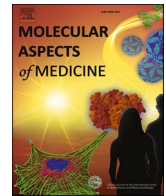




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Molecular allergology approach to allergic asthma

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ABSTRACT

Allergic asthma is a frequently encountered and well described asthma phenotype. However, its precise mechanisms are less known. The tools for targeted selection of patients for an optimal response to intervention (prevention or treatment) are also lacking. Here we explore the potential of the molecular allergology approach to achieve a better understanding of allergic asthma mechanisms, a precise diagnosis and an optimal management of these patients.

1. The concept of asthma phenotype, endotypes, biomarkers and theratypes

Asthma is a complex heterogeneous disease of the airways characterized by lung inflammation, airway hyperreactivity (AHR), mucus overproduction, and remodelling of the airways. Asthma phenotype reflects a cluster of visible properties trying to individualize disease expression in groups of patients (Agache, 2019; Agache and Akdis, 2019; Kaur and Chupp, 2019; Chung and Adcock, 2019). A comprehensive set of visible properties is usually used and includes: clinical parameters (age, gender, race, disease onset, triggers, symptoms, etc); physiologic properties (lower airways patency and hyperreactivity, ventilation pattern), morphologic features (airway smooth muscle hypertrophy, shedding epithelium, mucus production, imaging (bronchial thickness); clinical relevant outcomes (exacerbations, lung function decline); inflammatory parameters (eosinophilic or neutrophilic inflammation); treatment response (responder/non-responder, corticosteroid sensitive or resistant) (Agache, 2019; Kaur and Chupp, 2019; Chung and Adcock, 2019; Agache et al., 2012). The compilation of asthma molecular mechanisms explaining disease expression in groups of patients is an asthma endotype (Agache and Akdis, 2019). Biomarkers are measurable indicators linking asthma endotype with the phenotype (Agache and Akdis, 2019; Breiteneder et al., 2020). Prediction of treatment response based on the disease endotype is coined as asthma theratype. Theratypes facilitate the selection of a patient population in which a successful intervention effect is more likely than in an unselected population (enrichment), the corner stone of the stratified approach in asthma

opening the gate towards precision medicine (Agache, 2019; Agache and Akdis, 2019).

Molecular mechanisms leading to asthma are still ill-defined. For approximately half of the asthma cases evidence indicates a type-2 (T2) immune response orchestrated by epithelial cell cytokines such as interleukin (IL)-25, IL-33, and thymic stromal lymphopoietin (TSLP), type 2 innate lymphoid cells (ILC2s) and antigen-specific T helper (Th) 2 cells and their cytokines IL-4, IL-5, and IL-13 (Woodruff et al., 2009; Peters et al., 2014; Cheng et al., 2014; Muraro et al., 2016; Agache and Akdis, 2016). For the other half of asthma cases other T cell-related cytokines such as IL-17A and IL-22 or the activation of the inflammasome were described (Agache, 2019; Zounemat Kermani et al., 2020; Sze et al., 2020).

For T2 asthma 3 major pathways were described, not mutually exclusive: the IL-5 pathway, the IL-4/IL-13 pathway and the immunoglobulin E (IgE) pathway. These pathways generate over-lapping T asthma phenotypes characterized by increased blood and/or sputum eosinophils and increased fractional exhaled nitric oxide (FeNO) (Agache, 2019; Agache and Akdis, 2016, 2019).

2. The allergic asthma phenotypes and endotypes

Allergic asthma is a frequent asthma phenotype encountered in up to 90% of the paediatric asthma population and in up to 50% of the adult asthma population. Its severity ranges from mild to severe (Arabkhaali et al., 2015; Ballardini et al., 2016; Knudsen et al., 2009; Pearce et al., 1999). Several asthma consortia reported increased asthma severity associated with allergy (Lu et al., 2016; Fleming et al., 2015; Del Giacco

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Abbreviations

ABPA	Allergic Broncho-Pulmonary Aspergillosis
AECs	Airway Epithelial Cells
AHR	Airway hyperreactivity
AIT	Allergen Immunotherapy
AR	Allergic Rhinitis
BAT	Basophil activation test
Breg	B regulatory cell
CRD	Component-resolved diagnosis
CRISPR	Clustered regularly interspaced short palindromic repeats
crRNA	CRISPR-targeting RNA
CRSwNP	Chronic rhinosinusitis with nasal polyps
CTLA4	cytotoxic T-lymphocyte-associated protein 4
DEP	Diesel exhaust particles
DNAm	DNA methylation
DSB	Double strand break
EAACI	European Academy of Allergy and Clinical Immunology
EWASs	Epigenome-wide association studies
FeNO	fractional exhaled nitric oxide
HDM	House dust mite
ICOSL	ICOS ligand
IFNR	Interferon response signature

Ig	Immunoglobulin
IL	Interleukin
ILC	Innate lymphoid cell
ILCreg	Regulatory innate lymphoid cell
MID1	Midline 1
miRNAs	MicroRNAs
NPC2	Cat-Niemann pick type C2
OVA	ovalbumin
PAR	protease-activated receptor
PEBP1	PE-binding protein-1
RA	Retinoic Acid
RNA-seq	RNA sequencing
SCIT	Subcutaneous immunotherapy
scRNA-seq	single cell RNA sequencing
sgRNA	Single-guide RNA
SLIT	sublingual immunotherapy
T2	Type 2 immune response
TGF	transforming growth factor
Th	T helper cell
TRAIL	tumor necrosis factor-related apoptosis-inducing ligand
Treg	T regulatory cell
TSLP	thymic stromal lymphopoietin

et al., 2017). Allergic asthma is defined by the presence of the specific IgE sensitisation to one or more aero-allergens in association with evidence of an allergen as the major driver of asthma symptoms and control (Agache et al., 2019). Allergic rhinitis (AR) is associated in up to 90% of cases (Bousquet et al., 2008) and other allergic diseases, such as atopic dermatitis or food allergy, might be present as well. In severe cases T2 chronic rhinosinusitis with nasal polyps (CRSwNP) is reported (Bachert et al., 2020). The levels of total serum IgE are usually elevated but not in all cases. The systemic production of IgE might be absent but there is local production in the nose and/or the airways, a conditioned coined as local allergic respiratory disease (local AR or local allergic asthma) (Campo et al., 2019; Eguiluz-Gracia et al., 2019).

The development of targeted, rational treatment approaches for allergic asthma requires a thorough understanding of disease mechanisms (Agache, 2019; Agache and Akdis, 2019). This requires a meticulous evaluation of the role of the allergic sensitisation in cooperation with the T2 immune response and the resident cells, especially the airway epithelial cells (AECs), mucus production and airway smooth muscle.

Although allergens are frequent and commonly recognized triggers of asthma severity and exacerbations, the majority of patients with asthma are not investigated for their underlying aeroallergen sensitizations, despite the potentially preventable consequences and therapeutic options. These options include allergen avoidance measures and allergen immunotherapy (AIT) (Pfaar et al., 2019). AIT administered either subcutaneous (SCIT) or sublingual (SLIT) is recommended in patients with controlled allergic asthma as an add-on to asthma regular treatment if an allergen extract with proven efficacy is available (as AIT is not a class effect and is dependent on the quality of allergen extract used) (Agache et al., 2019). The aim of adding AIT to asthma background medication is to decrease both background and rescue medication use and improve quality of life (Agache et al., 2019; Pfaar et al., 2019). Up until now only house-dust mites (HDM) vaccines have been tested in allergic asthma trials large enough to provide solid evidence for guidelines recommendations. HDM SLIT are recommended as well in partially control asthma to facilitate achieving asthma control, however special precautions are needed such as a specialized facility supervising SLIT, an FEV1 >70% predicted and no severe asthma exacerbation in the last year (Agache et al., 2019).

Both the adaptive and the innate immune response are involved in the pathogenesis of allergic asthma (Agache, 2019; Agache and Akdis, 2019; Kubo, 2017) (Fig. 1). The initiation of T2 responses is regulated by the airway epithelial cell-derived cytokines IL-25, IL-33, TSLP, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and midline 1 (MID1) (Agache, 2019; Byers, 2014; Laulajainen-Hongisto et al., 2020). ILC2s play critical roles in induction and exacerbation of allergic airway inflammation. In response to allergen, pollutants or viral infection, damaged airway epithelial cells secrete IL-33, IL-25, and thymic stromal lymphopoietin (TSLP), which activate ILC2s to produce T2 cytokines such as IL-5, IL-13, and IL-9 (Smith et al., 2016a; Kato, 2019; Chen et al., 2017). Furthermore, ILC2s coordinate a network of cellular responses and interact with numerous cell types to propagate the inflammatory response and repair lung damage. ILC2s display functional plasticity in distinct asthma phenotypes, enabling them to respond to very different immune microenvironments. ILC2 maturation and plasticity is facilitated by the transcription factor GATA-3, IL-4, IL-1 β and IL-12 (Smith et al., 2016a; Ohne et al., 2016). Regulation of pulmonary ILC2s is a major area of investigation aimed at dampening the allergic lung inflammation. ILC2s are counteracted by inducible regulatory ILCs (ILCregs) and T regulatory cells (iTregs). A unique immune regulatory and anti-inflammatory pathway converting ILC2s to ILCregs via retinoic acid (RA) was recently described (Morita et al., 2019). The pathway is dependent on interactions between airway epithelial cells and ILC2s as enzymes for RA synthesis were upregulated in airway epithelial cells during type 2 inflammation in vivo and by IL-13 in vitro. iTregs effectively suppress the production of ILC2-driven, pro-inflammatory cytokines IL-5 and IL-13 (Aron and Akbari, 2017). The inhibitory effects of iTregs are blocked by preventing direct cellular contact or by inhibiting the ICOS-ICOS-ligand (ICOSL) pathway, suggesting that both direct contact and ICOS-ICOSL interaction are important in the regulation of ILC2 function. Also, cytokines such as IL-10 and TGF- β 1 significantly reduce cytokine secretion by ILC2s. Other innate immune cells (notably, airway macrophages and neutrophils) play essential roles in the generation of steroid-resistant inflammation and AHR secondary to allergen and pathogen-induced exacerbations (Hadjigol et al., 2020; Plumb et al., 2012). Toll-like receptor 4 (TLR4) has a strong inhibitory role on the initiation of HDM-induced allergic disease, however the effect is dependent on the CD14 allelic variation (Rodríguez et al., 2003;

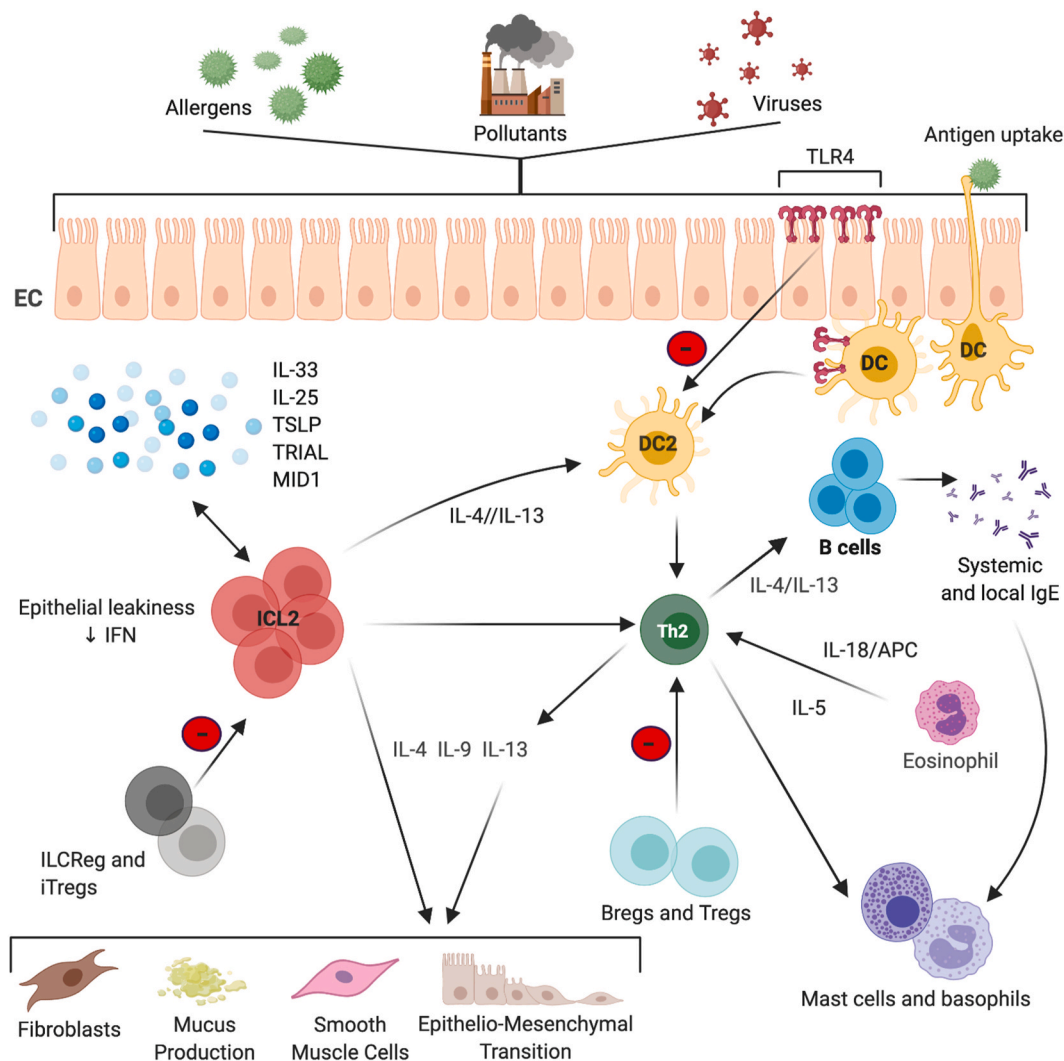


Fig. 1. Mechanisms of allergic inflammation in asthma

Both the adaptive and the innate immune response are involved in the pathogenesis of allergic asthma. The initiation of T2 responses is regulated by airway epithelial cell-derived factors in response to stimuli including allergens, pollutants and viruses. Upregulation and secretion of TSLP, IL-25, IL-33, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and midline 1 (MID1) leads to subsequent activation of ILC2s. Production of T2 cytokines IL-5, IL-13 and IL-4 from ILC2s acts directly to propagate allergic inflammation and also promotes DC2 and Th2 cells. Secretion of IL-4/IL-13 from Th2 cells promotes class switching of B cells to produce allergic specific IgE that circulates systemically or is located within the local milieu. IgE binding to the FcεRI receptor on granulocytes enables cross-linking of IgE upon allergen exposure leading to degranulation and the release of histamine, prostaglandins and other inflammatory mediators. IL-5 secretion from Th2 cells promotes airway eosinophilia. Tolerogenic mechanisms to overcome allergic inflammation include ILCregs and iTregs suppressing ILC2s through soluble factors such as TGF-β, IL-35 and IL-10. Similarly, Bregs and Tregs also act upon Th2 cells. TLR4 activation upon LPS ligation can also suppress DC2s driving Th2 cell expansion.

Haapakoski et al., 2013; Kljaic-Bukvic et al., 2014). Allergen-specific CD4⁺ Th2 lymphocytes and their associated cytokines (IL-4, IL-5 and IL-13) are hallmark features of allergic asthma. Classical Th2 cytokines (IL-4, IL-5 and IL-13) together with CCL11 (eotaxin-1) regulate critical aspects of eosinophil recruitment, allergic inflammation and AHR. T2 cytokines could operate in parallel, but their signals are usually integrated through crosstalk with resident and migrated inflammatory cells. These cytokines also had distinct temporal roles in the development of inflammation and AHR (e.g. IL-4 promoted the early phase of inflammation and IL-13 and IL-5 acted as late-stage effector molecules) (Walker and McKenzie, 2018; Mattes et al., 2002). Bidirectional signalling between eosinophils and Th2 cells regulates T cell cytokine production (Sawada et al., 2013; Kandikattu et al., 2019). IL-18 derived from eosinophils is a factor regulating IL-13 production. In addition, eosinophils and ILC2 could act as antigen presenting cells and directly promote allergic inflammation by activating Th2 cells in an antigen-specific manner (Schuijs et al., 2019). Tregs have been shown to

be critical in hampering the allergic asthmatic inflammation (Aron and Akbari, 2017). Both cell-cell contact (through membrane bound transforming growth factor (TGF)-beta or via suppressive molecules such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)) and soluble cytokine-(TGF-beta and IL-10) dependent mechanisms have been shown to contribute to the ability of Tregs to operate effectively. The transcription factor Foxp3, a member of the forkhead-winged helix family, appears to be critical in the suppressive abilities of regulatory T-cells (Deng et al., 2019). Clinical improvement seen after AIT for asthma has been associated with the induction of IL-10 and TGF-beta producing Tregs cells as well as Foxp3 expressing CD4⁺CD25⁺ T-cells (Kappen et al., 2017). Activation and expansion of antigen-specific CD4⁺CD25⁺ Tregs in vivo using adjuvants such as IL-10 or pharmacological agents such as low dose steroids or vitamin D3 could represent novel approaches to induce allergen-specific tolerance in asthma (Karagiannidis et al., 2004; Cantorna et al., 2015). IL-10-producing regulatory B cells (Bregs) were widely ascribed with potent immune regulatory functions

(van de Veen et al., 2016). A recent study reported on phenotypic and functional alterations of Breg subsets in adult allergic asthma patients showing that oral corticosteroids significantly affects the frequency as well as their ability to express IL-10 (Wiest et al., 2019).

3. The molecular allergology approach

The molecular allergology approach in allergic asthma allows (Fig. 2):

- A better understanding of disease mechanisms (molecular characterisation of allergens relevant to asthma, genetics and epigenetics studies, animal models, single cell analysis, CRISPR/Cas9 targeted intervention, T cell and receptor editing, novel mediators)
- Precise diagnosis through the description of the molecular allergen sensitisation profile
- Optimal selection of responders to the targeted treatment, either with AIT, or with biologicals
- Novel approaches to allergic asthma (adoptive cell therapies)

3.1. A better understanding of allergic asthma mechanisms

3.1.1. A1Molecular characterisation of allergens relevant to asthma

Several aerollergens (HDM, pollens, cat, moulds) have been linked to asthma inception, morbidity and severity.

HDMs are the most common indoor aeroallergen worldwide. The occurrence of sensitisation to Der p 1 or Der p 23 at age 5 years or less predicted the development of asthma at school age. The authors reported parental hay fever and early exposure to *D pteronyssinus* allergens as risk factors for IgE polysensitization to several *D pteronyssinus*

molecules. Der p 20 is a cross-reactive HDM allergen that may serve as a diagnostic marker for HDM-induced allergic asthma(53). Der p 20 was found to occur mainly in HDM bodies, but not in faecal particles. Thirty percent of clinically well-characterized HDM allergic patients showed IgE reactivity to Der p 20. IgE reactivity to Der p 20 was more frequently associated with lung symptoms. Der p 20 showed IgE cross-reactivity with arginin kinase from shrimp, thus shrimp consumption might elicit asthma symptoms (Sarzsinszky et al., 2021).

Altered pollen and moulds production caused by climate change could affect allergen-induced asthma morbidity (D'Amato et al., 2020). The impact on asthma symptoms and emergency department (ED) visits of the pollen concentrations of *Betulaceae*, *Cupressaceae*, *Quercus*, *Pinaceae*, *Poaceae*, and *Ambrosia* species was evaluated after controlling for covarying pollen taxa and ambient pollutant concentrations. A 2%–3% increase in asthma- and wheeze-related ED visits per SD increase in *Quercus* species and *Poaceae* pollen and a 10%–15% increased risk on days with the highest concentrations (comparing the top 5% of days with the lowest 50% of days) was reported. The association for *Quercus* species pollen was strongest for children aged 5–17 years. Effects of *Ambrosia* species pollen were difficult to assess because of possible confounding by the steep increase in circulating rhinoviruses every September (Darrow et al., 2012). Thunderstorm asthma is an uncommon but dramatic outbreak of asthma attacks occurring during a thunderstorm in the pollen and moulds season. Furthermore, in individuals affected by thunderstorm asthma, there is evidence of ongoing loss of asthma control in those with previously well controlled asthma, and the persistence of symptoms suggestive of asthma in those with no history or symptoms suggestive of prior asthma, even 36 months after the acute attack. However, low rates of inhaler adherence and asthma action plan ownership may confound these findings (Foo et al., 2020).

Pet-derived allergens are common indoor inhalant allergens and pets

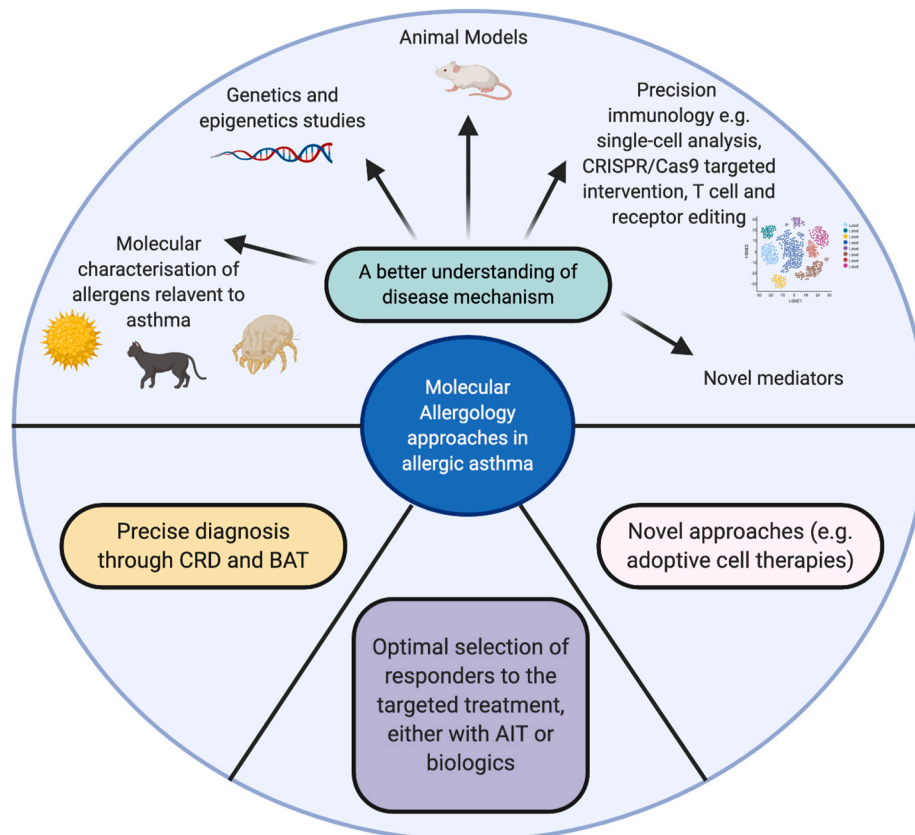


Fig. 2. Molecular allergology approaches in allergic asthma
Graphical summary of how molecular allergology can be used to improve allergic asthma research and management.

are the third-leading cause of IgE-mediated allergic asthma after HDM and pollens. Among them, cat and dog allergens constitute more than 80% of animal allergic patients, which greatly affect the quality-of-life of patients and increase the burden of social health care.

The immunological processes leading to the development of the allergic disease are strongly associated with the structural features of each allergen (Mueller, 2017; Cui, 2013). Molecular biology, crystallographic studies and molecular modelling shed new light on the allergen molecule regions and the amino acid residues involved in immunological functions and interactions with other molecules of the immune system (Aalberse and Stadler, 2006). They also revealed the structural basis of immunological cross-reactivity and the structural determinants conferring the strong sensitisation potential of major allergens (Breiteneder and Mills, 2006).

Various mite allergens, contained primarily in mite fecal particles but also in shed mite exoskeletons and decaying mite body fragments target multiple cellular protective mechanisms and promote local inflammation dependent on proteolysis, lipid-binding activity and interactions with polysaccharides and polysaccharide molecular recognition systems (Agache et al., in press). Mite allergens have proteolytic activity, homology with the lipopolysaccharide-binding component of Toll-like receptor 4, chitin-cleaving and chitin-binding activity. Mite proteases breach epithelial tight junctions and stimulate protease-activated receptors (PARs) 2 and 4, which in turn activates store-operated Ca^{2+} channels, a common signalling module in numerous cell types in the lung. This augments the epithelial dysfunction and promotes epithelial cytokines release (Lin et al., 2018). Der p1 also activates the human receptor mas-related G-protein-coupled receptor MRGPRX1 with release of IL-6 (Reddy and Lerner, 2017). Epithelial stimulation by HDM selectively increased the proliferation of asthmatic ASM cells and not that of non-asthmatic. The mechanism involved epithelial PAR-2-dependent production of leukotrienes C4 associated with an overexpression of leukotrienes receptor CysLTR1 by asthmatic ASM cells (Triantafyllidis et al., 2015). Other components, including chitin, unmethylated mite and bacterial DNA, and endotoxin, activate pattern recognition receptors of the innate immune system and act as adjuvants promoting sensitisation to mite and other allergens. The acidic mammalian chitinase plays an important role as uncleaved chitin promoted the release of IL-33, whereas cleaved chitin could be phagocytosed and could induce the activation of caspase-1 and subsequent activation of caspase-7 with resolution of T2 immune responses (Kim et al., 2015). Mite endotoxin reduced epithelial cell cytokines that activate DCs, thus suppressing T2 immunity to HDMs and loss of the ubiquitin-modifying enzyme A20 in lung epithelium abolished this protective effect. A single-nucleotide polymorphism in the gene encoding A20 was associated with allergy and asthma risk in children growing up on farms (Schuijs et al., 2015). Group 1 HDM allergens have a basic structure, comprising a substrate-binding groove separating two globular domains, which is archetypally defined by the extensively studied representatives, papain, actinidin, and cathepsin K (Cui, 2013). Understanding how the proteolytic activity drives the immunological events lead to the discovery of a new class of drugs called “allergen delivery inhibitors”, demonstrated to be able to interfere with the ability of HDM allergens to engage with antigen-presenting cells (Zhang et al., 2018).

Mechanisms which make the pollen able to enter the deeper airways and provoke severe asthma symptoms are still unclear. Acute severe attacks may be triggered when thunderstorm moisture ruptures pollen grains through osmotic shock to release respirable ($<3 \mu\text{m}$) allergen-impregnated granules which then trigger bronchoconstriction. Sub-pollen particles obtained after the osmotic shock of grass, pellitory and olive tree pollen, contain allergens and therefore they can induce severe asthma attacks (Cecchi et al., 2021). Immunologically, the two most prevalent major allergens of *Lolium perenne* are Lol p 1 and Lol p 5. Lol p 1 is initially presented in the cytosol of pollen grains, from which it is subsequently secreted to coat their surface. It is then readily leached in soluble form from grains lodged in the upper airways, triggering nasal

symptoms. In contrast, Lol p 5 resides in the starch granules (amyloplasts) that are released following pollen grain rupture and thus might be inhaled into the lower airways during thunderstorms. Thus, predominant with Lol p 5 sensitisation might identify patients at risk of developing thunderstorm asthma (Hew et al., 2020).

To date, eight cat-origin allergens have been identified (Fel d 1 to Fel d 8). A novel cat allergen, belonging to the NPC2 protein family, was identified as Cat-Niemann pick type C2 (NPC2) protein, a homologue of Can f 7 (Zhu et al., 2021). At least 14.5% cat-allergic patients displayed high specific IgE recognition of Cat-NPC2. IgE cross-reactivity was demonstrated between Cat-NPC2 and dog and HDM (Can f 7/Der f 2). Fel d 1, the major cat allergen, is a four-subunit (tetrameric) secretoglobulin composed of two covalently linked heterodimers, each of which contains two distinct chains (Kaiser et al., 2003). The three-dimensional structure of Fel d 1 is unique and complex, with an internal cavity and two external calcium-binding sites (Kaiser et al., 2003). The heterodimer structure is important in allergenicity of Fel d 1 because IgE reactivity directed against the individual chains is far less than the level of reactivity against the heterodimer, suggesting the importance of protein conformation in Fel d 1-IgE binding. Polyclonal binding is necessary to neutralize Fel d 1's allergenicity. Novel strategies have been developed to treat Fel d 1-induced allergy. One approach is immunizing cats against their own major allergen using a conjugate vaccine consisting of recombinant Fel d 1 and a virus-like particle derived from the cucumber mosaic virus containing the tetanus toxin-derived universal T-cell epitope tt830-843 (CuMVT) (Thomsen et al., 2019). However, clinical studies to confirm the efficacy of reducing the production of Fel d 1 are still needed. In addition, the biological function of Fel d 1 is currently unknown, thus the potential health effects of ceasing its production are also unknown. Another approach fed cats with neutralising avian egg yolk-derived immunoglobulin Y (IgY) directed against Fel d 1 and decreased the quantity of Fel d 1 secreted in the saliva (Satyraj et al., 2019).

Mice develop strong T2 immune responses when exposed to ovalbumin (OVA) or ragweed in the presence of *Alternaria* extract. Extracts of other fungi, such as *Aspergillus* and *Candida*, showed similar T2 adjuvant effects, albeit not as potently. *Alternaria* stimulated bone marrow-derived DCs to express MHC class II and costimulatory molecules and inhibited IL-12 production by activated DCs. Furthermore, adoptive airway transfer of DCs pulsed with OVA in the presence of *Alternaria*, lead to enhanced IgE production and T2-like airway responses to OVA in the recipient mice (Kobayashi et al., 2009). Currently, 23 allergenic components with different biochemical functions have been described for *Aspergillus fumigatus* (Allergen.). Allergic bronchopulmonary aspergillosis (ABPA) affects about 1%–2% of asthma patients and is associated with asthma exacerbations and a worsening of lung function (Agarwal et al., 2013; Knutsen, 2017). It has been shown that asthma patients with ABPA have higher amounts of specific IgE to *Aspergillus fumigatus* allergen than asthma patients with a simple *Aspergillus fumigatus* allergy.

3.1.2. A2Animal models

Animal models of allergic asthma combined with precision immunology tools significantly contributed recently to the understanding of molecular mechanisms of allergic asthma, from the role of combined exposure triggering allergic sensitisation to novel innate and adaptive immune pathways (Table 1). Nevertheless, differences between mice and humans should be carefully considered and limits of extrapolation should be acknowledged when translating experimental results into clinical use.

3.1.3. A3Single cell analysis

Transcriptomics analyses are affordable and frequently used in allergic asthma research. Initially, RNA analysis was previously restricted to identifying individual transcripts by Northern blots or quantitative PCR. High throughput transcriptomics was made

Table 1

Examples of contribution of animal models combined with precision immunology tools to the improved understanding of mechanisms in allergic asthma.

Allergic asthma pathogenic pathway	Key results from the animal model	What the animal model brings novel	Reference
IL-33 and its receptor ST2	ST2 deficiency diminished HDM-induced AHR in the peripheral lung, while AHR in the central airways was unaffected. Inflammatory responses to HDM were also reduced in ST2(−/−) mice.	in addition to promoting Th2 inflammation, this animal model suggests a new role for the IL-33/ST2 pathway for the induction of peripheral inflammation and mucus production that causes AHR in the peripheral lung.	Zoltowska AM, Lei Y, Fuchs B et al. Clin Exp Allergy. 2016; 46(3):479-90
	Combined exposure to diesel exhaust particle (DEP) and HDM increased IL-33 and ST2 expression in lung, elevated inflammatory responses and AHR. Interference with the IL-33/ST2 signalling pathway impaired the DEP-enhanced allergic airway inflammation with minimal effects on AHR. Recombinant soluble ST2 did not modulate the DEP-enhanced allergic airway responses.	IL-33/ST2 pathway contributes to the onset of DEP-enhanced allergic airway inflammation	De Grove KC, Provoost S, Braun H et al. Clin Exp Allergy. 2018; 48(12):1665-1675
Type 2 innate lymphoid cells	Mice lacking PKC-θ had reduced ILC2 numbers, Th2 cell numbers and activation, AHR, and expression of the transcription factors IRF4 and NFAT1. Adoptive transfer of ILC2s restored eosinophil influx and IL-4, IL-5 and IL-13 production in lung tissue, as well as Th2 cell activation. Pharmacologic PKC-θ inhibition administered during allergen challenge reduced ILC2 numbers and activation, airway inflammation and IRF4 and NFAT1 expression.	PKC-θ is a critical factor for ILC2 activation that contributes to Th2 cell differentiation, which is associated with IRF4 and NFAT1 expression.	Madouri F, Chenuet P, Beuraud C et al. J Allergy Clin Immunol. 2017; 139(5):1650–1666.
Type 2 and 3 innate lymphoid cells	High fat diet feeding exacerbated allergic airway disease features, including humoral response, airway eosinophilia, AHR,	Obesity exacerbates allergic airway inflammation through mechanisms involving ILC2s and ILC3s	Everaere L, Ait-Yahia S, Molendi-Coste O et al. J Allergy Clin Immunol. 2016; 138(5):1309-1318

Table 1 (continued)

Allergic asthma pathogenic pathway	Key results from the animal model	What the animal model brings novel	Reference
	and Th2 and Th17 pulmonary profiles. The numbers of total and cytokine-expressing lung ILC2s and ILC3s further increased in HDM-challenged obese mice accompanied by high IL-33 and IL-1β levels. Depletion of ILCs followed by T-cell reconstitution, led to a profound decrease in allergic airway inflammatory features, including Th2 and Th17 infiltration.		
T2 helper cells		B-T cell interaction is required for robust Tfh and Th2 cell induction, but not essential for eosinophilic airway inflammation	Vroman H, Bergen IM, Li BW et al. Clin Exp Allergy. 2017; 47(4):551-564

achievable with the use of microarrays, which detect nucleic acids in a sample by hybridization to probes on microchips. Microarrays allow detection of a fraction of all mRNA transcripts that are predefined by probes, and they cannot be used for discovery on new genes. Moreover, there are limitations, as background hybridization and probe saturation can interfere both with low-level and high-level detection. RNA sequencing (RNA-seq) is a newer technology, and it can assemble transcripts in an unbiased way with high accuracy.

The currently known endotypes of asthma, namely T2-high/T2-low, were identified based on a microarray study on bronchial brushings. In this study, Th2-high endotype was associated with an atopic profile, airway remodelling and responsiveness to corticosteroids response compared to Th2-low endotype (Woodruff et al., 2009). Another example is the transcriptomic analyses performed in blood samples from the U-BIOPRED study. Asthmatics had a high number of genes differentially expressed compared to non-asthmatics (Bigler et al., 2017). Moreover, transcriptomic analysis in sputum yielded four distinct clinical clusters associated with unique pathophysiological response. The authors also reported that distinct transcriptomic profiles were associated with adult-onset asthma or asthma with fixed airflow restriction. The findings from this study underscored the differential gene expression varied between sputum, nasal brush, endobronchial brush and endobronchial biopsy (Hekking et al., 2017, 2018). Moreover, sputum transcriptomics identified IL-4, IL-5 and IL-13 genes (T2GM) profile that did not decrease following corticosteroid treatment in severe asthmatics (Peters et al., 2014).

Recently, studies have started to evaluate the asthma transcriptome at a single-cell level using single-cell RNA sequencing (scRNA-Seq). First, scRNA-Seq was used to chart the cellular landscape of upper and lower airways and lung parenchyma in healthy lungs, and lower airways in asthmatic lungs. A location-dependent airway epithelial cell states was described, together with a novel subset of tissue-resident memory T cells. In the lower asthmatic airways, mucous cell hyperplasia and goblet cell hyperplasia were shown to stem from a novel mucous ciliated cell state. Th2 cells were identified in asthmatic lungs together with

evidence for T2 cytokines in maintaining the altered epithelial cell states. Unbiased analysis of cell-cell interactions identified a shift from airway structural cell communication in healthy lungs to a Th2-dominated interactome in asthmatic lungs (Vieira Braga et al., 2019). Another single-cell study evaluated the pathogenic role of Th2 cells in allergic asthma. scRNA-seq evaluation of T helper cells from lymph node, lung, and airways in the HDM model of allergic airway disease showed distinct transcriptional profiles of naive CD4⁺, Th1, Th2, and Treg cells, together with a CD4⁺ T cell population responsive to type I interferons. Th2 cells in the airways were enriched for transcription of many genes, including CD200r1, IL6, Onzin, and Insulin-like growth factor-binding protein 7. Their mRNA profile was supported by analysis of chromatin accessibility and flow cytometry. Pathways associated with lipid metabolism were enriched in Th2 cells, and experiments with inhibitors of key metabolic pathways supported roles for glucose and lipid metabolism (Tibbitt et al., 2019). The analysis of the single-cell transcriptome of ~50,000 HDM allergen-reactive Th and Treg cells from asthmatics with HDM allergy in comparison with three control groups (asthmatics without HDM allergy and non-asthmatics with and without HDM allergy) showed that HDM allergen-reactive T helper and Treg cells are highly heterogeneous, with certain subsets quantitatively and qualitatively different in individuals with HDM-driven allergic asthma. IL-9-expressing HDM-reactive Th cells were higher in HDM-driven allergic asthma compared with non-asthmatics with HDM allergy, and the IL-9-expressing Th subset displayed enhanced pathogenic properties. HDM-reactive Th and Treg cells expressing the interferon response signature (THIFNR and TregIFNR) were increased in asthmatics without HDM allergy compared with those with HDM allergy. Of interest, in THIFNR and TregIFNR positive cells the expression of TNFSF10 encoding for tumor necrosis factor-related apoptosis-inducing ligand which dampens activation of Th cells, was enriched. These findings suggest that the THIFNR and TregIFNR subsets may dampen allergic responses, which may help explain why only some people develop Th2 responses to nearly ubiquitous allergens (Seumois et al., 2020). Another recent scRNA-Seq study assessed the effects of IL-13 on human airway epithelial cell cultures and showed that IL-13 generates a distinctive transcriptional state for each cell type. A mucus secretory program induced by IL-13 in all cell types which converts both mucus and defence secretory cells into a metaplastic state with emergent mucin production and secretion, ER stress and cell death in ciliated cells was described. The IL-13-remodeled epithelium secretes a pathologic, mucin-imbalanced, and innate immunity-depleted proteome that arrests muco-ciliary motion. Signatures of IL-13-induced cellular remodelling were mirrored by transcriptional gene signatures characteristic of the nasal airway epithelium within Th2-high versus Th2-low asthmatic children (Jackson et al., 2020). In another study scRNA-Seq identified a set of cytokines and intracellular transduction regulators associated with asthma exacerbations and shared across multiple cell clusters, forming a complicated molecular framework (Li et al., 2020).

3.1.4. A4CRISPR/Cas9 directed interventions

The identification of clustered regularly interspaced short palindromic repeats (CRISPR), as well as the CRISPR-associated protein (Cas), have been reported to play a crucial function in gene alterations and has transformed many scientific fields (Mojica and Rodriguez-Valera, 2016). The engineered CRISPR/Cas system utilises a chimeric single-guide RNA (sgRNA) to direct the *S. pyogenes* Cas9 (SpCas9) endonuclease or other Cas9 orthologs such as *S. aureus* Cas9 (saCas9) and Cpf1 to target DNA sequence. A double strand break (DSB) is induced using its RuvC and HNH nuclease domains. The sgRNA is a fusion of a CRISPR-targeting RNA (crRNA) array, containing a 20-nucleotide guide sequence, a short direct repeat, and an auxiliary trans-activating crRNA (tracrRNA) (Makarova et al., 2020). CRISPR can be used to screen for novel disease drivers or drug targets in allergic asthma or can correct genetic mutations and edit epigenetic marks that contribute to asthma pathogenesis. Examples of the use of CRISPR

technology are the creation of an asthma animal model deficient in transient receptor potential (TRP) A1 (Reese et al., 2020), modulation of the immune response in asthma by enhancing T regs (Simeonov et al., 2017) or evaluation of the epithelial-mesenchymal transition (Jiang et al., 2019).

3.1.5. A5Genetic and epigenetic mechanisms

Epigenetics can be referred to as nuclear information that complements genomics and can alter gene expression. Epigenomics consist of DNA methylation, histone modifications and various forms of noncoding RNA. Epigenome-wide association studies (EWASs) identify the association of epigenomics with asthma, its severity and therapy response.

Epigenomics studies have revealed prenatal and perinatal asthma routes related to allergic sensitisation. Moreover, the differentiation of Th2 cells in early-onset asthma has been attributed to epigenetic regulation, with the prenatal and perinatal exposome modelled by maternal smoking and diet (Peng et al., 2018, 2019; Brand et al., 2012). It is noteworthy that the lack of reproducibility and validation in studies have put epigenomics under uncertainty in asthma research (Edris et al., 2019). A major limitation of many of the current studies of asthma epigenetics is that they evaluate epigenetic modifications in both allergic and non-allergic asthma, making it difficult to distinguish the epigenetic modifications specific for allergic asthma. Novel integrated transcriptomic and epigenetic chemistries at single-cell level and functional assessment of the impact of the epigenetic modification with novel gene editing tools such as CRISPR/Cas9 will allow cross-validation as well as strengthen the utility of epigenetic analyses. In addition, most DNA methylation studies in asthma use peripheral or cord blood due to poor accessibility of airway cells or tissue. Epigenetic biomarkers are tissue-specific, thus changes found in circulating blood may be discordant from the airway. Recently, epigenetic signatures in the nasal epithelium, which is a primary interface with the environment and an accessible proxy for the bronchial epithelium, provided valuable insights into mechanisms of allergic asthma.

MicroRNAs (miRNAs) are small non-coding RNAs that act as endogenous sequence-specific regulators of gene expression and control many cellular pathways. miRNAs are approximately 22 nucleotides in length and can act by directly blocking the translational machinery or by regulating mRNA stability. miRNAs exert multiple roles in allergic asthma (Fig. 3). Inhibition of miR-126 function in the lung with antagomir significantly inhibited HDM-induced AHR, recruitment of eosinophils and neutrophils into the lung and reduced mucus hypersecretion. However, blocking miR-126 function was less dramatic in a mouse model of chronic asthma suggest that miR-126 is likely to regulate acute inflammatory events and other miRNAs may regulate the chronic aspects of disease (Mattes et al., 2009). miR-145 is one of the highly expressed miRNAs within the trachea and airway wall. From all the miRNAs investigated only anti-miR-145 treatment could suppress features of chronic asthma. Anti-miR-145 treatment reduced eosinophilic inflammation, mucus hypersecretion, T2 cytokine production (IL-5 and IL-1) and AHR with levels of inhibition similar to corticosteroid treatment alone (Collison et al., 2011). miR-145 has also been linked to the regulation of innate immunity by inhibiting IFN- β production (Witwer et al., 2010). Smooth muscle development and the differentiation of fibroblasts into smooth muscle cells have also been shown to be regulated by miR-145 (Cordes et al., 2009). In an experimental chronic asthma mouse model miR-155 deficiency resulted in diminished eosinophilic inflammation and mucus hypersecretion in the lungs together with a reduction in Th2 cell numbers and airway T2 cytokine levels and with complete abrogation of allergen-induced airway eotaxin-2/CCL24 and periostin levels (Malmh  ll et al., 2014). The transcription factor PU.1, a negative regulator of Th2 cytokine production, was upregulated. Recent reports show a role of miR-155 in ILC2s and IL-33 signalling (Johansson et al., 2017). IL-13 dose-dependently inhibited the expression of miR-155-5p in SMCs. Mechanistic studies showed that inhibition of miR-155-5p promoted the stimulatory effects of IL-13, whereas

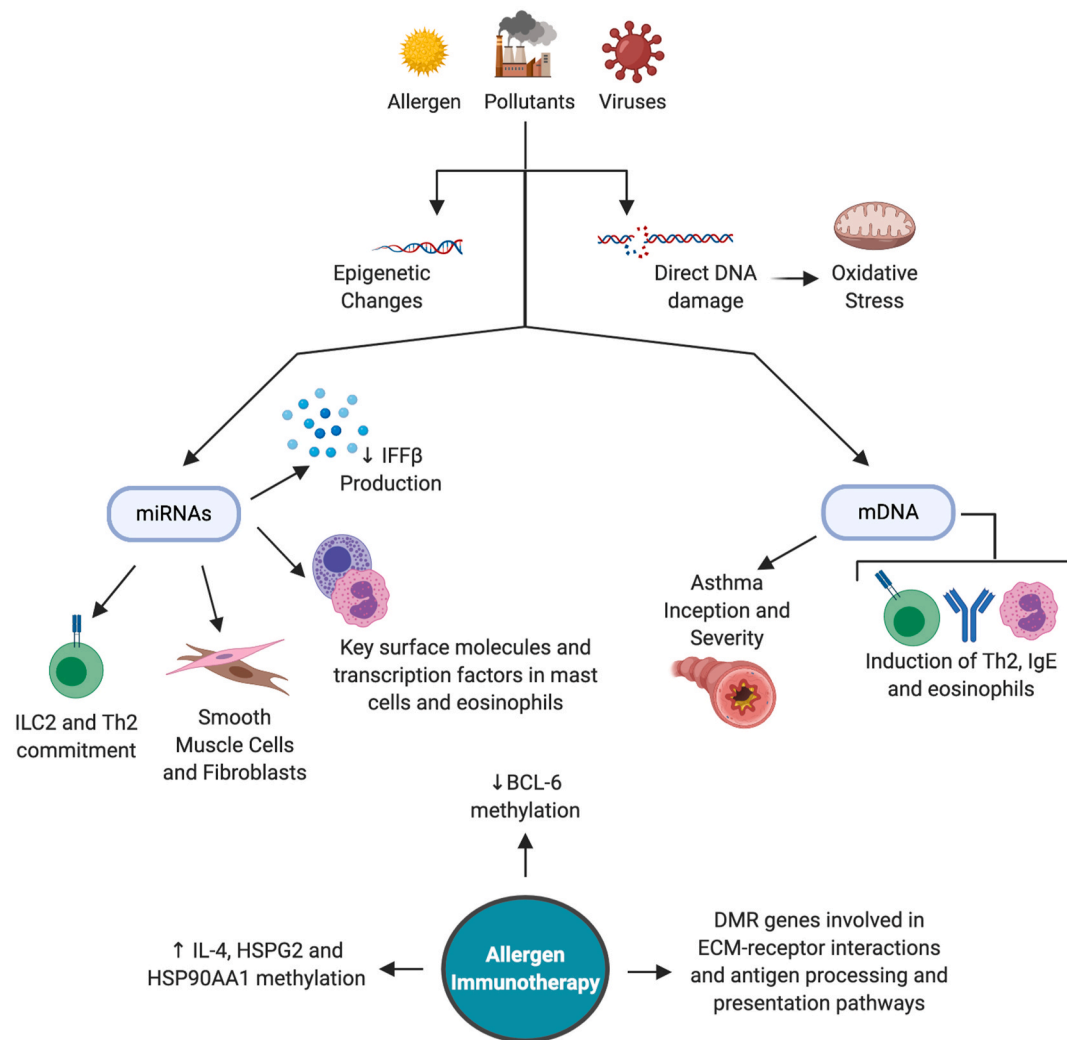


Fig. 3. Epigenetic and genetic mechanisms in allergic asthma and the modifications induced by AIT.

Exposure to allergens, pollutants or pathogens can lead to epigenetic changes or to direct DNA damage. Direct DNA damage induces oxidative stress. miRNAs have been observed to promote commitment of ILCs and Th cells to ILC2 and Th2 phenotypes, support the phenotypic changes in smooth muscle cells and fibroblasts, decrease IFN- β production and alter the expression of key surface molecules and transcription factors in granulocytes. DNA methylation has been demonstrated to contribute to asthma inception and can be a determinant of severity, while also promoting a T2 phenotype. During AIT, tolerogenic methylation profiles have been observed that promote immune modulation and reduce inflammation.

overexpression of miR-155 significantly inhibited these effects (Shi et al., 2018). miR-21 is as an important regulator of the recruitment and activation of inflammatory cells in the lung, which may also play a role in the development of T2 responses by decreasing IL-12 levels (Lu et al., 2011). Altered expression of 86 miRNAs during the in vitro differentiation of bone marrow-derived mast cells was described. Target prediction analysis identified subsets of altered miRNAs with potential roles regulating key mast cell surface receptors, transcription factors, signaling molecules and secretory proteins. Seven specific miRNAs were directly associated with the expression of the mast cell surface receptors c-Kit and the high affinity IgE receptor (Fc ϵ RI α). A further 18 miRNAs promote expression of MITF, GATA-1 and c/EBP α ; three core transcription factors that direct mast cell differentiation. Furthermore, 11 miRNAs may regulate the expression of STAT-3, -5a/b, GATA-2 and GATA-3 during differentiation, along with 13 miRNAs that target transcripts encoding Ndst2, mast cell protease 4 (mMCP4) and mMCP6, and thus may regulate biosynthesis of mast cell secretory mediators. Networks of miRNAs target key eosinophil surface molecules and transcription factors, as well as potential regulation of TLR-4/13 expression. 13 miRNAs were associated with the expression of IL-5R α and CCR3 and 14 miRNAs were associated with regulation of the transcription factors

GATA-1/2, PU.1 and C/EBP ϵ . A further 17 miRNAs may regulate translation of TLR-4/13 (Xiang et al., 2014; Yang et al., 2014).

DNA methylation (DNAm) contributes to allergic asthma pathogenesis, from increased risk of developing asthma to increased severity of the disease. A recent evaluation of the nasal epigenome showed differentially methylated regions (DMRs) annotated to genes implicated in allergic asthma, Th2 activation and eosinophilia (EPX, IL4, IL13) and genes previously associated with asthma and IgE in EWAS of blood (ACOT7, SLC25A25). Asthma, IgE and FeNO were associated with nasal epigenetic age acceleration (Zhu et al., 2020; Cardenas et al., 2019).

Traffic-related air pollution contributes to the increasing prevalence and severity of allergic asthma. The evaluation of DNAm changes may elucidate these adverse health effects of environmental exposures. Exposure to black carbon (BC) drives asthma proinflammatory gene demethylation that in turn may link to airway inflammation. Atopic children, especially those sensitized to cockroach allergens, may be more susceptible to the effect of acute BC exposure on epigenetic changes (Jung et al., 2017). A human exposure challenge with allergen and diesel exhaust particles (DEP) showed that the response to allergen challenge was associated with key DNAm regulation enzymes, however this relationship was not influenced by DEP co-exposure but was

dependent on AHR status (Jung et al., 2017).

Desensitisation-associated methylomes were described as biomarkers of AIT in allergic asthmatic children. HDM AIT induced significant BCL6 hypomethylation, while IL-4, HSPG2 and HSP90AA1 were hypermethylated. KEGG pathway analysis of AIT modulated gene expression further revealed DMR genes involved in extracellular matrix-receptor interactions and antigen processing and presentation pathways (Wang et al., 2018, 2020).

Exposure to environmental allergens is a major risk factor for asthma development. Allergens possess proteolytic activity that is capable of disrupting the airway epithelium. Although there is increasing evidence pointing to asthma as an epithelial disease, the underlying mechanism that drives asthma has not been fully elucidated. High levels of reactive oxygen and nitrogen species can induce cytotoxic DNA damage. Nevertheless, little is known about the role of allergen-induced DNA damage and DNA repair as modulators of allergic asthma. HDM challenge increased lung levels of oxidative damage to proteins (3-nitrotyrosine), lipids (8-isoprostane), and nucleic acid (8-oxoguanine). There was immunohistochemical evidence for HDM-induced DNA double-strand breaks (DSB) foci in bronchial epithelium associated with increased levels of DNA repair proteins and apoptosis. Notably, the DSB repair inhibitor NU7441 augmented DNA damage and cytokine production in the bronchial epithelium and apoptosis in the allergic airway, implicating DSBs in allergic asthma pathophysiology. Expression of nuclear factor erythroid 2-related factor 2-dependent antioxidant genes was reduced immediately after HDM exposure and HDM-induced DNA damage was prevented by the antioxidants glutathione and catalase, suggesting that HDM altered antioxidant responses (Chan et al., 2016, 2017).

3.1.6. A5Vesicular trafficking in immune cells

The small GTPase Rab27 is known to regulate intracellular and intercellular vesicle traffic (Fukuda, 2008; Ostrowski et al., 2010). Exophilin-5 is a Rab27-binding protein which regulates extracellular superoxide release, intracellular ROS production, and phosphoinositide 3-kinase activity by controlling intracellular trafficking of Nox2-containing vesicles. It was shown to be highly expressed in lung epithelial cells and in the specialized IL-5 and IL-13 producers pathogenic Th2 cell population. Exophilin-5 deficiency increased IL-33 secretion by lung epithelial cells and IL-5 and IL-13 production in response to TCR and IL-33 stimulation from pathogenic Th2 cells (Okunishi et al., 2020).

3.1.7. A6Novel mediators

Timely elimination of damaged or unnecessary organelles and cells through programmed death is a prerequisite of inflammation resolution. Under T2 inflammatory conditions, human airway epithelial cells generate proferroptotic phospholipids as death signals dependant on activation of 15-lipoxygenase-1 (15LO1) in complex with PE-binding protein-1 (PEBP1). However, the cellular membrane damage induced by these proferroptotic phospholipids triggers compensatory pro-survival pathways, in particular autophagic pathways. PEBP1 is pivotal to driving dynamic interactions with both proferroptotic 15LO1 and the autophagic protein microtubule-associated light chain-3 (LC3). In T2 asthma, high levels of both 15LO1-PEBP1 and LC3-II are expressed in airway epithelial cells are correlated with low bronchoalveolar lavage fluid mitochondrial DNA and more severe disease. The concomitant activation of ferroptosis and autophagy by 15LO1-PEBP1 complexes is a novel pathobiologic pathway relevant to allergic asthma and a potential therapeutic target (Zhao et al., 2020).

In an allergic asthma mouse model prevention of increases in lung ceramide levels mitigated allergen-induced apoptosis, reactive oxygen species, and neutrophil infiltration. In contrast, dietary supplementation of the anti-oxidant α -tocopherol decreased reactive oxygen species but had no significant effects on ceramide elevation or apoptosis, indicating that the increases in lung ceramide levels in allergen-challenged mice

are not mediated by oxidative stress. Moreover, specific ceramide species were altered in bronchoalveolar lavage fluid from patients with severe asthma compared to non-asthmatics. Ceramide might be the trigger of formation of Creola bodies found in the sputum of patients with severe asthma and could be a biomarker to optimize diagnosis and to monitor and improve clinical outcomes in this disease (James et al., 2020).

3.2. A better diagnosis through the description of the molecular allergen sensitisation profile

Immunoglobulin E is pivotal for the manifestation and persistence of allergic asthma. Unfortunately, just a small fraction of total IgE can be assigned to specific target allergen (specific IgE). Specific IgE might be evaluated in vivo or in vitro. In vivo IgE testing is done via skin prick test (SPT), and rarely by intradermal testing. SPT has high sensitivity, results available within 10–20 min and the method have low costs. However, it is a manual method thus results are highly dependent on the skills of the examiner, and are influenced by medication and skin lesions. In addition, systemic or distant site reactions are possible (<1% of the cases) and thus might be risky in severe allergic asthma. Moreover, it is based only on natural allergen extract, with wide variations in allergen content and in additional contaminants (Casset et al., 2012; Klimek et al., 2020), and is not suitable for sensitisation monitoring. In vitro IgE measurement can be done with either natural extracts or with recombinant allergens. It has high sensitivity but has high costs and results available within days to weeks. A well-established and frequently used test system is the ImmunoCAP® (Thermo Fisher Scientific) assay.

Component-resolved diagnosis (CRD) has revolutionized allergy diagnosis as it provided detailed information on the patient's sensitisation patterns, possible cross-reactions, and the severity of anticipated clinical reaction. CRD makes use of individual allergen components/proteins, therefore identifying the exact molecule against which specific IgE (sIgE) is being produced (Matricardi et al., 2016). Singleplex or multiplex immunoassays are used for CRD. An advantage of most of the microarray approaches is the statistical power of assaying replicates in parallel. Immunoblots represent another in vitro approach to investigate the patient's molecular sensitisation patterns. A novel analytical platform for unbiased IgE target epitope detection relying on chemically produced random peptide libraries immobilized on polystyrene beads capable to simultaneously present millions of different peptide motifs to immunoglobulins from biological samples was recently described (Krause et al., 2020).

A CRD model investigated the mite molecular signature in subjects with T2 asthma. Major allergens (Der p 2, Der p 23, and Der p 1) were present in >70% of all subjects, with mid-tier allergens (Der p 5, Der p 7, and Der p 21) reaching up to 51% in the present cohort, thus confirming their dominant role in T2 asthma. A complex repertoire of HDM molecules recognized by IgE was depicted, with 38 distinct profiles (González-Pérez et al., 2020). In the Nationwide Swedish study on severe childhood asthma 89% of those with severe asthma and 86% of those with controlled asthma were sensitized to Fel d 1, with Fel d 2 sensitisation present in 3% of those with severe asthma and 4% of those with controlled asthma and lipocalin Fel d 4 sensitisation in 38% of those with severe asthma and 21% of those with controlled asthma (Konradsen et al., 2014). Fel d 4 sensitisation may lead to cross-reactions with Can f 6 and Equ c 1 (Nilsson et al., 2012). In contrast with asthma, in paediatric allergic rhinitis patients, considerably fewer sensitizations to cat components were found (17% to Fel d 1, 1% to Fel d 2, and 2% to Fel d 4) (Araujo et al., 2016). The presence of sIgE to Fel d 1 was more frequent in asthmatic patients than in those with rhinoconjunctivitis (Gronlund et al., 2008).

Moreover, Fel d 1 sensitisation in childhood can be used as a predictive marker for cat allergy and associated symptoms in adolescence (Nordlund et al., 2012; Asaranoj et al., 2016). In the same Swedish study 92% of patients with severe asthma and 86% of those with controlled

asthma were positive to dog-derived allergen extracts. The most frequent sensitisation against dog components in those with severe childhood asthma was against Can f 5, which is present in urine of male dogs. Can f 2 has been found positive only in severe asthmatic children (Konradsen et al., 2014). Like Fel d 1, Can f 1 sensitisation in childhood and polysensitisation to dog allergens predict dog allergy at adolescence, with Can f 1 being a better prognostic marker than dog extracts (Nordlund et al., 2012). Sensitisation to the major horse allergen Equ c 1, a lipocalin, was also significantly more prevalent and characterized by higher sIgE levels in severe vs controlled paediatric asthma (Konradsen et al., 2014). The components Asp f 4 and Asp f 6 have been found positive only in asthma patients with ABPA, whereas Asp 1 and Asp f 3 sensitisation occurred in asthma patients regardless of the presence of ABPA (Lukaszewicz et al., 2021; Cramer et al., 1998).

In addition to clinical history, skin prick test, and specific IgE determination, basophil activation test (BAT) can be a part of the diagnostic evaluation in allergic asthma. BAT measures basophil response to allergen cross-linking IgE on between 150 and 2000 basophil granulocytes in <0.1 ml fresh blood. BAT may be used to monitor patients on AIT, anti-IgE treatment or to evaluate the role of co-factors increasing asthma severity such as *Staphylococcus aureus* enterotoxins (SEs) (Pereira Santos et al., 2015; Noga et al., 2008; Macglashan and Saini, 2013; Flora et al., 2020). BAT may use fewer resources and be more reproducible than challenge testing. An important next step is to standardize BAT and make it available in diagnostic laboratories.

Today's most widely applied is the "top-down" diagnostic approach starting with the patient's clinical history, followed by an SPT or extract-based IgE testing, and using CRD only as an additional source of information. This is due to the fact that CRD, especially by means of microarrays, are deemed to be too complex and detailed. When CRD is performed, it is usually through singleplex assays, and clinicians decide which allergen component should specifically be looked at. However, polysensitization is frequent in allergic asthma, and in vitro IgE assays, particularly those based on allergen components, provide additional useful information, especially the possibility of distinguishing between genuine sensitisation and cross-reactivity. The "bottom-up" approach starts with the detailed information that CRD provides and moves from that to the clinical history to allow a comprehensive evaluation of the allergic disorder. The reasoning behind this approach is the value of the comprehensive evaluation of the patient's IgE repertoire in predicting a future allergic manifestation, for example the development of asthma in patients with atopic dermatitis or allergic rhinitis. In addition, detailed information regarding the sensitisation profile of a patient is essential for a successful allergen-specific immunotherapy. The European Academy of Allergy and Clinical Immunology (EAACI) proposes the "U-shaped" approach starting with the patient's clinical history, physical examination, and SPT or extract-based IgE testing, with CRD performed for allergen components of sources to which the previous diagnostics have detected a clinically relevant sensitisation. Additionally, when IgE sensitisation to highly cross-reactive allergens has been found, CRD diagnostics should be broadened to include molecules of the same family, and clinical history should be expanded by actively investigating for the presence of possible symptoms caused by other allergen sources of the index protein family (Matricardi et al., 2016). A broader use of CRD may lower costs on the manufacturer's side, as well as for the healthcare systems because of fewer patient-doctor-laboratory encounters.

3.3. A better selection of responders to the targeted treatment, either with AIT, or with biologicals

Disease phenotypes cluster together relevant visible properties such as age at onset, triggers, co-morbidities, physiologic traits, remodelling, inflammation type (eosinophilic and non-eosinophilic), and treatment response (Agache, 2019; Agache and Akdis, 2019; Kaur and Chupp, 2019; Chung and Adcock, 2019; Agache et al., 2012). Asthma phenotypes defined based on the predominant inflammatory cell identified in

induced sputum (eosinophilic, neutrophilic, mixed granulocytic, and paucigranulocytic phenotypes) or in blood (eosinophilic asthma) are currently being used for a stratified approach to severe asthma (Agache, 2019). Phenotypes do not necessarily relate to or give insights into the underlying pathogenetic mechanism (Agache et al., 2012). In addition, they frequently overlap and are subject to change over time. Thus, defining disease endotypes based on key pathogenetic mechanisms has become a rational development. The current indication for AIT in asthma is based on the demonstration of the allergic phenotype (Agache et al., 2019). However, there are still many non-responders, thus a more accurate sensitisation profile would help in the selection of the proper AIT vaccine (Agache et al., 2019; Pfaar et al., 2019). Similarly, biologicals currently available for T2 asthma target over-lapping phenotypes, eg eosinophilic and allergic asthma, thus the selection of a particular biological is difficult for the clinician (136.). No biomarkers are yet available to indicate the predominant pathway in T2 asthma (IL-5, IL-4/IL-13 or the IgE pathway) (Breiteneder et al., 2020; 136.). The presence of specific IgE against perennial allergens is required by the regulatory authorities for prescribing omalizumab, thus the sensitisation profile needs to move centre stage into the asthma diagnostic plan.

3.4. Novel approaches to allergic asthma

Adoptive cell therapies with genetically engineered T cells have revolutionized the oncology field, bringing curative treatment for leukaemia and lymphoma, while therapies exploiting the suppressive functions of Tregs have been developed for non-oncological settings, such as in transplantation, autoimmune diseases, cardiovascular disease, obesity and chronic inflammatory disorders. Several experimental models support Treg-based approaches for severe IgE-mediated responses and chronic respiratory airway diseases. CAR T-cells are genetically engineered T cells comprising of a recombinant receptor targeting a specific antigen. Engineered TCRs are generated by transferring α and β chains from a known TCR to react with an antigen that can be presented in the context of the MHC molecule. These cells can be expanded ex vivo and re-infused into patients. CARs are recombinant receptors which are incorporated with co-stimulatory and regulatory molecules. To generate CAR-T cells, autologous PBMCs are isolated and activated with anti-CD3 and anti-CD28 monoclonal antibodies and then engineered to express CARs using viral vectors. CAR-T cells are subsequently expanded ex vivo using cytokines and antibody-coated beads and re-infused to patients following lymphodepletion (Agache et al., 2020; Smith et al., 2016b).

A recent study has revealed that CAR-T cells can potentially have utility in the treatment of fungal allergy. The authors explored Treg-based therapy for the treatment of aspergillus induced allergic airway inflammation. Adoptively transferred autologous CAR Tregs accumulated in the lung and tracheobronchial lymph nodes, reduced AHR, inhibited pulmonary mucus production, diminished eosinophilic airway inflammation, allergen-specific IgE and T2 cytokine levels. These findings pave the way for cell therapy of severe allergic asthma (Gross et al., 1989).

4. Integrating individual genetic or molecular data for improved geno-/phenotyping and proper selection of personalised asthma treatment

A huge amount of omics data has been developing during the few years. It can only be analysed in-depth by stimulating the cooperation between immunologists, data scientists and healthcare professionals. Translational bioinformatics had been proposed and developed as a new field recently. Deep learning and machine-learning algorithms are currently being tested in asthma research (Chung and Adcock, 2019; Skuljec et al., 2017; Agache et al., 2018). The use of artificial intelligence is expected to facilitate the evaluation of very complex non-linear

relationships between features in an unbiased fashion.

5. Future perspectives

A revised taxonomy of asthma based on endotype profiling is necessary for the targeted approach, together with the identification of biomarkers that can predict patient theratype. Allergic asthma evaluation should involve the interconnected concepts of phenotype-biomarker-endotype, while expand towards new targets such as the sensitisation profile and genetic and epigenetic modifications. At the early stages of the disease, allergic asthma endotypes may be easier to differentiate, while as the disease progresses, other cell types and structures would be affected making the distinction of each endotype more difficult.

Last but not least collaboration between scientific organizations, governmental regulatory agencies, pharmaceutical and biotechnical companies is essential to move the field forward.

COIs

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