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Stratified approaches to the treatment of asthma

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While asthma is a chronic inflammatory disorder that is managed with inhaled controller and reliever drugs, there remains a large unmet need at the severe end of the disease spectrum. Here, a novel stratified approach to its treatment is reviewed, based upon identification of causal pathways, with a focus on biologics. A systematic search of the literature was made using Medline, and publications were selected on the basis of their relevance to the topic. Despite strong preclinical data for many of the more recently identified asthma targets, especially those relating to the T-helper 2 allergic pathway, clinical trials with specific biologics in moderate to severe asthma as a group have been disappointing. However, subgroup analyses based upon pathway-specific biomarkers suggest specific endotypes that are responsive. Application of hypothesis-free analytical approaches (the 'omics') to well-defined phenotypes is leading to the stratification of asthma along causal pathways. Refinement of this approach is likely to be the future for diagnosing and treating this group of diseases, as well as helping to define new causal pathways. The identification of responders and nonresponders to targeted asthma treatments provides a new way of looking at asthma diagnosis and management, especially with biologics that are costly. The identification of novel biomarkers linked to well-phenotyped patients provides a stratified approach to disease management beyond simple disease severity and involving causal pathways. In order to achieve this effectively, a closer interaction will be required between industry (therapeutic and diagnostic), academia and health workers.

Historical perspective

In 1860, Henry Hyde Salter first identified asthma as a distinct disease entity, separating it from the previously broad use of the Greek term meaning 'shortness of breath'. His experiences are related in his treatise 'On Asthma: Its Pathology and Treatment'; he carefully separated asthma from other obstructive diseases of the airways by identifying contraction of smooth muscle as the primary cause of the airway obstruction [1]. As a physician practising in London, over many years he was able to collect 50 patients who provided the basis for his observations including, purportedly, having asthma himself. With the recognition of the importance of 'bronchospasm' in the symptomatology of the disease, treatment was directed towards bronchodilators. In the 19th century this included *Datura stramonium*, which was smoked to release anticholinergic alkaloids. Asthma cigarettes were available in the UK up to 1985. Much earlier, the Chinese had identified Ma huang, a herb used as an oral treatment for respiratory disease, from which ephedrine was subsequently identified and synthesized [2]. Salter also recognized that black coffee offered some relief to asthma sufferers, but it was not until the 1930s that theophylline, a methylxanthine, was identified as the active agent, along with other xanthines, such as caffeine and theobromine [3]. Theophylline and its ethylenediamine salt, aminophylline, were widely adopted as asthma therapies up to the end of the 20th century, administered orally for chronic disease control and intravenously or rectally for acute asthma [3]. Theophylline and its analogues are still widely used, especially in low- and middle-income countries, because it is a cheap and efficacious therapy; however, its popularity is waning, largely on account of potentially serious side-effects, especially cardiac arrhythmias, convulsions and diuresis, as well as nausea and vomiting. Slow-release preparations and blood

drug level monitoring were introduced to increase the therapeutic index of theophylline, but use of these preparations also is now falling [3].

A major breakthrough in asthma treatment came in 1901 with the identification of adrenaline [4], which was shown to be a powerful bronchodilator and cardiovascular stimulant when administered systemically [5]. Over the following 100 years, the potent bronchodilator action of adrenaline was separated from cardiovascular effects by separating β- from α-adrenoceptor activity, leading to the introduction of isoprenaline, followed by subdivision of β-receptors into β_1 and β_2, the latter carrying the bronchodilator response, leading to the introduction of salbutamol and related β_2 agonists, such as terbutaline [5] (Figure 1). Finally, the identification of key molecular characteristics that produce bronchodilatation led to the introduction of salmeterol and formoterol as long-acting β_2-agonists (LABAs) [6]. Ultra-long-acting bronchodilators are now appearing (e.g. indacaterol) [7], suggesting that molecular manipulation of adrenergic bronchodilators has probably reached the maximum that can be reasonably achieved.

The rise in asthma mortality in the 1960s and 1970s that was seen in those countries where inhaled β-agonists became available without prescription and in high doses [8] led to an intense series of studies that pointed to β-adrenoceptor tolerance as the most likely cause [9]. A second peak of asthma deaths in New Zealand in the late 1980s has been attributed to the introduction of a high-dose formulation of inhaled fenoterol [8]. While controversial, these peaks in asthma mortality have driven research into the underlying causes of asthma. The recognition that airway inflammation is a common feature of asthma [10] and the discovery that inhaled corticosteroids can both control asthma and release airway inflammation [11] has been the driving force for using anti-inflammatory drugs to control asthma. Beginning with the discovery of the potent anti-inflammatory activity of cortisone by Phillip

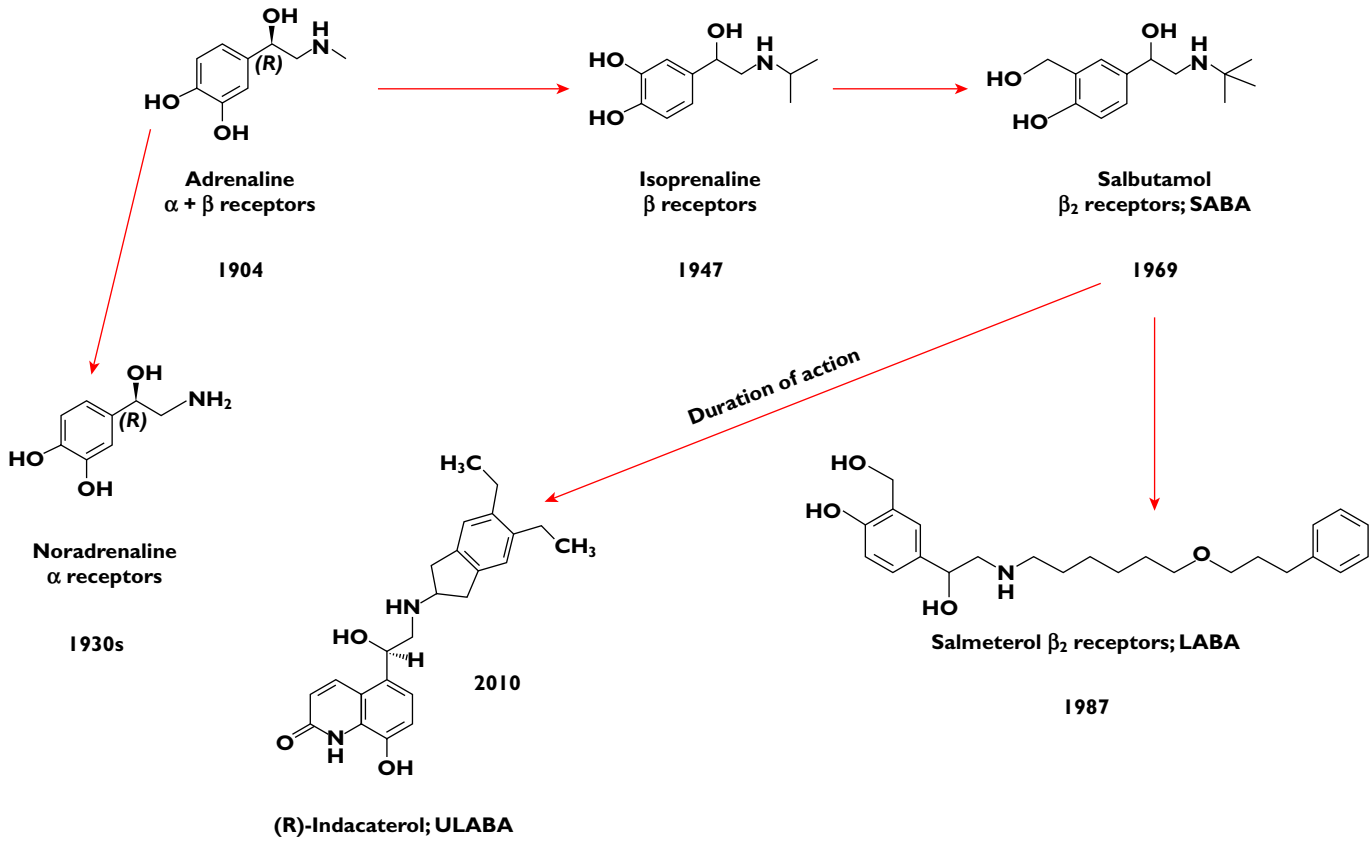

Figure 1

The evolution of inhaled β-adrenoceptor bronchodilators for the treatment of asthma. The discovery of adrenaline in 1901 and its powerful bronchodilator effect, initially when administered by injection but later as a nebulized aqueous aerosol, was to stimulate a relentless search for improved specificity, potency and duration of action. Application of medicinal chemistry and classical structure–activity pharmacology led to the sequential separation of α- (noradrenaline) from β-receptor activity (isoprenaline), followed by the selection of β_2 activity, mediating airway smooth muscle relaxation and anti-bronchoconstriction, from cardiovascular β_1 effects (e.g. salbutamol, turbutaline). The next discovery was to incorporate structural properties that extended the duration of action to 12 h (salmeterol, formoterol) and finally to 24 h (indacaterol) following a single inhalation

Hench in 1947 [12] and the discovery of inhaled beclomethasone disproprionate in the early 1970s as a highly active 'controller' drug for asthma [13], there have been many attempts to improve potency and safety, with limited success. The main problem has been producing anti-inflammatory drugs free of endocrine activity and, at the same time, maintaining desirable potency, bioavailability and pharmacokinetics [14]. The search continues, with the important discovery that corticosteroids produce much of their suppressive effect on inflammation by interfering with transcription factor activation of pro-inflammatory and prosurvival genes in immune and inflammatory cells (transrepression), whereas their endocrine effects result from the corticosteroid–receptor complex activating selective genes (transactivation) [15].

In parallel with these developments, there has been development of inhaler devices, from the hand-held nebulizer through to the pressurized metered-dose aerosol to inhaled dry powder devices and those that time and meter dose administration [16]. The vast range of different inhaler devices now available has its problems in causing patient confusion as well as operating difficulties and, along with the social stigma accompanying their use and fear of side-effects, this could be an important factor contributing to lack of adherence to prescribed therapies [17]. Accepting this as an ongoing problem in asthma management, corticosteroid refractoriness is increasingly being recognized as an unmet problem in asthma, especially in those patients with more severe disease, and constitutes the majority of patients with 'difficult-to-treat' asthma [18]. A range of factors have been incriminated in such corticosteroid refractory disease [19], but one that has recently come to the forefront is tobacco smoking [20].

To avoid the inevitable endocrine side-effects of ever-increasing doses of inhaled corticosteroid (ICS) for uncontrolled asthma, combination therapy is advocated, in which a LABA and an ICS are combined in a single inhaler. However, even this approach has 'responders' and 'nonresponders'. It should also be recognized that ICS started at the inception of asthma has no effects on the natural history of the disease despite effectively suppressing airway inflammation [21].

Anti-allergic approaches to asthma

All of the treatments so far referred to act on the secondary consequences of asthma (i.e. bronchospasm and inflammation) rather than treating the underlying mechanistic and aetiological causes of the disease [21]. Atopy and allergic mechanisms have long been recognized as contributing to asthma in a high proportion of patients, leading to its classification as an atopic disorder. While allergen exposure is a powerful trigger for early (mast cell-driven) and late (inflammatory cell-driven) airway narrowing in asthma [22], somewhat counterintuitively, attempts at allergen avoidance have had limited success, even with such common allergens as those from dust mites [23]. Likewise, while there has been some success with allergen-specific immunotherapy, either systemically by subcutaneous injections or sublingually, this is in large part in those with single-allergen sensitization, e.g. cat or pollen, rather than multiple allergens, which is the situation in most asthmatics [24]. Moreover, allergen-reduction strategies in young children genetically at risk of asthma have had little success [25] combined with other interventions (e.g. breast-feeding, environmental tobacco smoke avoidance) [26].

Recognition that mediator release from mast cells is important in the acute asthmatic response with allergen challenge but also that mast cell activation is a feature of more chronic asthma [27] has stimulated interest in agents that can inhibit mast cell activation and mediator release. The first of these was sodium cromoglicate (SCG), derived from the flavonoid khelin extracted from the herb *Ammi visnaga* by Altounyan in the 1950s. Inhaled sodium cromoglicate was shown to inhibit both allergen-induced early and late asthmatic responses and exercise-induced asthma [28]. *In vitro*, SCG inhibited IgE-dependent mast cell mediator release and, in 1968, was shown to be effective as a treatment for asthma when inhaled regularly [29]. Nedocromil sodium is a second-generation drug with similar properties but is more active [30]. Even during its early development, it was recognized that only a proportion of patients responded well to SCG, especially children [31]. However, subsequent meta-analysis and a Cochrane Review concluded that 'there was insufficient evidence to be sure about the efficacy of SCG over placebo' [32] and, as a consequence, it has been withdrawn from the World Health Organization list of drugs, despite some concerns over the methods used and the limited number of trials selected for the analysis [33]. For those asthmatics who benefited from SCG as a safe and effective anti-allergic therapy, this was a loss. If only it had been possible at the time of its development to have a clear idea of the asthma phenotype most likely to respond to SCG, then the drug might be available today. Another stumbling block was the lack of an underlying pharmacological mechanism. Although there was some evidence that SCG inhibited the chloride flux associated with mast cell activation [34], the precise ion channel involved eluded discovery. Both SCG and nedocromil sodium have recently been found to be potent and selective inhibitors of the G protein-coupled receptor 35 that recognizes its natural ligand 2-acyl-lysophosphatidic [35, 36]. G protein-coupled receptor 35 is one of several lysophosphatidic acid receptors that are involved in mast cell development and activation [37]. Identification of this receptor class on mast cells might stimulate a search for compounds more active than SCG and nedocromil sodium because, while these drugs were active at suppressing mast cell activation *in vitro* and *in vivo*, they lacked potency and were also subject to tachyphylaxis, so that dose estimation was difficult [38].

Mast cell-derived histamine is a powerful bronchoconstrictor in asthma, acting via the H_1-G protein-coupled receptor, which has led to its use as a provocation test to assess 'nonspecific airway hyperresponsiveness' (AHR). Given the importance of histamine as a contractile agonist of airway smooth muscle, it is somewhat surprising that H_1-antihistamines, particularly the more potent selective inverse agonists, such as cetirizine, loratidine and fexofenadine, are not effective in asthma, in stark contrast to their proven efficacy in allergic rhinoconjunctivitis [39]. Although there may be certain types of asthma that are responsive to H_1-receptors, especially asthma associated with acute pollenosis [40], this lack of efficacy is puzzling if the mast cell is so important in driving AHR. One possible explanation is that in airways the smooth muscle H_1-receptors activate an alternative cellular signalling mechanism from the one utilized by vascular endothelial cells in the nasal mucosa (responsible for much of the sympomatology of allergic rhinitis). This would require inhibitors to bind to a different component of the H_1-receptor for effective inhibition, as has recently been proposed for β_2-adrenoceptor functions with repeated dosing [41].

The situation of low efficacy is different for inhibitors of a second mast cell mediator class, the cysteinyl leukotrienes (cyst-LTs). The discovery that slow reacting substance of anaphylaxis (SRS-A), first identified by Kellaway and Trethewie in 1940, is a powerful smooth muscle contractile agent released upon allergen challenge that cannot not be inhibited with antihistamines led to a 50 year search for its structure. In 1989, Samuelsson identified SRS-A with a new family of lipid mediators, the cyst-LTs, of which LTC_4 was the secreted form [42]. Subsequent extracellular processing of LTC_4 into LTD_4 and eventually LTE_4, in which the peptide side-chain was progressively shortened, provided the molecular basis for the biological effects of SRS-A, which has smooth muscle contractile activity almost 1000 times greater than that of histamine [43]. The development of cyst-LT receptor antagonists (LTRAs; most notably, montelukast, zafirlukast and pranlukast) has provided the first orally active anti-asthma controller drugs beyond corticosteroids and xanthines. Shortly after their development, the receptor via which LTC_4 and LTD_4 contracts airway smooth muscle, the cyst-LTR_1, was identified [44]. With a remarkably good safety record, cyst-LTR_1 antagonists are now widely used in asthma treatment, although head-to-head trials with inhaled corticosteroids have generally shown them to be less efficacious, and for most asthma guidelines ICSs are the first-line controller drugs. However, this may be an over simplification, because in head-to-head trials in which patient-related outcome measures have been used the difference in efficacy between ICSs and LTRAs is far less apparent [45]. Moreover, in effectiveness studies conducted in the community (as opposed to efficacy studies in highly selected patients), montelukast used as first-line therapy was not different from ICS, and as add-on therapy to ICS, not different from the LABA salmeterol [46]. This may be in part because adherence to treatment with once daily oral montelukast is greater than with inhaled drugs [47]. In addition, montelukast is more active than ICS in asthmatic patients who smoke [48]. It is salutary to know that <4% of asthmatic patients are represented in efficacy trials for drug registration [49]. However, this greatly underestimates the spectrum of patients who eventually receive the drug in the 'real world', once it is approved [50].

The assessment of responsiveness to LTRAs is also greatly influenced by whether this mediator pathway is dominant in causing airway dysfunction in different patients. A responder analysis of head-to-head comparator trials revealed that the mean response to both drugs masks a remarkable heterogeneity of responsiveness [51] (Figure 2). For both the ICS arm and the montelukast arm, there are clear differences from placebo in a range of asthma outcome measures. However, within each active arm there were patients who experienced dramatic and

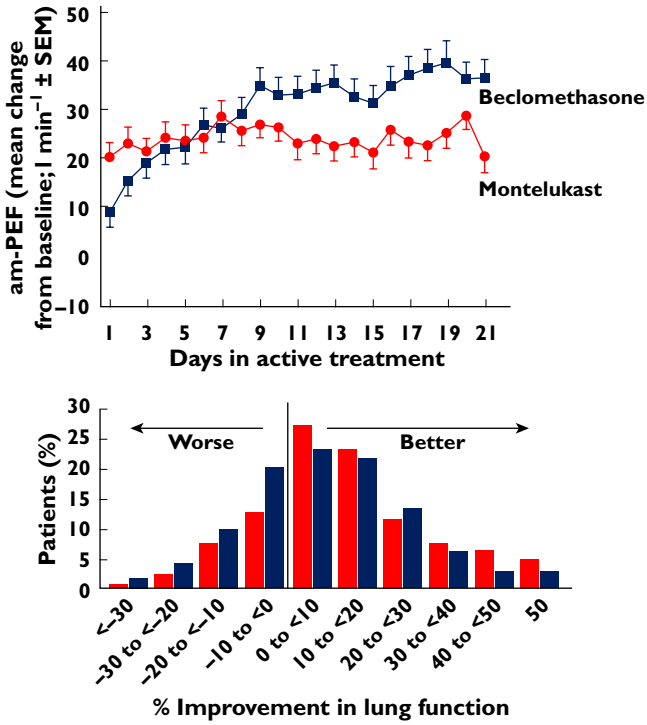

Figure 2

Top panel shows results from a randomized controlled trial of oral montelukast (10 μg twice daily) and inhaled beclomethasone (200 μg twice daily) against the asthma outcome measure of morning peak expiratory flow (am-PEF), over 21 days of treatment of moderate asthma. Improvement in am-PEF was more rapid and initially greater with montelukast compared with beclomethasone, but after day 8, the beclomethasone treatment effect surpassed that of montelukast. Bottom panel shows results from the same clinical trial, but displayed as the percentage of individuals achieving changes in peak expiratory flow at week 12, showing the large range of responders and nonresponders for both drugs (Adapted from [53]). ■, beclomethasone (n = 246); ■, montelukast (n = 375)

consistent improvements, whereas others experienced no change or even deteriorated (Figure 2). In the past, such variability has been brushed aside as being part of the 'normally expected spectrum of response' to any drug; however, recent research suggests that there may be very specific reasons why one drug may work well in one patient but not in another. The 5-lipoxygenase pathway responsible for generating cyst-LTs is selectively upregulated in patients with aspirin-intolerant asthma [52, 53]; in particular, there is a selective overexpression of the terminal synthetic enzyme, LTC_4 synthase, that overrides the suppressive effects of locally generated inhibitory prostaglandin prostaglandin E_2 [54, 55]. Thus, both the 5-lipoxygenase inhibitor zileuton [56] and the cyst LT_1R antagonist montelukast [57] are especially efficacious in this subgroup who exhibit increased airway LTC_4 and airway LTE_4 levels at baseline and following aspirin challenge commensurate with overexpression of this pathway in pathogenesis [58]. In a broader range of asthma, the urinary LTE_4/exhaled nitric oxide (eNO) ratio predicts a superior response to montelukast compared with the inhaled corticosteroid fluticasone propionate in children with mild to moderate asthma [59]. Exhaled NO is generated by epithelial inducible NO synthase, which is upregulated in asthma and is suppressed by corticosteroids [60] and has proved to be a sensitive biomarker of responsiveness to inhaled corticosteroids in mild to moderate disease [61]. Exhaled NO, as a biomarker of corticosteroid responsiveness, is much less useful in severe asthma, possibly owing to alternative cellular sources and the fact that most severe asthmatics are already receiving high doses of inhaled corticosteroids [62].

Mechanism-directed treatment of asthma

Anti-IgE monoclonal antibody (mAb)

Over the last 50 years, there has been an explosion in knowledge about the cells and mediators involved in the allergic tissue response. Prominent in this has been the identification of the T-helper 2 (Th2)-type T cell as the 'orchestrator' of allergic responses [63], culminating in the interleukin (IL)-4- and IL-13-dependent generation of IgE by dedicated follicular B cells and plasma cells, and represents the principal trigger of the allergic response. Allergen-specific IgE is the mechanism through which the acute mast cell/basophil-mediated early response is generated, by cross-linking of IgE bound to its high-affinity receptors ($Fc_\varepsilon R1$). When allergen binds to cell-bound IgE, it undergoes a major conformational change [64] to initiate the secretory response, involving the noncytotoxic release of preformed mediators, cytokines, chemokines and growth factors and the generation of newly formed products, including prostaglandin D_2 and LTC_4, as well as cytokines such as IL-4, IL-5, IL-13, thymic stromal lymphopoietin, tumour necrosis factor-α (TNFα) and a range of chemokines [65].

Although IgE was structurally identified as the fifth immunoglobulin class in 1968 [66], it took a further 35 years before a therapeutic agent targeting IgE was developed. Omalizumab, a noncomplement-fixing IgG mAb binds to the $Fc_\varepsilon 3$ of IgE, thereby blocking the binding to the α-chain of $Fc_\varepsilon R1$ and to the low-affinity receptor $Fc_\varepsilon R2$ (CD23) [67]. The small tri- and hexameric complexes formed are taken up by the reticuloendothelial system and rapidly eliminated. In addition to blocking the IgE-dependent mechanism involved with the acute allergic response, omalizumab blocks IgE-dependent facilitated uptake of allergens by mature myeloid dendritic cells [68] and, through this mechanism, is also able to modify the more chronic allergic response. After administration of intravenous or subcutaneous omalizumab as a once monthly or 2 weekly subcutaneous injection (according to an algorithm calculated from the total serum IgE and bodyweight), free circulating IgE levels fall precipitously, but tissue cell-bound IgE levels decrease more slowly over 12–16 weeks [69]. At this time, the allergen-induced early and late allergic response are both almost ablated, and there is a reduction of the influx of eosinophils into the airways [70]. Clinical trials in adults and children have confirmed clinical efficacy of omalizumab in moderate to severe allergic asthma, but the responses have not been uniform across patients or asthma-related end-points [71]. Over 12 months of treatment in severe allergic asthma, omalizumab exerts a far greater impact on patient-related outcome measures (e.g. asthma control and quality of life) than over lung function (assessed as forced expiratory volume in 1 second or AHR) [72]. In mild to moderate asthma, omalizumab efficacy was shown to be accompanied by a dramatic loss of mast cell-associated IgE and $Fc_\varepsilon R1$ and reductions in eosinophils, T cells and B cells [73].

Another feature of omalizumab treatment is its dramatic life-transforming effect in some patients (~30%), while in others (~30%) only moderate effects have been observed despite there being no apparent differences in asthma phenotypes [71, 74]. For those who response well to omalizumab, the relationship between free serum IgE and asthma outcome measures after beginning and stopping therapy are closely paralleled, whereas for those who do not respond, there is no such relationship during therapy induction or withdrawal [75]. There has been much speculation about why such a variable response exists when targeting the principal activation pathway of the allergic response. Although genetic studies conducted by Novartis failed to find any associations of polymorphisms along the IgE–receptor–signalling pathway that could explain even part of the variability, there may be considerable subtlety in the way in which omalizumab is able to interact with different IgE species in relationship to their affinity for binding allergen [76] and the relative

importance of IgE signalling *vs*. other non-IgE Th2-related inflammatory pathways in the disease [77]. There is clearly a need to understand more about the immunopathology and physiology of the airways in omalizumab responders *vs*. nonresponders and the propensity of the mAb to bind and eliminate subtypes of IgE. Another possible explanation for lack of efficacy in certain subjects is lack of potency/tissue penetration; these possibilities will be testable for the more potent form of anti-IgE (QGE031) currently in clinical development (http://clinicaltrials.gov/ct2/show/NCT01451450).

Targeting T cells in asthma

From extensive observational studies, *in vitro* experiments and animal models, the Th2 cell has been identified as central in driving the allergic response [77]. It is entirely reasonable to consider this as an attractive therapeutic target. Experience with immunosuppressants, such as cyclosporine, tacrolimus, methotrexate, azathioprine and cyclophosphamide, has been mixed [78]. These drugs are usually given as oral corticosteroid-sparing agents, and some patients respond well, with T-cell reductions in airway biopsies [79], whereas other patients experience either severe side-effects or lack of efficacy that precludes their use.

Given that major histocompatibility antigen class II-dependent allergen presentation to the T-cell receptor (CD3) is critical to propagation of the allergic cascade, more specific immunotherapeutics have been tried. Blockade of CD4 with the mAb keliximab initially looked promising for treatment of severe corticosteroid-refractory disease, but while a small clinical trial revealed significant effects on lung function, other asthma outcomes were either minimally affected or unchanged [80], despite finding that over the three doses used the T-cell proliferation in response to allergen was markedly suppressed [81]. More recently, the mAb daclizumab, which is directed at the T-cell activation marker CD25, has been trialled in moderate to severe asthma on the basis of its powerful immunosuppressive effect in organ transplant rejection [82]. While some benefit was shown, this was not statistically significant until 6 months of therapy had been given and, again, was restricted to a limited number of end-points [83]. Immunosuppressive side-effects were also a problem. As CD25 is an important marker for forkhead box P3 (FOXP3) regulatory T cells, there was concern that its blockade with daclizumab might break any allergen-specific tolerance [84]. However, it has now been shown that the apparent decrease in $CD25^+$ regulatory T cells observed with daclizumab therapy reflected lack of detection of the cells, as a result of antibody allosterically blocking the immunoreactive epitopes on the CD25 protein [85]. These somewhat disappointing clinical results for anti-T-cell therapies in chronic asthma are in stark contrast to the efficacy that might be expected if T cells were obligatory in driving the asthmatic response in more severe disease. However, the recognition that a myriad of different T-cell subtypes, as well as Th2 cells, are involved in asthma as it becomes more severe (e.g. Th1, Th9, Th17, Th21, γδT, iKT and $CD8^+$ cells) mitigates against specific T-cell therapies unless it is possible to endotype (i.e. defined by a distinct functional or pathobiological mechanism) asthma better, according to specific causative T-cell subsets [86].

An alternative approach has been to target the proliferation and activation of T cells by interfering with dendritic cell–T cell co-stimulation (the 'second signal' in the immunological synapse). Two targets have come to the forefront: CD28/CD80/82 and OX40/OX40 ligand, the former being regulated by the negative signalling molecule CTLA-4 on T cells [87], the latter by the positive regulator epithelial and mast cell-derived thymic stromal lymphopoietin [88]. The Ig-CTLA-4-Ig fusion protein, abatacept, has proven effective in rheumatoid arthritis (RA) [89], but the response is variable. A clinical trial of CTLA-4-Ig in allergen-induced airway inflammation is in progress (http://clinicaltrials.gov/ct2/show/NCT00784459), while a trial on the use of mAbs to block OX40 ligand has also been completed (http://clinicaltrials.gov/ct2/show/NCT00983658), although the outcome is currently not published.

Cytokines and their receptors as therapeutics

The cluster of cytokines genetically encoded on chromosome 5q31 and secreted by Th2 cells, as well as mast cells and basophils, have been strongly implicated in the causal pathway of the allergic cascade in *in vitro*, animal and human studies. Indeed, this cytokine gene cluster encoding IL-3, IL-4, IL-5, IL-9, IL-13 and granuloctye macrophage-colony stimulating factor (GM-CSF) has almost become synonymous with asthma [90]. It is therefore hardly surprising that each of these cytokines has become the target for new therapeutics, especially biologics.

Interleukin-5 Given that most asthma, whether allergic or non-allergic, is characterized by eosinophilic inflammation of the airways and sputum eosinophilia (which is a sensitive index for assessment of disease control and corticosteroid responsiveness) the factors that influence the influx, maturation and survival of eosinophils are obvious therapeutic targets [91]. Eosinophils mature from $CD34^+$ precursors, both in the bone marrow and resident in the airways, and mature under the influence of IL-3, IL-5 and GM-CSF. However, based on strong evidence from gene-manipulated mice and blocking antibodies in rodents and nonhuman primates [92], IL-5 was selected as the optimal target, in part because of its relatively selective actions on eosinophils in promoting their terminal maturation and survival. Interleukin-5 binds to the IL-5 receptor α chain and signals via a common β chain. Blockade of IL-5, using the IgG1 mAb mepolizumab administered intravenously, had a dramatic effect in almost ablating circulatory and sputum eosinophils in asthma but, somewhat surprisingly,

had no significant effect on the late allergic response [93] following allergen challenge, nor on any clinical outcomes in moderate to severe asthma [94]. Subsequent studies showed that mepolizumab decreased tissue [95] and bone marrow eosinophils [96] by ~50%, possibly because some eosinophils lose their IL-5α chain as they migrate into the airways. Partial depletion of airway eosinophils was argued to be responsible for the unexpected lack of efficacy of mepolizumab in asthma, with mepolizumab-resistant eosinophils being dependent upon other factors for their survival, e.g. GM-CSF.

Recently, two small clinical trials have shown that mepolizumab markedly reduced exacerbations of asthma in patients with severe disease who exhibited persistent sputum eosinophilia and elevated eNO despite moderate- to high-dose oral corticosteroid treatment (representing ~1% of the asthmatic population) [97, 98]. Thus, sputum eosinophilia in refractory asthma could be used as a biomarker for determining anti-IL-5 responsive patients. Similar results have been obtained with a second mAb, reslizumab (CTx55700), which initially showed no overall response in moderate to severe asthma apart from a possible beneficial trend in baseline lung function [99]. In a larger trial of eosinophilic asthma poorly controlled by high-dose ICS, when compared with placebo those receiving reslizumab exhibited improved lung function and a trend towards improved asthma control in parallel with a reduction in sputum eosinophilia [100]. The beneficial response to reslizumab was especially noted in those patients with concomitant nasal polyposis. Indeed, in a separate randomized controlled trial, reslizumab significantly suppressed corticosteroid-resistant polyps in proportion to IL-5 levels in nasal lavage [101]. Given that eosinophil infiltration is common to both asthma and nasal polyposis, a recent small placebo-controlled trial with mepolizumab has likewise shown efficacy against nasal polyposis [102].

As with anti-IgE, a further possible explanation for variable efficacy is incomplete tissue penetration and removal of bioavailable IL-5. The development of an antibody-dependent cell-cytotoxic mAb against the IL-5 receptor α chain by the removal of fucose from the Fc portion of IgG [103] (MEDI 563, Immunex) will definitively test the role of IL-5 signalling and eosinophils in asthma. An initial proof-of-concept and safety study of MEDI 563 administered as single, escalating, intravenous doses (0.0003-3 mg/kg) to patients with mild asthma has demonstrated a dose-dependent reduction in blood eosinophils, with total ablation lasting for 8–12 weeks occurring at the highest dose [104]. At lower doses, circulating eosinophils were reduced by of those remaining; their ability to secrete cytokines and mediators *ex vivo* was markedly reduced.

Interleukin-4 and interleukin-13 Interleukin-4 and IL-13 are of particular interest in asthma based upon the ability of these two cytokines to drive Th2 (IL-4) cell differentiation and activation, to enhance inflammatory responses by upregulating Vascular cell adhesion protein 1 (in concert with TNFα) involved in eosinophil and basophil recruitment (IL-4 and IL-13) [105] and to prime inflammatory cells for secretion of mediators (IL-4 and IL-13) [106]. Interleukin-4 and especially IL-13 also drive aspects of airway remodelling, including mucous metaplasia, fibroblast activation and smooth muscle development [107]. Animal models (especially involving mice) of acute and chronic Th2-type lung inflammation have reinforced the importance of these two cytokines as candidates involved in the pathophysiology of both airway inflammatory and remodelling responses [107]. There is accumulating genetic evidence incriminating these two cytokines in human asthma [108]. Interleukin-4 and IL-13 signal through a complex set of receptor subunits, some of which are shared by the two cytokines (Figure 3). Interleukin-4 binds to IL-4 receptor alpha (IL-4Rα), leading to phosphorylation of signal transducer and activator of transcription factor-6 (STAT-6) by the second subunit, γC (common to the interferon receptors) to activate Janus kinase (JAK) 3. Alternatively, IL-4 can bind to IL-4Rα associated with the IL-13Rα$_1$ rather than the γC chain to activate JAK2 and tyrosine kinsae (TYK) 2 as a way of phosphorylating STAT-6 [109]. Finally, a second IL-13 subunit, IL-13α$_2$, has a higher affinity for IL-13 than the IL-13Rα$_1$ and serves as a decoy, while the greatly shortened cytoplasmic tail of IL-13α$_2$ may interfere with the association or activation of signalling molecules, such as JAK1, on IL-4Rα to provide an inhibitory feedback mechanism [110]. There is also evidence that in certain conditions soluble IL-4Rα has the potential to stabilize binding of IL-13 to its receptor to augment IL-13-mediated responses [111]. Thus, the cellular disposition of IL-4Rα and the IL-13α$_2$ subunit is able to regulate IL-13 agonist signalling activity tightly.

Initial studies using an inhaled formulation of an IL-4R Ig fusion protein (Nuvance) showed initial promise in two small trials [112, 113]; however, in a large phase II study, efficacy in moderate to severe asthma could not be confirmed (http://www.clinicaltrials.gov/ct2/show/NCT00001909). These initial disappointing results might be due to rapid breakdown of Nuvance by proteolysis in the asthmatic airway, although, following inhalation, circulating levels of Nuvance could be detected, as well as activity in induced sputum. Attention, therefore, has moved to systemic administration of anti-IL-4 and anti-IL-13 biologics. The first of these to reach clinical trial was an IL-4Rα double mutein (pitrakinra) involved in both IL-4 and IL-13 signalling [114]. When administered intravenously (and also by inhalation), pitrakinra attenuated the allergen-induced late allergic reaction, as well as reducing sputum and circulating eosinophilia [115]. Trials are now in progress in clinical asthma. It is worth noting, however, that the late allergic reaction was only partially attenuated and there was no effect on allergen-induced AHR. Nevertheless, there were

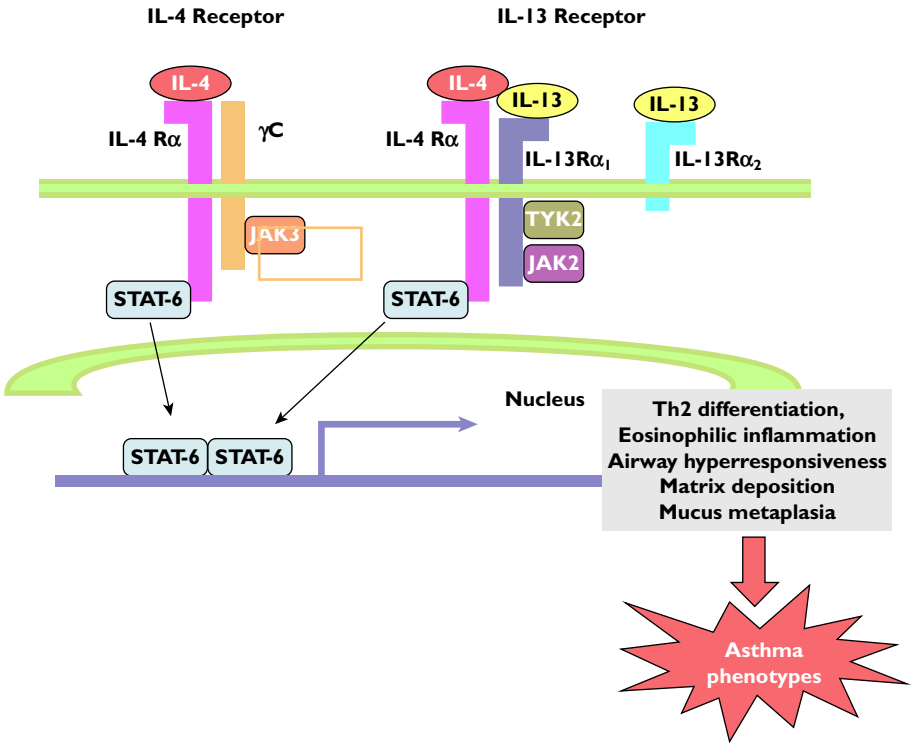

Figure 3

Schematic diagram of the interleukin (IL)-4/IL-13 signal transducer and activator of transcription factor (STAT)-6 signalling pathways linked to T-helper 2 (Th2)-type inflammation. Interleukin-4 and IL-13 are recognized by IL-4Rα, a component of the IL-4 type I (IL-4Rα and γC) and type II receptors (IL-4Rα and IL-13Rα$_1$). Interleukin-4 signals through both type I and type II receptor pathways, whereas IL-13 signals only through the type II IL-4R. Interleukin-13 also binds to the IL-13Rα$_2$ chain with greater affinity, lacking a transmembrane-signalling domain, but functions to interfere with janus kinase (JAK) 2 activation in the IL-4Rα/ IL-13Rα$_1$ complex as well as functioning as a decoy receptor to down-regulate IL-13 signalling. γC activates JAK3, while IL-13Rα$_1$ activates tyrosine kinase 2 (TYK2) and JAK2. Activated JAKs phosphorylate STAT-6 which, upon dimerization, translocates to the nucleus, where it binds to the promoters of the IL-4- and IL-13-responsive genes associated with Th2 cell differentiation, airway inflammation, airway hyperresponsiveness, fibrosis and epithelial mucous metaplasia (Adapted from reference [105]; reproduced with permission of Trends in Immunology)

encouraging results validating the IL-4/IL-13 target in human asthma.

Most of the subsequent attempts to interrupt the STAT-6 pathway have been through mAbs targeting the IL-4Rα (AMG317, Amgen), IL-13 itself (QAX-5676; Novartis, CAT-354; Astra Zeneca/Immunex, IMA-638 and IMA-026; Pfizer/Wyeth, lebrikizumab; Roche, TNX-650; Tanox) and IL-13Rα (Merck) or by using mAb fragments (IL-13, DOM 100P; IL-4/IL-13 DOM-0910; both Zenyth Therapeutics and UCB) [116]. As with pitrakinra, the anti-IL-13 mAb IMA-638 (Pfizer) attenuated the allergen-provoked late allergic reaction, but interestingly, a related mAb, IMA-026, directed to the same target but a different epitope, does not [117]. A search for the reasons for this difference has been informative with regard to how the IL-4/IL-13 receptor complex functions. IMA-638 binds IL-13 in such a way that it still allows it to bind to both the IL-13Rα$_1$ and IL-13Rα$_2$ subunits but inhibits the docking of the IL-4Rα to the IL-13/IL-13Rα$_1$ complex [118]. In contrast, IMA-026 binds to IL-13 at a point that blocks its interaction with IL-13Rα$_1$ and IL-13Rα$_2$. Thus, when compared with IMA-026, the efficacy of IMA-638 in depleting IL-13 indicates that IL-13Rα$_2$ on the cell surface is important for the removal of IL-13. This clearly has implications for the design of any future IL-13 inhibitors.

AMG-317 (Amgen), directed to IL-4Rα, was the first mAb targeting this pathway to enter clinical trial in moderate to severe asthma [119]. Three doses *vs.* placebo were assessed, with the Juniper Asthma Control Questionnaire used as the primary actions measure. No significant change in this or any other asthma end-point was observed, although at the highest tertile of disease severity there was a trend towards improvement in a number of end-points, as well as ~50% reduction in serum total IgE. Based upon the strong preclinical data supporting the STAT-6 pathway as a therapeutic target, this was a disappointing result. The second mAb for which there are reports is CAT-354 (MedImmune, Astra Zeneca), an IgG4 mAb directed to IL-13 [120, 121], again showing no overall benefit in either the Juniper Asthma Control Questionnaire or lung function over a range of three doses [122]. However, in a small subset of patients who had elevated

sputum IL-13 levels measured at baseline, CAT-354 did show efficacy against both end-points, suggesting that the sputum level of IL-13 might be a good biomarker for anti-IL-13 responsiveness.

Identification of biomarkers for Th2 responsiveness

Although there is mounting evidence that eosinophils and cytokines in sputum, as well as eNO, could serve as biomarkers for allergic-type disease of the airways, as well as responses to corticosteroid anti-IgE and LTRA treatment, what are needed are more precise biomarkers of the different types of inflammatory response to help direct treatment to causative pathways. In the past, subphenotyping of asthma has largely been in relationship to disease severity, although classification has included some causal associations, e.g. allergic asthma, aspirin-induced asthma, occupational asthma and reactive airways disease [123]. More recently, nonhierarchical statistical approaches, such as cluster analyses, have been applied to subdivide asthma. Up to six 'endotypes' of adult asthma and four endotypes of childhood asthma have now been identified, but to date none of these has been linked directly to causal pathways, although allergen sensitization, eosinophils and elevated NO predominate in some but not others [124–128].

Given that asthma is primarily an airway disease driven through the epithelium, this structure has provided some of the first insights into disease causality and responsiveness to specific treatments. For example, elevation of the FK506 binding protein (*FKBP51*) gene in epithelial cells has proved to be a highly sensitive marker of ICS sensitivity [129, 130], an observation also confirmed for this target in peripheral blood mononuclear cells when seeking a biomarker for oral corticosteroid responsiveness [131].

Applying this approach to a wider set of Th2-responsive genes, Woodruff and colleagues examined IL-13-responsive genes in epithelial cells obtained from bronchial brushings of asthmatic and normal airways [130, 132]. After examining a wide range of genes, they focused on the following three: *RSNT* (periostin), encoding an epithelial secreted matrix protein; *CLCAI*, encoding a chloride channel involved in mucus secretion; and *SERPINB2*, encoding a plasminogen activator inhibitor type II, on the basis of showing marked IL-13 upregulation and consistency of expression over time [132]. There was also a broad expression of these three genes across the asthmatic population. The asthmatic patients were subdivided into two groups designated $Th2^{high}$ ($IL\text{-}13^{+}$ response) and $Th2^{low}$ (little or no change in the expression level of the 13 responsive genes). $Th2^{high}$ asthmatics had a greater number of circulating eosinophils and bronchoalveolar lavage eosinophilia and, in bronchial biopsies, had increased expression of IL-5, IL-13 and $tryptase^{+}$ mast cells [132, 133]. The $Th2^{high}$ phenotype had great AHR, serum levels of total IgE, thickening of the epithelial basement membrane lamina reticularis and airway mucin (*MUC5AC*) gene expression. In separate studies, *POSTN* gene expression has been shown to correlate closely with thickening of the lamina reticularis [134], and in monolayer epithelial cultures, periostin protein was secreted into the basal medial in response to IL-13 [134, 135]. Periostin activates transforming growth factor-β to drive the secretion of 'repair' collagens, such as type I, by underlying myofibroblasts, as well as cross-linking collagen fibrils, which causes matrix stiffening [134]. Subepithelial matrix deposition is a characteristic feature of asthma as a possible marker of airway wall remodelling in this disease [136]. A proof-of-concept randomized controlled trial of the anti-IL-13 mAb lebrikizumab (Roche) revealed a small but significant improvement in baseline lung function over the 12 weeks of treatment, but it was of considerable interest that this was almost entirely restricted to those patients with elevated serum periostin levels [137]. However, in this trial other asthma-related end-points were not affected, including patient-related outcome measures.

Recently, sputum cells have been used as a source of transcriptomics. In moderate to severe asthma, three gene profiles have been described, one almost identical to the $Th2^{high}$ endotype and two with characteristics of the $Th2^{low}$ endotype, one being dominated by neutrophils and the other macrophages [138]. The neutrophil-dominant endotype had increased expression of IL-1-, TNFα- and nuclear factor-κB-associated genes, indicating activation of oxidant and inflammazone pathways [138], and was associated with greater systemic inflammation, as revealed by elevated circulating C-reactive protein and IL-6 and increased sputum IL-8 and neutrophil elastase and *CXCL-8* gene expression [139].

The identification of TNFα as being overexpressed at both gene and protein levels in severe corticosteroid-refractory asthma in which neutrophils are prominent has led to anti-TNF strategies as potential therapies [140]. While several small trials with the TNF-R1-Ig fusion protein etanercept (Wyeth/Pfizer) looked promising [140–143]), a phase II trial with etanercept in patients with rather less severe asthma on high-dose ICS showed no overall benefit [144]. A further trial in moderate to severe asthma with the anti-TNF mAb golimumab (Centecor) also showed no overall effect over 6 months of treatment, although sub-stratification into those displaying rhinosinusitis and >12% bronchodilator reversibility did identify a dose-dependent responsive subgroup [145]. However, concerns over increased infection have halted further development of this mAb for asthma, even though it is highly efficacious in RA.

Conclusions

Asthma can no longer be regarded as a homogeneous disorder, with increasing evidence for multiple endotypes

now emerging. Beginning with $Th2^{high}$ and $Th2^{low}$ asthma subtypes, it is increasingly clear that different causative pathways will become linked to different disease endotypes. The identification of such novel pathways will provide the opportunity to develop novel animal models beyond the allergen sensitization/challenge (Th2) model [145] and will form the basis for the stratified treatment of this disease, hopefully attacking those pathways high up the causal cascade. What is now required to achieve this is a close collaboration between academia, clinicians and industry to enable careful mapping of these causative pathways onto the distinct clinical, physiological and laboratory phenotypes that occur in humans.

Competing Interests

As sole author, I have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf and declare no support from any organization for the submitted work; I have listed on the form any potential organizations that might have an interest in the submitted work in the previous 3 years. No other relationships exist.

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