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# The Complex Type 2 Endotype in Allergy and Asthma: From Laboratory to Bedside

**Ioana Agache**[1] · **Kazunari Sugita**[2,3] · **Hideaki Morita**[2,3] · **Mübeccel Akdis**[2,3] · **Cezmi A. Akdis**[2,3]

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**Abstract** Better management of allergic diseases needs a sharpened understanding of disease heterogeneity and mechanisms in relation to clinically significant outcomes. Phenotypes describing observable clinical and morphologic characteristics and unique responses to treatment have been developed; however, they do not relate to disease mechanisms. Recently, extended heterogeneous and disease-related metabolic, inflammatory, immunological, and remodeling pathways have been described, and reproducible patterns are defined as disease endotypes. An endotype might consist of several intricated mechanisms that cannot be clearly separated into "pure single molecular mechanism" thus being a "complex endotype." The description of an endotype may rely on biomarkers, which can be the signature of a complex underlying pathway or a key molecule associated with or directly playing a role in a particular disease endotype. The Th2 type inflammation can be defined as a complex endotype in asthma and linked to mechanisms of disease development and response to treatment and to disease outcomes such as exacerbations and remodeling. The type 2 complex endotype in allergies and asthma includes innate lymphoid cells, T helper 2 cells, tissue eosinophilia, and IgE production. Currently, emerging endotype-driven strategies in asthma, particularly the development of biologicals that target a single molecular pathway, are being focused for solving individualized clinical problems on disease outcomes. Progress is also being made for endotyping rhinitis, chronic rhinosinusitis, and atopic dermatitis.

**Keywords** Allergy · Phenotype · Endotype · Th2 inflammation · Asthma · Rhinitis

**Abbreviations**

| | |
|---|---|
| ADMA | Asymmetric dimethylarginine |
| CLC | Charcot-Leyden crystal protein |
| CPA3 | Carboxypeptidase A3 |
| DNA-SE1L3 | Deoxyribonuclease I-like 3 |
| DC | Dendritic cells |
| Eos | Eosinophils |
| FeNO | Fractional exhaled NO |
| ICS | Inhaled corticosteroids |
| ILC | Innate lymphoid cells |
| L-Arg | L-Arginine |
| NKT cells | Natural killer T cells |
| PG | Prostaglandin |
| SARP | Severe Asthma Respiratory Program |
| Th | T helper cell |
| TSLP | Thymic stromal lymphopoietin |
| VOCs | Volatile organic compounds |

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✉ Ioana Agache
ibrumaru@unitbv.ro

[1] Department of Allergy and Clinical Immunology, Faculty of Medicine, Transylvania University of Brasov, Brasov, Romania

[2] Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos, Switzerland

[3] Christine Kühne – Center for Allergy Research and Education (CKCARE), Davos, Switzerland

## Introduction

Allergic diseases affect the lives of more than one billion people worldwide, and their prevalence is expected to reach up to 4 billion in 2050. Better understanding of disease heterogeneity and mechanisms in relation to clinically significant

outcomes, such as disease progression or response to treatment, is essential to improve their outcome (Fig. 1). In recent years, induction of immune tolerance has become a prime target for prevention and treatment strategies for allergic diseases. Immune tolerance to allergens can be defined as establishment of a long-term clinical tolerance against allergens, which immunologically implies changes in memory type allergen-specific T and B cell responses as well as mast cell and basophil activation thresholds that no longer cause allergic symptoms [1–3]. T and B regulatory cells and production of allergen-specific IgE-blocking IgG4 isotype antibodies play an essential role in allergen tolerance. Selection of patients for allergen-specific immunotherapy is based today on clinical judgment, and unfortunately, there are no good predictors of response. An endotype-driven approach to select responders might improve results if properly validated, predictive biomarkers were available.

## The Concept of Asthma Phenotypes, Endotypes, and Biomarkers

The heterogeneity of allergic diseases and asthma in relation to clinically significant outcomes, including response to treatment, has been established beyond any doubt. However, current guidelines ignore disease heterogeneity and causal pathways. These lead to unsuccessful clinical "bulk" trials or contradictory epidemiologic and genetic surveys, in which patient subgrouping and disease heterogeneity has not been taken into consideration.

At first, phenotypes describing clinical and morphologic characteristics as well as unique responses to treatment have been developed to address the complexities of the disease. Phenotypes may be clinically relevant in terms of presentation, triggers, and treatment response, but do not necessarily relate to or give insights into the underlying pathological mechanism [4]. For most of the allergic diseases, extended heterogeneous disease-related metabolic, inflammatory, immunological, and remodeling pathways have been described, and a reproducible underlying mechanism is defined as a disease endotype [5•, 6•]. Single molecular mechanism-linked endotypes can be defined such as periostin high, anti-IL-13 responsive asthma. In contrast, other endotypes involve concomitantly several pathways that might prevail one over the other (in a network model) and can be due to several modulators such as metabolic or different inflammatory pathways. Examples of such complex endotypes are Th2 inflammation or aspirin-intolerant asthma.

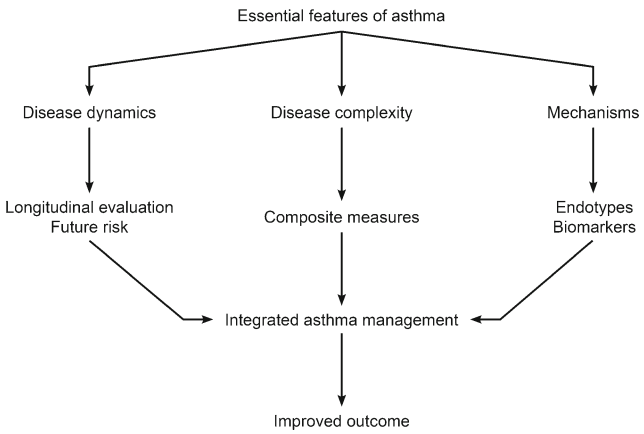


**Fig. 1** The concept of integrated asthma management is developing according to recent progress in asthma phenotypes and endotypes

There are several benefits of endotyping in a clinical setting such as stringent consideration of entry criteria for epidemiological, genetic, or drug-related trials. In the future, the endotype-tailored management (personalized medicine, stratified medicine, individualized medicine) may identify patient subgroups at risk for severe disease or with a particular response to existing or new treatments [5•, 7••, 8]. In addition, developing mouse models reflecting human endotypes would help the understanding of pathophysiological mechanisms of allergic disease.

To become clinically relevant, the endotype should be related to validated biomarkers that correspond to the underlying mechanism. The purpose of the biomarker is to identify disease endotype, predict onset and prognosis of a disease, measure exposure, monitor response to treatment, and forecast unfavorable evolution [6•]. The biomarker can be the signature of a complex underlying pathway or a key molecule of a particular disease endotype. To further complicate the picture, the predictive value of the same biomarker is highly variable across age groups, disease severity, and in relation to the outcome. The ideal biomarker should be pathway-specific, reproducible in the same individual and in an independent prediction cohort, and usable as a diagnostic test (easily measurable and affordable). New strategies for discovery and validation of biomarkers such as gene expression (microarrays) and omics provide combined signatures as per system medicine.

The concept of mechanism-tailored end points is surfacing in the field of allergic disease. In addition, the outcomes should be relevant both for the disease and for that particular patient. Unfortunately, no single intervention with a biological immune response modifier has been proven to improve disparate aspects of the disease, with significant differences within the same individual in terms of disease outcomes (the dissociated response). For example in asthma, one biological may well control exacerbations but has no effect on lung function, symptoms, or quality of life [1, 6•, 8].

## The Type 2 Inflammatory Endotype

### Physiologic Type 2 Inflammatory Responses

As part of the physiologic immune response, type 2 inflammatory responses are highly dynamic processes initiated by epithelial cell damage, resulting either in chronic injury or in healing of the tissue architecture. The type 2 milieu promotes wound healing processes, which are beneficial in parasitic infections or toxin exposure, but account for increasingly dysfunctional vital organs such as the lung in the case of asthma. This milieu is shaped by cytokines (IL-25, IL-31, IL-33, and thymic stromal lymphopoietin (TSLP)) released from epithelial cells, which stimulate Th2 cells, type 2 innate lymphoid cells (ILCs) and invariant natural killer T cells (NKT cells) to secrete Th2 cytokines and to activate dendritic cells, which results in further differentiation and clonal expansion of Th2 cells and further activation of ILC2 cells as well as tissue eosinophilia [9–14].

The type 2 inflammatory process requires communication between resident cells such as epithelial, endothelial, fibroblast, and muscle cells and the highly mobile cells of the innate and adaptive immunity. In the human airway mucosa, the TSLP receptor is constitutively and preferentially expressed by myeloid $CD1c^+$ dendritic cells (DCs) [15].

### The Type 2 Inflammatory Response in Allergic Diseases

The density of the DC subset expressing the TSLP receptor in the nasal mucosa increases significantly after allergen challenge. TSLP enhances the capacity of $CD1c^+$ DCs to activate allergen-specific memory $CD4^+$ T cells and induces CCR7 expression on $CD1c^+$ DCs with their recruitment from the lymph nodes. However, in an already established Th2-mediated inflammatory reaction, the Th2 cytokines attenuate TSLP-mediated CCR7 induction with retention of $CD1c^+$ DCs in the inflamed tissue to further exacerbate local inflammation by activating local antigen-specific memory Th2 cells [15]. Eosinophils also contribute to the initiating phase of the type 2-polarized pulmonary inflammation and to the recruitment of effector Th2 T cells while suppressing the Th1/Th17 pathways [16]. Epidermal γδ T cells might play an important role in the pathogenesis of Th2-dominant skin diseases because of their active production of IL-13 [17].

ILC2s are capable of producing high amounts of IL-5 and IL-13 in response to IL-25, IL-33, and TSLP, leading to eosinophilia and goblet cell hyperplasia at mucosal surfaces [18–20]. Moreover, ILC2s facilitate sensitization to local Th2-inducing allergen exposures and mediate Th2 cell differentiation via IL-33-dependent manner [21, 22].

In humans, the subset of ILC2 is expressed in peripheral blood, lung, skin, and inflamed tissues and release high amounts of important sources of IL-5 and IL-13 [23•, 24, 25]. IL-5- and IL-13-producing ILC2 have been found in the sputum of asthma patients [26], while overexpression of TSLP in the airway epithelium results in increased number of IL-13-producing ILC2s that correlated with severe asthma [27]. Taken together, these data indicate that ILC2s play a critical role for the induction of Th2-driven inflammation in asthma. In addition, ILC2s have been found in nasal polyps in patients with chronic rhinosinusitis [28–30].

In addition, ILC2 shares features with Th2 cells such as expressions of GATA3 transcription factor and CRTH2 [23•]. CRTH2 is also abundantly expressed on eosinophil and basophils [31–33]. It can directly stimulate ILC2s and can also potentiate IL-25/IL-33-mediated innate immune responses [34]. Nasal cat allergen challenge in allergic rhinitis induced an increased percentage of peripheral blood CRTH2+ ILC2 that express CD84 [35].

Effector Th2 cells release IL-4, IL-5, and IL-13. IL-4 and IL-13 are essential for class-switching to the ε immunoglobulin heavy chain in B cells and the production of allergen-specific IgE antibodies, while IL-5 is crucial for promoting and sustaining tissue eosinophilia. Other pathways involved in Th2 inflammation are the mast cell/IL-9 pathway and the $PGD_2$ pathway [36–38, 39•]. Th1 or Th17 cells may add to the Th2-driven inflammation, with their role in apoptosis of the epithelium in asthma and atopic dermatitis, formation of granulomas (Th1), and in promoting neutrophilic inflammation (Th17) [40–44]. Further influences may be added by the remodeling phenotype, by associated microbiota or by activation of peculiar metabolic pathways such as the eicosanoid pathway in aspirin-exacerbated respiratory disease or the L-Arg/asymmetric dimethylarginine (ADMA) or the lectin pathway in obesity (Fig. 2) [45, 46, 47•]. It has been shown that human eosinophils express leptin receptor Ob-Rb and that leptin induces eosinophils to produce inflammatory cytokines [48, 49]. Data from the SARP cohort showed a higher median plasma ADMA level and lower median plasma L-arginine in subjects with late-onset asthma compared with early onset. A reduced L-arginine/ADMA was associated with less IgE [47•].

The type 2 inflammation is characterized by a high cellular plasticity that enables the cells to adapt to a specific inflammatory milieu. Innate cytokines such as IL-33 and TSLP modulate the mast cell phenotype [50], while type 2 cytokines promote a particular phenotype of smooth muscle cells in asthma and influence permissiveness of epithelium for allergens and of the endothelium for the recruitment of inflammatory cells to inflamed tissues and are involved in the production of mucus [51].

## The Type 2 Complex Endotype in the Clinic

The type 2 inflammatory complex endotype has been related to response to treatment and to disease outcomes, such as

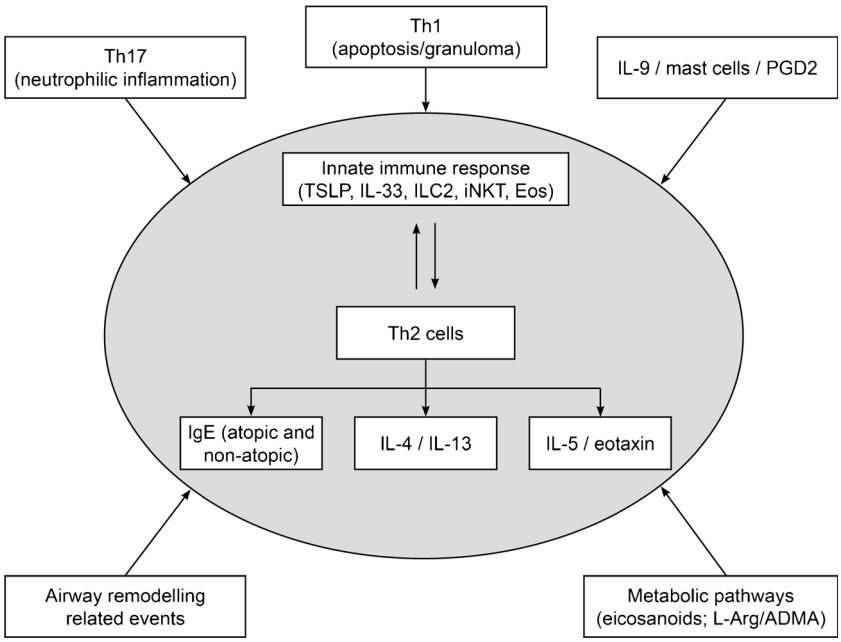


**Fig. 2** The complex network of Th2 endotype in allergic diseases involves the interaction between innate immune response and Th2 cells. Three major downstream effector pathways can be described: the IgE pathway, the IL-5/eotaxin pathway, and the IL-4/IL-13 pathway. Additional modulators of the Th2 endotype can be described such as Th17 or Th1 cells, the IL-9/mast cell axis, activation of the metabolic pathways, or the degree of airway remodeling

exacerbations, and may have a role in remodeling in asthma [52•]. Several type 2 biomarkers have been described: sputum and blood eosinophils, fractional exhaled NO (FeNO), serum periostin, the Th2 gene signature (serpin B2, periostin, CLCA1 or CLC, CPA3, DNA-SE1L3) in bronchial and nasal epithelial cells, the sputum cells Th2 gene mean (IL-4, IL-5, IL-13), TSLP and mast cells in bronchial biopsies, and the salivary inflammatory profile (Table 1). Each biomarker reflects a compartment or a pathway involved in type 2 inflammation related to Th2 cells and ILC2. For non-specific interventions, such as inhaled corticosteroids, all of the above can be used to predict response. However, for more targeted interventions, such as anti-IL-5 or anti-IL-13, each of these Th2 or ILC2 biomarkers needs to be related to the specifically targeted pathway. For example, FeNO and sputum eosinophils on the one hand and blood eosinophils on the other hand reflect different endotypes of Th2 or ILC2-mediated inflammation. In a cross-sectional study, FeNO and blood eosinophil values offered independent information with respect to the prevalence of wheeze, asthma diagnosis, and asthma events [70•]. The authors suggest that blood eosinophilia is a marker of more severe systemic inflammation driven by a strong chemokine signal (such as IL-5) and more extensive eosinophilic airway inflammation involving the small airways and therefore inhaled corticosteroids (ICS) non-responsive. It may also highlight the risk of asthma exacerbations requiring oral corticosteroids. Further evidence was provided in the DREAM trial, which indicated that blood eosinophils were most closely related to a positive response to mepolizumab (anti-IL-5) compared to sputum eosinophils [70•, 71••]. In contrast, an increased FeNO value indicates predominance of the IL-4/IL-13-mediated pathway with eosinophilc inflammation localized in the bronchial mucosa and thus responding to ICS or to IL-4/IL-13 blockade. Increased FeNO value was a good predictor of a clinical response to lebrikizumab in the MILLY trial, besides serum periostin [72••]. Also in support, serum periostin was the best predictor of lung eosinophilia in severe asthma, when compared with blood eosinophils, again depicting the two types of systemic versus local inflammation [73•]. Longitudinal analysis of trends of increase or decrease in biomarkers, including fluctuation analysis, might offer additional information (Table 1). Combination of multiple biomarkers, including omics approaches and gene expression microarrays, may provide an added value.

Endotype-driven therapeutic strategies have been increasingly successful in asthma. Selection of patients in the DREAM trial based on their eosinophilic profile proved the efficacy of anti-IL-5 interventions. However, the issues related to the dissociated effect and drug efficacy at the target site remain unresolved. Unfortunately, so far very few attempts were made for the endotype-driven management of allergic diseases.

Applying the same model to allergic and non-allergic rhinitis could prove as successful in promoting personalized approaches, especially for the severe forms of the disease. The well-recognized link between rhinitis and asthma should be integrated and tackled within the framework provided by endotypes. Rhinitis endotypes can be defined in relation to the background inflammation or in terms of treatment responsiveness. The following endotypes can be proposed for allergic rhinitis, although their clinical relevance remains to be proven and associated biomarkers validated: IL-5-, IgE-, or IL-4/IL-13-driven Th2 type inflammation which can be steroid-responsive, anti-IgE responsive, anti-IL-5 responsive,

**Table 1** Th2 biomarkers in asthma management

| | Biomarker | Availability | Reproducibility | Response to treatment | Risk prediction |
|---|---|---|---|---|---|
| Localized lung inflammation | Sputum eosinophils [53] | Research type | Questionable | Response to ICS | Exacerbations |
| | Exhaled NO [54, 55] | Easily available | Questionable | Response to ICS and anti-IL-13 | Exacerbations<br>LF decline |
| | Serum periostin [56] | Research type | Unknown | Response to anti-IL-13 | LF decline |
| Systemic inflammation/small airways disease | Blood eosinophils [57, 58] | Easily available | Low | Response to anti-IL-5 (mepolizumab) | Exacerbations<br>LF decline<br>Fixed airway obstruction |
| Combined biomarkers | Th2 gene signature (epithelial cells, sputum) [52, 59–61] | Research type | Good ($r$=0.93) for the sputum Th2 gene mean | Response to ICS | AHR<br>Airway obstruction<br>Reticular basal membrane thickening<br>Increased mucus production<br>Asthma control |
| | The salivary inflammatory profile (IL-5, eotaxin, RANTES) [62] | Research type | Unknown | | Nocturnal symptoms<br>Exercise-related symptoms<br>Asthma control<br>Exacerbations |
| | Blood eosinophils/periostin/FeNO [63•] | Research type | See above for each b iomarker | Response to anti-IgE | |
| | Blood eosinophils/BMI/high reversibility [64] | Easily available | Good for BMI and reversibility | Response to anti-IL-5 (mepolizumab) | |
| Longitudinal evaluation of biomarkers | Persistent high FeNO [65] | Easily available | Unknown | | LF decline<br>Fixed airway obstruction |
| | Fluctuation analysis of FeNO [66] | Research type | Unknown | | Pediatric asthma severity and control |
| | Fluctuation analysis of lung function [67] | Research type | Unknown | | Difficult pediatric asthma |
| | Highly variable sputum Eos [68] | Research type | Unknown | | LF decline |
| Other biomarkers | TSLP in bronchial biopsies [27] | Research type | Unknown | | Asthma severity |
| | Mast cells in bronchial biopsies [36] | Research type | Unknown | Response to ICS | |
| | Exhaled air metabolomics (VOCs) [69] | Research type | Unknown | Response to ICS | |

and anti-IL-4/IL-13 responsive. For non-allergic rhinitis, the definition of endotypes (Th2 or non-Th2 type inflammation) should include the driving cause: superantigens, local IgE production, and autoantibodies.

A predominant Th2 type of inflammation characterized by increased levels in tissue homogenates of IgE, specific IgE to *Staphylococcus aureus* enterotoxin, ECP, and IL-5 was related to relapse after surgery for chronic rhinosinusitis (CRS) with nasal polyps, while a mixed profile with significant higher levels of IFN-γ and lower concentrations of IgE, ECP, and IL-5 was encountered for non-recurrent cases [74]. In the same line, a PRACTALL document described several endotypes for CRS characterized by differences in responsiveness to treatment, including topical intranasal corticosteroids and biological agents, such as anti-IL-5 and anti-IgE. The described CRS endotypes were based on different biomarkers linked to underlying mechanisms [75•].

Atopic dermatitis (AD) is a chronic inflammatory skin disease with complex genetic and immunological mechanisms. Several endotypes can be proposed according to the inflammatory background such as Th2/IL-22/periostin high or Th17/Th1 high or in relation to the expression of fillagrin, MATT, or vitamin D pathway gene mutations. For the Th2 type AD, serum periostin is related to disease severity [76, 77].

Targeting both the inflammatory immune-dysregulated pathways and the barrier defect in AD holds future promise. Several new targets such as toll-like receptors, type 2 ILCs, and tight junction proteins are emerging. Promising new therapeutic agents in the near future are sphinganine, cannabinoids, and highly targeted monoclonal antibodies.

## Conclusion

The type 2 endotype was described for all major allergic diseases: asthma, rhinitis, chronic rhinosinusitis, and atopic dermatitis. This complex endotype is mainly mediated by ILC2 and Th2 cells as well as Th2 cytokine-producing NKT cells, whereas their individual contribution is not known. Major progress has been obtained in some clinics for endotype-guided treatment of asthma, but still, there are unsolved issues pending such as the dissociated effect on disease outcomes and the drug efficacy at target site.

The description of an endotype relies on biomarkers. Several biomarkers have been described for the Th2 endotype, such as exhaled NO, sputum or blood eosinophils, total serum IgE or specific IgE, serum periostin, sputum or epithelial cell (bronchi or nasal) gene signature, or the saliva inflammatory profile. Until now, none of these biomarkers matched the ideal profile: pathway-specific, reproducible, and affordable as diagnostic test in the clinic. The variance across age, asthma severity, and with the treatment target is still an unsolved issue. The use of a composite index of clinical biomarkers such as BMI, sputum or blood eosinophils, exhaled NO or derived from omics approaches, and gene expression microarrays seems to have a better predictive value.

Pending identification of validated biomarkers reflecting the disease mechanism and predicting the natural course and response to treatment of the disease, an endotype-driven approach should be identified also for allergic rhinitis, chronic rhinosinusitis, and atopic dermatitis, especially for the severe forms of the disease. Selecting responders for allergen-specific immunotherapy might also benefit from an endotype-oriented strategy.

**Compliance with Ethics Guidelines**

**Conflict of Interest** Ioana Agache, Kazunari Sugita, Hideaki Morita, Mübeccel Akdis, and Cezmi A. Akdis declare that they have no conflicts of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance


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