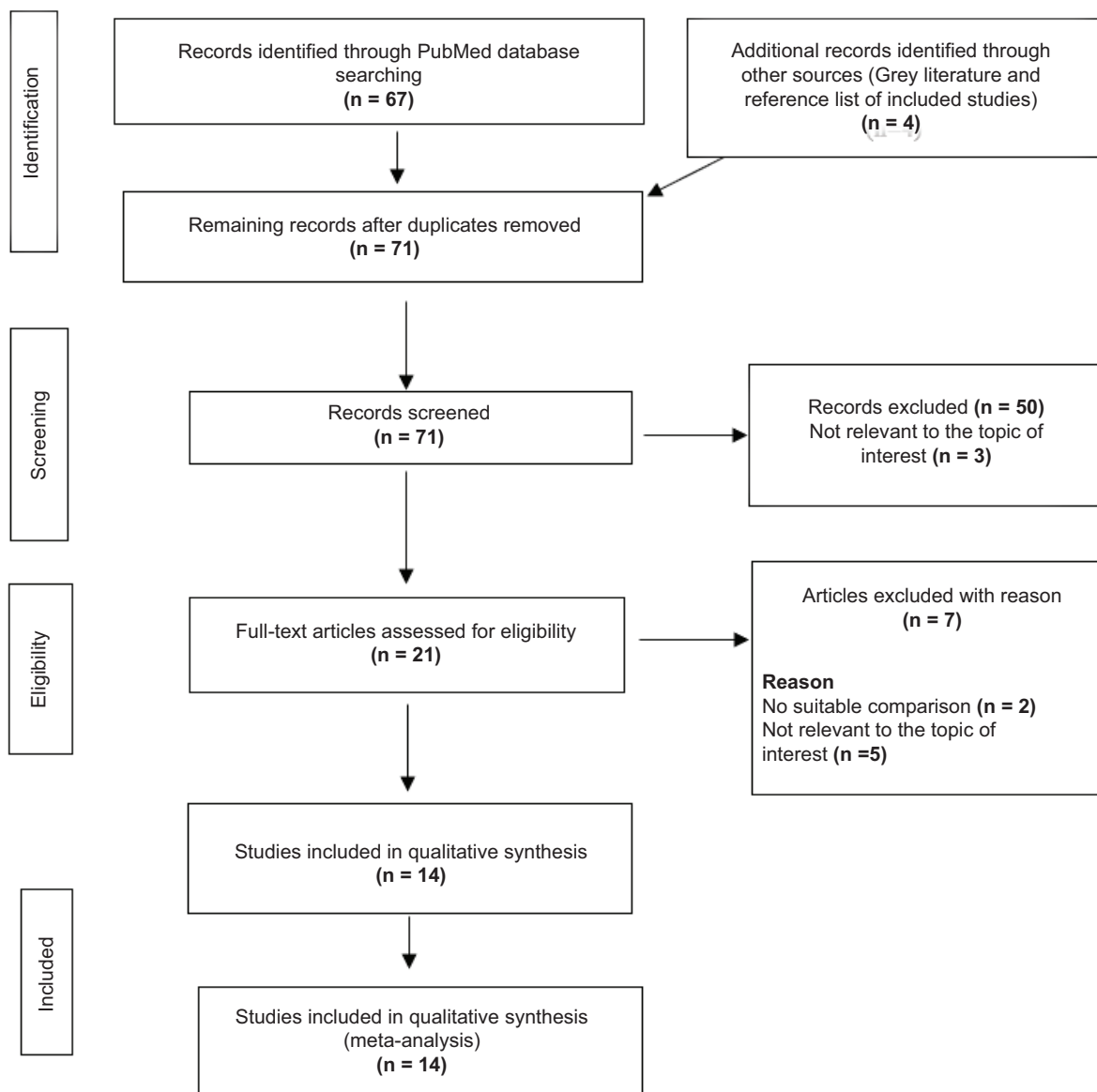


Figure 1 PRISMA flow diagram showing the study selection process.

Different types of allergen influence IL-5 levels in patients with allergies

A total of 43% of the included studies^{12,16-18,28,33} reported IL-5-weighted immune responses in patients with allergy, whereas 14% reported on comparable or undetectably low levels.^{14,15} The elevated levels of IL-5 in patients with allergy were also associated with increased levels of IgE,¹⁷ eosinophils^{12,13} and disease severity.^{28,33} A comparison between patients allergic to HDM and pollen showed a significant increase in IL-5 levels in the former group.¹⁷ We, therefore, performed a meta-analysis to assess the levels of IL-5 in patients with allergies. Similarly to IL-4, the quantitative synthesis of included studies revealed that IL-5 levels are dependent on the type of allergen and not participants' characteristics. The test for subgroup differences in the

allergen subgroup was significant ($P = 0.01$), suggesting that allergens modified the reported effect size of T_H2 immune responses. Studies involving participants allergic to HDM (Hedges' g : 0.80 [95% CI: 0.02, 1.59], $P = 0.044$) and unspecified allergens (Hedges' g : 2.04 [95% CI: 1.10, 2.98], $P < 0.01$) had a large increase in the levels of IL-5 versus healthy controls (Figure 3). However, studies including patients allergic to pollen ($P = 0.857$) or a mixture of patients allergic to pollen and HDM ($P = 0.082$) did not modify the effect estimate. Comparisons of standardised means of IL-5 levels amongst the allergens showed significant differences in unspecified antigens versus pollen ($P < 0.0001$) versus HDM ($P = 0.0086$) versus pollen and HDM ($P = 0.0001$) (Supplementary Table S3). The sensitivity analysis showed that only the cohort study changed the direction of the effect size (Supplementary Table S4).

Table 1 Characteristic features of included studies reporting on interleukin (IL)-4 and IL-5 in children with allergies (n=7).

Study	Country	Study design	Age (years)	Male n (%)	Allergen/s	Main findings	Risk of bias
Benson et al. ¹³	Sweden	Cohort study involving 38 patients with allergic rhinitis and 19 healthy controls.	Allergic (8 ± 2.5) Control (13-19)	Allergic 20 (52%) Control 11 (58%)	Grass and birch pollen	The levels of both IL-4 and IL-5 were exacerbated during pollen season when compared to pre-pollen season. The increment of both cytokines during pollen season was associated with an elevation in eosinophil counts. Notably, treatment with budesonide, a corticosteroid, induced a significant expression of soluble IL-4 receptor and reduced the levels of eosinophils.	Medium
Smart et al. ¹⁷	Australia	Cross-sectional study involving 46 children patients with atopy and 23 healthy controls.	Allergic (6.9 ± 3.6) Control (7.3 ± 4.2)	Not reported	House dust mite and rye grass pollen	<i>Staphylococcal enterotoxin B</i> (SEB)-induced plasma levels of IL-4 and IL-5 were significantly increased in the symptomatic group when compared to the non-atopic group, which were comparable with the asymptomatic group. Activation of T-helper (T _H) cells with house dust mite and rye grass pollen, induced a significant release of IL-5 and IL-13 in both groups when compared to the controls. The levels of IL-5 were higher in children allergic to house dust mites when compared to RYE grass pollen. The levels of IL-4 were however, below the lower limit of detection in both groups. The elevated levels of IL-5 were concomitant with elevated immunoglobulin (Ig) E levels.	Medium
Rastogi et al. ³²	USA	Cross-sectional study involving 60 patients with atopy and 30 healthy controls.	Allergic (9.2 ± 1.2) Control (9.3 ± 1.5)	Not indicated	House dust mite	Serum levels of IL-4 and IL-13 were higher in the lean asthmatic group than the obese group. Activation of T _H cells from children with atopy using the house dust mite-induced allergic rhinitis model released significantly increased levels of IL-4 in the lean asthmatic group vs. their lean counterparts.	Medium
Wisniewski et al. ¹⁰	USA	Non-randomised controlled trial (RCT) involving 21 patients allergic to peanuts and 8 control patients.	Allergic (9.59 ± 5.0) Control (9.75 ± 4.9)	Allergic 9 (43%) Control 2 (25%)	Ara h1 and Ara h2 (peanuts)	Ara h2 is the most common predictor of peanut allergy. More than 85% of the children were sensitised to both Ara h1 and Ara h2. The levels of IL-4, IL-5 and IL-13 were elevated in children with atopy vs. controls. Notably, Ara h2 specific IgE levels were higher than that of Ara h1 specific antibodies. In addition, high levels of peanut specific IgE were associated with IL-4 skewed T _H cell responses. Interestingly, both allergens induced similar degree of T-cell activation between children who received oral immunotherapy and those that did not. Nevertheless, oral immunotherapy reversed the IL-4-biased T-cell response.	Medium

(continues)

Table 1 Continued

Study	Country	Study design	Age (years)	Male n (%)	Allergen/s	Main findings	Risk of bias
Rastogi et al. ¹⁴	USA	Cross-sectional study involving 84 patients with asthma and 44 healthy controls.	Allergic (15.70 ± 1.8) Control (16.3 ± 1.7)	Allergic 44 (54%) Control 16 (36%)	House dust mite	Patients with asthma had poor pulmonary function when compared to the non-asthmatic group. House mite activated T _H cells in the lean group expressed increased levels of IL-4 in comparison to the obese group. Notably the IL-4 levels in the obese group were significantly higher than that of the healthy controls. However, the levels of IL-5 were below detectable levels.	Medium
Wambre et al. ¹⁸	USA	RCT involving 80 patients with atopy and 34 healthy controls.	4 to 26	Not reported	Peanuts	Allergy-associated T _H 2 (T _H 2A) cells are nearly non-existent in individuals without atopy and unlike conventional T _H 2, they co-express CD161 and CR45RB or CD27 on their cell surface. Notably, activated T _H 2A expressed elevated levels of IL-5 in comparison to convectional T _H 2 cells. However, the levels of IL-4 were comparable between the two T _H 2 subsets.	Medium
Wang et al. ¹⁵	Taiwan	Non-RCT involving 12 patients with asthma and 12 healthy controls.	Allergic (10.49 ± 1.7) Control (11.1 ± 3.5)	Allergic 8 (67%) Control 8 (67%)	House dust mite	A subgroup analysis of patients with peanuts allergy showed that oral desensitisation immunotherapy reduced the levels of T _H 2A when compared to pre-treatment. Activation of peripheral blood mononuclear cells with house dust mite was associated with increased levels of IL-4 and IL-13 in the asthmatic allergic group when compared to the healthy controls. However, the levels of IL-5 were comparable between the groups. Notably, the levels of IL-4 and IL-5 were significantly lower in the allergen-specific immunotherapy group in comparison to the asthmatic allergic group.	Medium

Table 2 Characteristic features of included studies reporting on interleukin (IL)-4 and IL-5 in adult patients with allergies (n=7)

Study	Country	Study design	Age (years)	Male n (%)	Allergen/s	Main findings	Risk of bias
Li et al. ¹⁶	Canada	Cross-sectional study involving 20 patients with allergic rhinitis and 20 healthy controls.	18 to 35 (All participants)	Not reported	Grass pollen	Activation of peripheral blood mononuclear cells with grass pollen antigens elevated the levels of IL-4, IL-5 and IL-13 in patients with allergy when compared to healthy controls. Notably, CD4 ⁺ T-cell responses mediated by IL-13 was positively associated with IL-5 and not IL-4 levels.	Medium
Oda et al. ¹²	Japan	Non-RCT involving seven patients with house dust mite sensitive asthma and three healthy controls.	18 to 26 (All participants)	Not reported	House dust mite	The activation of T-cells by house dust mite specific allergen skewed the immune response towards IL-4 and IL-5 weighted. The elevation of IL-5 and IL-4 in these patients was associated with poor pulmonary function and disease severity. Notably, increased levels of IL-5 were directly associated with an elevation of eosinophils. Interestingly, there was a significant reduction in the secretion of both cytokines by the T-cells following a modified allergen-specific immunotherapeutic procedure (rush immunotherapy). This reduction was associated with an increase in interferon (IFN)- γ levels.	Medium
Tang et al. ³³	Australia	Cross-sectional study involving 28 patients with atopy and 10 healthy controls.	Allergy (40.36 \pm 13.1) Control (29.3 \pm 7.8)	Allergy 21 (75%) Control 7 (70%)	Rye grass pollen and house dust mite	Unstimulated T-cells had a slight increase in IL-4 and significant release of IL-5 when compared to healthy controls. Notably, there was no significant changes in the levels of both cytokines released by T-cells post specific allergen stimulation. However, subgroup analysis showed that patients with atopy and non-asthmatic had significantly elevated levels of IL-5 vs. the control group. Increased levels of IL-5 were associated with increased airways obstruction and decreased pulmonary function.	Medium
Movérare et al. ³¹	United Kingdom	Cross-sectional study involving seven patients allergic to birch pollen and six healthy controls.	Allergy 24 to 38 Control 26 to 47	Allergy 2 (29%) Control 1 (17%)	Birch pollen	Activation of T-cells with birch-pollen extract skewed the immune response towards IL-4, IL-5 and IL-13 and reduced the levels of IFN- γ . Elevated levels of IL-4 and IL-13 directly correlated with increased levels of birch-pollen-specific IgE levels.	Medium

(continues)

Table 2 Continued

Study	Country	Study design	Age (years)	Male n (%)	Allergen/s	Main findings	Risk of bias
Lee et al. ²⁸ 2001	South Korea	Cross-sectional study involving 80 patients with asthma and 10 healthy controls	Allergy (53.83 ± 16.7) Control (48.6 ± 10.2)	Allergy 48 (60%) Control 6 (60%)	Not indicated	The serum levels of IL-4, IL-5, and IL-13 were elevated, whereas IFN- γ levels were reduced in patients with asthma than healthy controls. Subgroup analysis showed that patients with acute asthma had exacerbated levels of both IL-4, IL-5 and IL-13 in comparison to asymptomatic group. Notably, the increased levels of IL-5 were directly associated with severe airway obstruction in patients with acute asthma.	Medium
Bullens et al. ³⁰	Belgium	Cross-sectional study involving 15 adults allergic to pollen and 10 healthy controls.	Not reported for all participants	Allergy 7 (47%) Control 4 (40%)	Birch pollen	Activated T-cells with birch pollen antigen significantly released elevated levels of IL-4, IL-5, and IL-13 in comparison to unstimulated levels. Notably, activation of T-cells with IL-4 augmented IL-5 and IL-13 levels in comparison to without IL-4.	Medium
Horiguchi et al. ²⁹	Japan	RCT involving 67 patients allergic to cedar pollen.	Treated (26.8 ± 5.4) Placebo (26.4 ± 5.9)	Treated 22 (51%) Placebo 11 (46.8)	Cedar pollen	In comparison to before pollen season, the levels of IL-4 were significantly increased post pollen season only in the untreated group. The level of IL-5 remained comparable between both seasons. Immunotherapy reduced the increase fold of IL-4 and IL-5 during the pollen season which was concomitant with mild clinical symptoms and elevated serum IgG4 activity during the pollen season.	Low

The effect of treatment on the levels of IL-4 and IL-5 in patients with allergies

As expected in allergic inflammation, a comparison of IL-4 levels between symptomatic patients and healthy controls showed a large effect size (Hedges' *g*: 1.30 [95% CI: -0.10, 2.70], *P* = 0.068) (Figure 2) whilst revealing a small effect size in IL-5 levels (Hedges' *g*: 0.38 [95% CI: -0.14, 0.90], *P* = 0.150) (Figure 3). Hence, treatment strategies are designed to antagonise the effect of these cytokines in allergic responses. A total of 43% of included studies reported on the effect of treatment on these T_H2-type cytokines. Treatment with corticosteroids,¹³ oral immunotherapy^{12,15,19} and rush immunotherapy reduced IL-4 and IL-5 levels.^{10,18} This decrease was associated with reduced eosinophil counts and increased levels of T_H1-type cytokines. Notably, treatment lowered IL-4 levels to almost like that of healthy controls (Hedges' *g*: 0.11 [95% CI: -0.34, 0.56], *P* = 0.638) (Figure 2). Only one study reported on the levels of IL-5 in the treated group, hence we could not perform a meta-analysis. Nonetheless, there was a large effect size in the levels of IL-5 between untreated and treated individuals with allergies (Hedges' *g*: 0.82 [95% CI: -0.89, 2.53], *P* = 0.346) (Figure 3).

Discussion

This systematic review and meta-analysis aimed to comprehensively assess available literature reporting on T_H2-mediated inflammation in allergic reactions. The primary objective of this study was to determine whether the levels of IL-4 and IL-5, the key cytokines that mediate

inflammation in allergic reactions, are influenced by the type of allergen. Moreover, the secondary objective was to evaluate the effect of various treatment strategies on the levels of these cytokines. The pooled estimates showed that the levels of IL-4 and IL-5 are allergen-dependent. Notably, HDM elicited greater IL-4 and IL-5 mediated immune responses when compared to pollen. Moreover, treatment with the anti-inflammatory corticosteroid drugs or allergen-specific immunotherapy (rush immunotherapy) was effective in normalising the levels of T_H2-cytokines and ameliorating the allergy-associated symptoms.^{10,13,15,18,30} the total nasal symptom scores (TNSS).

The secretion of IL-4 and IL-13 by activated T_H2 cells drive the immune responses triggered by invading allergens in immediate reactions. The subsequent binding of IL-4 and IL-13 to their respective receptors, IL-4Rα and IL-13Rα, activates Janus kinase/signal transducers and activator of transcription-6 (JAK/STAT6) signalling transduction pathway.³⁵ This results in the activation of various promoter genes such as transforming growth factor-beta 1 (TGF-β1) and signalling pathways which are essential for the activation and differentiation of naïve T-cells into T_H2 and B-cells to produce IgE antibodies.³⁶⁻³⁸ Notably, the IL-4 and IL-13-induced STAT6 signalling is enhanced in patients with atopic asthma compared to healthy individuals.³⁹ The activation of STAT6 signalling in allergic responses is associated with increasing levels of IL-4 and IgE, and a decrease in T_H1 cytokines.^{39,40} Interestingly, in an animal study of asthma, STAT6^{null} mice had reduced airway inflammation congruent with impaired T_H2 differentiation and reduced T_H2 immune responses.^{41,42} Although the studies included in our meta-analysis did not assess STAT signalling, our data synthesis showed that the levels of IL-4 and IL-13 were increased in patients with allergy and are associated with increased allergen-specific

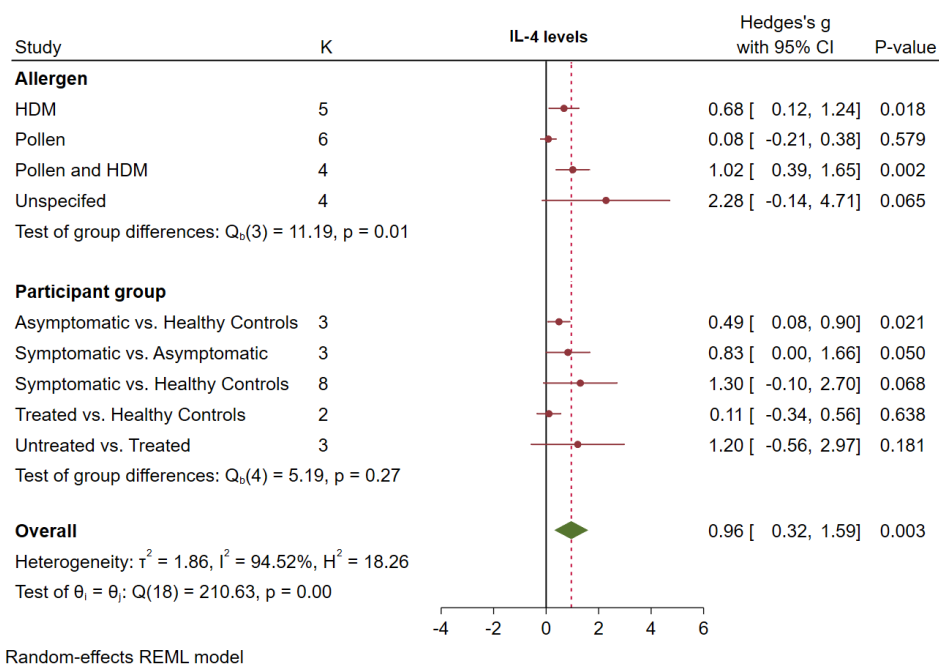


Figure 2 The levels of IL-4 in patients with allergies as an effect measure of T_H2 immune response.

IgE, and disease severity,^{10,12,31} and reduced IFN- γ levels.^{28,31} This highlights the shift towards T_H2 in the T_H1/T_H2 paradigm in allergic responses, possibly induced by enhanced IL-4/IL13-mediated JAK/STAT6 signalling.

The increased activation of eosinophils is also associated and implicated in T_H2 inflammation in allergic responses.⁴³ Even though various cytokines such as IL-4 and IL-3 may induce the differentiation and activation of eosinophils, IL-5 also facilitates these processes.⁴⁴ Notably, the binding of IL-5 to its receptor results in the downstream activation of the PI3K-AKT and MAPK signalling pathways, various STAT transcription factors and tyrosine kinases, which collectively promotes cell differentiation, activation and proliferation and inhibit apoptosis.⁴⁵ As expected, we report on increased levels of IL-5 in allergic responses, which were concomitant with elevated eosinophil counts and increased airways obstruction.^{12,13,28,33}

The use of allergen-specific immunotherapy and anti-inflammatory drugs in patients with allergies is effective in lowering the levels of allergy-associated T_H2 cells, IL-4, IL-13 and IL-5.^{10,12,13,15,18,29} However, because of the heterogeneous nature of allergic responses and confounding factors such as obesity that alter immune responses,³ there is an increased interest in cytokine-targeting biologics as an alternative treatment strategy for allergic responses.¹¹ In that context, while both IL-4 and IL-5 play pivotal roles in mediating type 2 inflammation in allergic patients, our meta-analysis showed that IL-4/IL-5 immune responses are weighted towards IL-4 when compared to IL-5 as indicated by a larger effect size in the levels of the former cytokine. Thus, cytokine-targeting biologics that directly antagonises IL-4/IL-13 may be effective in ameliorating allergic responses. Most importantly, for optimal therapeutic benefit, the type of

antigen should be considered when determining dosage. However, it should be noted that utilisation of IL-4/IL-13 or IL-5 biologic antagonists should be done with caution as it requires a delicate balance in order to correct and maintain the T_H1/T_H2 paradigm, as reported elsewhere.^{35,37}

The main strength of our study is its uniqueness. To our knowledge, this is the first systematic review and meta-analysis to assess the effect of allergen type on the levels of IL-4 and IL-5 in patients with allergies. In addition, the comprehensive literature search and the subsequent data extraction were independently carried out by two reviewers, thus minimising the risk of errors. Moreover, the inter-rater reliability scores were high in the study selection and the risk of bias assessment. Even though the included studies had a medium risk of bias overall, the studies scored poor in the external validity domain. As a result, the findings must be adopted with caution in a population outside this study's populace. Nonetheless, the findings are a true representation of T_H2 responses in allergies as denoted by a high score in internal validity. The main limitation of our study is that some of the included studies did not report on the exact levels of IL-4 and IL-5, so we estimated some levels from the reported graph values using the GetData Graph Digitizer software.

Conclusion

T_H2 -mediated inflammation in patients with allergies is allergen dependent and treatment with anti-inflammatory corticosteroid drugs or allergen-specific immunotherapy is effective in normalising the levels of T_H2 -cytokines and ameliorating the allergy-associated symptoms. Therefore,

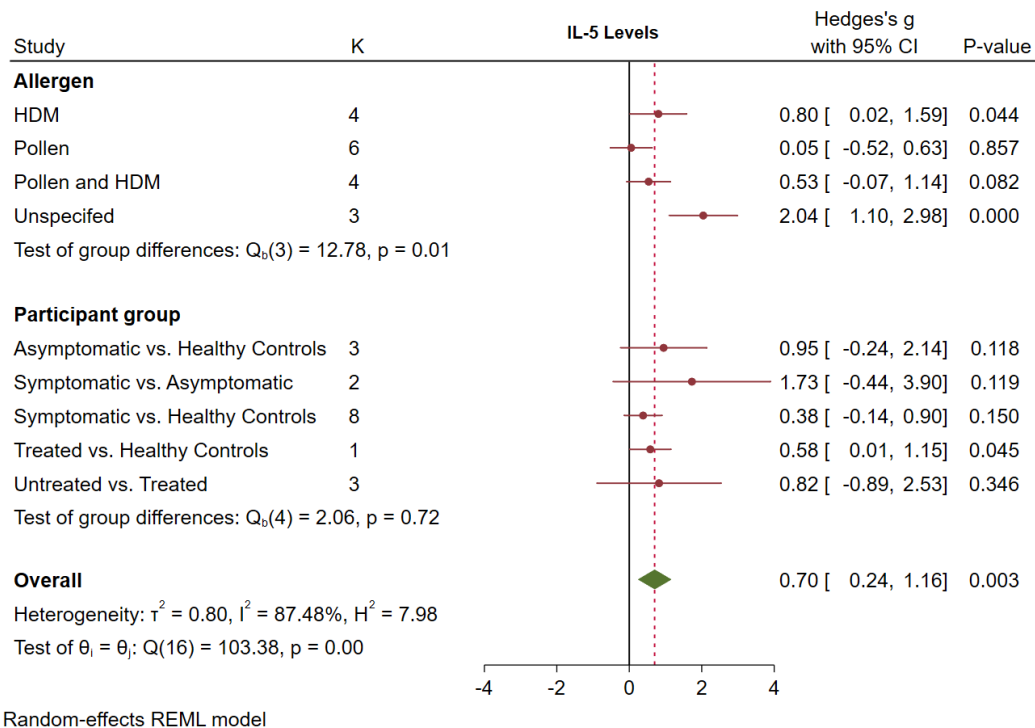


Figure 3 Effect estimates of T_H2 immune response in patients with allergies measured by the levels of IL-5.

to further improve the efficacy of treatment in allergic reactions, the type of allergen should be considered when planning therapeutic strategies, particularly those involving the usage of cytokine-targeting biologics that antagonise the activities of T_H2 signature cytokines.

Abbreviations

IL: interleukin; cardiovascular diseases; JAK/STAT: Janus kinase/signal transducers and activator of transcription; TH2: T helper 2

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable

Availability of data and materials: The authors confirm that the data supporting the findings of this study are available within the article and its supplementary files.

Conflict of interests: We declare no conflict of interests associated with this manuscript.

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Authors' contribution: EPN, BBN and TMN conceptualised, designed and drafted this manuscript. EPN and TMN - search strategy; EPN and FN - study selection; EPN, FN and TMN - data extraction; EPN, FN and PVD - study appraisal; EPN, BBN and TMN - statistical analysis, EPN, BBN, PVD, EN and TMN - editing and final approval of manuscript. TMN is the guarantor of this systematic review and meta-analysis

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Supplementary

Table S1 Search strategies

Terms	Search terms
#1	(T helper cells [MeSH Terms]) =45,274 hits
#2	(IL-5[MeSH Terms]) =5,938 hits
#3	(IL-4[MeSH Terms])= 22,802 hits
#4	(allergen [MeSH Terms])= 41,348 hits
Combined #1 and #2 or #3 and #4	((T helper cells[MeSH Terms]) AND (IL-5[MeSH Terms])) OR (IL-4[MeSH Terms]) AND (allergen[MeSH Terms]) =46 hits (Age: 2-12 years)
Combined #1 and #2 and #3 and #4	((T helper cells[MeSH Terms]) AND (IL-5[MeSH Terms])) AND (IL-4[MeSH Terms]) AND (allergen[MeSH Terms]) =67 hits

Table S2 Modified Downs and Black check list scores for included studies (n = 14)

Author	Domain	Benson 1997	Smart 2002	Rastogi 2012	Wisniewski 2015	Rastogi 2015	Wambre 2017	Wang 2018	Li 1998	Oda 1998	Tang 1998	Moverare 2000	Lee 2001	Bullens 2004	Horiguchi 2008	
Reporting bias	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	3	1	1	1	1	0	0	0	1	1	1	1	1	0	1	
	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	6	1	1	1	1	1	1	1	1	0	1	1	1	1	1	
	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	8	0	0	0	1	1	0	0	0	0	0	0	0	0	1	
	9	0	0	0	0	0	0	1	0	0	0	0	0	0	1	
	10	0	1	1	0	1	1	1	0	0	0	0	1	1	1	
	Score	7	8	8	8	8	7	8	7	6	7	7	8	7	10	
External validity	11	1	1	1	1	0	0	1	0	1	1	1	0	1	1	
	12	0	0	0	0	0	0	0	0	0	0	1	1	0	1	
	13	1	0	0	1	0	0	0	1	0	0	0	0	0	0	
		Score	2	1	1	2	0	0	1	1	1	1	2	1	1	2
Internal validity	14	0	0	1	0	0	1	0	0	0	0	0	0	0	1	
	15	0	0	0	0	0	1	0	0	0	0	0	0	0	1	
	16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	17	0	0	0	0	0	1	1	1	1	1	1	0	1	1	
	18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	19	0	1	1	1	0	0	0	0	0	0	0	1	0	0	
	20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
		Score	3	4	5	4	3	6	4	4	4	4	4	4	4	6
	Selection bias	21	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		22	1	1	1	1	1	0	0	1	1	0	0	0	1	1
23		0	0	0	0	0	1	0	0	0	0	0	0	0	1	
24		0	0	0	0	0	1	0	0	0	0	0	0	0	1	
25		0	1	1	0	1	1	1	1	1	1	1	1	0	1	
26		0	0	0	0	0	0	0	0	0	0	0	0	0	1	
	Score	2	3	3	2	3	4	2	3	3	2	2	2	2	6	
Overall score		14	16	17	16	14	17	15	15	14	14	15	15	14	24	

Table S3 Comparisons of standardised means of interleukin 4 and 5 levels based on the type of allergen

Allergens	Standardised means differences	95%CI	p-value
Interleukin 4 Levels			
HDM vs Pollen	0.60	0.02 to 1.18	0.0430
Pollen and HDM vs Pollen	0.94	-0.02 to 1.90	0.0009
Unspecified vs Pollen	2.20	1.07 to 3.33	0.0002
HDM vs Unspecified	1.60	0.57 to 2.63	0.0024
Pollen and HDM vs Unspecified	0.94	-0.02 to 1.90	0.0544
HDM vs Pollen and HDM	0.34	-0.50 to 1.18	0.4232
Interleukin 5 levels			
HDM vs Pollen	0.75	-0.11 to 1.61	0.0878
Pollen and HDM vs Pollen	0.48	-0.21 to 1.17	0.1730
Unspecified vs Pollen	1.99	1.40 to 2.57	<0.0001
HDM vs Unspecified	1.24	0.32 to 2.16	0.0086
Pollen and HDM vs Unspecified	1.51	0.75 to 2.28	0.0001
HDM vs Pollen and HDM	-0.27	-1.26 to 0.72	0.5923

Table S4 Sensitivity analysis of interleukin 4 and 5 levels based on study design

Study design	Number of studies	Omitted studies	Hedges's g [95% CI]	p-value
Interleukin 4				
All	8	0	0.96 [0.32, 1.59]	0.003
Cross-sectional	6	2	1.54 [-0.31, 3.40]	0.103
Cohort	1	7	0.18 [-0.34, 0.90]	0.619
Non-RCT	1	7	1.00 [0.19, 1.80]	0.016
Interleukin 5				
All	8	0	0.70 [0.24, 1.16]	0.003
Cross-sectional	6	2	0.56 [0.04, 1.07]	0.034
Cohort	1	7	-0.96 [-1.70, -0.24]	0.012
Non-RCT	1	7	0.57 [-0.21, 1.34]	0.151