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REVIEW ARTICLE

Current and future biomarkers in allergic asthma

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Abstract

Diagnosis early in life, sensitization, asthma endotypes, monitoring of disease and treatment progression are key motivations for the exploration of biomarkers for allergic rhinitis and allergic asthma. The number of genes related to allergic rhinitis and allergic asthma increases steadily; however, prognostic genes have not yet entered clinical application. We hypothesize that the combination of multiple genes may generate biomarkers with prognostic potential. The current review attempts to group more than 161 different potential biomarkers involved in respiratory inflammation to pave the way for future classifiers. The potential biomarkers are categorized into either epithelial or infiltrate-derived or mixed origin, epithelial biomarkers. Furthermore, surface markers were grouped into cell-type-specific categories. The current literature provides multiple biomarkers for potential asthma endotypes that are related to T-cell phenotypes such as Th1, Th2, Th9, Th17, Th22 and Tregs and their lead cytokines. Eosinophilic and neutrophilic asthma endotypes are also classified by epithelium-derived *CCL-26* and *osteopontin*, respectively. There are currently about 20 epithelium-derived biomarkers exclusively derived from epithelium, which are likely to innovate biomarker panels as they are easy to sample. This article systematically reviews and categorizes genes and collects current evidence that may promote these biomarkers to become part of allergic rhinitis or allergic asthma classifiers with high prognostic value.

With the increasing prevalence of asthma, the continuous lack of novel therapies and inefficient disease prevention, the demand for predictive biomarkers for allergic rhinitis and asthma is steadily increasing. Following the current clinical guidelines, it is essential to identify allergen sensitization patterns as well as lung function parameters.

The aim of this review was to extend the biomarker portfolio, summarized in previous reviews (1–6), and to provide a structured and comprehensive overview of future biomarkers both in terms of their origin and in view of their predictive value in different phases of the disease. The perspective is to define molecular patterns of rhinitis and asthma as basis for endotype definitions, rather than using clinical disease phenotypes following the definition of Wenzel (7). Thus, the definition of an endotype is a molecular phenotype that supports a clinical outcome. The current review summarizes the biomarkers along with the disease stages and generates a categorized overview of biomarkers in rhinitis and asthma. The categorization is provided in Tables 1–3 (see columns on the right) and mirrored in the sections 2 through 6. A second important categorization approach is the grouping according to the producer of the biomarker, which may be blood-borne or about to infiltrate, or may reside in the tissue in course of the inflammation (Fig. 1).

Early life endotypes

Due to diagnostic procedure restrictions, the determination of disease prognosis in early life faces the unique challenge of a limited biomarker repertoire (Tables 1–3; column 1). Critical for the emerging treatments and monitoring strategy of children is the definition of asthma endotypes (8).

Genetic dispositions and their associated endotypes

Genetic associations with genes such as *ORMDL3* or *NPSR1* have not led to clinically applicable asthma endotyping, as it has been initiated for *filaggrin* mutations in allergic skin

Table 1 Recently described biomakers. Potential biomarkers are listed according to cellular sources (epithelium and/or infiltrate derived) as well as the matrices where they were described. References are described on the coloured side of the table. For better visualization and categorization, the references are segmented into stages of disease. Asterisks indicate that the expression/secretion level of the referred marker has been reported from the cell culture experiments

		sputum	BALF	biopsies	bronchial exhalates	nasal secretions	nasal cells	tear fluid	blood	Early life endotypes	Disease onset and asthma prevention	Established disease and prevention of remodelling	Response to environment and treatment
										Literature references			
Epithelium& infiltrate derived	CCL-3	+	+	+	+	+		+	+	216			123, 202, 214
	CCL-17	+	+	+	+	+	+	+	+	221	129	128, 222	
	IL-1α	+	+	+	+	+			+			199	199, 200
	IL-1β	+	+	+	+	+	+	+	+	26	140	201	201, 202
	IL-6	+	+	+	+	+	+	+	+	26	203	204, 205*	164, 168, 205*, 206*
	IL-8	+	+	+	+	+	+	+	+	26, 56	171		113,171,179,206*-209
	IL-10	+	+	+	+	+	+	+	+	26, 56, 210		122	110, 143, 209
	IL-15	+	+	+	+	+	+	+	+	22, 23		134	211, 212
	IL-16	+	+	+	+	+	+	+	+		213	214	213, 214
	IL-20	+	+	+	+	+	+	+	+			136	
	IL-24					+			+		99	99	
	IL-25	+	+	+			+	+	+		103		178, 215
	NOS2	+	+	+	+			+	+	223	224	104	225*
	Osteopontin	+	+	+		+		+	+	217	142	109	109, 112, 113
	TGF	+	+	+	+	+	+	+	+	25*		25*, 135	113, 129, 144, 209, 220
	VEGF	+	+	+	+	+	+	+	+	25*	218	25*, 117	117, 219
	CHI3L1 (YKL-40)	+	+	+		+	+	+	+	107	99	107	
Epithelium derived	CCL-11	+	+	+	+	+	+	+	+	11	124, 125, 138		166, 167
	CCL-24	+	+	+		+	+		+	227	125	228	
	CCL-26	+	+	+		+	+		+		76, 99, 110, 138	99	166
	CCL-27			+					+			104	
	CXCL-10	+	+	+		+	+	+	+	24, 221	229	128, 205*	158, 181, 205*, 230
	Fibrinogen	+							+			199	199
	GCSF (CSF3)	+	+	+		+	+		+		99	99	209
	IL-1RL1 (IL-33R)	+					+		+	226	19, 99	99	213
	IL-33	+	+	+		+	+		+	12, 159	19, 99, 159	99	159, 160
	IL-36G			+								104	
	MMPs	+	+	+	+	+	+		+	235	236	108,114-116,120,121,199	199
	Periostin	+	+	+	+	+	+		+	25*		231, 232	233, 234
	TIMPs	+	+	+		+	+		+	235	236	108, 114-116, 121	237
	TSLP	+	+	+		+	+		+	11		128	
Infiltrate derived	CCL-2	+	+	+	+	+		+	+	249			169, 250
	CCL-5	+	+	+	+	+	+	+	+	227	251		158, 206*
	CCL-22	+	+	+	+	+	+		+	24	254	128	255
	CRP								+	256		257	209
	CXCL-4								+		258	32	259
	ECP	+	+					+	+	24		260	113, 181,
	IFN-α	+	+				+	+	+	21, 57		248	
	IFN-γ	+	+	+	+	+	+	+	+	21, 26		86, 238*	206*
	IL-2	+	+	+	+	+		+	+	26		122	
	IL-4	+	+	+	+	+	+	+	+	26, 56, 57	76, 94, 175	95, 136, 238	144 - 146, 178
	IL-5	+	+	+	+	+	+	+	+	26, 58	76, 110	136, 238*	145, 146, 166, 167, 178
	IL-9	+	+	+		+	+		+	239		93	
	IL-12	+		+			+		+	22, 23, 26	110		165
	IL-13	+	+	+	+	+	+	+	+	56	76, 110	77, 95, 127, 136	113,144,165,211,220,240
	IL-17A	+	+	+	+	+	+	+	+	241	19, 94	95, 238*	178,179,219
	IL-18	+	+	+		+	+		+	242	243	244	
	IL-22	+	+	+	+			+	+	245		86	215
	IL-31	+				+			+		19, 246 247*	247*	240
	SCF			+			+		+		246		
	TNF-α	+	+	+	+	+	+	+	+	26, 56, 210		238*, 252	123, 181, 253

Table 2 Recently described suface markers on innate immune cells. Surface markers of innate immune cells are listed according to the cellular sources. References are described on the coloured side of the table. For better visualization and categorization, the references are segmented into stages of disease

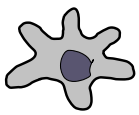
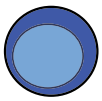
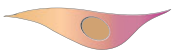
	Surface markers	Early life endotypes	Disease onset and asthma prevention	Established disease and prevention of remodelling	Response to environment and treatment
		Literature references			
Eosinophils 	C3AR			261	
	CD11b			262	
	CD13			263	
	CD25			152, 264	
	CD29			32	
	CD32			265	
	CD44			262, 264	
	CD52		266		
	CD54			262	
	CD62L			152, 262, 265	
	CD63			267	
	CD66e			264	
	CD67			267	
	CD69			50, 262	
	C5AR/CD88		268	261	
	CD108			269	
	CD125			153	
	CD193		270		
	CD244		148		
	CD294		271	272*	
	FcεR1α		149, 150	151, 265	
	Galectin-3			273	
	HLA-DR			267	
	Siglec-8		274	275	
Neutrophils 	CD11b		139, 152	50	276
	CD14	277		278	
	CD35			276	
	CD62L		152	50	
	CD63		139		
	CD64		279		
	CD66b		139, 152		
	CD88				280
	CD274			50	
	CXCR2			281	
	TRL2			278	
	TLR4			278	
Basophils 	CD44			188, 282*	
	CD54			282*	
	CD63		162, 163, 283		
	CD69			282*	
	CD193/CCR3		163, 284	284	
	CD203c		162, 163, 283	152	
	CD294/CRTH2		285		
	FcεR1α		150	286	
Mast cell 	CD63		287*		
	CD117		288		
	CD203c			152	
	CXCR3		289	289	
	FcεR1α		150, 290	286	
Macrophages 	CD11a		132, 154		
	CD11b		132, 291		
	CD14		132, 154	152, 154, 155	
	CD16		132	152, 154	
	CD18		132		
	CD23		132		
	CD54			154	
	CD58			154	
	CD63		132		
	CD64		132		
	CD83		154	154	
	CD86		154, 156	152, 154	
	CD163		157		
	CD206		157		
	HLA-DR		132	154	
	HLA I		132		

Table 3 Recently described surface markers on innate and adaptive immune cells and fibrocytes. Surface markers of innate and adaptive immune cells and fibrocytes are listed according to the cellular sources. References are described on the coloured side of the table. For better visualization and categorization, the references are segmented into stages of disease

	Surface markers	Early life endotypes	Disease onset and asthma prevention	Established disease and prevention of remodelling	Response to environment and treatment
		Literature references			
T cells 	CD4	13, 82, 308	309, 310	13, 81, 223, 311	83, 172, 312
	CD8			84, 320	315
	CD25	82, 308, 313			312
	CD26		313	314	
	CD39			84, 318	319
	CD73			84	
	CD103			78	
	CD154		310		
	CD161			189	172
	CD194/CCR4		161, 310		
	CD196/CCR6		310		
	CD197/CCR7			315	
	CD294/CRTH2	316	80		317
	CD366/TIM-3			81	
	CCR8			315	
	CXCR1				315
	CXCR3		161		
	GITR				83
	HLA-DR	301			312
	TIM-1		300		
B cells 	CD19		69, 72		
	CD20		69, 72		
	CD21	301			
	CD23		68	302*	
	CD24		69, 303*		
	CD27		69, 303*	70	
	CD38		69, 72	71	
	CD80		68		
	CD86		68		
	CD138		73		
	CD252			304	
	CD268		305		
	CD279		306		
	IgD		69		
	IgM		307		
Fibrocytes 	CD163	96			
	CD204	96			
	CD206	96			
	CCR7			183	
	CXCR4			182, 183	
ILCs 	CD16			292, 293	
	CD25			294, 295	
	CD56			292, 293, 296	
	CD69		297		
	CD90			294	
	CD117			15	
	CD127			15, 294	
	CD161			20	
	CD294/CRTH2			15	
	CD314		298		
	CD335			299	
	ICOS			295	
	KLRG1			295	
	SCA1			295	
	ST2			294	

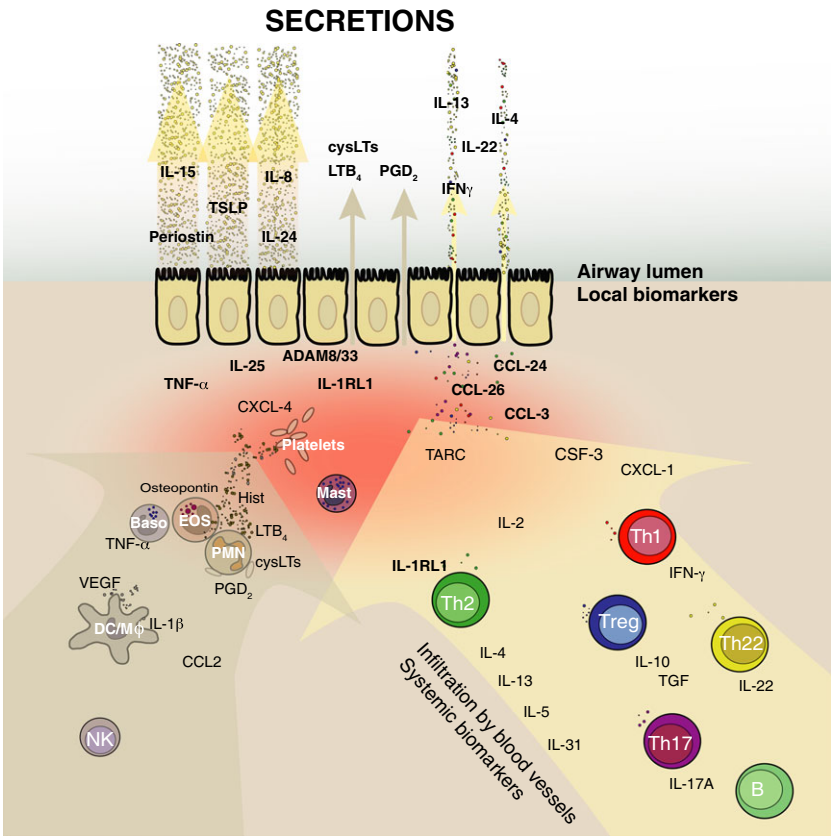


Figure 1 Schematic overview on local biomarkers relevant in allergic rhinitis and allergic asthma in the airways. The airway lumen unifies cytokines and mediators both of different origins. This covers epithelial-derived cytokines as well as mediators of granulocytes (left half) or lymphocytes (right half). While cytokines produced by infiltrating cells (infiltrate derived; eosinophils, basophils, neutrophils, dendritic cells, macrophages and T cells) have a passage through the epithelial barrier, epithelial-derived cytokines have a more direct access. The infiltration-derived biomarkers are also present in the peripheral blood.

manifestation in childhood. This mutation can be used to define specific endotypes of atopic dermatitis characterized by an early onset of the disease and a more severe course (9). Clinically defined phenotypes include nonatopic wheezers, viral-induced asthma or multitrigger wheezers that may differentially respond to treatment or may even undergo spontaneous remission and therefore require personalized therapy (10). In fact, rhinovirus infection-induced asthma exacerbations in children were associated with elevated TSLP and CCL-11 (eotaxin-1) levels that can be noninvasively detected in nasal secretions (11). The repeated report of genetic associations of *IL-33* and *TSLP* (12) with asthma risk is particularly interesting as TSLP, IL-25 (IL-17E) and IL-33 (13) are considered to play a role in the early commitment towards Th2 immunity and type 2 innate (ILC2) lymphocytes (14). They are considered to be recruited to the site of allergic inflammation and produce IL-4, IL-5, IL-13 and IL-6 (15). Little is known about ILC2s during allergic sensitization *in vivo*, but the role of ILC2 was demonstrated in the pathophysiology of chronic rhinosinusitis (15) with nasal polyps (14, 16) and asthma exacerbation (17), also suggesting a role of ILC2 as amplifiers of type 2 cytokine-mediated inflammatory processes. In the peripheral blood, higher frequencies of ILC2 (18) and higher IL-33 levels (19) are observed in asthma patients (18). Similarly increased frequency of iNKT (CD16/CD56/CD161) cells is found in asthmatic children upon exacerbation (20) (Table 3). As the acquisition of non-invasive cord blood samples for life-long stem cell storage becomes increasingly more common, these samples could at the same time be used for the analysis of immunological functionality. These analyses may provide additional information for a lack of Th1 immunity represented by lower levels of interferons (21), IL-12 and IL-15 (22, 23) and increased Th2 markers CCL-17 (TARC), CCL-22 (MDC) and ECP (24).

Stratification of novel paediatric endotypes

Besides genetic predisposition, immunological changes in the airways may provide important information for allergic disease endotypes. However, sampling is restricted for ethical reasons. Therefore, semi-invasive analysis of nasal brushings has been experimentally performed and has revealed increased mRNA levels of *VEGF*, *TGF-β2* and *periostin*.

These biomarkers are an option in addition to the skin prick test that helps to distinguish atopic from nonatopic asthma endotypes (25). Another minimally invasive option available from school age is the analysis of saline-induced sputum, which separates atopic from nonatopic asthma on the basis of increased infiltrate-derived IL-2, IL-4, IL-5, IL-12 and IFN-γ levels, in comparison with higher IL-10 levels in healthy individuals (Table 1; section infiltrate-derived biomarkers) (26). Furthermore, sputum cell counts are validated markers of lower airway inflammation, which generated reproducible data, when standardized protocols (27) for induction and processing were applied for the quantification of sputum eosinophil and neutrophil counts in adults (28) or children (29).

Characteristic differential sputum cell counts

Basophils, eosinophils and neutrophils are considered key effector cells infiltrating the asthmatic airway together with platelets (30). Platelets are carrying multiple mediators such as leukotrienes (31), platelet factor 4 (CXCL-4) (32), β-thromboglobulin (33), CCL-5 (RANTES) (33), thromboxane (34) or serotonin (35). These mediators are directly correlated with severe asthma (32), and therefore, CXCL-4 may serve as a biomarker. Eosinophil and neutrophil counts are used to discriminate between eosinophilic and neutrophilic asthma (36). In severe persistent asthma, their numbers are related to disease severity (37–41), and spontaneous or induced asthma exacerbations, for example, during allergen-induced late-phase responses or during tapering of corticosteroids (40, 42). The reduction in sputum eosinophils by anti-IL-5 treatment was associated with a mild improvement in symptom scores (43, 44) and decrease in the number of exacerbations (44, 46), but not improvement in lung function parameters (45, 46).

Disease management based on sputum eosinophils was shown to improve the prevention of asthma exacerbations in an open-label study (47). Sputum eosinophil and neutrophil counts can be used to distinguish asthma phenotypes (48); however, severe asthmatic conditions may trigger the co-occurrence of eosinophils and neutrophils in the sputum (49). Therefore, eosinophil-based diagnosis benefits from additional assessment of surface markers such as CD69 (50) and sputum mediator profiles (e.g. cysteinyl leukotrienes, prostaglandin D_2, IL-13), which facilitate the differentiation between asthma phenotypes or endotypes and predicted responsiveness to treatment with current leukotriene modifiers such as montelukast or potential future therapeutics, for example enzyme inhibitors or certain monoclonal anticytokine antibodies (51–53). As leukotrienes are recognized as central mediators in neonatal and childhood phenotypes, the measurement of sputum leukotriene levels may be particularly promising to monitor the development of asthmatic inflammation early in life (54, 55).

Disease progression in children

Besides disease classification, the monitoring of disease progression and development such as degree of inflammation and tissue remodelling are essential for personalized treatment strategies. For this purpose, noninvasive sampling in preschool age is particularly important, using, for example, exhaled breath condensate (EBC) to detect asthma development-associated biomarkers of the infiltration type (Table 1; section infiltrate-derived biomarkers), such as IL-4, IL-8, IL-10, IL-13 and sICAM1 (56). CCL-5 significantly correlates with both reduction in FEV1 and increase in airway resistance, while increased levels of TNF-α and TGF-β significantly correlate with nonspecific bronchial hyperresponsiveness. In addition, ratios of IL-4 to IFN-γ in exhaled breath condensate are higher in children with asthma compared to asthmatic children under inhaled steroid therapy (57). Also, the eosinophilic factor and Th2 cytokine IL-5 was elevated in EBC of children with atopic dermatitis, allergic rhinitis and asthma in comparison with healthy children, whereas the nasal IL-5 concentrations are higher in asthma and allergic rhinitis than in healthy children (58). Recently, the assessment of leukotrienes in noninvasive EBC has been extended to paediatric asthma, using fractionated EBC sampling to show selectively increased leukotriene B_4 levels in the small airway and alveolar fraction of EBC in children with more severe airway obstruction (59).

Minimally invasive biomarkers, such as peripheral blood measurements, allow both cellular and soluble mediator analyses. However, the analysis of cytokines secreted from allergen-specific Th2 cells without additional *in vitro* culture is technically challenging and does not allow asthma endotype discrimination (60). In summary, local, noninvasive methods are applicable for the diagnostic separation of asthma endotypes, assessment of steroid response and correlation with lung function or bronchial hyper-responsiveness. However, these biomarkers are only based on infiltrate or the mixed infiltrate/epithelial type (Table 1), while pure epithelium-derived biomarkers remain to be discovered and validated along with the infiltrate type.

Disease onset and asthma prevention

In patients with manifested disease (Tables 1–3, column 2), it is critical to prevent the spread of sensitization (61), the shift from rhinitis to asthma and further disease progression (62, 63), for example, by specific immunotherapy.

B-cell-associated biomarkers

The assessment of the disease status and its classification into mono-, oligo- and polysensitized (atopic) is already providing important prognostic information for clinical outcomes. For instance, polysensitized endotypes are more frequently prone to hospital admissions (64). Profiling of immunoglobulin specificities is currently performed on the level of IgE (multicomponent protein array analysis). Most recent studies demonstrate that distinct patterns of IgE responses to different protein families are associated with different clinical symptoms (65). Furthermore, the balance of Ig subclasses may complete the overall picture of the B-cell response, such as IgG4 investigated in specific immunotherapy (66). Possibly

underestimated are immunoglobulin (Ig) IgG1, IgG2, IgG3, IgM and IgD as well as IgA (67) that may provide important insights into the overall response of the B-cell immunological memory on systemic level. The early differentiation of B cells is difficult to diagnose in clinical settings; however, a Th2 influence can be assessed by CD86 on $CD23^+$ B cells (68) (Table 3). Generally, increased $CD19^+CD20^+$ B-cell counts are found in local allergic inflammation and in the blood of patients with atopic dermatitis (69). This study also demonstrates the systemic expansion of transitional and chronically activated $CD27^+$ IgE^+ memory subsets, which was also described in rhinitis patients and suggested as potential IgE^+ blast precursor cell (70). $CD38^+$ or $CD138^+$ plasmablasts were found both locally and in peripherally (71–73). Importantly, too, local allergen-specific immunoglobulins have been reported to play a pivotal role in symptomatic allergic reactions (74, 75).

T-cell-associated biomarkers

T cells are also infiltrating the affected tissue and are anticipated to play an important role in disease development and progression. Their assessment in the peripheral blood is difficult due to the low frequency of allergen-specific Th2 cells, while local Th2 endotypes are most visible in sputum (76, 77). However, sputum T lymphocytes are predominantly of activated intraepithelial phenotype ($CD103^+CD69^+$) that are known to belong to the long-lived memory pool, which rapidly responds to antigen challenge (78).

IL-4, IL-5 and IL-13 are detectable in the serum (1–50 pg/ml range) of asthma patients in an acute episode (79). However, once recruited to the local tissue, T cells are more likely to reflect changes in immunopathology. Among the infiltrating T cells, the impact of Th2 cells is well characterized and used in biomarker panels (Table 1, column 2; section infiltrate-derived biomarkers); however, Th2 surface markers (CRTH2/CD294 and TIM-3) are not covering all IL-4-producing T cells (80, 81).

Regulatory T cells (Tregs) are characterized by intracellular FoxP3 expression and surface expression of CD25, CTLA-4, GITR, TLR4 and are negatively correlated with IgE levels in serum (82, 83). The expression of CD39 and CD73 on the surface of $CD4^+$ T cells and Tregs in asthma patients was negatively correlated with the number of IL-17-producing T cells (Th17) (84). Both Th17 and Th22 cells (85) were described to be important for allergic inflammation. Th22 cells are present in lung biopsies of asthma patients where they play an anti-inflammatory role (86). We speculate that Th22 cell frequency could provide an indication for disease remission and/or tissue repair (87, 88). The Th22 frequency is currently considered as a biomarker for disease progression in several diseases (89–92). This finding illustrates that not only Th2 cells are important for allergy, rhinitis and asthma, but also other subsets such as Th9 cells (93), which remain to be further characterized.

Another recently discovered subset of Th2 cells has the plasticity to express IL-17 in addition to IL-4. The frequency of this Th2-IL-17^+ subset in the BAL fluid correlated with BAL and blood eosinophilia with PC20 following metacholine provocation (94). This study also showed that the more plastic T-cell phenotype expressing IL-17 in addition to IL-4 occurs mainly in rather established, severe disease exhibiting steroid resistance. Because steroid resistance is a critical determinant in guideline classifications, it appears possible that memory lymphocytes may have to be taken into consideration in the definition of asthma endotypes. Furthermore, IL-4, IL-13 and IL-17A synergistically promote the proliferation of $CD34^+COL1^+$ fibrocytes and collagen expression (95). Fibrocytes constitutively expressed the scavenger receptors CD163 and CD204 as well as the mannose receptor CD206 (96). Taken together, the analysis of biomarkers derived from local, infiltrating cells of the immune memory cells (T and B cells) is rewarding because of their disease-modifying capacity, but the detection of such biomarkers may be limited by the low frequency of antigen-specific source cells (97, 98).

Epithelium-associated biomarkers

The role of tissue cells in the early phase of disease is largely unknown, but could provide important information about the pathologic development and could help to identify the causal relationships (99). The use of lining fluids such as nasal secretions (100), sputum supernatants (101) and tear fluids (102) may increase assay sensitivity compared to systemic biomarkers diluted in the peripheral fluids. In fact, most genetic associations with allergic diseases point to genes in structural or barrier contexts rather than classical immune contexts, for example *ORMDL3* expressed by epithelial cells and *filaggrin* expressed by keratinocytes. Furthermore, allergen challenge induces IL-25 (IL-17E) and its receptor in the asthmatic bronchial mucosa and skin dermis of atopic subjects (103). Like biomarkers in early disease, epithelium-derived, infiltration-independent biomarkers are still unknown.

Established disease and prevention of remodelling

Once disease has manifested for several years and therapies failed to interrupt the allergic march, the diagnostic attention should be drawn to the prevention of further damage and irreversible remodelling of the tissue (Tables 1–3; column 3). For atopic dermatitis, the ongoing inflammation is associated with keratinocyte apoptosis and spongiosis of the epidermis. Most recently, a discrete molecular signature including *NOS2*, *IL36G* and *CCL-27* (*CTACK*) that takes advantage of highly discriminative gene expression measured in the biopsies of atopic dermatitis and psoriasis patients was described (104). However, it is currently not known whether these genes can also be used to distinguish airway manifestations.

Local microbiome as biomarker source

Future biomarker development may benefit not only from the mediators released of the tissue-infiltrating T cells (Th1,

Th2, Th9, Th17, Th22), but also from the antimicrobial repertoire of the epithelial defence (e.g. defensins, S100, cathelicidins). This broad panel of genes selectively determines the composition of the local epithelial microbiome. The microbiome itself is considered as a potential biomarker source in interstitial pulmonary fibrosis (IPF), because colonization with *Staphylococcus aureus* correlates with disease progression of IPF (105). In addition, the antimicrobial peptides play a critical role in the mucosal defence, and it can be speculated that distinct defence patterns relate to different microbiome and rhinitis/asthma endotypes.

Biomarkers of airway remodelling

Airway remodelling is associated with local matrix deposition, vascularization, epithelial hyperplasia and changes in the submucosa, such as smooth muscle cell hyperplasia and fibroblast proliferation. The development of biomarkers predictive for airway remodelling is very important in order to adjust therapy and to reassure the maintenance of lung function. However, serological remodelling markers such as YKL-40 (106, 107), MMPs (108) and osteopontin (109) are under validation process in multiple studies. Of note, YKL-40 levels showed in this study remarkable correlations with subepithelial basal membrane thickening. Their use as local biomarkers might further improve biomarker-based diagnosis due to higher concentrations at the site of inflammation. However, the access to brushings, bronchoalveolar lavage fluid (BALF) or lung biopsies is restricted due to ethical reasons, but their analysis showed a correlation of tissue Th2 signatures with serum IgE as well as BALF and blood eosinophil frequency (110). Furthermore, periostin is a well-investigated, systemic biomarker of eosinophilic inflammation in asthmatic patients that is superior to blood eosinophil counts (111). In contrast, minimally invasive sputum fluids show better amplitude and facilitate the discrimination of the osteopontin levels between mild, moderate and severe/refractory asthma (112). In addition, theses studies showed a highly significant correlation with smoke-induced neutrophilic infiltration, but not with bronchial hyper-responsiveness (113). Furthermore, the MMP-9/TIMP ratio was demonstrated to correlate with disease severity (114, 115) or airway remodelling (116), whereas VEGF correlates with airway vascular permeability index even in post-treatment asthmatics (117). VEGF and angiogenin are found in increased amounts in the sputum supernatants of patients with acute asthma attacks (118); however, their correlation with the degree of vascularization remains to be demonstrated. In contrast, indicators of vascular endothelial cell perturbation, specifically von Willebrand factor (vWF) and P-selectin, but not CXCL-4, correlated with airway structural changes and ventilation defects (119). MMP-9 also correlates with eosinophil frequency, but not with bronchial hyper-responsiveness (120), and can even be noninvasively detected in EBC (121). In addition, levels of IL-2 and IL-10 in EBC were increased in asthmatic patients, but only IL-2 levels significantly correlated with predicted reductions in FEV1 in nonallergic asthma (122).

Surrogate biomarkers for lung function

In addition to diagnostic quantification of remodelling mediators, it is critical to determine the markers that are associated with airway functions. Simultaneous increase in IL-4, IL-6, IL-8, IL-10, TNF-α, TGF-β, CCL-3 (MIP-1α), CCL-4 (MIP-1β) and CCL-5 was identified in EBC among adult steroid-naïve asthmatic patients (123). Sputum levels of CCL-11 (eotaxin-1) and CCL-24 (eotaxin-2) correlate positively with bronchial hyper-responsiveness (124, 125), while CCL-11 and CCL-26 (eotaxin-3) correlate with decreased FEV1, asthma exacerbations and frequencies of sputum eosinophils (126). In addition, increased levels of IL-13 in sputum and bronchial biopsy specimens are features of severe asthma (127); TSLP and CCL-17 expression correlates with airway obstruction (128, 129). Increased levels of CCL-11 induce eosinophilic accumulation to the airway wall, their activation as well as degranulation. Patients with uncontrolled asthma showed higher CCL-11 levels compared to stabilized asthmatic patients (130). Furthermore, reduction in CCL-11 levels after omalizumab treatment among patients with severe asthma reflected remission of eosinophilic inflammation (131). The degree of airway hyper-responsiveness correlates with macrophage activation markers such as CD14, CD16, CD18, CD29, CD32, HLA class I and HLA DQ (132).

The extent of local infiltration can be monitored in exhaled breath condensate (EBC) on the basis of TNF-α (133), which correlates with methacholine inhalation challenge threshold (123).

In biopsies, multiple genes and cells were demonstrated to play a confirmatory role for the selection of future biomarkers. The expression of IL-15 was shown to be associated with Th1-mediated chronic inflammatory diseases of the lung (134). Furthermore, IL-22 might control the extent of IFN-γ-mediated lung inflammation and therefore plays a tissue-restricted regulatory role (86), whereas TGF-β isoform expression was increased after allergen challenge (135). Also, high expression levels of IL-20 in the airway epithelium of asthma patients were found as well as a positive correlation between IL-20 and Th2 cytokines IL-4, IL-5 and IL-13 (136). Correlations were also observed between TSLP, CCL-17 and CCL-22 expression and airway obstruction, which were most tightly associated in the epithelium (128).

Surrogate biomarkers of the upper airways

In contrast to lower airway biopsies, the upper airways allow minimally invasive access to epithelial cell. Nasal epithelial cells can be used as surrogate marker source for bronchial epithelial cells (137), as they were shown to express significantly higher levels of pro-remodelling factors VEGF and TGF-β compared to healthy individuals even after air liquid cell culture (25). Furthermore, they mirror the increased local expression of CCL-11 and CCL-26 (eotaxin-3) in allergic rhinitis and asthma (138). In these patients, nasal mucosa is showing seasonal changes such as increased neutrophil levels expressing CD11b, CD66b and CD63 (139). In contrast, nasal mucosal expression upon rhinovirus exposure showed

expression changes in IL-1β, IL-24, MMP-10, but also in lysyl oxidase-like 2 (LOXL2) expression, indicating that lipid mediators are involved in early activation of the epithelium (140). The Th2-promoting alarmin TSLP was increased in polyps and chronic rhinosinusitis (141), and also, osteopontin was detectable in nasal tissues both in infiltrating cells and within the epithelial layer (142). Taken together, CCL-11 and CCL-26 are epithelium-derived indicators of Th2-driven, eosinophilic asthma and correlate with airway function, while osteopontin appears to be related to a neutrophil asthma phenotype and indicates disease severity.

Response to environment and treatment

Monitoring environment and treatment is highly variable with responding cells and pathways compared to defined causal relationships (Tables 1–3; column 4).

Response to environmental challenge

The dynamics of disease pathology in allergy, rhinitis and asthma were investigated by allergen challenge in skin, nose and lung and were found to be not only characterized by an increase in Th2 cytokines, but also by potentially regulatory (143) or remodelling factors such as TGF-β detected in skin biopsies, nasal secretions, BALF and sputum (144–146) or ADAM metalloproteinase family members (147). In the lung, the response to allergen challenge can be characterized by the infiltration of eosinophils expressing CD244 (148) and FcεR1α (149–151) and in the late-phase response also CD25 and CD62L (152). Of note, the IL-5Rα (CD125) expression drops on eosinophils following allergen challenge (153). In contrast, neutrophils of the late-phase response are expressing increased surface levels of CD11b, CD11b/18, CD16, CD32, CD35, CD62E, CD62L, CD64 and CD66b (152). Also, the macrophage phenotype is influenced by allergen challenge, displaying increased surface expression of CD14 and CD86 (154–156). It is not yet clear whether the M2 macrophage phenotype (CD163, CD206) (157) can be utilized for Th2 endotyping.

Some of the ADAM family members are genetically associated with asthma (147). Environmental exposure to smoke increases IL-6, IL-7 and IL-12 and thus lacks a Th2 fingerprint (158). In contrast, fungal sensitization with *Alternaria alternata* or rhinovirus-triggered asthma exacerbations are reported to stimulate increases in IL-33-mediated ILC2 numbers and Th2 cell numbers (159, 160). CCR4 and CXCR3 are up-regulated in *Aspergillus fumigatus*-specific T cells from non-ABPA-allergic asthmatics (161).

Drug hypersensitivities are commonly tested by *ex vivo* basophil activation assays (BAT) to predict drug reactions. Here, the expression of inside–outside markers is used to determine degranulation (CD63, CD69, CD203c, CCR3) (162, 163).

Biomarker usage in treatment monitoring

The control of environmentally triggered inflammation by systemic glucocorticoids (GC) leads to reduced CCL-5 and CXCL-10 (IP10), while CCL-4 is increased in nonsmokers (158). Because CCL-5 is infiltrate derived, while CXCL-10 is most likely of epithelial origin, it can be speculated that the impact of GC is based both on local and on systemic effects on the immune cells. Furthermore, steroid resistance was reported both on the level of resident cells (airway smooth muscle cell-derived IL-6, CCL-11) and on the level of infiltrated cells (macrophage-derived IL-6, IL-13/IL-12 ratio, IL-5, IL-15) (134, 164–168). Taken together, systemic treatment effects influence local and infiltrating cells, while steroid resistance has been investigated so far only with respect to infiltrating cells.

Interestingly, topical treatment also affects infiltrating cells despite the insoluble property of inhalative GC, which prevents systemic side-effects. It has been demonstrated that inhalative GC reduced Th2 cytokines in nasal fluids (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, CCL-11, CCL-5, CCL-2 (MCP1), CCL-3, CXCL-10, GM-CSF, IFN-γ and TNF-α) (169), but increased the relative frequency of Tregs that express the latent form of TGF-β1 (83, 170). Also, systemic treatments with herbal drugs reduced nasal levels of IL-8 and leukotriene B4 (171). Furthermore, phospho-STAT6 unresponsiveness in central $CD4^+CD161^+$ T cells identified steroid-resistant asthma patients (172). As shown for GC therapy, infiltrate-derived levels of IL-4, IL-9, IL-13, CCL-11 and mast cell tryptase were reduced in nasal secretions after allergen challenge and correlated with improvements in clinical symptoms in patients who had received immunotherapy as compared to allergic controls (173). Up-regulation of serum osteopontin after venom immunotherapy has diagnostic potential comparable to asthma biomarkers (174). Nevertheless, there is currently no predictive indicator for treatment success. Allergen-specific IgG4 is induced and its avidity is correlating with symptom-rescue medication scores (66). We could recently show that allergen-specific Th2 immunity can be long lastingly diminished by coadministration of an anti-IL-4 biological together with a grass-specific immunotherapy (175). Stand-alone anti-Th2 therapies such as anti-IL-4 receptor (dupilumab) or anti-IL-13 (lebrikizumab) treatment show efficacy even without immunotherapy, when patients are stratified for Th2 phenotypes or endotype by eosinophil or periostin levels (176, 177).

Biomarkers for disease severity and exacerbation

Overall, a great body of information is available on biomarkers derived from local, infiltrating cells, while the response of tissue cells is currently underrepresented and might further improve therapy and exposition monitoring (Table 1; section infiltrate-derived biomarkers). Disease severity is monitored by cytokines of infiltrative cells, with the exception of CXCL-10: patients with an 'IL-5, IL-17A, IL-25-high' airway inflammatory pattern are typical among uncontrolled asthma patients (178, 179). Sputum levels of uric acid, ECP, CXCL-10 and TNF-α are elevated upon asthma exacerbation (180, 181). In the peripheral blood, $CD45^+Col1^+CXCR4^+$ fibrocytes were more frequently found in patients suffering from

severe asthma (182). CXCR4 and CCR7 mediated fibrocyte transmigration in acute asthma exacerbation and in chronic obstructive asthma, respectively (183).

Due to the systemic involvement, asthma exacerbation can also be monitored or even predicted by urinary metabolites including arachidonic derivatives such as leukotrienes (184, 185) and urinary trypsin inhibitor (186). Exacerbated allergic asthma is accompanied with increased BAL levels of quinolinic acid, tryptophan, ECP, eosinophils (187), decreased CD29 (32) and CD44 (188) on eosinophils and elevated CD203c expression on basophils, which decreases significantly during remission (101). In contrast to this Th2-type eosinophilic response, $CD161^+$ central memory T cells are displaying an activated $CD69^+$ phenotype predominantly of a Th1 (IFN-γ^+) phenotype in acute asthma attacks (189). While early or mild conditions are well-reflected local biomarkers, it appears that moderate to severe disease conditions are even visible in the peripheral blood.

Future directions

Exosomes and microvesicles in various body fluids

In addition to cells and their locally produced mediators, body fluids can contain extracellular vesicles (exosomes or microvesicles (EMVs)), which are very stable and can be found even weeks after their 'parent' cells have emigrated or died. EMVs contain potentially disease-associated molecules, including microRNAs and lipid mediators that have been suggested as future biomarkers for a variety of diseases (190). In the last decade, potential pathological roles of exosomes in airway inflammation have been suggested by work showing the capacity of these vesicles to carry pro-inflammatory mediators (191, 192). Moreover, eosinophils from asthmatic patients were recently shown to release increased amounts of exosomes (193). Thus, profiling the content of EMVs from all the body fluids discussed above using current 'omics' approaches may result in the identification of specific molecular signatures in vesicles from distinct populations of allergy and asthma patients.

Novel lipidomics approaches

While studies of the past mainly used immunoassays and simple HPLC or GC methods to quantify eicosanoid biomarkers such as LTs and 8-isoprostane (54, 194), more comprehensive lipid mediator profiling of local and systemic body fluids by state-of-the-art LC-MS/MS approaches holds great potential to identify disease-specific eicosanoid signatures in the near future (195–197). Notably, recent 'fluxomic' approaches should enable clinicians to monitor eicosanoid profiles over time and during treatment (198). New data analysis tools will also facilitate the integration of lipidomic, proteomic and transcriptomic data sets, which will likely result in more precise and meaningful biomarker applications in future.

Conclusion

For historic reasons, allergy biomarkers are mainly blood-borne, while local biomarkers or those derived from cells infiltrating the site inflammation are only more recently studied with increased interest. However, genetic association studies mainly revealed tissue-specific genes such as *filaggrin*, *ORMDL* or *IL-33* highlighting the important role of tissue and epithelium. This example highlights that the quality of a predictive biomarker may also depend on polymorphisms or to the extent it drains into body fluids or occurs on cell surfaces. In addition to these genetic markers, this review illustrated multiple potential biomarkers for early life, disease onset and established disease that originate from local inflammation processes, of which it is currently not clear whether they can be translated into preferable systemic indicators. Future studies are required to translate this panel of at least 161 potential biomarkers into validated sets that display an optimal ROC profile of false-positive rates (specificity) to true-positive rates (sensitivity).

An exception to this situation are asthma exacerbations that can be more easily monitored by systemic parameters such as urine-based mediators and blood-borne biomarkers. Recently described epithelium-derived mediators have diagnostic potential for asthma endotyping, disease progression and treatment monitoring. B- and T-cell biomarker classifiers may characterize the disease endotypes; in fact, Th2 fingerprints have frequently been observed. About 20 genes are exclusively expressed by epithelial cells, of which many are chemokines, matrix related and only a smaller fraction are cytokines. Some of these genes have great potential also for paediatric assessment, as the epithelial secretion may allow noninvasive sampling by nasal lining fluid or EBC.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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