Early View

Review

Identification and treatment of T2-low asthma in the era of biologics

Chris Kyriakopoulos, Athena Gogali, Konstantinos Bartziokas, Konstantinos Kostikas

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Identification and treatment of T2-low asthma in the era of biologics

Chris Kyriakopoulos, Athena Gogali, Konstantinos Bartziokas, and Konstantinos Kostikas

Respiratory Medicine Department, University of Ioannina School of Medicine, Ioannina, Greece

Correspondence:

Dr. Konstantinos Kostikas

Respiratory Medicine Department

University of Ioannina School of Medicine, 45110 Ioannina, Greece

Tel: +30-6944780616

E-mail: ktkostikas@gmail.com

Abstract

Currently, and based on the development of relevant biologic therapies, T2-high is the most well-defined endotype of asthma. Although much progress has been made in elucidating T2-high inflammation pathways, no specific clinically applicable biomarkers for T2-low asthma have been identified. The therapeutic approach of T2-low asthma is a problem urgently needing solution, firstly because these patients have poor response to steroids, and secondly because they are not candidates for the newer targeted biologic agents. There is, thus, an unmet need for the identification of biomarkers that can help the diagnosis and endotyping of T2-low asthma.

Ongoing investigation is focusing on neutrophilic airway inflammation mediators as therapeutic targets, including IL-8, IL-17, IL-1, IL-6, IL-23, TNF-a; molecules that target to restore corticosteroid sensitivity, mainly mitogen-activated protein kinase inhibitors, tyrosine kinase inhibitors and phosphatidylinositol 3-kinase inhibitors; PDE3 inhibitors that act as bronchodilators and PDE4 inhibitors that have an anti-inflammatory effect; and airway smooth muscle mass attenuation therapies, mainly for patients with paucigranulocytic inflammation.

This manuscript aims to review the evidence for non-eosinophilic inflammation being a target for therapy in asthma, discuss current and potential future therapeutic approaches, such as novel molecules and biologic agents, and assess clinical trials of licensed drugs in the treatment of T2-low asthma.

Background

Asthma is a chronic inflammatory disorder of the airways, characterized by reversible expiratory flow limitation and bronchial hyperresponsiveness to a variety of triggers, which is an increased sensitivity of the airways resulting in bronchoconstriction. It presents with symptoms such as wheezing, shortness of breath, cough and chest tightness that vary over time and in severity [1]. This is a rough description though, as asthma is not a homogenous disease in terms of disease natural history, severity and response to treatment. It has variable clinical presentations (phenotypes) and distinct underlying pathophysiological pathways (endotypes) responsible for this heterogeneity [2]. Based on the presence of T-helper cell type 2 (Th2) driven inflammatory responses (IL-4, IL-5 and IL-13 mediated), two major asthma endotypes, Th2 and non-Th2 had been described. The recent discovery of innate lymphoid cells type-2 (ILC2) and the fact that they can release Th-2 cytokines (predominantly IL-5 and IL-13), contributing to the T2-high signature, led to the better categorization of asthma into Type-2 high (T2-high) or Type-2 and Type-2 low (T2-low) or non-Type-2 (non-T2). Currently, based on the existence of T2 biomarkers and the development of relevant biologic therapies, T2-high asthma is the most well-defined endotype [3]. T2-low asthma presents with either neutrophilic or paucigranulocytic inflammation, tends to be more resistant to inhaled corticosteroids, and it involves various asthma phenotypes, related to obesity, smoking, late onset (usually after 40 years of age) or occupational exposures [2]. There is, thus, an unmet need for the identification of biomarkers that can help the diagnosis and the endotyping of T2-low asthma. The therapeutic approach of T2-low asthma is a problem urgently needing solution for two main reasons: first, these patients have poor response to steroids, the cornerstone of asthma treatment [4], and second, they are not candidates for the newer targeted treatment with biologic agents such as anti-IgE or anti-IL-5 [5]. This review aims to discuss older as well as more recent therapeutic approaches, such as novel small molecules and biologic agents, in the treatment of T2-low asthma.

Definition of T2-low asthma

At present, a solid definition of T2-low asthma has not been established. Although some emphasis has been placed on noninvasive biomarkers for the detection of T2-high asthma (i.e. T2 cytokines, such as IL-4, IL-5, and IL-13, exhaled nitric oxide (FeNO), serum periostin, total IgE, blood and sputum eosinophils), the exact cut-off points for these markers are debatable [6]. Furthermore, these T2 markers may have low concordance when measured in the same patient [7, 8] and may be subject to significant variability over time and in response to asthma treatments [9]. The most important characteristic of these biomarkers is that they are characterized by high specificity, but low sensitivity, rendering them more useful in the identification of T2-high patients. In clinical practice, the most useful tool to identify T2-low asthma phenotype is the absence of any evidence of increased values in the above-mentioned biomarkers of T2-high asthma. Additionally, it seems that there is an association of T2-low asthma with obesity, smoking, pollutants, viral or bacterial infections and advanced age. In terms of pathophysiology, T2-low asthma is usually characterized by neutrophilic (NA) or the paucigranulocytic (PGA-absence of sputum eosinophilia or neutrophilia) phenotype of inflammation [10] and Th1 and/or Th17 cells seem to be the key effector cells in this setting.

Several studies have related asthma severity to airway inflammation. Sy Duong-Quy et al. has related eosinophilic inflammation to asthma management, asthma control and to predicting ICS response, through fractional exhaled nitric oxide (FeNO) measurement [11]. Kuo et al. have shown that type 2 inflammation, as reflected by FeNO, serum immunoglobulin E (IgE), and blood eosinophils plays a central role in small airways dysfunction in adults with moderate to severe persistent asthma [12]. Luis et al. demonstrated the connection between clinical activity, bronchial hyperresponsiveness, and lung function to eosinophilic, and to a lesser degree, neutrophilic inflammation [13]. In a recent study Scioscia et al. showed a positive correlation between 17β-estradiol concentrations and neutrophil levels in in the sputum of female patients with postmenopausal severe asthma, and their results confirmed the central role of neutrophils in the pathogenesis of some forms of severe late-onset asthma [14]. It is possible that increased airway remodelling is an important future risk factor for asthma, inducing further decline in pulmonary function, increased BHR, and exacerbation rate [15].

Neutrophilic phenotype

The percentage of neutrophils in the sputum of healthy controls ranges from 0-64% [16, 17], the aetiology of which may be shear stress in the mucosa during the induction process, the influence of specific resident microbiota or prior exposures to environmental agents. Airway neutrophilia may be associated with pollution [18], smoking [19], workplace agents [20], acute infections [21], chronic airway infections (e.g. in bronchiectasis) [22] or with an altered airway microbiome [23]. NA is common in smokers with asthma [19], in obese asthmatics [24] and occupational asthma [25, 26]. The NA phenotype is characterized by a neutrophilic proportion of ≥64%in induced sputum [27, 28], although different cut off values have been used in different studies ranging from 40-76% [29]. It is uncertain which cut off value correlates with activated neutrophils promoting the pathogenic process [30], nevertheless,sputum neutrophil counts have been associated with disease severity [31]. It is prudent to mention that sputum total cell count, $>15 \times 10^6$/g with a neutrophil percentage greater than 64% is a strong indication of lower airway tract infection [32].

The NA phenotype accounts for 5-22 % of the asthmatic patients [33] often related to more severe disease with worse pulmonary function and poor asthma control [13]. Patients with NA are less responsive to ICS [34]; however, it is unclear if this corticosteroid insensitivity is related to the presence of neutrophils or if it is induced by the mechanisms underlying neutrophil recruitment [35].

Although blood eosinophils correlate with sputum eosinophilia, this is not applicable in NA in which blood neutrophilia cannot predict airway neutrophilia [30]. There are several candidate biomarkers to define NA, however, apart from the differential sputum cell counts; they are not applicable in clinical daily practice. Several cytokines are associated with neutrophilic airway inflammation. IL-6 is a pleiotropic cytokine produced by various cell types in response to a wide range of inflammatory stimuli. It is considered an indicator of metabolic dysfunction as well as asthma severity and has been identified as a potential candidate biomarker in a study with obese asthmatic patients [36]. Increased levels of IL-8, which is known to activate neutrophils, have been observed in patients with NA [37] and were linked to sputum neutrophil counts [38]. Other important biomarkers of neutrophil

induction are myeloperoxidase and neutrophil elastase which can be discovered in induced sputum samples [39]. TNF-α [40], IL-23 [41] and IL-17 [42] are also related to neutrophilic inflammation in asthma. Maes et al. have suggested that certain micro-RNAs in sputum might be able to accurately identify neutrophilic airway inflammation, but most clinicians are unable to obtain satisfactory sputum samples from these patients [43]. Transcriptomic analysis of airway samples in severe neutrophilic asthma found a strong upregulation of mucins, IL-17-inducible chemokines (CXCL1, CXCL2, CXCL3, and CSF-3) and the neutrophil chemoattractants CXCL8 (IL-8), CCL3 and Galectin-3 (LGALS3) [44]. Signatures of antibacterial responses including CD14, JUN and TLR2, implicate airway bacteria in driving the neutrophilia [45]. NA is associated with airway colonisation by bacteria including Haemophilus influenza [46] and Moraxella catarrhalis [47], which might induce Th17 responses [48]. Neutrophilic asthma is also associated with upregulation of oxidative stress responses and matrix metalloproteases [49], including MMP-9, a type IV collagenase involved with CXCL8-induced neutrophilia [50]. MMPs are increased in airway samples from asthmatic smokers and in severe neutrophilic asthma and are implicated in tissue remodelling [51].

Paucigranulocytic phenotype

Asthmatic patients with both neutrophil levels <64% and eosinophil levels <2% are classified as having PGA [29]. The prevalence of this phenotype in asthmatic patients ranges from 17%-48% in different studies [30, 37]. Although little is known about the PGA phenotype, in the majority of patients with PGA the absence of inflammatory cells is usually related to appropriate anti-inflammatory treatment that achieves adequate asthma control [30]. Even though PGA is related to better lung function, a substantial proportion of patients with PGA (21.7%) is characterized as having severe refractory asthma, and 14.8% of patients with PGA has an Asthma Control Test score of less than 19, suggesting that only this subpopulation of PGA is not well controlled despite the absence of inflammatory cells in their sputum [38]. Surprisingly, in a cohort, patients with PGA with severe refractory asthma presented with higher levels of FeNO and sputum IL-13 and IL-8 compared with those with mild/moderate asthma [37, 41]. Animal models

demonstrate that processes stimulating airway hyperresponsiveness and airway smooth muscle thickening occur independently from inflammation and are a consequence of a loss of negative homeostatic processes, advancing eventually to the pathogenesis of asthma in PGA phenotype [52].

The main clinical and pathophysiological characteristics that can help distinguish T2-low from T2-high asthma are shown in Table 1, while possible pathogenetic pathways; current drug targets and those in the development pipeline are shown in Figure 1.

Table 1. Characteristics of T2-low vs. T2-high asthma

Differential Diagnostic Characteristics of T2-asthma	T2-low asthma	T2-high asthma
Onset	Late	Early
Symptoms	Mostly significant	May be significant
Obesity	Often present	May be present
Smoking	Often present	May be present
Response to inhaled corticosteroids	Often poor	Usually good
Severity of asthma	Often difficult to treat	Mild to severe
Asthma Control	Often poor	Variable
Sputum eosinophils	Absent	Normal or high levels
Sputum neutrophils	Frequently present	May be present
Exhaled nitric oxide	Usually normal	Elevated or normal
Airways	Often present	May be present

Current therapeutic options

Presently, there are no specific therapeutic interventions for patients with PGA. A very crucial and low cost, but often overlooked intervention that may dramatically improve asthma control also in patients with NA, is smoking cessation and avoidance of exposure to environmental/occupational pollutant agents. In neutrophilic asthmatic smokers, smoking cessation and avoidance of second-hand smoke resulted in the reduction of neutrophilic inflammation and improved asthma control and lung function [53].

Other treatment approaches currently available for T2-low asthma, address non-inflammatory pathways possibly involved in its pathogenesis. Tiotropium has been recently included as a new add-on treatment for GINA Steps 4 and 5 in patients aged ≥18 years with a history of exacerbations. Its clinical efficacy has been demonstrated in patients with fixed airway obstruction, an asthma phenotype usually associated with neutrophilic airway inflammation [54] and in patients with low sputum eosinophils (and possibly T2-low asthma) [55].

Macrolides are often administered in refractory NA, although these antibiotics promote an enhanced risk of adverse events and promote bacterial resistance [56]. Macrolides exhibit their beneficial effects in NA with three mechanisms: 1) they exert antibacterial [57] and antiviral actions [58]; 2) they demonstrate immunomodulatory effects and anti-inflammatory activity through inhibition of transcription factors such as NFκB, reduction of activation and migration of neutrophils [59], revitalization of corticosteroid sensitivity by inhibiting the Phosphatidylinositol 3-kinase (PI3K) pathway and restoring histone deacetylase 2 (HDAC2) [60] and by attenuating TNF-α and IL-17 immune responses [59]; and 3) they display prokinetic properties, thus diminishing the pernicious consequences of reflux and aspiration events [61]. Although not explicitly highlighted, macrolides reduce the sequence of reflux effects that include: micro and macro aspiration of gastric contents; bronchoconstriction mediated by vagal reflexes [62] and neurogenic inflammation in the lung [63]. Erythromycin has been shown to promote gastric and small bowel motility [64], increase lower oesophagal sphincter pressure [65] and affect gallbladder function [66]. Azithromycin also exhibits action as a motilin receptor agonist [67] and enhances gastric motility in healthy subjects [68]. In patients with impaired gastrointestinal

motility, azithromycin has shown comparable positive effects on gastric and duodenal motility to erythromycin [67]. In one study which included smoking asthmatics, the use of azithromycin did not induce amelioration of asthma control or lung function [69]. In other reports, azithromycin significantly improved asthma exacerbations and quality of life in both eosinophilic and non-eosinophilic asthma [70, 71]. Moreover, the use of clarithromycin in patients with NA resulted in the reduction of neutrophilic inflammation followed by improvement in asthma control [72]. In a large randomized, double-blind study in moderate-to-severe asthma in Australia (AMAZES), azithromycin reduced exacerbations and improved Asthma Quality of Life Questionnaire (AQLQ) scores [71]. Moreover, current ERS/ATS severe asthma guidelines recommend the use of azithromycin in severe asthma that does not respond to treatment [73].

Bronchial thermoplasty (BT) could be a possible therapeutic approach, but its long term effectiveness or adverse events have not been fully assessed in PGA [74]. BT administers targeted thermal energy to the airway wall ablating airway smooth muscle (ASM) cells with a subsequent decrease in the ASM mass [75] while it has been verified that thermoplasty also regulates mucosal inflammatory responses and collagen deposition [76]. This intervention has been observed to reduce asthma exacerbations and to improve lung function and health-related quality of life. Moreover, macrolides may be an effective option in PGA since the potentialeffects of azithromycin have been observed irrespective of the underlying airway inflammation [70].

The use of statins in clinical trials demonstrated alterations in several inflammatory markers but failed to show improvement in asthma control or lung function [77].

Given the high prevalence of obesity in patients with T2-low asthma, weight reduction along with exercise and dietary modification should be included in the therapeutic plan of those patients. Several clinical studies have shown that weight loss in obese patients not only improves asthma symptoms but also contributes to a reduction in doses of rescue medication. It also reduces the risk for severe asthma exacerbations and improves asthma-related quality of life, perhaps through improvements in lung function [78–80].

Future therapeutic targets

Although no specific treatments have been made available so far, according to the pathophysiology of T2-low asthma, some potential targets for therapeutic interventions are summarized below [81]. An overview of the major biologic approaches currently in development for type 2 low asthma management is shown in Table 2.

1) Cysteine X cysteine chemokine receptor 2 (CXCR2)/IL-8

IL-8 is a potent chemoattractant that regulates activation and migration of neutrophils to the site of inflammation through the high-affinity CXCR2 receptor. Increased levels of IL-8 in sputum often precede asthma exacerbations in severe asthmatics and coincide with the development of late-phase airflow obstruction in patients with atopy [82]. IL-8 is also elevated in the serum of those patients, but its role as a biomarker demonstrating disease activity remains disputable. CXCR2 is a common chemokine receptor, which is expressed on the surface of neutrophils. The tripeptide PGP, which is generated from extracellular matrix proteins through the action of matrix metalloproteinases, is also a neutrophil chemoattractant (matrikine) that activates CXCR2 [83]. These chemokines also attract circulating monocytes, which differentiate within the lung to macrophages that are thought to guide neutrophilic inflammation [84]. This implicates that blocking CXCR2 could be a tempting therapeutic approach to the treatment of neutrophilic inflammation since small molecule inhibitors to these G-protein receptors have now been developed.

In a phase 2 study of patients with moderate to severe asthma with high neutrophils count at baseline (>40%), CXCR2 antagonist SCH527123/Navarixin demonstrated a significant reduction in sputum neutrophils by 36.3% compared to a 6.7% increase in the placebo group (p=0.03), but no significant improvement of asthma control was established and no change to FEV1 was proved [85, 86]. In a subsequent randomized clinical trial, using a CXCR2 antagonist (AZD5069) as add on therapy for patients with severe asthma 45 mg BID (n=161) for up 12 months resulted in a sustained ~25%, reversible on discontinuation of treatment and reduction in blood neutrophils. In severe asthmatics, 5, 15 or 45 mg BID for 6

months did not reduce the rate of exacerbations nor improvedFEV1 or total asthma symptom score. A statistically significant reduction of sputum neutrophils by 69% was found in bronchiectasis patients after 80 mg BID for 4 weeks. There was no consistent increase in the rate of infections [86]. Another phase 1 clinical trial treatment of AZD8309, a CXCR2 antagonist, demonstrated a mean 77% reduction in total sputum cells ($p < 0.001$) and 79% reduction in sputum neutrophils ($p < 0.05$) compared with placebo after LPS challenge. There was also a reduction in neutrophil elastase activity ($p < 0.05$) and CXCL1 ($p < 0.05$) and trends for a reduction in sputum macrophages (47%), leukotriene B_4 (39%) and CXCL8 (52%).The clinical trial was discontinued for asthma [87]. Overall, although CXCR2 antagonists seemed to be promising molecules as an add-on therapy for severe uncontrolled NA, they were not able to improve clinical outcomes and exacerbation rates [85–87].

2) Interleukin 17

IL-17A and IL-17F are produced by Th17 cells and trigger the production of IL-8 and CXCL1 from epithelial cells. Moreover, they are neutrophil chemoattractants [88], while Th1 cells are connected to neutrophilic inflammation, by producing INF-γ and TNF-α. Neutrophilic inflammation generated by this pathway leads to airway injury and remodelling, hyperplasia and hypersecretion of the mucous gland and corticosteroid insensitivity [77]. It has been recorded that severe NA is related to higher levels of Th17 cytokines primarily CXCL1, CXCL10, CCI-2, IL-6 and IL-8 in comparison to other inflammatory endotypes [89]. In patients with asthma, elevated levels of IL-17A and IL-17F are found in BAL and airway tissue biopsies and positively correlate with the level of disease severity and neutrophil inflammation [90]. Furthermore, IL-17A acts on airway smooth muscle cells and mediates airway hyperresponsiveness. IL-17A and IL17F bind to receptor complexes with IL-17RA serving as the common subunit. Nevertheless, a recent study with mice models suggested that IL-17F, rather than IL-17A, underlies airway inflammation in steroid-insensitive asthma [91].

Secukinumab, an IL-17A blocker, administration in healthy volunteers who evolved acute neutrophilic airway inflammation following an ozone challenge did not demonstrate a significant change in the total number of sputum neutrophils

from baseline. In a subsequent phase 2 study of AIN457 (secukinumab) in patients with uncontrolled asthma, there was no difference in ACQ score leading to its termination [92]. Brodalumab is a human monoclonal antibody that is blocking the activity of IL-17A, IL-17B, and IL-25 through binding to IL-17RA. In a randomized controlled trial recruiting patients with uncontrolled, moderate to severe asthma on inhaled corticosteroids, brodalumab failed to show a change in ACQ score, asthma symptoms or lung function in the overall study population. However, it did show a trend towards improvement in ACQ score in a small subgroup of patients with high bronchodilator reversibility [93]. However, this drug led to significant mental health issues in clinical trials, thus resulting in discontinuation of further development for asthma. Another formulation of secukinumab (CJM112), an anti-IL17A antibody, is now in phase 2 randomized clinical trial for patients with inadequately controlled moderate to severe asthma with low IgE and low circulating eosinophil levels, but no results have been announced yet [94].

3) Phosphodiesterases (PDE)

Most PDE isoenzymes are expressed in lung tissue, including PDE1, PDE2, PDE3, PDE4, PDE5, PDE7, PDE8, and PDE9; however, PDE3 and PDE4 have been the primary targets for drug therapy. The PDE4 isoenzyme is found in most inflammatory and immune cells, including T-cells, eosinophils, neutrophils, B-cells, monocytes, macrophages, and dendritic cells. In several preclinical studies, PDE4 inhibitors have been shown to suppress bronchial hyper-responsiveness, eosinophil infiltration, and production of histamine, leukotrienes, and cytokines, however with significant side effects when administered orally. PDE3 inhibitors act as bronchodilators, while PDE4 inhibitors have an anti-inflammatory effect [95]. PRL-554, an inhaled phosphodiesterase 3/4 inhibitor is now in a phase 1 trial for severe asthma. It is an analogue of trequinsin and, like trequinsin, is a highly selective inhibitor of the phosphodiesterase enzyme, PDE3. Indeed, it is >3000-times more potent against PDE3 than against PDE4. Ensifentrine (RPL554), a nebulized PDE3/4 inhibitor, demonstrated a significant dose-dependent bronchodilator response in asthma patients [77, 96, 97]. CHF6001, an inhaled PDE4 inhibitor, was evaluated in a phase 2 trial; the drug was safe and well-

tolerated at daily doses up to 4800μg for 14 days in healthy volunteers. No results have been announced yet about drug efficacy [98].

4) Tumour necrosis factor-alpha (TNF-a)

TNF-a is secreted by lymphocytes, mast cells, and macrophages and promotes bronchial hyperresponsiveness and sputum neutrophilia thus was considered as an attractive target for severe asthma [99]. Etanercept (anti TNF-a) showed short term and modest clinical efficacy for severe and mild asthma respectively [100]. Mild reduction in the number of exacerbations and improvement in wheezing was observed in patients with moderate uncontrolled asthma, who were under monotherapywith inhaled corticosteroids (ICS), after administration of infliximab or adalimumab [101]. Golimumab lowered risk of asthma exacerbations, nevertheless, its trial on severe persistent asthma ended at phase II due to incidences of malignancies and infections such as pneumonia [99]. Given the possible beneficial role of TNF-a in severe NA, future studies may need to evaluate the long-term risk-benefit profile of these agents in such patients.

5) Interleukin 1 (IL-1)

IL-1β and IL-1 receptor antagonist (IL-1 RA) genes have both been associated with asthma risk [102]. In humans, IL-1β is increased in bronchoalveolar lavage (BAL) fluid and sputum of asthmatics compared to normal volunteers [103], with airway macrophages from asthmatics also having increased expression of IL-1β [104]. IL-1β is also increased in BAL fluid from persons with symptomatic asthma vs. those with asymptomatic asthma [105], and there is an increased expression of this cytokine in the airways' epithelium of asthmatics [105]. IL-1β also appears to be involved in the recruitment of airway neutrophils by LPS in rat models [106] and is upregulated in the airway after LPS inhalation challenge in humans [107]. Canakinumab, a fully human immunoglobulin IgG1k monoclonal antibody against IL-1β was originally developed as a potential treatment for asthma but is no longer listed for that indication [108]. A study of anakinra, an IL-1βantagonist, in NA demonstrated a significant reduction of airway neutrophilia compared to placebo; subjects tolerated the treatment well, without an increased frequency of infections that were attributable to anakinra [109].

7) Interleukin 6 (IL-6)

IL-6 in sputum is associated with a lower FEV_1 in patients with mixed eosinophilic-neutrophilic bronchitis [110]. In mice, allergen exposure increased lung IL-6, and IL-6 was produced by dendritic cells and alveolar macrophages. Loss of function of IL-6 signaling abrogated elevations of eosinophil and neutrophil recruiting cytokines/ chemokines and allergen-induced airway inflammation in mice. The association of pleiotropic cellular airway inflammation with IL-6 using human and animal data suggests that exacerbations of asthma, particularly those with a combined eosinophilic and neutrophilic bronchitis, may respond to therapies targeting the IL-6 pathway [111]. Sirukumab, an IL-6 antagonist for patients having uncontrolled severe asthma despite the use of high dose ICS in combination with LABA is now in a phase 2a trial [112].

8) Interleukins 22 and 23 (IL-22, IL-23)

Currently, one phase 2 ongoing clinical trial for anti-IL-23 monoclonal antibody (risankizumab) attempts to investigate the efficacy and safety profile of this agent in T2-low severe asthmatic patients (NCT02443298). There are no ongoing studies on IL-22 targeted therapy in asthma.

9) Protein Kinases

Several p38 mitogen-activated protein kinases (MAPK) inhibitors have shown to restore corticosteroid sensitivity in peripheral blood mononuclear cells from patients with severe asthma [113]. A phase 2 study of PF03715455 was conducted and was terminated due to business reasons, without any safety or efficacy concerns [114]. A post hoc analysis of a 6-month clinical trial with losmapimod (GW856553) in COPD, but not in asthma, reported a reduction in exacerbations in a subgroup of patients with a blood eosinophil count ≤2%, which may suggest a preferentially beneficial effect of p38 MAPK inhibitors in non-eosinophilic inflammation [115]. AZD7624, an inhaled p38α and p38β inhibitor, in COPD, significantly reduced additional inflammatory biomarkers from sputum (IL-6, IL-8, and MIP-1β) and blood (neutrophil percentage, IL-6, MIP-1β, and CRP), but demonstrated no benefit concerning the reduction of exacerbations [116].

Inhibition of the stem cell factor /c-kit receptor pathway leads to a significant decrease of the mast cell population, histamine levels, interleukin-4 production and airway hyper-responsiveness. Growth factors such as platelet-derived growth factor (PDGF) contribute to bronchial remodelling, a characteristic of severe asthma [117]. Masitinib and imatinib are tyrosine kinase inhibitors, targeting stem cell factor receptor/c-kit and PDGF receptor. A phase 2a study of masitinib in severe, uncontrolled, corticosteroid-dependent asthmatics was conducted, showing no significant difference to the corticosteroid weaning process. Contrary to that, a significant improvement in asthma control was observed [118].

Phosphatidylinositol 3-kinase (PI3K) and Janus-activated kinases (JAK) modulate lymphocyte activation. JAK and PI3K inhibitors reduced the cytokine level through direct effects on T-cell activation in both asthma and healthy BAL cells. Nemiralisib (GSK2269557), a once-daily inhaled PI3Kδ inhibitor underwent a Phase 2 clinical trial for patients with severe, uncontrolled asthma. It exhibited a significant reduction in sputum IL-5, IL-13, IL-6, and IL-8 level; however with no discernible difference in trough FEV_1 and ACT score [119]. PI3K δ and γ isoforms are involved in inflammatory cell recruitment and activation and dual PI3Kδ/γ inhibitors, such as TG100-115 and IPI145 reduces airway inflammation induced by allergen or cigarette smoke in murine models [120, 121] and restored corticosteroid sensitivity in the smoke model [121]. A phase 2 study of RV1729, an inhaled PI3Kγ/δ inhibitor, demonstrated attenuation of airway inflammation, promotion of bronchodilation and reversal of β2 adrenoreceptor tachyphylaxis [122]. Both IPI-145 (duvelisib) and RV-1729 are PI3Kδ/PI3Kγ combination inhibitors that are being developed for clinical use [120, 121]. Furthermore, the combination of dexamethasone with either a JAK or PI3Kδ inhibitor showed an additive anti-inflammatory effect [123]. Recently, an inhaled inhibitor of Janus kinase 1 (JAK1) and intravenous administration of human mesenchymal stem cells attenuated neutrophilic inflammation in an experimental murine of allergic asthma [124]. Overall, the inhibition of PI3K blunts mucus production, prevents mast cell degranulation, deters immune cell recruitment and facilitates bronchodilation, all of which are potentially beneficial in asthma [125], but these need to be proven in future clinical trials.

Conclusions

Treating T2-low asthma is a therapeutic challenge, mainly because its mechanisms are not fully understood. Although much progress has been made in elucidating T2-high inflammation pathways, no specific biomarkers for T2-low asthma have been identified. Neutrophilic airway inflammation mediators targets, including IL-8, IL-17, IL-1, IL-6, IL-23, TNF-aor airway smooth muscle mass attenuation therapies (for paucigranulocytic inflammation) are under investigation. Failed previous attempts with some of the agents targeting these mechanisms may have been due to the poor characterization of severe T2-low asthma. Further studies are necessary to accurately characterize T2-low pathogenetic processes to assess novel evidence-based treatments.

Abbreviations

Airway smooth muscle: ASM; Asthma Control Questionnaire: ACQ; Asthma Control Test: ACT; bronchial hyperresponsiveness: BHR; bronchial thermoplasty: BT; bronchoalveolar lavage: BAL; cysteine X cysteine chemokine receptor 2: CXCR2; fractional exhaled nitric oxide: FeNO; histone deacetylase 2: HDAC2; Innate lymphoid cells: ILCs; inhaled corticosteroids: ICS; janus-activated kinases: JAK; long-acting beta-agonist: LABA; Interleukin: IL; Galectin-3:LGALS3; macrophage inflammatory protein-1β: MIP-1β; mitogen-activated protein kinases: MARK;neutrophilic asthma: NA; nuclear factor kappa-light-chain-enhancer of activated B cells: NF-κB; paucigranulocytic asthma: PGA; phosphatidylinositol 3-kinase: PI3K; platelet-derived growth factor: PDGF; and tumour necrosis factor-a: TNF-a.

Table 2. Overview of the major biologic approaches currently in development for type 2 low asthma management

Target patient population	Studied biomarker	Biologic (drug name)	Drug type	Sponsor	Main effects	Ref.
Add-on therapy in severe uncontrolled neutrophilic asthma	IL-8	SCH527123 /Navarixin	CXCR2 antagonist	AstraZeneca	Reduction in sputum neutrophils and in blood neutrophils No improvement in clinical outcomes and exacerbation rates	[85–87]
Add-on therapy in uncontrolled moderate to severe asthma	IL-17	Brodalumab	Humananti-IL-17receptorAm onoclonalanti body	LEO Pharma A/S	Did not improve ACQ score, lung function or asthma symptoms	[93]
Add-on therapy in severe uncontrolled neutrophilic asthma	PDE4	PRL-554 CHF6001 (inhaled)	Dual phosphodiest erase PDE3 and PDE4 inhibitors	Verona Pharma ChiesiFar maceutici S.p.A	Bronchodilatatory and anti-inflammatory effect	[96] [97]
Severe and mild asthma Severe, refractory, or steroid-resistant asthma	TNF-α	Etanercept Golimumab	Human monoclonal antibody against TNF-a	Pfizer Janssen Biologics B.V.	Treatment was associated with substantial adverse reactionssuch as infections and malignancies	[100] [99]
Add-on therapy in	IL-1	Anakinra	Human IL-1 receptor	SwedishOr phanBiovit	Reduced neutrophilic airway	[109]

severe uncontrolled neutrophilic asthma			antagonist (IL-1 Ra)	rum AB	inflammation and sputum levels of IL-1b, IL-6 and IL-8	
Severe neutrophilic asthma	p38 MAPK Tyrosine kinase	Losmapimod AZD7624 Masitinib, imatinib	Protein kinase inhibitors Mitogen-activated protein kinase (MAPK) inhibitors Tyrosine kinase inhibitors	Glaxo SmithKline AstraZeneca AB-Science, Novartis	Reduced exacerbations Reduced inflammatory biomarkers from blood (IL-6, neutrophil percentage, and CRP) and sputum (IL-6, and IL-8) Improvedasthmacontrol	[115] [116] [118]
Severe uncontrolled asthma Severe neutrophilic asthma	Phosphoinositide 3 (PI3)-kinase	Nemiralisib IPI-145 RV-1729(duvelisib)	Phosphoinositide 3 (PI3)-kinaseinhibitors	Glaxo SmithKline	No discernible difference in trough FEV1 and ACT score Attenuation of airway inflammation, promotion of bronchodilation and reversal of β2 adrenoreceptor tachyphylaxis	[119] [120],[121] [122]

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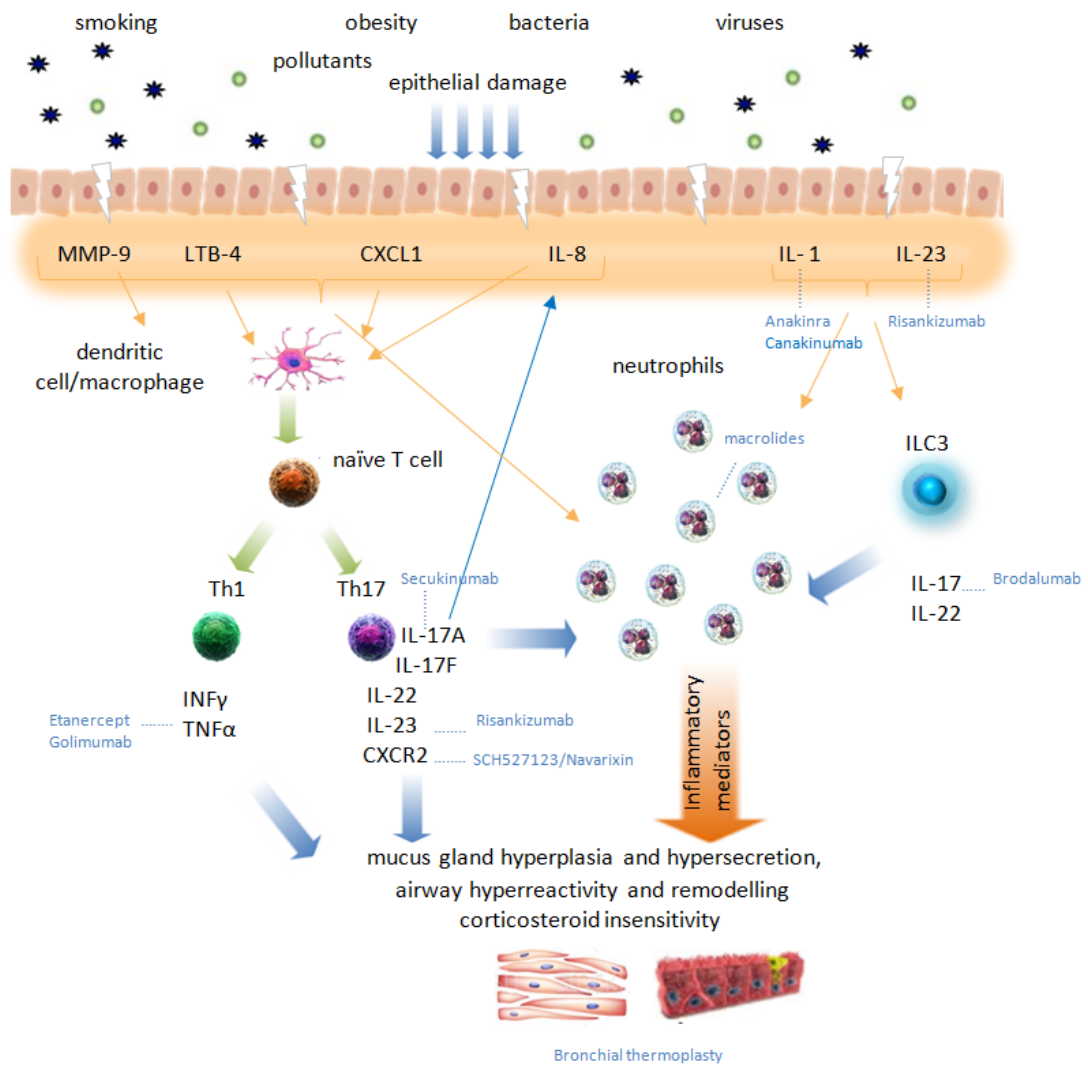
Figure 1: T2-low asthma pathways and potential therapeutic targets