

THE ADVANCE OF PERSONALIZED AND STRATIFIED THERAPIES IN BRONCHIAL ASTHMA: PHENOTYPES – ENDOTYPES – BIOMARKERS

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ABSTRACT

Bronchial asthma (BA) is a chronic inflammatory condition with increasing incidence and prevalence worldwide. BA is currently the most prevalent chronic disease in pediatric patients. The majority of BA patients are therapeutically well controlled with guideline based anti-inflammatory therapies; however, there is also a clinically recognized proportion of patients who do not benefit from currently available medication for several reasons. This is the starting point for further investigation into the complexity of the inflammatory phenotype of BA. Recently, the heterogeneity in terms of cellular and molecular pathways underlying BA has been recognized and established. These different pathogenic mechanisms are defined as 'endotypes'. The best studied endotype so far is the association with T-helper type 2 (TH2) cell eosinophilic airway inflammation. Recently, a number of different therapeutic strategies have been clinically explored which target certain mediators of this pathway, including the interleukins IL-4, IL-5 and IL-13. It is now clear that patients with the TH2-endotype largely benefit from novel biologicals in this area. However, the challenge for diagnostics is to identify patients exhibiting this endotype, and this is the starting point for the search for new biomarkers. One biomarker that has recently been selected based on differential gene expression analysis, and which seems to be strongly associated with the TH2 endotype, is periostin. In this article we will provide a state of the art update on the definition of clinical phenotypes, pathogenetic endotypes and biomarker development for improving BA treatment.

INTRODUCTION

In the middle of the 20th century, a significant increase in the prevalence of bronchial asthma (BA) was reported worldwide. This rise particularly referred to BA of pediatric onset, therefore asthma became one of the most prevalent chronic inflammatory diseases in pediatrics (1–3). BA is described as a chronic inflammatory disorder of the airways with inflammatory symptoms causing a variable degree of airflow limitation and is accompanied by an increased sensitivity to multiple pharmacological and non-pharmacological stimuli. The bronchial obstruction seen is often reversible, either spontaneously or with treatment.

BA is considered a complex disorder based on a multifactorial interaction of genetic predisposition and diverse environmental influences (4, 5). However, there are global differences in the frequency as well as the increase in prevalence. After a significant rise during the last centuries in Western industrial countries, a plateau seems to have been reached in some areas. Overall, Eastern European countries show a lower prevalence of BA than Western European countries. Asia and South America are emerging regions showing a lower prevalence of BA than industrialized regions despite the presence of particularly high levels of environmental pollution. This rise cannot be explained by a change in the genetic predisposition of the population, but it can instead be ascribed to changes in the environment. Changes in life style conditions contribute to disease susceptibility – these include changes in qualitative and quantitative exposure to microbes (referred to as the hygiene hypothesis), dietary habits, lack of outdoor activities and exercise, psychological stress and others (4, 6–9).

ALLERGIC AND NON-ALLERGIC ASTHMA

The multifactorial syndrome of BA is caused by a diverse set of environmental triggers against the background of genetic predisposition (4, 5). Historically it can be divided into extrinsic, IgE-mediated, allergic asthma caused by allergen contact, and non IgE-mediated, non-allergic BA which is exacerbated by infections, toxic or chemical-irritative substances or gastro-esophageal reflux. Often, non-allergic or intrinsic BA develops from a bronchial infection of the upper or lower airways. It has also been proposed that an autoimmune reaction may play a contributing role. As well as respiratory (viral) infections, a number of other factors including obesity, physical exercise, cold air, stress and emotion, noxious inhalations such as cigarette smoke, ozone, nitrous gas, sulfur dioxide, and other noxious substances (analgesics, beta-blockers) have been identified as triggers of BA exacerbations (10–19).

More recent data indicate that the categorization into allergic IgE-mediated and non-allergic non-IgE-mediated phenotypes is oversimplifying the complex situation in BA patients. Although the majority of asthma patients are well controlled with anti-inflammatory medications including inhaled corticosteroids (ICS) or leukotriene receptor antagonists together with short and/or long acting beta-adrenergic drugs, there is a substantial need for additional and new therapeutic approaches. Several studies indicate that about a third to a quarter of patients do not respond adequately to ICS, or are steroid resistant or develop side effects to conventional anti-inflammatory therapy, thus limiting their use (20, 21). This situation has stimulated a series of investigations aiming to better understand the complex pathophysiology of the disease in order to target medication in a more specific way. This approach requires the identification of distinct signaling pathways and, subsequently, biomarkers, to test new drug developments which target and interfere in a highly specific and selective fashion with these cellular and molecular phenotypes. Here we describe the progress made in the area of BA research.

THE EXTENDED CONCEPT OF THE PATHOGENESIS OF ALLERGIC ASTHMA: THE TH1 AND TH2 CONCEPT

The chronic inflammatory reaction is based on a complex interaction of different cell types, all of them belonging to the adaptive or innate immune system and including T-lymphocytes, mast cells, eosinophils and antigen-presenting cells and structural cells such as macrophages, dendritic cells, bronchial epithelium and myofibroblasts and their products.

As central cells in the regulation of allergic inflammation, eosinophils and T lymphocytes have the ability to differentiate from naïve TH0 cells to effector T cells under special conditions. The subsets of T-helper type 1 (TH1) and T-helper type 2 (TH2) cells are prototypical. An enhanced production of proinflammatory cytokines such as the interleukins IL-4, IL-5 or IL-13 by the TH2 subset mediates pulmonary allergic inflammation. These T cells control the effector response at the level of the innate immune system (granulocytes, mast cells). Furthermore, they control the level of antibody production by the B-lymphocytes – in this case especially the IgE isotype. The TH2 subset plays a central role in regulation of the allergen-driven phenotype of BA (22, 23).

The TH1–TH2 concept: differentiation of T lymphocytes

Activation of epithelial cells leads to the secretion of cytokines IL-33 and IL-25, which subsequently promotes the release of IL-13 and IL-5 from the IL-25-receptor-positive natural helper cells, which themselves induce the differentiation of naïve T cells into TH2 cells (23–26). The induction of TH2 cells is prototypical for the development of atopic disorders; the preferential differentiation into the TH2 endotype is mediated through the interaction of genetic predisposition and environmental influences such as the microbiological milieu (22, 90).

For the development of TH2 effector cells, the transcription factors STAT6 and GATA binding protein 3 (GATA3), which is induced in the presence of IL-4 and IL-6, play a critical role. GATA3 itself activates genes which encode for IL-3, IL-4, IL-5, IL-9, IL-13 and GM-CSF (22). GATA3 inhibits STAT-4 as well T-bet (the transcription factor which regulates the TH1-endotype), which results in TH2 polarization. Reversely, T-bet can inhibit GATA3 expression (22, 90). The differentiation of T cells is essentially modulated by the prevalent milieu of cytokines. At the other end of the spectrum, the differentiation of TH0 cells into TH1 cells takes place

under different conditions. In the presence of IL-12 the TH1 machinery is started, which is activated through binding of the transcription factors STAT-4 and subsequently T-bet (T-box expressed in T-cells) to their receptors on the naïve TH0 cell (9, 27–32).

Therefore IL-4 and IL-13 play a prominent role in IgE isotype switching in plasma cells. The allergen-specific IgE antibodies bind to high-affinity receptors expressed on basophils and mast cells, which degranulate after a second allergen contact following crosslinking of membrane-bound antibodies. This leads to a release of mediators of inflammation such as histamine, prostaglandins and leukotrienes from preformed granulae as well to the de novo synthesis of soluble mediators of inflammation. This represents the allergic early-phase reaction, which develops within just a few minutes of allergen contact and which presents clinically as an acute bronchial constriction that returns back to baseline levels within the next few hours. In the late-phase response, newly synthesized lipid mediators such as prostaglandins and leukotrienes appear together with an influx of eosinophils and T cells (33–35).

A further aspect in a TH2-dominated effector response is the development of goblet-cell metaplasia, increased production of mucus and the development of fibrosis in the airways. Further consequences include hyper-responsiveness of the smooth muscle fibers. All of these are part of so-called airway remodeling (36–42).

Eosinophils play an essential role in the TH2-inflammatory response. In contrast to the early-phase allergic reaction, the asthmatic late-phase response is characterized by the dominance of this cell type. IL-5 causes the sustained development and activation of eosinophil granulocytes in the bone marrow. After accumulation in the bronchial tissue, eosinophils release a broad spectrum of inflammatory mediators. The inflammation is maintained by different lipid mediators, cytokines, and other highly toxic mediators such as oxygen-free radicals, major basic protein (MBP), eosinophil-derived neurotoxin (EDN), platelet-activating factor (PAF), prostaglandins and especially the highly toxic eosinophilic cationic protein (ECP). Following chronic activation these mechanisms (Figure 1) form the basis for airway remodeling, which is characterized by the destruction of the bronchial epithelium and hyperplasia of the smooth myocytes and glandular cells, as well as an enlargement of the bronchial basement membrane (35, 43). The consequence of these processes is the development of peribronchial fibrosis of the airways and thereby chronic constriction of the airway profile, a reduction in airway resilience and a fixation of bronchial obstruction with persisting symptoms. This process is essentially disease-limiting.

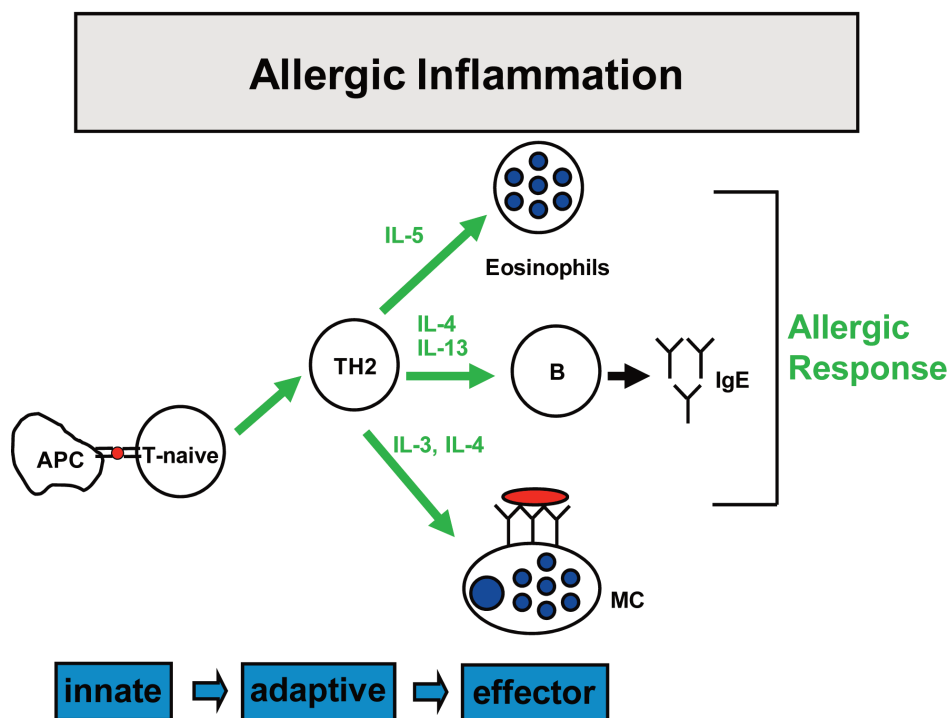


Figure 1
Cellular network of allergic asthma.

CLINICAL BRONCHIAL ASTHMA PHENOTYPES

Early-onset allergic asthma

It is evident that most asthmatic patients have their first asthma episode before the age of 6 years. At the age of 11, the prevalence in male and female children is similar at 7.7% and 7.4%, respectively, whereas a gender difference has been described at the age of 16, with female subjects more frequently being affected by asthma than males (6.2% versus 4.3%, respectively). A family history of atopic diseases and allergies, based on sensitization to allergens and smoking exposure in early life have been shown to be a risk factor for childhood asthma. From a clinical perspective, the early-onset asthma phenotype is more prone to recurrent infections and eczema in early childhood compared with those children with later onset BA. The co-appearance of eczema leads to the hypothesis that this phenotype may be TH2 driven, but cases with low IgE and limited response to ICS suggest that there are forms of early asthma not related to TH2. Viral infections are co-modulators of early onset BA. Early infection with the respiratory syncytial virus (RSV) modulates regulatory T-cell function and, via the IL-4 receptor pathway, increases susceptibility to allergic asthma (44–56).

Adult-onset (severe) asthma

This phenotype shows an association with TH2 cytokines as well as TH2 inflammatory cells. Some patients often show severe reactions after cyclooxygenase inhibitor intake. This phenotype is often severe from disease onset. Anti-IL-5 may be a promising therapeutic option for these patients (44, 57, 58)

Late-onset non-allergic asthma of the elderly

The prevalence of asthma in individuals beyond 65 years is about 8–10%. This phenotype is often misdiagnosed. Atopy and elevated IgE levels are less frequent. The symptoms of cough, wheezing and dyspnea and the histopathology are comparable to those seen in younger patients. Comorbidities (e.g. chronic obstructive pulmonary disease [COPD] or heart insufficiency) that lead to polypharmacy might play an important role since some drugs may also cause asthma symptoms (59–64).

Virus-induced asthma

Virus-induced asthma is a phenotype which is characterized by a sudden onset and sometimes a severe clinical course. Each viral infection may alter the course of preexisting asthma, or can affect the immune system and subsequently modify the susceptibility to allergen sensitization and asthma in childhood. RSV, influenza and parainfluenza viruses may affect the airways and may cause inflammation, increased airway responsiveness and obstruction. A range of possible mechanisms have been suggested: increased airway hyper-responsiveness, altered neural control mechanisms, disturbed small airway geometry caused by airway wall thickening and mucus plugging and airway inflammation may also play a role. In terms of inflammation, it has been shown that human rhinovirus (HRV) causes a neutrophilic inflammation. Patients with asthma exacerbations showed an approximately 5-fold higher sputum neutrophil count compared with that seen in stable asthmatics (10–13, 64–68).

Exercise-induced asthma

In exercise-induced asthma, the symptoms usually develop 10–15 minutes after starting exercise and diminish 30–45 minutes after ending it. Symptoms occur more frequently under cold and dry conditions. A TH2 association with elevated sputum eosinophils has been described in a subgroup of athletes, although individuals with no marked eosinophilic inflammation were also described (14–18).

Other phenotypes (obesity-related asthma, neutrophilic asthma, brittle asthma, menstrual-linked asthma, smoking-associated asthma)

Obesity-induced asthma is characterized by a lack of atopy, female predominance and late onset of the disease. The role of obesity itself in asthma is, however, controversial as the symptoms caused by obesity can be misdiagnosed as asthma. A link between obesity and BA seems to exist regarding non-TH2 asthma, but in TH2 asthma a correlation between disease duration and body mass index (BMI) has also been shown. Higher BMI levels are usually linked to higher production levels of inflammatory mediators.

Asthmatics with elevated peripheral neutrophils and neutrophilic airway inflammation display another phenotype of asthma. Neutrophilia is often seen after steroid treatment and cigarette smoking. Thus a relationship between neutrophilic asthma and smoking has also been discussed. Brittle asthma is characterized by recurrent severe asthma attacks (19, 69–81)

Cellular bronchial asthma phenotypes

The relative distribution of eosinophils and neutrophils in induced sputum has been proposed as a criterion for the differentiation of cellular phenotypes. Classification as eosinophilic (eosinophils in the sputum >2%) and non-eosinophilic (eosinophils in the sputum <2%) BA does not necessarily correlate with the degree of disease severity, but is associated with differences in qualitative clinical characteristics, especially different responses to therapeutic intervention. Several cellular subgroups could be distinguished

based on the quantitative and qualitative assessment of eosinophils and neutrophils in induced sputum, in particular an eosinophilic type, a neutrophilic type, a mixed-cell type and a paucigranular type (absence of granulocytes) (82–84).

A comparison of patients with and without sputum eosinophilia showed that many non-eosinophilic BA patients responded inadequately to treatment with ICS. With regard to the degree of BA severity, in many cases eosinophilic BA presented with a worse forced expiratory volume in 1 minute (FEV1), a lower number of mast cells and less subendothelial fibrosis (85). Eosinophilia is often found in cases of classic atopic BA in association with allergen-mediated inflammation and usually shows a good response to ICS except for severe cases (82, 85–89).

In contrast, neutrophilic BA (neutrophil granulocytes >60%) is frequently associated with acute and chronic infection, obesity, smoking, a higher degree of asthma severity and more frequent exacerbations as well as exposure to environmental toxins. Neutrophilia is often associated with reduced lung function as measured by FEV1 (82, 85, 86).

Mixed-cell phenotypes are associated with refractory clinical courses in association with the poorest lung function and the highest frequency of exacerbation. Several studies have shown that a low number of eosinophils and a high number of neutrophils correlate with a reduced response to ICS (82, 83).

The key question is whether the classification of cellular phenotypes has a therapeutic impact on BA treatment. In the case of eosinophilic BA, interference at the level of the TH2 signaling pathway may represent a potentially promising strategy. IL-5 plays a central role in the biology of eosinophil development, maturation, chemotaxis and survival. The scientific rationale for treatment with anti-IL-5 antibodies is based on the central function of IL-5 with regard to the induction and activation of eosinophilic granulocytes.

In several clinical studies stratification of patients was performed on the basis of their cellular phenotypes. These studies revealed that focusing on this target (IL-5) in a selected patient population showed significant positive results (83, 90, 92). Patients with severe asthma of the eosinophilic type (eosinophils in the sputum >3%) who were treated with ICS and sometimes required oral steroids, showed a significant improvement in their asthma following treatment with anti-IL-5 monoclonal antibodies (91, 92) (Figure 2).

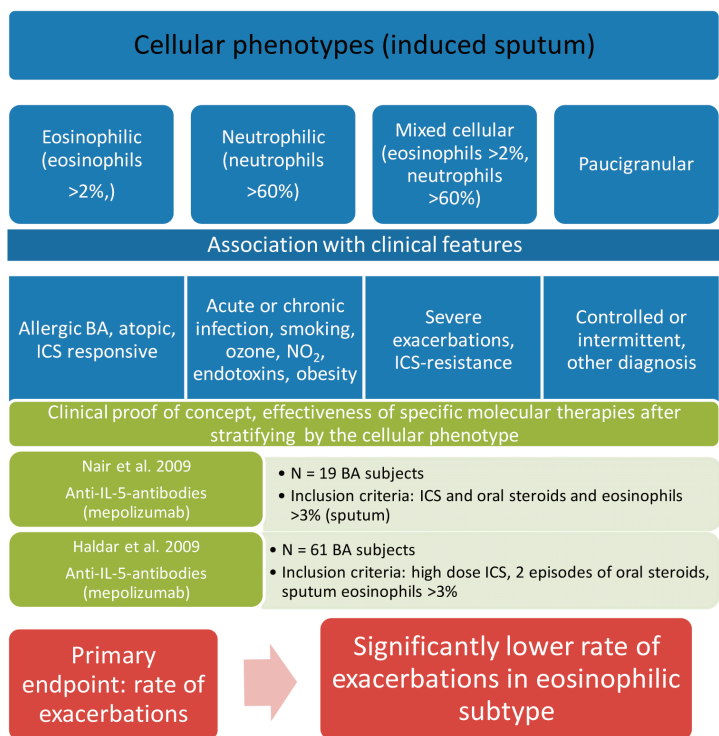


Figure 2 Differential cell typing in induced sputum, association with clinical features, and cell-type specific therapeutic approaches. Haldar et al. (82, 91); Nair et al, (92).

Molecular immunological phenotypes / endotypes

Recently, great progress has been made with regard to new insights into the heterogeneity of asthma on a molecular-biological level using unsupervised hierarchical clustering. Through molecular-immunological endotyping, a change of gene expression was identified, with the gene products representing potential biomarkers (73).

In a study by Baines et al. (73) gene expression analysis was used to perform endotyping at a transcriptional level. mRNA was extracted from induced sputum of BA patients and gene expression was examined using whole-genome microarray analysis. In total, 330 genes were found to be differentially expressed in these patients as compared with healthy controls. Three subgroups could be identified by the use of hierarchical clustering (Figure 3). According to clinical and cellular findings, the first translational asthma phenotype 1 (TAP1) was associated with a lower FEV1/forced vital capacity (FVC) ratio (FEV1%), higher levels of fractional exhaled nitric oxide (FeNO), and a high rate of eosinophils in the sputum. It seems to be associated with the eosinophilic phenotype. With regard to clinical and cytological information, TAP2 was also associated with a lower FEV1%, together with a higher proportion of sputum-eosinophils compared to healthy controls. This phenotype was associated with a neutrophilic dominance, particularly with an up-regulation of genes of the IL-1 and TNF-alpha/NF-kB-signaling-pathway. In comparison to the other phenotypes, in terms of inflammatory parameters, TAP 3 lies in the range of healthy controls with a slightly higher amount of macrophages in the sputum. It approximately resembles the paucigranular and mixed-cell type and the healthy controls (73).

Woodruff et al. (93, 94) performed bronchoscopies in order to obtain epithelial cells from mild to moderate asthmatics, smokers, and healthy controls before and after a randomized, placebo-controlled, double-blind treatment with ICS. In particular, gene expression profile was analyzed in the different subgroups through genome-wide profiling after isolation of mRNA and microarray evaluation. The analysis led to the identification of a panel of differentially expressed genes, which were strongly over-expressed in subjects with asthma compared with healthy controls, smokers and ICS-treated asthmatics. The genes that were most strongly-induced in asthma were:

- Chloride channel, calcium activated, family member 1 (CLCA1), (6.2 fold)
- Periostin (POSTN), (4.4 fold)
- Serine peptidase inhibitor, clade B (ovalbumin), member 2 (Serpina2) also known as plasminogen activator inhibitor-2 (PAI 2), (3.5 fold)

Validation of gene-expression was performed by PCR, and validation of protein expression was performed by immunostaining and enzyme-linked immunosorbent assay (ELISA).

In order to obtain further information regarding the regulation of these genes, additional invitro experiments were carried out on human respiratory epithelium, which showed that IL-13 induced the identified genes while dexamethasone prevented this effect or inhibited the gene expression (93–96).

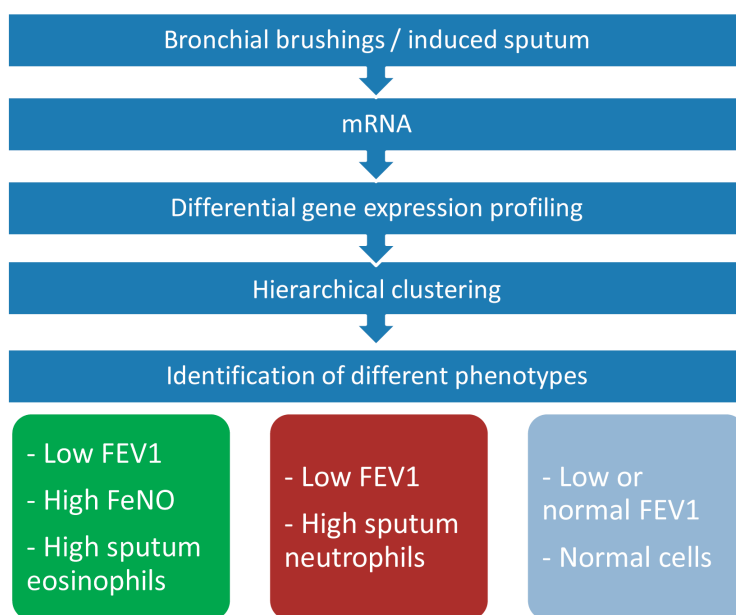


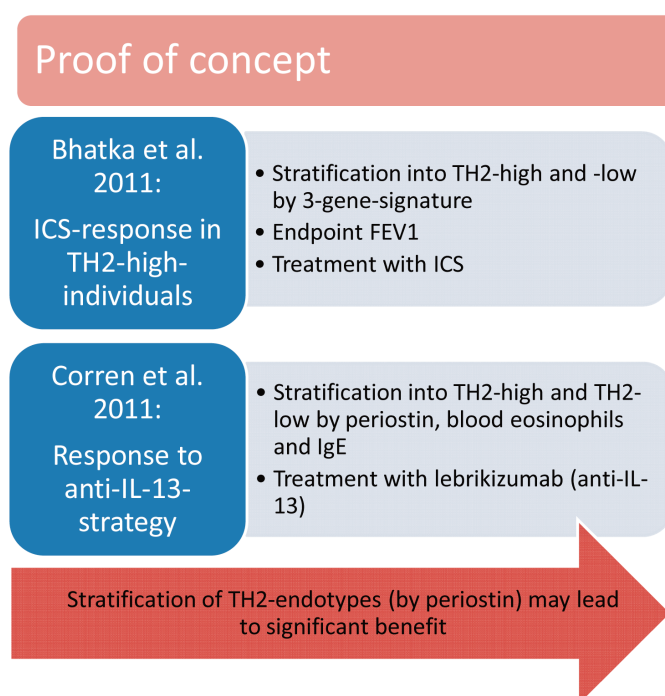
Figure 3 Clustering of asthmatic patients based on differential gene expression profiling from bronchial brushings and induced sputum samples reveals at least three distinct (clinical) phenotypes. Baines et al. (73).

TH2-high and TH2-low gene signatures and endotypes

Based on the above findings suggesting that the products of the POSTN, CLCA1 and SERPINB2 genes could serve as a surrogate marker for a TH2-driven immune-response, Woodruff et al. (93, 94) examined whether this gene signature can contribute to the identification of different endotypes within the whole population of BA patients. The experiments and studies revealed that these three genes were over-expressed only in a subset of asthmatics. To define this subset precisely, unsupervised hierarchical clustering was applied to the findings of the gene array analysis based on the expression level of the genes POSTN, CLCA1 and SERPINB2.

Almost half of the subjects showed high expression level TH2-induced genes, while the other half exhibited a normal expression level. A comparison of all BA subjects and healthy controls revealed a general over-expression of the three genes. However after hierarchical clustering about half of the asthma-patients remained undistinguished from the healthy controls using these surrogate markers. As a result the following two clusters could be separated: in cluster 1 the markers POSTN, CLCA1 and SERPINB2 were highly up-regulated, which was not the case in cluster 2, representing the other half of the participants and the healthy controls (Table 1). These studies together with additional analysis of the characteristics of airway inflammation helped to define the TH2-high and the TH2-low endotypes. At one end of the spectrum it is possible to define an endotype with an up-regulated TH2 response mediated by the typical TH2 cytokines IL-5, IL-13 and IL-4 measured by the surrogate-markers POSTN, CLCA1 and SERPINB2; and at the other end resides the endotype which is not associated with a TH2 response. This provides further evidence that the population of asthmatics is heterogeneous on a molecular level (94). It has been suggested that the TH2 endotype shows a higher degree of bronchial hyper-reactiveness, higher serum IgE concentrations and a higher eosinophilia in blood and bronchoalveolar lavage (BAL). There is also a higher number of mast cells in the TH2 endotype (82). Patients with the TH2-endotype benefit from ICS treatment as measured by improvement of the FEV₁, while the TH2-low subset is more resistant to steroid treatment (23).

Molecular endotyping obviously has important therapeutic implications. While the TH2 endotype, in particular the eosinophilic subtype, largely contains responders to steroid-treatment, TH2-low endotype asthma, which probably is consistent with the neutrophilic phenotype, is less understood with regard to the molecular pathogenesis. Based on this concept, Corren et al. (97) undertook a randomized placebo-controlled study using a monoclonal antibody against IL-13 (lebrikizumab), a prototypic TH2 cytokine, which is pathogenetically important in the TH2 endotype. A total of 219 asthmatics were stratified according to TH2 endotype (high versus low) using serum periostin level as a surrogate marker of IL-13 (and hence TH2 status). The primary endpoint was FEV₁. Treatment with anti-IL-13 monoclonal antibody was given once a month for 6 months, with the result that when compared with placebo, there was a significant improvement in FEV₁ only in the TH2-high endotype ($p=0.03$) which was not seen in the TH2-low endotype ($p=0.61$) (97) (Figure 4).

**Figure 4**

Molecular target-based therapies significantly improve outcomes in patients stratified on the basis of the TH2-endotype. Bhatka et al. (23); Corren et al. (97).

Potential biomarkers and candidates for TH2-endotype asthmatics	
CLCA1	Woodruff et al. PNAS 2007 Choy et al. J Immunol 2011
Periostin	Woodruff et al. PNAS 2007 Blanchard et al. Mucosal Immunol
Serpin B2 (PAI 2)	Woodruff et al. PNAS 2007
Carboxypeptidase A3	Woodruff et al. PNAS 2007
Ratio MUC5AC/MUC5B	Choy et al. J Immunol 2011
CCL26 (MIP4)	Choy et al. J Immunol 2011
Osteopontin	Samitas et al. Eur Respir J 2010
Tryptase alpha/beta1	Woodruff et al. PNAS 2007
Tryptase beta2	Baines et al. J Allergy Clin Immunol 2011 Woodruff et al. PNAS 2007
NOS A2	Choy et al. J Immunol 2011
ALOX15	Choy et al. J Immunol 2011

Table 1

Potential biomarkers and candidates for the identification of TH2 endotype asthmatics.

PERIOSTIN AS A POTENTIAL NOVEL BIOMARKER OF A TH2 INFLAMMATION /AS BIOMARKER OF A TH2 ENDOTYPE

Biological/physiological basics of periostin, inducibility and effects

Periostin is a POSTN-gene encoded, disulfide bridge-bearing, 90 kDa, matricellular protein, which was originally isolated from osteoblasts and described as osteoblast-specific factor 2. Periostin is secreted into the extracellular matrix of the bronchial tissue, and not via the apical membrane into the lumen of the airway, which allows systemic measurement. Periostin is inducible in the airway epithelium by IL-4 and IL-13 (96–98) and is secreted by lung fibroblasts into the extracellular matrix (99, 100). Furthermore, there seems to be inducibility by the anti-inflammatory cytokine TGF-beta in fibroblasts (96).

Four isoforms have been identified, which differ in the length of the C-terminal domain, originating from alternative splicing (100). Periostin is a matricellular protein, which contains a cysteine-rich domain at the N-terminal end and four repetitive fascilin-1-domains and, therefore, belongs to the fascilin family (99). Periostin functions as an adhesion molecule. It interacts with different integrin molecules on the cell surface such as alpha-V/beta1, alpha-V/beta3 and alpha-V/beta5, and facilitates signals for tissue differentiation, growth and remodeling (101). As a ligand for the mentioned integrins it supports cell adhesion and migration (102).

It seems that periostin has a modulating function in bone and teeth development, as well as the development of the cardiac valves in mesenchymal tissues (103, 104). Periostin is able to induce the proliferation of cardiomyocytes and has a modulating function in the remodeling process after myocardial infarction or acute stress (105–107). In addition it functions as a mediator in epidermal wound healing (105, 108). It seems to be involved in the tumorigenesis of different tumors via the integrin/PI3K/Akt-signal pathway (109). Furthermore it is a highly inducible product in lung fibroblasts following increased IL-4 and IL-13 and plays a role in the development of fibrosis in asthma (110, 96). Furthermore, Blanchard et al. (111) found that periostin is overexpressed in the esophagus of patients with eosinophilic esophagitis. Periostin directly regulates the accumulation of eosinophils in TH2-associated mucosal inflammation, also in the airways.

Periostin enhances the emergence of fibrosis through binding to proteins of the extracellular matrix. It is also involved in the genesis of collagen fibers through activation of lysyloxidase (96, 112, 113). As a matricellular protein, there is evidence that periostin influences inflammation of the airways through an increase of chemokine production in fibroblasts as well as through recruitment of eosinophils. Periostin can also promote TGF-beta production in the airway epithelium (111, 114, 115). Another effect of periostin is the counter-regulation of mucus production. It was found that a POSTN-deficiency leads to an enhancement of the expression of Muc5ac and Gob5, and, consequently to an enhanced mucus production.

These novel observations suggest that periostin not only promotes inflammation. This is further supported by the observation that periostin can increase the production of IFN-gamma (116). How these pro- and anti-inflammatory effects of periostin are regulated and controlled requires further study and whether periostin is important in the pathogenesis of other allergic conditions, or even in other chronic inflammatory diseases also needs to be investigated.

Periostin as biomarker of TH2 inflammation

Is it possible to use markers of TH2 inflammation as biomarkers for stratification prior to therapeutic interventions? A distinct eosinophilia is a reliable predictor for response to ICS. Particularly with regard to molecular-based therapies, differentiation in TH2-high and TH2-low profiles is required and studies of mepulizumab and reslizumab (two monoclonal antibodies against the TH2 cytokine IL-5) demonstrate the importance and the benefit of selection of patients to obtain a clinically significant effect of treatment with targeted therapeutics (117).

There is recent evidence (84, 118, 119) that periostin levels in serum are significantly increased in patients with asthma. This was found particularly for the eosinophilic type of airway inflammation compared with patients with only minimal eosinophilic airway inflammation. Periostin levels seem to correlate well with the TH2-high gene signature, and with the TH2 endotype. This leads to the question of whether serum periostin levels may serve as a good predictive parameter for stratifying patients prior to asthma treatment.

The next important step is the development of reliable assay systems for such new biomarkers. For this purpose Jia et al. (117) established an ELISA for serum periostin. The assay was tested in a variety of asthmatic patients, and significantly elevated serum periostin levels were found in a subset of asthmatics together with a correlation with eosinophilic airway inflammation.

Although treatment with ICS has been found to reduce periostin levels as well as the airway eosinophilia in patients with mild-to-moderate asthma (93, 120), a subset of asthmatics showed persistent eosinophilic airway inflammation and symptoms despite high doses of ICS. This subset could be divided into a subpopulation with sputum- or tissue-eosinophilia (ensured by biopsy) and a subpopulation without these criteria (sputum eosinophils <3% and tissue eosinophils <22/mm²). The median periostin levels were significantly higher in individuals with high sputum- or tissue-eosinophilia as compared with individuals with lower eosinophil counts. Individuals with a periostin level above a cut-off of 25 ng/mL had a greater than 85% risk of having an increased amount of eosinophils either in sputum or in bronchial tissue. These eosinophil-low and eosinophil-high subpopulations could be distinguished by means of a periostin cut-off level of 25 ng/mL with a positive predictive value of 93%. These analyses may serve as a starting point for further investigation in order to establish periostin as a systemic biomarker of eosinophilic airway inflammation.

Of all previously examined non-invasive biomarkers such as blood eosinophils, FeNO or IgE, periostin shows the highest probability as a predictor of a TH2-driven inflammation (117) (Table 2). The ability of periostin to serve as a suitable biomarker

Structure	90 kDa matricellular protein
	4 isotypes by alternative splicing
	Fascillin family member
	Coded by POSTN-gene
Induced by	IL-4
	IL-13
	TGF-beta
Localization	Secreted by fibroblasts into extracellular matrix (ECM)
Interaction / ligands	Interaction with integrin molecules on cell surfaces as alpha-V/beta1, alpha-V/beta3 and alpha-V/beta5
Biological effects	Modulates tissue differentiation, growth, and remodeling
	Interaction between periostin and ECM proteins: pathogenesis of fibrosis
	Migration, chemotaxis and adhesion of eosinophils: enhancement of TH2
	Inflammation
	Inhibition of mucus production

Table 2
Periostin: biological characterization.

for the identification of the TH2-endotype was illustrated in the study by Corren et al. (97) in which 219 BA patients were stratified into a TH2-high and a TH2-low-endotype as measured by their peripheral periostin levels. After treatment with lebrikizumab, an anti-IL-13 monoclonal antibody, subjects with a high peripheral periostin level showed a significant improvement in FEV1 compared with placebo that was not observed in subjects with low periostin levels.

CONCLUSIONS

Recognition of the heterogeneity of BA together with the definition of clinical phenotypes, pathogenetic endotypes and the development of novel biomarkers has opened a new chapter in BA clinical care. Although the vast majority of BA patients are well controlled with guideline based anti-inflammatory therapies, there is a strong medical need for improved therapies in the remaining portion of BA patients. This goes hand in hand with recognition of the complexity of BA pathogenesis. Certain well-defined pathogenetic cellular molecular networks underlying the disease in a defined group of patients are defined as 'endotypes'. The best studied endotype so far is the TH2-eosinophilic endotype of airway inflammation. Patients suffering from this endotype seem to benefit from targeted biological anti-inflammatory therapies. Such biologicals interfere, for example, with the cytokine network IL-4, IL-5 and IL-13. Whether periostin is a suitable biomarker for identifying this particular endotype in clinical practice requires further studies. Additional research is also required to develop further biomarkers, not only for the TH2 endotype, but also for the other endotypes. Therefore, the field of allergy and asthma research has reached a new chapter in the area of personalized, individualized therapeutic strategies. Many more studies are needed to improve this situation, but the right avenue has been taken so far.

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